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Estradiol-induced enhancement of fear extinction in female rats: The role of NMDA receptor activation

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ABSTRACT

Converging cross-species evidence indicates that fear extinction (the laboratory basis of exposure therapy for anxiety disorders) in females is modulated by endogenous and exogenous estradiol. The mechanisms underlying estradiol's influences on fear extinction are largely undefined. However, one likely candidate is the NMDA-receptor (NMDAr), activation of which is necessary for estradiol-mediated enhancements in structural and functional neural plasticity, as well as extinction consolidation in males. Here, we demonstrate that systemic co-administration of the non-competitive NMDAr antagonist, MK801, blocked the enhancement of fear extinction by systemic estradiol in ovariectomized rats. In intact rats, MK801 during diestrus (rising estradiol) prevented the enhancement in extinction recall in rats that received extinction training during proestrus (peak estradiol). Systemic administration of the partial NMDAr agonist D-cycloserine (DCS) prior to extinction training facilitated extinction in ovariectomized rats, mimicking the effects of estradiol. In intact rats, DCS administered on the afternoon of proestrus and the morning of estrus (declining estradiol) facilitated extinction in rats that received extinction training during metestrus (low estradiol). Finally, DCS also facilitated extinction in ovariectomized rats when administered immediately after extinction training. Combined, these findings suggest that endogenous and exogenous estradiol enhance fear extinction via NMDAr-dependent mechanisms. Moreover, these findings raise the possibility that fear extinction deficits during periods of low endogenous estradiol levels can be reversed by increasing NMDAr activation via DCS administration, either well prior to, or immediately after, extinction training.

1. Introduction

Anxiety disorders are more prevalent, burdensome, and associated with poorer treatment outcomes, in women relative to men (Li and Graham, 2017; McLean et al., 2011). Despite this, current neurobiological theories of fear extinction, the learned reduction in fear that occurs following repeated exposure to a fear-eliciting conditioned stimulus, are founded on preclinical research that has mostly been conducted in males (Lebron-Milad and Milad, 2012). Studies of extinction and the pharmacological adjuncts that augment it provide important insights about how to improve exposure therapy, the recommended psychological treatment for anxiety disorders that was based on extinction (Graham and Milad, 2011). It is therefore critical that contemporary models of extinction are informed by studies conducted in females.

The sex hormones estradiol and progesterone substantially modulate extinction in females. Extinction is enhanced during phases of the reproductive cycle characterized by high hormonal levels, and impaired during low hormonal phases, an effect observed in rats, non-anxious women, and clinically anxious women (reviewed in Glover et al., 2015; Li and Graham, 2017). Furthermore, estradiol administration, or pharmacological enhancement of estrogen receptors (at the time of extinction training), facilitates extinction in rats and women (Graham and Milad, 2013; Graham and Scott, 2018; Zeidan et al., 2011). Finally, we have recently demonstrated that higher estradiol levels during exposure therapy for spider phobia are associated with improved treatment efficiency and effectiveness, suggesting that the relationship between estradiol and extinction in laboratory studies translates comparably to clinical treatment settings (Graham et al., 2018). Estradiol enhancements in fear extinction are associated with alterations in ventromedial prefrontal cortex (vmPFC), hippocampus, and amygdala activity, key components of the extinction neurocircuitry (Zeidan et al., 2011). Beyond this, the mechanisms underlying hormonal modulation of extinction are poorly understood. The acute effects of estradiol on extinction are likely driven by rapid facilitation of cell signaling, long-term potentiation (LTP), and epigenetic modifications supporting

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memory consolidation (Frick, 2015). However, cyclic fluctuations in extinction may also be attributable to longer-term effects of estradiol that are subject to biphasic regulation by progesterone, as highlighted by our recent study in which we subjected ovariectomized (OVX) rats to a hormonal replacement regime mimicking cyclic fluctuations in sex hormones (Graham and Daher, 2016). We demonstrated that two treatments of estradiol alone facilitated extinction, whereas progesterone augmented and then attenuated estradiol's effects in a time-dependent manner. As extinction training took place 48–72 h following estradiol treatment, the augmentation of extinction could not be attributed to rapid effects of estradiol on cell signaling processes. Moreover, in intact rats, pharmacological inactivation of the progesterone receptor during proestrus (peak estradiol and progesterone) maintained extinction at optimal levels 48 h later during metestrus (basal estradiol and progesterone), when extinction is typically impaired. This suggests that, when unopposed by progesterone, cyclic increases in estradiol facilitate long-term enhancements in extinction that are sustained after estradiol levels decline.

One candidate pathway by which estradiol could enhance extinction is via the *N*-methyl-ᴅ-aspartate receptor (NMDAr). Estradiol facilitates NMDAr binding, phosphorylation, and transmission in the hippocampus and PFC (Bi et al., 2001; Galvin and Ninan, 2014; Smith and McMahon, 2005; Weiland, 1992; Woolley et al., 1997), and pharmacological blockade of NMDAr prevents estradiol's impact on brain functioning (e.g., facilitated LTP; Smith and McMahon, 2006; Smith et al., 2016) and morphology (e.g., augmented dendritic spine density; Woolley and McEwen, 1994; Smith and McMahon, 2005). Extensive evidence demonstrates that extinction in male rats is dependent on NMDAr activation within the mPFC, hippocampus, and amygdala (reviewed in Singewald et al., 2015); surprisingly, the role of NMDAr in extinction in females has not been systematically examined. However, estradiol's enhancement of other kinds of learning, such as object recognition, is NMDAr-dependent (Lewis et al., 2008; Vedder et al., 2013). Therefore, the present study examined the role of NMDAr in the long-term effects of estradiol on extinction in female rats. Similar to our past work (Graham and Daher, 2016), we took a two-pronged approach in which, to achieve optimal experimental control, initial experiments examined the role of NMDA in extinction in OVX rats treated with an estradiol replacement regime that mimics natural increases in estradiol during the estrous cycle (Woolley and McEwen, 1993). We subsequently validated these outcomes in intact, cycling rats to examine the role of NMDA in extinction during natural increases in estradiol during the estrous cycle. Experiment 1 assessed whether enhanced extinction in estradiol-treated OVX rats was blocked by co-administration of the NMDAr antagonist, MK801, 48 h prior to extinction training. Experiment 2 assessed whether enhanced extinction in intact rats extinguished during proestrus (peak estradiol) was blocked by MK801 administered 24 h prior to extinction training during diestrus (rising estradiol). Experiment 3 assessed whether the partial NMDAr agonist, D-Cycloserine (DCS), mimicked estradiol's effects on extinction in OVX rats when administered 48 h prior to extinction training. Experiment 4 assessed whether extinction was enhanced during metestrus (basal estradiol) by administering DCS on the afternoon of proestrus, and the morning of estrus (declining estradiol), 24 h prior to extinction training. Note that in experiments involving OVX rats (i.e., 1 and 3), extinction training was timed to take place 48 h after hormone and drug treatments because past research has demonstrated that the effects of estradiol on extinction (Graham and Daher, 2016) and brain morphology/functioning (Smith and McMahon, 2005; Woolley and McEwen, 1993) are evident in OVX rats at this time point. Thus, the timing of treatments in OVX rats mimicked the effects of the natural rise in estradiol during proestrus in intact rats. Combined, Experiments 1–4 demonstrated long-term effects of NMDAr modulation on extinction. To determine whether NMDAr modulation also has immediate effects, Experiment 5 assessed whether DCS administered immediately after extinction training enhanced extinction recall in OVX rats.

2. Materials and methods

2.1. Subjects

Experimentally naïve Sprague Dawley-derived female rats, aged 10–12 weeks, obtained from a commercial supplier (Animal Resources Centre, Perth, Australia), were used. Rats were housed in groups of eight in plastic cages (67 × 30 × 22 cm) in a 20–22 °C colony room. They were maintained on a 12 h light-dark cycle (lights on at 7 am) with food and water available ad libitum. In Experiments 1, 3 and 5 rats received bilateral ovariectomy, and in Experiments 2 and 4 vaginal smears were conducted daily (between 8 and 10 am) to determine estrous cycle phase in intact rats; as detailed previously (Graham and Daher, 2016). Procedures were approved by the Animal Care and Ethics Committee at UNSW Australia and followed guidelines outlined in *The Australian Code Of Practice For The Care And Use Of Animals For Scientific Purposes* (8th edition, 2013).

2.2. Pharmacological manipulations

2.2.1. Estradiol

In Experiment 1, OVX rats received two s.c. injections of β-Estradiol, spaced 24 h apart (Sigma-Aldrich, Australia; 10 μg in 150 μL sesame oil), or an equivalent volume of sesame oil. We have previously demonstrated that this quantity and dose, which mimic the rise in natural estradiol across the cycle (Woolley and McEwen, 1993) augment extinction in OVX rats (Graham and Daher, 2016).

2.2.2. MK801

In Experiments 1 and 2, OVX and intact rats received s.c. injections of MK801 dissolved in saline (Sigma-Alrich, Australia; at a dose of 0.2 mg/kg of body weight, in a volume of 1 mL/kg of body weight), or an equivalent volume of saline. This dose counteracts the impact of estradiol treatment (identical to that described above) on NMDAr transmission, LTP, and CA1 dendritic spine density in OVX rats (Smith and McMahon, 2005; Woolley and McEwen, 1994).

2.2.3. D-Cycloserine (DCS)

In Experiments 3, 4, and 5, OVX and intact rats received s.c. injections of DCS dissolved in saline (Sigma-Alrich, Australia; at a dose of 15 mg/kg of body weight, in a volume of 1 mL/kg of body weight). This dose enhances extinction in male rats (reviewed in Singewald et al., 2015).

2.3. Apparatus

2.3.1. Conditioning and extinction chambers

Two sets of four Med Associates experimental chambers, designated Context A and B, were used for conditioning, extinction training, and recall. Contexts differed in visual and tactile features, and were identical to those described previously (Graham and Daher, 2016; Milligan-Saville and Graham, 2016).

2.3.2. Conditioned and unconditioned stimuli

The CS was a white noise (4 dB above background noise) delivered through the sidewall speaker, and the US was a scrambled foot-shock (1 s, 0.6 mA) delivered through the floor.

2.4. Procedure

2.4.1. Handling and context pre-exposure

Rats were handled for 4–5 min each day for three consecutive days; after handling on each day rats were individually placed in Context A for 10 min.