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Effects of systemic estradiol on fear extinction in female rats are dependent on interactions between dose, estrous phase, and endogenous estradiol levels

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ABSTRACT

Administering estradiol to females during periods of low endogenous estradiol enhances their ability to extinguish fear, the laboratory basis of exposure therapy for anxiety disorders. It has therefore been proposed that estradiol could be a useful adjunct to enhance exposure therapy outcomes. The present study aimed to clarify the boundary conditions under which estradiol could be used for this purpose, by assessing whether the impact of estradiol, administered systemically prior to extinction training, differs depending on dose and estrous phase in adult female rats. Results demonstrated that in rats extinguished during metestrus (naturally low estradiol), a low dose of estradiol reduced freezing during extinction training and augmented extinction recall the following day, whereas a high dose of estradiol had no effect on either extinction training or recall. In rats extinguished during proestrus (naturally high estradiol), a high dose of estradiol impaired extinction recall, whereas a low dose of estradiol had no effect, or impairing effects, on extinction recall in different experiments. A subsequent analysis revealed that estradiol-treated proestrus rats that exhibited impaired extinction recall had significantly higher pre-treatment serum estradiol levels than those that exhibited good extinction recall. Together, these results indicate that systemically administered estradiol interacts with endogenous estradiol to produce an inverted U shaped dose effect on fear extinction, where low and high estradiol levels lead to poor extinction recall, and moderate estradiol levels lead to good extinction recall. These results highlight potential limitations to the use of estradiol as an adjunct to exposure therapy in clinical settings.

1. Introduction

Anxiety disorders are the sixth leading cause of disability (Baxter et al., 2014), and are highly prevalent, with current global estimates indicating that one in nine people will experience an anxiety disorder in any given year (Baxter et al., 2013). Cognitive behavioral therapy (CBT) is the recommended first-line psychological treatment for anxiety disorders (Craske and Stein, 2016). A fundamental component of CBT is exposure therapy, during which the individual gradually confronts fear-eliciting stimuli and situations without avoidance until anxiety reduces. Although exposure therapy is successful for many, a proportion of individuals fail to show complete symptom remission or exhibit symptom relapse over time (DiMauro et al., 2013; Holmes et al., 2014), demonstrating a need to improve treatment efficacy for those who are less responsive.

Fear extinction is a laboratory procedure that forms the basis of exposure therapy, and therefore holds great translational value as a means of advancing the treatment of anxiety (Graham and Milad, 2011). In this procedure, subjects are first trained to fear a conditioned stimulus (CS; e.g., a noise) that is paired with an aversive unconditioned stimulus (US; e.g., shock). Extinction training involves repeatedly presenting the CS without the US until fear responses reduce. Long-term recall of extinction is indicated by low fear responses when the extinguished CS is presented the next day. Rodent studies of fear extinction have led to the identification of numerous pharmacological agents that enhance the acquisition of extinction learning, the consolidation of the extinction memory, or both, several of which have been demonstrated to augment exposure therapy in human clinical trials (Singewald et al., 2015). Such findings represent a new era of drug discovery, in which rather than using pharmacological agents that mask psychiatric symptoms (and often counteract the benefit of psychological treatments), the focus has shifted towards the development of agents that augment the underlying processes of CBT (Graham et al., 2014).

One such agent that is receiving increasing attention is the sex hormone estradiol. Accumulating evidence suggests that estradiol

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modulates fear extinction. For example, female rats and non-anxious women exhibit poor extinction recall if they receive extinction training in the metestrus phase of the estrous cycle (rats) and the early-follicular phase of the menstrual cycle (humans), when estradiol levels are low, and they exhibit good extinction recall if they receive extinction training in the proestrus phase (rats) or late-follicular/mid-luteal phase (humans), when estradiol levels are high (Chang et al., 2009; Graham and Milad, 2013; Graham and Daher, 2016; Gruene et al., 2015; Milad et al., 2009, 2010; Milligan-Saville and Graham, 2016; Pineles et al., 2016; Rey et al., 2014; White and Graham, 2016; Wegerer et al., 2014; Zeidan et al., 2011). A similar relationship between endogenous estradiol levels and extinction outcomes has been reported in women with spider phobia (Li and Graham, 2016), and posttraumatic stress disorder (PTSD; Glover et al., 2012, but see Pineles et al., 2016, who reported that a combination of high progesterone and low estradiol predicted poorer extinction recall in women with PTSD). Systemic administration of estradiol prior to or immediately after extinction training abolishes extinction impairments in female rats and non-anxious women (Graham and Milad, 2013; Zeidan et al., 2011), whereas an estrogen receptor antagonist prior to extinction training impairs extinction in female rats (Milad et al., 2009), suggesting that fear extinction depends on estrogen receptor activation. Similarly, hormonal contraceptives, which suppress endogenous estradiol, are associated with impaired extinction in female rats and non-anxious women (Graham and Milad, 2013), and reduced discrimination between threat and safety cues following extinction in non-anxious women (Lonsdorf et al., 2015). In rats, administration of estrogen receptor agonists prior to extinction training abolishes hormonal contraceptive-associated impairments in extinction recall (Graham and Milad, 2013). Estradiol administration also enhances extinction in ovariectomized rats (Chang et al., 2009; Graham and Daher, 2016). Combined, these findings have led to suggestions that phasic or chronic reductions in endogenous estradiol may reduce women's responsiveness to exposure therapy, and that estradiol could be a novel pharmacological enhancer of exposure therapy (Glover et al., 2015; Li and Graham, 2017).

Studies examining the effect of estradiol administration on fear extinction in intact rats have only administered estradiol during the metestrus phase, or in rats treated with hormonal contraceptives, both of which have low endogenous levels of estradiol (Milad et al., 2009; Graham and Milad, 2013; Zeidan et al., 2011). Similarly, the only human study examining the effects of estradiol administration on extinction examined women within 7 days of menstruation, during the lowest levels of endogenous estradiol (Graham and Milad, 2013). The reason for these methodological choices is clear, as rats in proestrus and women with higher endogenous estradiol generally exhibit optimal extinction performance (i.e., very low levels of fear at extinction recall), and so any beneficial impact of estradiol on fear extinction would be difficult to detect in these subjects due to floor effects. However, the need to assess the impact of estradiol on fear extinction in females with higher levels of estradiol becomes apparent when the logistics involved in using estradiol as an adjunct to exposure therapy are considered. For example, one option would be to administer estradiol only prior to exposure sessions that occur during periods of low estradiol, such as at the beginning of the menstrual cycle. At a minimum, this approach requires that the patient's menstrual cycle be carefully tracked and a decision be made as to whether the patient is likely to benefit from adjuvant estradiol, based on the number of days since menstruation onset. This approach is fraught with potential sources of error, including unreliability in menstrual cycle monitoring, difficulties in determining appropriate cut-off points and in making decisions regarding irregular cycles, and the unknown impact of large individual differences in estradiol levels both across and within menstrual cycle phases (Becker et al., 2005). Moreover, this approach assumes that women with higher endogenous estradiol levels would already exhibit optimal responsiveness to exposure therapy and therefore not require an adjuvant. However, this is unlikely, as exposure therapy is a more elaborate process than extinction that requires anywhere between a single prolonged three hour session (in the case of specific phobia treatment), and multiple sessions over several months in the case of more complex disorders like PTSD (Craske and Stein, 2016; Öst, 1989). Therefore, even if women exhibit stronger responsiveness to exposure therapy during times of higher endogenous estradiol levels, there would likely remain scope to improve treatment efficiency and effectiveness.

A simpler approach than the one described above would be to administer estradiol once prior to each exposure session in all individuals, irrespective of their endogenous hormonal state. Such an approach rests on the assumption that very high levels of estradiol (for example, resulting from a combination of heightened endogenous estradiol and systemic estradiol administration) will either enhance exposure therapy outcomes further, or lead to ceiling effects, in which case no further enhancement of exposure therapy can occur. Currently, there is no evidence to support this assumption, as the effect of estradiol administration on fear extinction in rats during proestrus, or in women with higher endogenous estradiol levels during the late-follicular/mid-luteal phases of the menstrual cycle, has never been assessed. Moreover, the effects of varying doses of estradiol on fear extinction have not been assessed even amongst females with low endogenous estradiol. In order to identify the conditions under which estradiol might be suitable as an adjunct to exposure therapy, the present study examined the impact of differing doses of estradiol administered during different phases of the estrous cycle in rats prior to extinction training. In Experiment 1, we compared the effects of estradiol on fear extinction in rats extinguished during metestrus or proestrus using the standard low dose of estradiol that has been used in past research (Milad et al., 2009; Zeidan et al., 2011). In Experiments 2a–b, we compared the effects of a low or a high dose of estradiol on fear extinction in rats extinguished during metestrus or proestrus. Across both experiments, we observed substantial variability in extinction recall amongst rats that received the low dose of estradiol prior to extinction training during proestrus, with some exhibiting good extinction recall, and others exhibiting poor extinction recall. In Experiment 3, we assessed whether this variability was attributable to within-estrous phase variations in endogenous estradiol. Blood samples were taken from rats during proestrus, and then the low dose of estradiol or vehicle was administered prior to extinction training. Following extinction recall, estradiol-treated rats were divided into those that showed comparable freezing to vehicle-treated rats (i.e., good extinction recall), versus those whose freezing was greater than one standard deviation away from that exhibited by vehicle-treated rats (i.e., poor extinction recall). Serum concentrations of estradiol were then compared between the two subgroups to determine whether the impact of systemically administered estradiol on fear extinction differed depending on pre-treatment endogenous estradiol levels.

2. 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. To control for potential influences of systematic differences in social interaction between cages, group assignment (with respect to estrous phase during extinction training and drug treatment) was distributed equally across rats within a given cage, such that all cages in each experiment contained at least two rats from each experimental group. Rats were maintained on a 12 hr light-dark cycle (lights on at 7 am) with food and water available ad libitum. 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).