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Roles of testosterone and amygdaloid LTP induction in determining sex differences in fear memory magnitude

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ABSTRACT

Women are thought to form fear memory more robust than men do and testosterone is suspected to play a role in determining such a sex difference. Mouse cued fear freezing was used to study the sex-related susceptibility and the role of testosterone in fear memory in humans. A 75-dB tone was found to provoke weak freezing, while 0.15-mA and 0.20-mA footshock caused strong freezing responses. No sex differences were noticed in the tone- or footshock-induced (naïve fear) freezing. Following the conditionings, female mice exhibited greater tone (cued fear)-induced freezing than did male mice. Nonetheless, female mice demonstrated indistinctive cued fear freezing across the estrous phases and ovariectomy did not affect such freezing in female mice. Orchidectomy enhanced the cued fear freezing in male mice. Systemic testosterone administrations and an intra-lateral nucleus of amygdala (LA) testosterone infusion diminished the cued fear freezing in orchidectomized male mice, while pretreatment with flutamide (Flu) eradicated these effects. Long-term potentiation (LTP) magnitude in LA has been known to correlate with the strength of the cued fear conditioning. We found that LA LTP magnitude was indeed greater in female than male mice. Orchidectomy enhanced LTP magnitude in males' LA, while ovariectomy decreased LTP magnitude in females' LA. Testosterone decreased LTP magnitude in orchidectomized males' LA and estradiol enhanced LTP magnitude in ovariectomized females' LA. Finally, male mice had lower LA GluR1 expression than female mice and orchidectomy enhanced the GluR1 expression in male mice. These findings, taken together, suggest that testosterone plays a critical role in rendering the sex differences in the cued fear freezing and LA LTP. Testosterone is negatively associated with LA LTP and the cued fear memory in male mice. However, ovarian hormones and LA LTP are loosely associated with the cued fear memory in female mice.

Introduction

Women are more vulnerable to several mood disorders than men (Piccinelli and Wilkinson, 2000). The occurrence of these mood disorders in women is greatest during the premenstrual and perimenopausal periods (Rapkin et al., 2002; Yonkers, 1997). These findings suggest that the sexually dimorphic brain structures and gonadal hormones may be associated with the prevalence and severity of certain mood disorders in humans. Post-traumatic stress disorder (PTSD), defined by the development of abnormal behavioral and physiological responses after traumatic events, is more prevalent in women than men (Peters et al., 2006). Two reports document that women exhibit more severe PTSD symptoms than men (Moser et al., 2007; Peters et al., 2006). In fact, the symptoms of PTSD tend to persist for a long period of time (Green, 2003). Traumatic event-related memory is long-lasting and critical to the maintenance of PTSD symptoms (Brewin, 2011). Thus, it is reasonable to suspect that women can form traumatic event-related memory more rapid and/or robust than men. Animal studies have shown that female rats outperform male rats in the eyeblink conditioning, cued and context fear startle (Dalla et al., 2009; de Jongh et al., 2005; Leuner et al., 2004). Likewise, female mice display stronger cued fear conditioning as compared to male mice in many inbred strains (Bolivar et al., 2001). Nonetheless, some investigators claim that males exhibit more context fear conditioning than females do in rat and mouse models given that certain shock delivery procedures are used (Maren et al., 1994; Wiltgen et al., 2001). Due to consistent findings in sex difference in cued fear conditionings with female rodents exhibiting more robust memory, mouse cued fear conditioning may provide a reasonable animal model to study the sex differences in fear memory in humans and the gonadal hormone mechanisms for causing such sex differences. Accordingly, we decided to assess the sex difference in the magnitude of cued fear freezing responses and to study the

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modulating effects of gonadal hormones on such a sex difference in adult mice.

Individuals with PTSD are observed to display symptoms, including anxiety, insomnia, distressing and recurrent dreams, flashback imagery and intrusive thoughts, irritability, poor concentration, avoidance behavior and detachment (Green, 2003). These symptoms are directly or indirectly due to the learned fear memory (Brewin, 2011). Thus, we hypothesized that female mice may exhibit a more robust cued fear memory than male mice. Although sex differences in human naïve fear level remain argumentative, many studies involving healthy volunteers indicate that males seem to exhibit more intense fear-related physiological responses to a range of acute laboratory challenges than females do (Dorn et al., 1996; Earle et al., 1999; Kirschbaum et al., 1999; Seeman et al., 2001; Zimmer et al., 2003). In contrast, a classic study documents that male mice display lower levels of naïve emotional responses than female mice (Archer, 1977). In this study, 75-dB tone-, 1.5- and 2-mA footshock-induced acute freezing responses were obtained for revealing the likely baseline differences in naive fear level in both sexes of mice. In the cued fear conditioning procedure, the 75-dB tone was used as the conditioned stimulus (CS) and the 1.5- or 2-mA footshock was used as the unconditioned stimulus (US). After the conclusion of the CS–US conditionings, CS-induced freezing response was used to indicate the magnitude of the learned fear responses (that is, cued fear memory) in both sexes of mice. It was of importance to stress that we measured the freezing response after the termination of each tone (CS), rather than during the presence of the tone as in other studies. Conditioned response peaks are found to cluster within 10 s after the onset of the CS (acoustic stimulus) even a 20-s inter-trial interval is used in a trace conditioning paradigm (Marchand and Kamper, 2000). Since the conditioned freezing response was observed at a 2–2.5-min interval following the termination of the CS in the test using a delay conditioning paradigm, freezing response could be both a fear response to an incoming CS (or fear anticipation) and a conditioned fear response in this study. Although we found that female mice exhibited greater cued fear freezing than male mice, females' cued fear freezing magnitude was not altered by their ovary removal or estrous phase. Thus, the impact of testosterone on determining such a sex difference in the cued fear freezing was further assessed in this study.

Since neurons in the lateral nucleus of the amygdala (LA) can respond to both acoustic and footshock stimuli, LA neurons have long been hypothesized for their roles in mediating the acoustic fear conditioning (Romanski et al., 1993). Long-term potentiation (LTP) of synaptic activity, currently the best characterized form of synaptic plasticity, is suggested as a physiological substrate of learning and memory (Bear and Malenka, 1994; Bliss and Collingridge, 1993; Rioult-Pedotti et al., 1998; Whitlock et al., 2006). Quirk et al. (1995) demonstrate that LA neurons show significant increase in firing in response to an acoustic CS following pairing such CS with a footshock. In fact, LTP-like changes are noticed following the formation of various forms of Palovian fear conditioning (McKernan and Shinnick-Gallagher, 1997; Rogan and LeDoux, 1995; Rogan et al., 1997). Moreover, a previous study demonstrates that blockade of LTP induction can prevent the formation of a Pavlovian fear conditioning (Bauer et al., 2002). Thus, we decided to study the LA LTP magnitudes in both sexes of mice by using an LTP induction protocol. Since female mice displayed greater cued fear freezing responses than male mice, we hypothesized that the LTP magnitude in female mice' LA may also be greater than that in male mice' LA. Given the possibility that the cued fear freezing magnitude can be modulated by gonadal hormones in male mice, we suspected that the LTP magnitude in the LA-containing slices from male mice may be decreased by testosterone, whereas increased by the removal of the testes. Since GluR1, GluR2, NR1, and synaptophysin expressions are involved in the LTP induction (Hayashi et al., 2000; Schmitt et al., 2009; Shi et al., 1999, 2001; Weisskopf et al., 1999), male and female mice were speculated to exhibit differential expression of these proteins in LA. Likewise, the expression of these LTP induction-related proteins could be modulated by testosterone in male LA.

Material and methods

Animals

Male and Female C57BL/6 mice [bred and vended by the National Cheng Kung University (NCKU) Lab Animal Center, Tainan, Taiwan], aged 8–9 weeks old, were housed 4–6 per cage by sex since weaning with free access to food (Purina Mouse Chow) and tap water in the colony room maintained on a 12 h light/dark cycle (lights on at 07:00). All experiments were conducted in a temperature (23 ± 1 °C)- and humidity (70%)-controlled laboratory. This study was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised in 1996. All procedures were approved by the local Animal Care Committee at NCKU College of Medicine.

Acoustic stimulus (tone)- and footshock-induced freezing responses

In an attempt to test whether male and female mice may exhibit similar baselines in spontaneous, tone- and footshock-induced freezing responses, a series of acoustic or footshock stimuli were given and the freezing responses were measured thereafter in male and female mice. A total of 19 (10 male and 9 female) mice were used for the tone-induced freezing experiment. In brief, the mice were allowed to individually explore in a Plexiglas training chamber (18 cm L × 10 cm W × 10 cm H) for 6 min for acclimation purpose. The Plexiglas training chamber was situated in a sound-attenuating chest (San Diego Instruments, San Diego, CA, USA). During the 6-min period, mouse spontaneous freezing was observed every 10 s through a peeping hole in the chest by a rater. The percentage of the spontaneous freezing for the last 3 min was used as their spontaneous baseline of freezing. The mice, then, received four consecutive tones (75 dB and lasting for 10 s for each) at 90–120-s intervals. The percentage of freezing response was obtained from the following 15 observations (1 observation/10 s) immediately after the conclusion of the 4th tone delivery by the same rater. Freezing (response) was defined as a complete cessation of all movement except for respiration during the observation. Likewise, footshocks were delivered to both sexes of mice and the freezing responses were measured thereafter. A total of 44 (22 male/female) mice were used for the footshock-induced freezing experiment. Both sexes of mice, as mentioned previously, were allowed to explore in the afore-mentioned training chamber for 6 min and the percentage of spontaneous freezing for the last 3 min was recorded as their spontaneous freezing baselines by a rater. The floor of the Plexiglas training chamber consisted of 12 stainless-steel rods (0.4 cm in diameter) spaced 0.4 cm apart. The rods were wired to a commercial shock generator (SR-LAB™, San Diego Instrument) for delivering footshocks. Immediately after recording of the spontaneous freezing baselines, mice received four consecutive footshocks [0.15 mA (10 male/female mice) or 0.2 mA (12 male/female mice), both lasting for 1 s in duration] at 90–120-s intervals. The percentage of the freezing response for each sex was obtained from the following 15 observations (1 observation/10 s) immediately after the conclusion of the 4th footshock delivery by the same rater. It was of importance to stress that the rater was always blind to the sex of the mice in these experiments assessing the naïve fear levels in both sexes of mice. In order to examine the likely modulating effects of gonadal hormones on spontaneous freezing baseline, tone- and footshock-induced freezing, 41 female (21 for tone and 20 for 0.15-mA footshock) and 41 male (21 for tone and 20 for 0.20-mA footshock) were used.

Cued fear conditioning and test

In order to examine the likely sex difference in the cued fear conditioning, 38 male (20 for 0.15-mA and 18 for 0.20-mA footshock) and 81 female (36 for 0.15-mA and 45 for 0.20-mA footshock) mice were used.