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D-cycloserine reverses the detrimental effects of stress on learning in females and enhances retention in males

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Abstract

Exposure to acute, inescapable stress produces a facilitation of subsequent classical eyeblink conditioning in male rats. The same stress exposure produces a profound deficit in classical eyeblink conditioning in females. Activation of *N*-methyl-D-aspartate receptors (NMDAr) is necessary for the effect of stress on learning in males while the contribution of NMDAr activation to the deficit in learning after stress is unknown. Here, we tested the influence of D-cycloserine (DCS), a positive modulator of the NMDAr, in stressed or unstressed male and female rats. Groups of males and females were exposed to an acute stressful event. One day later, they began training with four sessions of trace eyeblink conditioning. Each day before training, they were injected with DCS (15 mg/kg) or saline. Females treated with DCS during training responded similarly to those that were untreated. However, those that were stressed and the next day treated with the drug during training did not express the typical learning deficit, i.e. they learned to time the CR very well. Because the drug was administered well after the stressor, these data indicate that DCS reversed the negative effects of stress on learning in females. In males, the effect of DCS was subtle, resulting in higher asymptotic responding, and enhanced retention in a drug-free retention test. Thus, as shown previously, training in the presence of an NMDA receptor agonist enhances associative learning and memory retention. In addition, it can reverse learning deficits that have already been induced.

Keywords

Stress; Sex differences; Eyeblink conditioning; D-cycloserine; Pavlovian conditioning; Glutamate

1. Introduction

The effect of acute, inescapable stress on aversive associative learning differs greatly between male and female rats. Inescapable stress can facilitate acquisition of future aversive learning, such as fear and eyeblink conditioning, in male rats (Maier, 1990; Mineka, Cook, & Miller, 1984; Shors, Weiss, & Thompson, 1992). Activation of *N*-methyl-D-aspartate receptors (NMDAr) is necessary for the facilitatory influence of stress in males (Shors & Servatius, 1995). Systemic antagonism of NMDAr at the time of stress exposure prevented the stress-induced facilitation of eyeblink conditioning (Shors & Servatius, 1995). The same stressful event can severely impair acquisition of eyeblink conditioning in females (Shors, Lewczyk, Pacynski, Mathew, & Pickett, 1998). The role of NMDAr in the female stress

response is not known. Normal acquisition of eyeblink conditioning requires activation of NMDARs (Servatius & Shors, 1996; Takatsuki, Kawahara, Takehara, Kishimoto, & Kirino, 2001; Thompson & Disterhoft, 1997b) and these receptors are critically involved in most types of learning (e.g., Campeau, Miserendino, & Davis, 1992; Collinridge, 1987). Thus, it seems likely that they would be involved in the modulation of learning ability by stress in females as well as males.

Acute administration of D-cycloserine (DCS), a partial agonist at the glycine site of the NMDA receptor, can enhance acquisition of many learning tasks in normal animals (Flood, Morley, & Lanthorn, 1992; Land & Riccio, 1999; Monahan, Handelsmann, Hood, & Cordi, 1989; Thompson & Disterhoft, 1997a; Thompson, Moskal, & Disterhoft, 1992; Walker, Ressler, Lu, & Davis, 2002). Administration of DCS drastically facilitates the acquisition of hippocampus-dependent trace eyeblink conditioning in rabbits (Thompson et al., 1992). Further, DCS can reverse learning deficits due to aging (Baxter et al., 1994; Thompson & Disterhoft, 1997a), sleep deprivation (Silvestri & Root, 2008), stress (Yamamoto et al., 2008) and pharmacological manipulations (Fishkin, Ince, Carlezon, & Dunn, 1993; Kawabe, Yoshihara, Ichitani, & Iwasaki, 1998; Ono & Wantanabe, 1996). DCS administration facilitates extinction of conditioned fear in rodents (Ledgerwood, Richardson, & Cranney, 2003, 2004; Walker et al., 2002; Woods & Bouton, 2006; Yamamoto et al., 2008) and has recently gained recognition as an efficacious adjunct to psychotherapies, such as exposure therapy and other cognitive-behavioral approaches (e.g., Ressler et al., 2004; Richardson, Ledgerwood, & Cranney, 2004).

In this study we directly assessed the effect of DCS on acquisition of the conditioned eyeblink response of stressed and unstressed male and female rats. Male and female rats, exposed to the stressor or not, were trained under the influence of DCS or saline, to assess whether DCS facilitates acquisition similarly to stress. Thus, in males and females, four conditions were used: Unstressed/Saline; Unstressed/DCS; Stressed/Saline; Stressed/DCS. These groups allowed us to directly assess the effect of DCS on learning in male and female rats, as well determine how modulation of the NMDAR interacts with stress and sex. Finally, we also conducted drug-free retention tests to assess the impact of DCS on retention.

2. Method

2.1. Surgery

Rats were anesthetized with sodium pentobarbital (50 mg/kg for males and 40 mg/kg for females). After being placed in the stereotaxic instrument, the scalp was cleaned with Betadine, and an incision was made. In preparation for eyeblink conditioning, a headstage was mounted to the skull. Three eyelid electrodes (insulated stainless steel wire, 0.005 in.) were implanted through the upper eyelid (orbicularis oculi muscle) and a fourth placed just outside of the muscle to serve as a ground wire. Rats were closely monitored during a minimum recovery period of 1 week before eyeblink conditioning began.

2.2. Vaginal cytology

Stages of estrous were monitored daily beginning the day following surgery. Sterile cotton swabs dipped in physiological saline were gently inserted into the vaginal canal, to collect loose epithelial cells. These cells were then applied to slides, fixed in 95% ETOH and stained with 1% Toluidine Blue for 15–20 min. Cells were rinsed and dehydrated with 95% ETOH. Each phase of the estrous cycle was identified using the following characteristics: proestrus was characterized by purple staining of epithelial cell nuclei, estrus was marked by blue clumped cornified cells, and diestrus was marked by dark leukocytes with scattered

epithelial cells. Animals that failed to exhibit a normal estrous cycle were eliminated from the study.

3. Conditioning chamber acclimation and stress exposure

Rats were placed in the conditioning boxes for an acclimation period. During this time, spontaneous eyeblinks were recorded. Following this acclimation, rats were transported to a separate room, placed in a restraint tube, and exposed to 30 inescapable stimulations to the tail at 60 s intervals. They were 1 s in duration, and 1 mA in magnitude. Rats in the No Stress condition were returned to the homecage. Eyeblink conditioning proceeded the following day. Females were exposed to stress during proestrus, when estrogen levels are rising. Therefore, training began 24 h later when estrogen levels remained elevated.

4. Trace eyeblink conditioning

Twenty-four hr following acclimation and stress exposure, rats were administered 15 mg/kg of DCS or saline, a dose known to facilitate extinction of fear in male and female rats (Ledgerwood et al., 2003; Woods & Bouton, 2006). Twenty minutes following intraperitoneal injections, rats were placed in the conditioning chamber. Rats were then given 10 presentations of the 250 ms conditioned stimulus (CS). Blinks were recorded for a 500 ms period after each CS. This was immediately followed by trace eyeblink conditioning, in which rats received 150 trials a day for four consecutive days (600 trials total). The CS was an 83 dB, 250 ms white noise. The US was a 100 ms periorbital shock (0.65 mA). The CS and US were separated by a 500 ms trace interval, in which no stimuli were delivered. Conditioned responses (CRs) were eyeblinks emitted during the 500 ms trace interval. Three days following the final conditioning day, all rats were injected with saline, and returned to the eyeblink conditioning chambers. To assess retention of the trace eyeblink task, rats were given 100 pairings of the CS and US, in the same manner as provided during the initial training condition.

5. Statistical analysis

The percentage of CRs emitted across blocks of 10 trials for the first 50 trials, and blocks of 50 trials thereafter was analyzed using repeated measures ANOVA. Using drug condition and stress exposure as between subject variables, and performance across trials as the within subjects variables, differences in rate of learning were assessed.

6. Classical conditioning and α -cycloserine treatment in females

The percentage of conditioned responses expressed across trials for females is presented in Fig. 1, left panel. The first 50 trials are presented in blocks of 10 trials, and in 50-trial blocks thereafter. Repeated measures ANOVA across the first five blocks of 10 trials (Trial) with Drug (DCS or saline) and Stress (stress or no stress) as the between subjects factor did not find a significant effect of Trial, $F(4, 96) = 1.78$, $p = .14$. Neither the interaction nor the main effect of Group was significant, largest $F = 1.83$. This suggests that there were no group differences during this early phase of conditioning.

Repeated measures ANOVA across the 16 blocks of trials (Trial) with Drug (DCS or saline) and Stress (stress or no stress) as the between subjects factor revealed a significant effect of Trial, $F(15, 360) = 20.48$, $p = .0001$. The Trial $\times$ Stress interaction was significant, $F(15, 360) = 3.49$, $p = .0001$, demonstrating that Stress impacted the pattern of responding across conditioning trials (Fig. 1, left panel). The Trial $\times$ Drug interaction was not significant, $F(15, 360) = 1.41$, $p = .14$, and the Trial $\times$ Drug $\times$ Stress interaction was also not significant, $F(15, 360) < 1$. The main effect of Drug just reached significance, $F(1, 24) = 4.07$, $p = .055$. The

main effect of Stress was not significant, $F(1, 24) < 1$, and the Drug $\times$ Stress interaction failed to reach significance, $F(1, 24) = 3.18, p = .08$. The failure to find a main effect of Stress is due to the fact that females in the Stress/Saline group did not express the typical increase in CRs across trials of training, whereas those that were stressed and trained in the presence of DCS did.

To confirm that stress exposure significantly decremented acquisition in the Stress/Saline group relative to the No Stress/Saline condition, these two groups were isolated and analyzed. Repeated measures ANOVA across the 16 blocks of acquisition revealed a significant effect of Trial, $F(15, 280) = 6.82, p = .0001$, as well as a significant Trial $\times$ Stress interaction, $F(15, 180) = 1.84, p = .03$. Further, the main effect of Stress was significant, $F(1, 12) = 4.55, p = .054$. This result indicates that stress exposure decremented acquisition in saline-treated, stressed females. To assess whether DCS facilitated acquisition itself, we compared the performance of unstressed saline-treated females to unstressed females treated with DCS. Repeated measures ANOVA failed to find a significant effect of Drug, $F(1, 12) < 1$. The effect of Trial was significant, $F(15, 180) = 21.19, p = .0001$. The Drug $\times$ Trial interaction did not reach significance, $F(15, 180) < 1$. Thus, DCS did not facilitate acquisition relative to Saline-treated females. To determine whether DCS influenced acquisition in the stress conditions, the Stress/Saline and Stress/DCS groups were isolated and compared. Repeated measures ANOVA across all 16 blocks of training revealed a significant effect of Trial, $F(15, 180) = 4.90, p = .001$. The Trial $\times$ Drug interaction did not reach significance, $F(15, 180) < 1$. The main effect of Drug was significant, $F(1, 12) = 5.33, p = .04$. Thus, DCS improved performance of females in the stress condition. To strengthen this conclusion, No Stress/DCS was compared to Stress/DCS. Repeated measures ANOVA across the 16 blocks of conditioning revealed a significant effect of Trial, $F(15, 180) = 15.32, p = .0001$ as well as a significant Trial $\times$ Stress interaction, $F(15, 180) = 2.03, p = .015$. The main effect of Stress was not significant, $F(1, 15) < 1$. DCS promoted acquisition in females that had been exposed to the stressor, and this was evidenced by a non-significant difference between the two groups treated with DCS.

Retention test data are presented in 50-trial blocks to the right of the dashed line in Fig. 1. These test data were assessed as percent conditioned responding on trials delivered in the drug-free session following 3 days with no training. Repeated measures ANOVA was used to analyze retention test data in two 50-trial blocks. Analysis was conducted with Trial as the within subjects variable, and Drug and Stress as between subjects variables. Analysis revealed a significant effect of Trial, $F(1, 24) = 12.67, p = .002$. The effect of Trial $\times$ Drug interaction was not significant, $F(1, 24) < 1$. The Trial $\times$ Stress interaction failed to reach significance, $F(1, 24) = 3.89, p = .06$. The Trial $\times$ Drug $\times$ Stress interaction did not reach significance, $F(1, 24) < 1$. The main effect of Drug or Stress did not reach significance, $F_s(1, 24) = < 1$. The Drug $\times$ Stress interaction was also not significant, $F(1, 24) < 2.09, p = .16$.

Because retention directly relates to the level of conditioned responding expressed at the end of conditioning, responding during the end of conditioning was isolated and compared to conditioned responding during the retention test (Fig. 2). Repeated measures ANOVA with the last 100 trials of conditioning and the 100 trials of retention were assessed (referred to as Phase) with Drug (DCS or Saline) and Stress (Stress or No Stress) as the between subjects factors found no effect of Phase $F(1, 24) = 1.67, p = .21$. The Phase $\times$ Drug interaction did not reach significance, $F(1, 24) = 3.75, p = .065$. No interactions were significant, $F_s(1, 24) < 1$. The main effect of Drug was significant, $F(1, 24) = 4.34, p = .055$. The main effect of Stress did not reach significance, $F(1, 24) = 1.45, p = .24$. The Drug $\times$ Stress interaction failed to reach significance, $F(1, 24) = 3.94, p = .059$. Therefore, DCS did not appear to

influence retention in females. Note that only saline treated, unstressed groups are depicted in Fig. 2 to simplify data presentation.

7. Classical conditioning and α -cycloserine treatment in males

Fig. 1, right panel, depicts the percentage of conditioned responses across the initial 4 days of conditioning. Repeated measures ANOVA across the first five blocks of 10 trials (Trial) with Drug (DCS or saline) and Stress (stress or no stress) as the between subjects factor found a significant effect of Trial $F(4, 104) = 4.15, p = .004$, as all groups exhibited an increase in CRs as training progressed (Fig. 1, right panel). Trial failed to significantly interact with either Drug or Stress, largest $F(4, 104) = .48$. The three-way Trial $\times$ Drug $\times$ Stress interaction did not reach significance, $F(4, 104) < 1$. The main effect of Stress was significant, $F(1, 26) = 7.41, p = .01$, demonstrating that males exposed to stress expressed a higher rate of CRs during early training trials. The main effect of Drug did not reach significance, $F(1, 26) < 1$. The Drug $\times$ Stress interaction also did not reach significance, $F(1, 26) < 1$.

Repeated measures ANOVA of the 16 blocks of conditioning trials (Trial), with Drug (DCS or saline) and Stress (stress or no stress) as a between subjects factors revealed a significant effect of Trial, $F(15, 390) = 20.99, p = .0001$, as conditioned responses increased across trials. The Trial $\times$ Drug interaction was not significant, $F(15, 390) < 1$. The Trial $\times$ Stress interaction was significant, $F(15, 390) = 1.74, p = .04$, indicating that rats exposed to stress did not exhibit the same pattern of responding across blocks of trials as those in the unstressed condition. As depicted in Fig. 1, right panel, rats in the stress condition expressed high levels of CRs early in conditioning, which persisted throughout training. Rats in the unstressed condition were slower to achieve a similar level of responding. The main effect of Drug was not significant, $F(1, 26) = 1.11, p = .30$. The main effect of Stress was significant, $F(1, 26) = 11.81, p = .002$, demonstrating that, as expected, stress exposure significantly facilitated acquisition. The Drug $\times$ Stress interaction was not significant, $F(1, 26) < 1$. An effect of DCS in addition to stress could not be detected during training.

To assess whether DCS administration alone influenced eyeblink conditioning, groups No Stress/Saline and No Stress/DCS were isolated and compared. Though the effect of Trial was significant, $F(15, 195) = 19.61, p = .0001$, the interaction, $F(15, 195) = 1.21, p = .27$, nor the main effect of Drug was significant, $F(1, 13) < 1$. Thus, DCS administration did not significantly change the rate of acquisition relative to saline treated controls. However, as is evident in Fig. 1, right panel DCS did produce a higher level of conditioned responding on the final day of conditioning. Isolation of this day, or the final three trial blocks of conditioning, found a significant effect of Drug, $F(1, 13) = 5.06, p = .033$, demonstrating that though DCS administration did not produce an observable facilitation early in conditioning, the drug did influence asymptotic responding by the end of conditioning. Isolation of Stress/Saline and Stress/DCS failed to find a significant effect of Drug, $F(1, 13) < 1$. Comparison of No Stress/DCS and Stress/DCS revealed that though Stress and DCS together appeared to elicit more conditioned responses relative to DCS treatment alone, the between subjects effect did not reach significance, $F(1, 13) = 4.23, p = .06$.

Though DCS did not produce a reliable facilitation of conditioning, we assessed its influence on retention. Repeated measures ANOVA was used to analyze retention test data in two 50-trial blocks. This analysis revealed a significant effect of Trial $F(1, 26) = 12.95, p = .001$. No interactions were significant, largest $F(1, 26) = 1.55$. The main effect of Drug was significant $F(1, 26) = 12.23, p = .002$. The effect of Stress nor the Drug $\times$ Stress interaction was significant, largest $F(1, 26) = 2.32$. Thus, rats trained under the influence of DCS expressed higher levels of responding during the drug-free retention test relative to

saline treated rats. This result suggests that though the influence of DCS on performance during eyeblink conditioning was subtle, the effect on retention of the CS-US memory was stable and reliable.

The final 100 trials of conditioning were isolated and compared to the 100 trials of the retention test. Repeated measures ANOVA with the last 100 trials of conditioning and the 100 trials of retention were assessed (referred to as Phase) with Drug (DCS or Saline) and Stress (Stress or No Stress) as the between subjects factors. This analysis found a significant effect of Phase, $F(1, 26) = 13.58, p = .001$. The effect of Phase did not significantly interact with Drug, $F(1, 26) = 1.08, p = .309$, or Stress, $F(1, 26) < 1$. The three-way interaction of Phase $\times$ Drug $\times$ Stress also did not approach significance, $F(1, 26) < 1$. The main effect of Drug was significant, $F(1, 26) = 14.61, p = .001$. The main effect of Stress did not reach significance, $F(1, 26) = 2.63, p = .117$. The Drug $\times$ Stress interaction was also not significant, $F(1, 26) = 2.29, p = .14$. Thus, DCS influenced retention, but Stress alone did not. This effect, as depicted in Fig. 2, is driven by the loss of conditioned responding in the No Stress/Saline condition, whereas conditioned responding was maintained in the DCS treated groups. Note that only saline treated, unstressed groups are depicted in Fig. 2 to simplify data presentation.

8. Discussion

Consistent with previous findings, females exposed to an acute stressful event exhibited impaired acquisition of eyeblink conditioning (e.g., Shors et al., 1998). DCS administration during eyeblink conditioning reversed this stress-induced learning deficit. DCS did not produce a clear or significant facilitation of learning in unstressed females although the drug did enhance asymptotic responding in the males. A similar facilitation in learning was observed between stressed males administered saline and unstressed males administered DCS. Drug-free retention tests confirm that DCS did not simply enhance performance, but rather strengthened long-term memory. This effect was most evident in the enhanced retention expressed by males treated with DCS during training.

Aside from the drug effects, we also observed that females that were not stressed or treated with the drug expressed more CRs than their male counterparts did following a three day period without training. Thus, the females expressed better long-term retention than males. To our knowledge, this sex difference in retention has not previously been demonstrated.

DCS modulates plasticity within the hippocampus and basolateral amygdala (BLA), areas critical for the influence of stress on learning in male and female rats (Bangasser & Shors, 2007; Waddell, Bangasser, & Shors, 2008). Stress differentially modifies hippocampal morphology in males and females (Leuner, Falduto, & Shors, 2003; Shors, Chua, & Falduto, 2001; Woolley & McEwen, 1994). For example, exposure to the stressor used here increases spine density within the hippocampus of male rats, an effect that parallels the stress-induced facilitation in eyeblink conditioning (Shors et al., 2001). In the female hippocampus, acute stress blocks the natural increase in spines evident during proestrus when estrogen levels are highest (Shors et al., 2001; Woolley, Gould, Frankfurt, & McEwen, 1990) suggesting that stress negatively impacts plasticity within the female hippocampus, while enhancing plasticity in the male hippocampus. This modulation of spine density observed in both males and females requires NMDAr activation (Shors, Falduto, & Leuner, 2004; Woolley & McEwen, 1994; Woolley, Weiland, McEwen, & Schwartzkroin, 1997). Thus, NMDArs are necessary for both stress and hormone induced changes within the hippocampus. As noted, the stress effect on learning in males and females is dependent on an intact hippocampus (Bangasser & Shors, 2007). DCS enhances NMDAr-mediated EPSPs in CA1 of the hippocampus performance during training on (Billard & Rouaud, 2007; Rouaud & Billard,

2003) and facilitates many hippocampus-dependent learning tasks (Lelong, Dauphin, & Boulouard, 2001; Quartermain, Mower, Rafferty, Herting, & Lanthorn, 1994; Thompson et al., 1992). Given the present data, we suggest that stress disrupts activation of the NMDA receptor and DCS restores its activation via these changes in NMDA mediated plasticity in the hippocampus.

DCS may also act within the BLA to modulate learning in stressed and unstressed animals. DCS either systemically or directly infused into the BLA facilitates extinction of fear (Walker et al., 2002) and the facilitatory influence of DCS on extinction has been directly tied to protein synthesis, AMPA receptor expression and MAPK activation in the BLA (Mao, Lin, & Gean, 2008; Yang & Lu, 2005), pathways also involved in acquisition of many Pavlovian tasks (e.g., Lin, Lee, & Gean, 2003). Similar to the hippocampus, stress enhances synaptic connectivity in the male BLA (Vyas, Jadhav, & Chattarji, 2006) and inactivation or enhanced inhibition of the BLA during acute stress blocks the stress-induced changes in aversive learning (Rodriguez Manzanares, Isoardi, Carrer, & Molina, 2005; Waddell et al., 2008). Little is known about the influence of stress and estrogen on morphology and cell excitability in the female BLA. However, it is possible that DCS restores NMDA activity within the BLA as well as the hippocampus and reverses the learning deficit exhibited in stressed females.

Acute stress maintains elevated estrogen levels (Shors, Pickett, Wood, & Paczynski, 1999). Estrogen increases the density of NMDA glutamate binding sites within the hippocampus (Weiland, 1992; Woolley et al., 1997) and increases NMDA activity by increasing calcium influx (Good, Day, & Muir, 1999; Pozzo-Miller, Inoue, & Murphy, 1999). Estrogen also transiently decreases GABAergic inhibition in CA1 of the hippocampus, suggesting a second means by which estrogen may disrupt inhibitory tone in the female hippocampus (Rudick & Woolley, 2001). The action of DCS on NMDA receptors may override this negative effect of estrogen, modulating cell excitation by increasing chloride influx, reversing the stress-induced impairment in acquisition (Monahan et al., 1989). Because the BLA and hippocampus are known targets of DCS action, it is possible that acute, uncontrollable stress disrupts NMDA-dependent plasticity in these areas, and elicits an anxious or aversive state that disrupts future conditioning only in females. This disruption of the BLA and hippocampus may cause pathological expression of fear that disrupts learning in females, but facilitates aversive learning in males.

The influence of estrogen on learning depends on the type of learning and memory task used as well as the hormonal status of the rat. Estrogen can improve performance during training on some learning tasks and not others (e.g., Holmes, Wide, & Galea, 2002; Korol, 2004; Sandstorm & Williams, 2004; Toufexis, Myers, Bowser, & Davis, 2007). Estrogen deprivation in adulthood through ovariectomy and its replacement suggest that estrogen's influence on learning interacts with working memory load (Luine, 2008; Sandstorm & Williams, 2004) as well as the aversiveness of the task (Korol, 2004; Luine, 2008). It is possible that whereas estrogen has positive effects on spatial and working memory tasks, it is disruptive when the task requires inhibition of fear. Toufexis et al. (2007) reported that through binding to estrogen receptors (ER α and ER β), estrogen antagonizes fear inhibition. Gonadectomized female rats administered estrogen were unable to suppress fear in a discrimination procedure, in which one CS explicitly signaled shock presentation, while a different CS signaled the absence of the US (Toufexis et al., 2007). However, ovariectomized sham-implanted female rats were capable of inhibiting expression of fear to the non-reinforced CS (Toufexis et al., 2007). Because stress enhances levels of estrogen (Shors et al., 1999), and estrogen disrupts the inhibition of fear (Toufexis et al., 2007), it appears that generalized fear interferes with future aversive learning in females. DCS may promote plasticity to reverse the interference caused by high levels of fear in stressed females.

Enhanced aversive learning following inescapable stress has been interpreted to parallel the hyper-vigilance exhibited in humans with symptoms of post-traumatic stress disorder (PTSD). During the course of this mental illness, a traumatic life event induces a change in emotional and cognitive responding that can persist for years. It is often epitomized by sensitized and otherwise abnormal responding to aversive cues and events (e.g., Foa, Zinbarg, & Rothbaum, 1992; Yamamoto et al., 2008). It is unclear how the female response to stress in rodents aligns with this interpretation. Most experiments examining the influence of stress on aversive conditioning has focused exclusively on male rats. Male rats exposed to inescapable shock show more fear than rats exposed to escapable shock, and this fear generalizes to neutral contexts (Maier, 1990; Mineka et al., 1984). Importantly, escapable stress does not influence future aversive conditioning in males (Maier, 1990) or eyeblink conditioning in males or females (Leuner, Mendolia-Loffredo, & Shors, 2004), implicating loss of control and generalized fear as critical factors in the effects of stress on learning (McAllister & McAllister, 1963). Generalized fear elicited by inescapable shock in males and females may produce opposing effects on subsequent learning. Perhaps exacerbated contextual fear potentiates aversive conditioning to an explicit CS in males, but produces interference of associative learning in females.

Exactly how the NMDA agonist reverses the effects of stress on learning in females is unknown. It may be that NMDA receptors are activated during the stressor which then prevents their subsequent activation during eyeblink conditioning. If true, it would be an outcome unique to females since stressed males appear to learn more rapidly than unstressed males. Again, the presence of estrogen may render the NMDA receptors less active after the stressful event. The presence of the agonist is thereby able to overcome their decreased activity. In general, these studies add to the many demonstrating the positive outcomes of DCS treatment and learning. They further point to its potential efficacy in treating women that suffer from the cognitive disturbances associated with PTSD.

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Fig. 1.

Left panel: percent CRs of female rats in each condition across eyeblink conditioning sessions. Systemic injections of DCS abolished the stress-induced disruption of eyeblink conditioning. Facilitation of eyeblink conditioning. Right panel: percent CRs of male rats in each condition across eyeblink conditioning sessions. DCS produced a slight enhancement of conditioned responding.

Fig. 2. Percent CRs of unstressed saline-treated female and male rats expressed in the last 100 trials of conditioning, and 100 trials of the drug-free retention test. Males exhibit a loss of conditioned responding during the retention test that is not evident in females.