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Roles of α- and β-estrogen receptors in mouse social recognition memory: Effects of gender and the estrous cycle

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ABSTRACT

Establishing clear effects of gender and natural hormonal changes during female ovarian cycles on cognitive function has often proved difficult. Here we have investigated such effects on the formation and long-term (24 h) maintenance of social recognition memory in mice together with the respective involvement of α- and β-estrogen receptors using α- and β-estrogen receptor knockout mice and wildtype controls. Results in wildtype animals showed that while females successfully formed a memory in the context of a habituation/dishabituation paradigm at all stages of their ovarian cycle, only when learning occurred during proestrus (when estrogen levels are highest) was it retained after 24 h. In α-receptor knockout mice (which showed no ovarian cycles) both formation and maintenance of this social recognition memory were impaired, whereas β-receptor knockouts showed no significant deficits and exhibited the same proestrus-dependent retention of memory at 24 h. To investigate possible sex differences, male α- and β-estrogen receptor knockout mice were also tested and showed similar effects to females excepting that α-receptor knockouts had normal memory formation and only exhibited a 24 h retention deficit. This indicates a greater dependence in females on α-receptor expression for memory formation in this task. Since non-specific motivational and attentional aspects of the task were unaffected, our findings suggest a general α-receptor dependent facilitation of memory formation by estrogen as well as an enhanced long-term retention during proestrus. Results are discussed in terms of the differential roles of the two estrogen receptors, the neural substrates involved and putative interactions with oxytocin.

Introduction

Apart from their key roles in reproductive behaviors estrogens can influence a number of neural growth, plasticity, learning and memory functions (Maggi et al., 2004). They can, for example, alter the organization and structure (Frankfurt, 1990; Wooley et al., 1990) and function (Gibbs, 1996; Kendrick, 1981; Wooley and McEwen, 1994) of brain areas involved in learning, such as the amygdala and hippocampus. Nevertheless, establishing robust correlations between physiological changes in, or exogenous treatment with, estrogen and cognitive performance has been more difficult. The problem is made more complex since estrogens have a variety of genomic and non-genomic actions (Hall et al., 2001; Toran-Allerand et al., 1999; Wise et al., 2001) and there are issues as to whether dynamic changes in physiological concentrations are important or just overall basal ones (Dohanich, 2002). Under the circumstances it is not surprising that attempts to establish estrogenic effects on cognitive performance have provided conflicting results, varying in both direction and magnitude (Dohanich, 2002). Discrepancies also arise with varying hormonal treatments (Gibbs, 1997) and the use of diverse learning tests involving different memory systems (for review see Daniel, 2006).

A few studies have investigated exogenous or endogenous estrogen effects on social recognition memory in female rodents. In rats, a proestrus facilitation of long-term (5 h) social recognition memory has been reported, but only following vaginocervical stimulation (Larrazolo-Lopez et al., 2008). Exogenous estradiol (E_2) treatment has been reported to prolong social recognition to 2 h in ovariectomized group-housed rats (Hlinak, 1993) and 24 h in ovariectomized mice (Tang et al., 2005). These effects are also dependent upon hormone dose and length of treatment. A recent study has also found enhanced habituation in a habituation/dishabituation social recognition paradigm in ovariectomized rats treated with estrogen and progesterone (Spiteri and Ågmo, 2009).

With other learning paradigms no specific proestrus facilitation effects have been reported, although in rats impaired spatial memory has been found in either proestrus (Warren and Juraska, 1997) or estrus (Healy et al., 1999) stages. In estrus, mice impaired learning in a footshock avoidance paradigm or spatial learning in the Morris maze (Frick and Berger-Sweeney, 2001) have also been reported. It is possible, however, that these results may have more to do with increased sensitivity to stress during proestrus/estrus in these types of

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tasks. Dose-related effects of exogenous treatments with E_2 on spatial memory and other learning and memory tasks have also been reported, sometimes with contradictory results (Di Paolo et al., 1985; Frick et al., 2002; Fugger et al., 1998; Leuner et al., 2004; Rissanen et al., 1999; Sawada et al., 1998). This dose-dependency could reflect an adaptation of the brain to naturally occurring fluctuations in estrogen levels during the ovarian cycle or pregnancy (Cyr et al., 2002; Rado et al., 1970). Differing hormonal levels may also influence learning strategies involved (Korol, 2004; Zurkovsky et al., 2007), with exogenous treatments producing high estrogen levels being associated with impaired learning whereas those producing lower ones with its facilitation (Holmes et al., 2002; Wide et al., 2004).

A further consideration is the roles of each of the two nuclear estrogen receptors, alpha (αER) and beta (βER), which are differentially expressed in brain areas associated with cognitive processing (Mitra et al., 2003; Osterlund et al., 1998; Shughrue et al., 1997a,b). This is further complicated by the fact that expression patterns of estrogen receptors can vary with different hormonal states (Alves et al., 1998; Greco et al., 2001; Nomura et al., 2003; Osterlund et al., 1998; Patisaul et al., 1999). Both male and female mice lacking functional expression of the βER show impaired learning on hippocampal-dependent spatial tasks such as the Morris water maze (Rissman et al., 2002), and do not show estradiol-dependent facilitation of long-term potentiation (Liu et al., 2008). Also, βER agonists can facilitate hippocampal long-term potentiation (LTP) and performance on spatial memory tasks in mice and rats (Liu et al., 2008). On the other hand, αER knockout mice do not show spatial memory deficits and have normal estradiol-dependent facilitation of LTP, and αER agonists do not facilitate spatial memory in rats or mice (Liu et al., 2008; Rissman 2008). Indeed, in some respects the two receptors may even be antagonistic in terms of estrogenic actions on hippocampal function and learning (Rissman 2008).

On the other hand, both αER and βER knockout male and female mice have been shown to have deficits in a short-term memory social recognition task based on a habituation/dishabituation paradigm (Choleris et al., 2003, 2004), although those in βER knockout mice are slightly less severe (Choleris et al., 2006). In the social recognition memory task estrogen, through activation of its receptors, is proposed to influence memory through modulating oxytocin (OT) and its receptor (OTR), with OT knockout animals showing similar social recognition memory deficits (Choleris et al., 2003, 2006; Ferguson et al., 2000). From these findings an interacting network involving four genes coding for OT, OTR, αER and βER has been proposed (Choleris et al., 2003, 2004). Estrogens may be acting on the OT system at a number of levels: through βER they regulate the production of OT in the hypothalamic paraventricular nucleus (Nomura et al., 2002; Shughrue et al., 1999, 2002), and through activation of αER they drive the transcription of OTR in the amygdala (Dekloet et al., 1986; Shughrue et al., 1999, 2002; Young et al., 1998). In the olfactory pathways involved in rodent social recognition, olfactory stimuli detected by the main and vomeronasal receptors are initially processed by the main and accessory olfactory bulbs which then project to the cortical and medial amygdala where OT via the OTR has been shown to be important for formation of social recognition memory (Choleris et al., 2007; Ferguson et al., 2001). There are also α and βER (Mitra et al., 2003) and OT and OTR (Broad et al., 1993, 1999; Yoshimura et al., 1993) in the main olfactory bulb and the action of OT within the olfactory bulb is important from prolonging the duration of social recognition following vaginocervical stimulation in proestrus female rats (Larrazolo-Lopez et al., 2008).

Previous studies using social recognition memory paradigms to investigate the roles of ERα and β have only used habituation/dishabituation testing protocols (Choleris et al., 2003, 2006; Gheusi et al., 1994; Imwalle et al., 2002) where a short-term memory (<60 min) for individuals is tested, and no ovarian cycle-dependent effects have been established. Possible gender differences in dependency on the different receptors have also not been considered. In mice, the duration of social recognition memory can be as long as 7 days in group-housed animals and that protein synthesis and cyclic AMP responsive element binding protein (CREB) function are necessary for the long-term form of this social memory (Kogan et al., 2000; Richter et al., 2005). Its consolidation also has two protein synthesis-dependent phases (Wanisch and Wotjak, 2008).

The present studies have therefore aimed to establish the influence of gender and the ovarian cycle on the formation and duration of social recognition memory and the respective roles of α and βERs for both the short-term and long-term components of this form of memory.

Materials and methods

Animals

All animal experiments received local Ethical Committee approval and were carried out under license in full compliance with the UK Animals (Scientific Procedures) Act, 1986. Homozygous αERKO and βERKO and wildtype control (C57/Bl6×129SV) mice were bred and housed in a full specific pathogen free barrier facility at The Babraham Institute. Founders of both ERKO colonies were kindly provided by Dr. Korach, NIH-NIEHS (USA). The ERKO mice had been backcrossed 10× onto a C57/Bl6 background. Mice were housed under temperature and humidity-controlled conditions with a 12 h light–12 h dark cycle (lights on at 07.00 h), with food (irradiated CRM(P) diet supplied by SDS, UK) and water available *ad libitum*. The diet used has very low levels of phytoestrogens (Genisten — 141 ppm, Daidzein — 78 ppm, and Coumestrol — undetectable) and well below those which might have produced any biological activity (Owens et al., 2003). Transgenic animals were bred from heterozygous male and females (trios of 1 male and 2 females) and were genotyped by PCR of tail biopsy DNA using a standard protocol (Couse et al., 2003). After weaning, around 31 days old, mice were housed in same-sex groups of between two to five animals in standard racks of M3 plastic cages containing enrichment aids (nesting material and fun tunnels) and were cleaned-out once a week.

16 αERKO and 13 αWT littermate, 18 βERKO and 14 βWT littermate intact adult male mice and 13 αERKO and 28 αWT littermate, 32 βERKO and 33 βWT littermate intact adult female mice were used in the experiments. Due to limited availability of ERKO mice and the fact that they might not be optimal socially attractive stimuli (Ågmo et al., 2008; Kavaliers et al., 2004) we used 24 male and 38 female gonadally intact C57/Bl6×129SV adult mice as stimulus animals (this strain has been successfully used as stimulus animals in this paradigm in our lab). At testing, all animals were 3–11 months old and test animals were weighed and handled for at least five days before testing commenced. Their cages were cleaned on the day prior to testing in order to avoid any stress effects on the test day and also to ensure a similar home cage background social odor intensity environment.

Smears were made daily (between 08.00 and 09.30) with sterile saline-soaked cotton swabs (toothpicks with the blunt end wrapped in a small amount of cotton wool) being used to harvest cells from the vaginal opening (swab sticks are gently twisted in the vaginal opening to remove cells and then rolled across glass slides for histological staining). Vaginal smears were taken from animals for at least five days before testing to confirm the presence of an ovarian cycle. Females that did not show a complete cycle during this period of smear testing were not used (about 1 in 8 females). Stages of the ovarian cycle were determined by microscopic analysis of vaginal cytology after staining with 1% toluidine blue (w/v) and using the guidelines in Allen (1922). Proestrus was determined by predominantly round nucleated epithelial cells that could be either dispersed or clumped, estrus by primarily the presence of only non-nucleated cornified cells and diestrus/metaestrus by a predominance of leukocyte cells with a few scattered epithelial