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Estrogens and their receptors in the medial amygdala rapidly facilitate social recognition in female mice

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ABSTRACT

Estrogens have been shown to rapidly (within 1 h) affect learning and memory processes, including social recognition. Both systemic and hippocampal administration of 17β-estradiol facilitate social recognition in female mice within 40 min of administration. These effects were likely mediated by estrogen receptor (ER) α and the G-protein coupled estrogen receptor (GPER), as administration of the respective receptor agonists (PPT and G-1) also facilitated social recognition on a rapid time scale. The medial amygdala has been shown to be necessary for social recognition and long-term manipulations in rats have implicated medial amygdalar ERα. As such, our objective was to investigate whether estrogens and different ERs within the medial amygdala play a role in the *rapid* facilitation of social recognition in female mice. 17β-estradiol, G-1, PPT, or ERβ agonist DPN was infused directly into the medial amygdala of ovariectomized female mice. Mice were then tested in a social recognition paradigm, which was completed within 40 min, thus allowing the assessment of rapid effects of treatments. 17β-estradiol (10, 25, 50, 100 nM), PPT (300 nM), DPN (150 nM), and G-1 (50 nM) each rapidly facilitated social recognition. Therefore, estrogens in the medial amygdala rapidly facilitate social recognition in female mice, and the three main estrogen receptors: ERα, ERβ, and the GPER all are involved in these effects. This research adds to a network of brain regions, including the medial amygdala and the dorsal hippocampus, that are involved in mediating the rapid estrogenic facilitation of social recognition in female mice.

1. Introduction

Social recognition is the ability of an animal to distinguish conspecifics, a fundamental skill for social species and one that is known to be facilitated by estrogens (rev. in Gabor et al., 2012). Estrogens exert their effects through at least three receptors, estrogen receptor (ER) α, ERβ, and the G-protein coupled estrogen receptor 1 (GPER). These effects can occur on both long-term and short-term timescales. The long-term, genomic effects take place over hours to days and involve direct ER regulation of gene transcription (rev. in Nilsson et al., 2001), whereas the short-term, non-genomic effects occur within minutes to hours and involve rapid changes to cell signaling cascades (rev. in Sheppard et al., 2017; Srivastava et al., 2011).

Estrogens have been shown to facilitate social recognition in female mice (Sánchez-Andrade and Kendrick, 2011; Tang et al., 2005) and rats (Hlinák, 1993; Spiteri and Ågmo, 2009). Studies with global knockout (KO) mice have consistently shown that ERαKO female mice have impaired social recognition, regardless of the paradigm used for testing (Choleris et al., 2003, 2006; Sánchez-Andrade and Kendrick, 2011), suggesting ERα is necessary for social recognition. In contrast, ERβKO mice showed impaired (Choleris et al., 2003), or not impaired (Choleris et al., 2006; Sánchez-Andrade and Kendrick, 2011) social recognition depending on the social recognition task employed, suggesting that ERβ may play a lesser role in social recognition than ERα (rev. in Ervin et al., 2015). To our knowledge, no research has been done implicating the GPER in social recognition on a longer-term time scale.

On a rapid time scale, systemic administration of 17β-estradiol improved social recognition in ovariectomized (OVX) female mice within 40 min of administration (Phan et al., 2012). This effect appears to be mediated by ERα and the GPER as systemic administration of an ERα agonist, PPT (Phan et al., 2011), or the GPER agonist, G-1 (Gabor et al., 2015) improved social recognition in OVX female mice within 40 min, whereas administration of an ERβ agonist impaired it (Phan et al., 2011). Therefore, social recognition appears to be rapidly facilitated by ERα and the GPER. Which brain regions might be involved in mediating these effects is only beginning to be investigated.

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Dorsal hippocampal administration of 17β-estradiol improved social recognition within 40 min of administration in OVX female mice (Phan et al., 2015). Similarly, dorsal hippocampal infusion of selective ERα (Phan et al., 2015) and GPER agonists (Lymer et al., 2017) also facilitated social recognition. However, a smaller dose range was effective in the dorsal hippocampus than with systemic treatment (Phan et al., 2011, 2015). In addition, when tested in an environment with minimal spatial and contextual cues, dorsal hippocampal administration of 17β-estradiol did not improve social recognition, whereas systemic administration of 17β-estradiol did (Phan et al., 2013). These results suggest that the estrogenic facilitation of social recognition in the absence of spatial cues is mediated by a brain region outside the hippocampus. In contrast, dorsal hippocampal administration of the GPER agonist, G-1 did improve social recognition in the absence of most spatial and contextual cues (Lymer et al., 2017). However, this effect appears to be less robust than that of the hippocampal GPER-mediated improvements in performance in social recognition in the home cage, where spatial and contextual cues are present, as a higher dose of G-1 was needed to see improvements in social recognition with minimal spatial cues (Lymer et al., 2017). Therefore, the GPER-mediated improvements in social recognition with systemic G-1 activation (Gabor et al., 2015) might be mediated by the dorsal hippocampus in combination with other brain regions.

A brain region that has been implicated in social recognition is the medial amygdala. Lesions to the medial amygdala impair social recognition in mice (Wang et al., 2014) and c-fos is upregulated in the medial amygdala following exposure to a social stimulus (Ferguson et al., 2001). Additionally, ERα, ERβ, and the GPER are expressed in the medial amygdala (Mitra et al., 2003; Hazell et al., 2009) and medial amygdala ERα has been shown to be necessary for the estrogenic facilitation of social recognition in OVX female rats in a study with prolonged inhibition of ERα gene expression (Spiteri et al., 2010). Whether the medial amygdala is also involved in the estrogenic facilitation of social recognition via rapid mechanisms is unknown.

The objective of this study is to investigate the role of estrogens and their receptors in the medial amygdala, in the rapid facilitation of social recognition in OVX female mice. To do this, we infused 17β-estradiol, the GPER agonist G-1, the ERα agonist PPT, or the ERβ agonist DPN directly into the medial amygdala and tested the mice in the social recognition paradigm as previously described (Gabor et al., 2015; Lymer et al., 2017; Phan et al., 2011, 2012, 2015). The social recognition paradigm was completed 40 min after treatment administration to investigate the rapid, improving effects of estrogens.

2. Methods

2.1. Subjects

Subjects were female CD1 mice (*Mus musculus*), purchased at 2 months of age (Charles River, QC). Mice were triple housed upon arrival and single-housed following ovariectomy and medial amygdala bilateral cannula implantation surgeries. Subjects were housed with corncob bedding and environmental enrichment in clear polyethylene cages (16 × 12 × 26 cm), on a 12:12-h reversed light/dark cycle (lights off at 0800 h) and received rodent chow (Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Teklad, WI) and water ad libitum. Ambient temperature was 21 ± 1C. All behavioural tests were conducted 10–15 days after surgery in the home cage during the dark phase of the light cycle under red light illumination. To establish a home cage territory, cages were not cleaned for at least 3 days prior to testing. Mice were moved into the testing room the night before the experiment and vaginal smears were taken and collected on microscope slides to ensure the ovariectomy was complete. Giemsa Stain (Sigma-Aldrich Canada Ltd., Oakville, ON) was used to stain the slides. The estrous phase of each mouse was then determined using descriptions and images by Byers et al. (2012). Two mice were removed because they were found to be in proestrus or estrus. Research was conducted in accordance with the Canadian Council on Animal Care and approved by University of Guelph's Animal Care and Use Committee.

2.2. Ovariectomy and cannulation surgeries

All mice were ovariectomized as described by Clipperton-Allen et al., (2012) and had bilateral guide cannulae (HRS Scientific, Montreal, QC) implanted 1 mm above the medial amygdala. Ovariectomy and cannulation surgeries were performed simultaneously (as described previously in Lymer et al., 2017; Phan et al., 2015) while mice were anesthetized with isoflurane (CDMV, St. Hyacinthe, QC) and after they had received systemic carprofen analgesic (50 mg/kg; Rimadyl, Pfizer Canada Inc., Kirkland, QC, Canada) and local lidocaine/bupivacaine (0.02 mL 0.17% bupivacaine [Hospira, Inc., Montreal, QC, Canada] and 0.67% lidocaine [Alveda Pharmaceuticals, Toronto, ON, Canada]) anaesthetic treatments. For the ovariectomy surgery, the lower back of the mouse was shaved, cleaned, and a 1 cm incision was made in the skin. On each side, 0.5 cm incisions were made in the muscles overlying the ovaries and each ovary was drawn out of the incision. The uterus was clamped just below the ovary and the ovary was then removed by cutting just above the clamp. The uterus was placed back into the abdominal cavity and the incision was stapled with 1 or 2 MikRon autoclip 9 mm wound clips (MikRon Precision Inc, Gardena, CA). For the cannulation surgery, an incision was made in the skin on the dorsal surface of the head and two holes were drilled into the skull for the guide cannulas, 2.5 mm lateral to the sagittal suture, each 1.5 mm posterior to Bregma. The injector (HRS Scientific, Montreal, QC) extended 1 mm below the guide cannula, reaching 5.3 mm ventrally and into the medial amygdala. The guide cannulae were held in place with Lang Jet Repair Acrylic (Central Dental, Scarborough, ON). Two additional holes, one in each parietal bone, were drilled to insert jeweler's screws (HRS Scientific, Montreal, QC) to anchor the dental cement in place.

2.3. Drugs

Mice received a bilateral medial amygdalar microinfusion of 17β-estradiol (Sigma-Aldrich, Oakville, ON, Canada), the GPER agonist G-1 (Tocris Biosciences, UK), the ERα agonist PPT (Sigma-Aldrich, Oakville, ON, Canada), or the ERβ agonist DPN (Sigma-Aldrich, Oakville, ON, Canada). The agonists each have a lower relative binding affinity to their respective receptors than 17β-estradiol. PPT has 50% relative ligand binding affinity to ERα compared to 17β-estradiol (Stauffer et al., 2000), DPN has 18% affinity to ERβ compared to 17β-estradiol (Carroll et al., 2012), and G-1 has an affinity for GPER of approximately 3 fold less than 17β-estradiol (Bologa et al., 2006). The vehicle was artificial cerebral spinal fluid (aCSF, 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4 \cdot 7H_2O$, 2.5 mM $CaCl_2$, 1 mM NaH_2PO_4, 26 mM Na_2HCO_3, and 11 mM Glucose in water) and 0.02% ethanol (as in Phan et al., 2015 and Lymer et al., 2017). The first experiment included five treatment groups for 17β-estradiol: vehicle, 10 nM, 25 nM, 50 nM, and 100 nM (0 pg, 1.362 pg, 3.405 pg, 6.810 pg, 13.62 pg per hemisphere respectively). Five other treatment groups were used for the G-1 study: vehicle, 25 nM, 50 nM, 200 nM, and 400 nM (0 pg, 5.154–82.456 pg per hemisphere). The treatment groups for the PPT study included: vehicle, 25 nM, 50 nM, 100 nM, 150 nM, and 300 nM (0 pg, 4.831–57.966 pg per hemisphere). Finally, the DPN treatment groups included: vehicle, 50 nM, 100 nM, and 150 nM (0 pg, 5.982–17.945 pg per hemisphere). The treatment (0.5 μL) was microinfused into the brain at a rate of 0.2 μL/min through each injector using a Harvard infusion pump (PHD 2000). Treatments were assigned using a random number generator. Each mouse received only one treatment and all animals were experimentally naïve.

After behavioural testing was completed, the mice received a bilateral medial amygdalar microinfusion of 1% Chicago Sky Blue dye