Title: Reproductive experience alters the involvement of N-methyl-D-aspartate receptors in fear extinction, but not fear conditioning, in female Sprague Dawley rats

Authors: Samantha Tang, Bronwyn M. Graham

Affiliations: School of Psychology, UNSW Sydney, NSW, 2052, Australia

Corresponding Author:

Samantha Tang

School of Psychology

UNSW Sydney

E: samantha.tang@unsw.edu.au

P: +61-2-93853886

F: +61-2-93853641

Key words:

Anxiety disorders

Fear extinction

Fear conditioning

NMDA receptors

Reproductive experience

Funding and Disclosure

This work was supported by grants from the Australian Research Council (DE140100243 and DP180101563) to BMG, and an Australian Postgraduate Award to ST. The authors have no conflicts of interest to declare.

Abstract

Recently, evidence has emerged showing that the behavioural and hormonal features of fear extinction are altered as a result of reproductive experience in both rats and humans. The current set of experiments sought to determine whether reproductive experience also alters the molecular features of fear extinction. In adult male rats, it has been widely demonstrated that the activation of N-methyl-D-aspartate receptors (NMDAR) is essential for fear extinction. We therefore compared the involvement of NMDAR in fear extinction between nulliparous (virgin) and primiparous (reproductively experienced) female rats. Nulliparous and primiparous females received systemic administrations of either MK-801 (a non-competitive NMDAR antagonist) or saline prior to extinction training. MK-801 was found to impair extinction recall in nulliparous females, but not primiparous females. When the same dose of MK-801 was administered prior to conditioning, both groups of rats showed impaired recall of conditioning the following day. The results of these experiments indicate that the extinction, but not the acquisition of fear, may become NMDAR-independent following reproductive experience.

Introduction

The inhibition of learned fear is commonly studied in the laboratory using Pavlovian fear extinction. This procedure involves presenting subjects with a fear conditioned stimulus (CS, e.g. a noise previously paired with a shock) in the absence of an aversive unconditioned stimulus (US, e.g. a shock). Subjects' recall of extinction can be tested by measuring their fear responses to the CS at a later time (extinction recall). Fear extinction has formed the basis of exposure therapy, the gold standard treatment for anxiety disorders which involves presenting patients with anxiety-provoking stimuli in the absence of a negative consequence (Foa and McLean, 2016; Graham and Milad 2011). As such, understanding the mechanisms underlying fear extinction can be helpful in optimising treatment outcomes among individuals with an anxiety disorder.

Given that women are twice as likely to be diagnosed with an anxiety disorder compared to men (McLean et al. 2011), it is promising that the past decade has seen a growing literature on the behavioural and molecular features of fear extinction in females. These studies have consistently demonstrated that estradiol, the major form of estrogen, facilitates fear extinction in this population (see Li and Graham 2017). When female rats and women undergo fear extinction during periods of high estradiol, they show good extinction recall the following day. Contrastingly, female rats and women that undergo fear extinction during periods of low estradiol show poor extinction recall (Chang et al. 2009; Graham and Daher 2016; Graham and Milad 2013; Graham and Scott 2018; Gruene et al. 2015; Li and Graham 2016; Milad et al. 2009; Milad et al. 2010; Pineles et al. 2016; Rey et al. 2014; Wegerer et al. 2014; White and Graham 2016; Zeidan et al. 2011). Notably, however, these studies remain limited in their lack of consideration of reproductive status. Most prior animal studies examining fear extinction in females rats have only used reproductively inexperienced subjects, while human studies have used young women, aged 18-30, whose reproductive status is unknown. This is problematic given that 84% of women will become mothers by the age of 44 (Monte and Ellis 2014). In administering treatment (and in modelling treatments in the laboratory using extinction), one cannot assume mothers will respond to exposure therapy in the same way as non-mothers, particularly given that pregnancy and motherhood are associated with numerous hormonal, neurobiological and behavioural changes that persist long after weaning, as described below.

For instance, reproductively experienced female rats have lower levels of circulating estradiol during proestrus (i.e. high estradiol phase), and increased levels of dendritic spine density in a number of brain regions, such as the hippocampus, amygdala and prefrontal cortex (Bridges and Byrnes 2006; Kinsley et al. 2006;

Leuner and Gould 2010; Pawluski and Galea 2006; Rasia-Filho et al. 2004). These brain regions are critically involved in the acquisition and consolidation of fear extinction (Quirk and Mueller 2008). Moreover, primiparous (i.e. one prior reproductive experience) female rats outperform nulliparous (i.e. virgin) female rats in a number of hippocampally-mediated tasks, including the dry land maze, object place task and radial arm maze (Kinsley et al. 1999; Kinsley et al. 2006; Lemaire et al. 2006; Love et al. 2005; Paris and Frye 2008). Given there is substantial overlap in the neural circuity altered by reproductive experience, and that which is involved in fear extinction, it is possible that fear extinction may also be altered by reproductive experience.

Indeed, we have recently reported evidence that key features of fear extinction differ between nulliparous and primiparous females (Milligan-Saville and Graham 2016). Consistent with previous findings (e.g., Milad et al., 2009), we found that nulliparous rats extinguished during metestrus (i.e. low estradiol phase) showed poor extinction recall (i.e. fear relapse) compared to those extinguished during proestrus (i.e. high estradiol phase). In primiparous females, however, extinction recall did not differ dependent on estrous phase. Similar findings were observed in women, whereby there was a positive correlation between estradiol levels and extinction recall in non-mothers, but no such correlation in mothers. Moreover, nulliparous female rats extinguished during proestrus showed fear relapse when presented with the CS outside of the extinction context (renewal) and when exposed to an unsignalled presentation of the US (reinstatement). Primiparous females, however, showed neither renewal, nor reinstatement, regardless of the phase in which they underwent extinction training. Together, these results indicate that both the hormonal and behavioural features of fear extinction are altered as a consequence of reproductive experience.

Since females undergo vast neuroendocrine changes over the course of pregnancy and motherhood, it is possible that such experiences may also affect the molecular features of fear extinction, which may underlie the changes in the hormonal and behavioural features of fear extinction observed after reproductive experience. In adult male rats, studies have consistently demonstrated that N-methyl-D-aspartate receptors (NMDARs) are critically involved in the acquisition and consolidation of extinction memories (Myers and Davis 2007; Singewald et al. 2015). The pharmacological blockade of NMDARs prior to or immediately after extinction training impairs extinction recall (Baker and Azorlosa 1996; Burgos-Robles et al. 2007; Liu et al. 2009; Sotres-Bayon et al. 2007), while the administration of an NMDAR agonist immediately post-extinction enhances extinction recall (Ledgerwood et al. 2003; Walker et al. 2002). The involvement of NMDARs in fear extinction has yet to be investigated in female rats. The aim of this study was therefore to examine the role of NMDARs in fear extinction in nulliparous female rats, and to determine whether the involvement of these receptors in fear

extinction is altered by reproductive experience. To do this, we investigated the effect of systemic MK-801 (a non-competitive NMDAR antagonist), or vehicle, administered prior to extinction training on subsequent extinction recall in primiparous and nulliparous female rats. If NMDARs are involved in fear extinction in females irrespective of reproductive history, then both groups of MK-801 treated rats should exhibit impaired extinction recall relative to vehicle-treated rats. In subsequent experiments, MK-801 was also used to investigate the involvement of NMDARs in cued and context fear conditioning in nulliparous and primiparous females rats.

Method

Subjects

Virgin, female, Sprague Dawley rats (8-10 weeks old) obtained from the Animal Resources Centre, Perth, WA, Australia were used in this study. The rats were randomly assigned to remain as virgins or to be mated. Breeding procedures were as previously described (Milligan-Saville and Graham 2016). Nulliparous and primiparous rats were age-matched in each of the experiments, and underwent fear conditioning at approximately 5 months of age. All rats were treated according to *The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (8th edition), and all procedures were approved by the Animal Care and Ethics Committee at The University of New South Wales.

Apparatus

The apparatuses were identical to those previously described (Graham and Daher 2016) and comprised two sets of chambers that served as distinct contexts (A and B). The CS was a 62 dB white noise delivered via high frequency speakers embedded to the right wall of each chamber, and the US was a 0.4 mA, 1.0s footshock. A computer running Med Associates Med-PC IV controlled presentations of both the CS and the US.

Vaginal cytology

Vaginal smears were conducted daily to determine estrous cycle phase as previously described (Graham and Daher 2016). Rats that did not exhibit a regular 4-5 day estrous cycle were excluded from the study. For this reason, primiparous rats were not fear conditioned until at least two weeks after weaning, when lactation had ceased and estrous cycling had recommenced (Leuner and Shors 2006).

In Experiments 1-3, rats were conditioned during diestrus, extinguished during proestrus and tested for extinction recall during estrus. Rats were extinguished during proestrus because this phase is associated with good extinction recall (Milad et al., 2009). In Experiment 4, half of the rats were conditioned during proestrus and tested during estrus, while the other half were conditioned during metestrus and tested during diestrus. This was to control for potential systematic differences in conditioning strength due to estrous stage; there is currently little consensus in the literature regarding the effect of estrous cycle on conditioning (Barha et al. 2010; Gupta et al. 2001; Markus and Zecevic 1997; Milad et al. 2009). In Experiment 5, rats were conditioned during metestrus and tested during diestrus since we did not find an effect of estrous cycle on conditioning in Experiment 4.

Procedure

Handling and context pre-exposure. Rats were handled for five minutes a day, for three consecutive days prior to the commencement of the experiment. In Experiments 1-4, rats were individually pre-exposed to Context A for ten minutes on the last two days of handling. Rats were not pre-exposed to Context A in Experiment 5.

Conditioning. In Experiments 1-4, rats were placed in Context A on Day 1. Following an adaptation period of two minutes, rats were exposed to 10-second CS presentations that co-terminated with a footshock. In Experiments 1, 2 and 4, rats received two CS-US presentations. The inter-trial interval (ITI) was 135 seconds. In Experiments 3a and 3b, rats received a single CS-US presentation. Conditioning in Experiment 5 was identical to that of Experiments 1, 2 and 4, except the two US presentations were not preceded by a discrete white noise CS.

Extinction training. In Experiments 1-3, rats underwent extinction training 24 hours after conditioning (Day 2) in Context B. Following an adaptation period of two minutes, rats received 10-second presentations of the CS, with an ITI of ten seconds. In Experiments 1 and 3b, rats received 30 trials extinction, while in Experiments 2 and 3a, rats received 45 trials of extinction.

Extinction recall. In Experiments 1-3, subjects were returned to Context B 24 hours after extinction training (Day 3). Following an adaptation period of one minute, rats were presented with a single 2-minute presentation of the CS.

Conditioning recall. In Experiment 4, subjects were tested for cue and context-elicited fear 24 hours after conditioning (Day 2). Cue-elicited fear was tested using the same procedure as extinction recall. Context-elicited fear was tested by placing subjects into Context A for a period of three minutes. The order of testing was counterbalanced across subjects and tests were conducted approximately one hour apart. In Experiment 5, rats were tested for context-elicited fear only on Day 2.

Scoring. Freezing, defined as the absence of all movement aside from that which is required for respiration (Fanselow 1980), was used to measure conditioned fear. Rats were hand scored as freezing or not freezing every three seconds during the adaptation periods prior to extinction and recall. These scores provided baseline levels of freezing. CS-elicited freezing was measured every three seconds throughout the CS presentations during extinction training and recall. Extinction trials were collapsed into blocks consisting of five trials each. A percentage of observed freezing was calculated for each block of extinction training, and for extinction recall.

Data analysis. The statistical software package, SPSS, was used to conduct all data analyses. In Experiments 1-2, two-way analyses of variances (ANOVAs) were used to assess group differences in baseline freezing during extinction training and extinction recall, as well as CS-elicited freezing during recall. Two-way ANOVAs with repeated measures assessed group differences in CS-elicited freezing during extinction training. Post-hoc Fisher's Least Significant Difference (LSD) tests were used when appropriate. In Experiments 3a and 3b, independent samples t-tests were used to assess group differences in baseline freezing during extinction training and extinction recall, and CS-elicited freezing during extinction recall. One-way ANOVAs with repeated measures assessed group differences in CS-elicited freezing during extinction training. In Experiment 4, two-way ANOVAs were used to assess group differences in baseline freezing during cued recall, and CS- and context-elicited freezing during conditioning recall. In Experiment 5, two-way ANOVAs were used to assess group differences in context-elicited freezing during conditioning recall. A significance value of $p = .05$ was applied to all tests. Since it is difficult to detect CS-elicited freezing when baseline levels of freezing are high, an exclusion criterion was applied. Rats that exhibited over 50% baseline freezing prior to recall were excluded from the analysis (n = 1 in Experiment 4). Furthermore, one statistical outlier (defined as >4 s.d. away from the mean) was removed from the analysis of Experiment 3.

Drug administration

Rats were administered either (+)-MK 801 maleate (0.1mg/kg, Sigma-Aldrich, Castle Hill, NSW) or saline ten minutes prior to extinction training (Experiments 1-3) or conditioning (Experiments 4 and 5). The volume of injection was 1ml/kg in body weight, administered subcutaneously in the nape of the neck using a 0.45mm x 13mm needle. The drug dose used was based on past work investigating the effect of systemic MK-801 on fear extinction in male rats (Baker and Azorlosa 1996; Langton et al. 2007).

Results

Experiment 1

Experiment 1 examined the role of NMDAR activation in extinction in nulliparous and primiparous rats. Both groups were administered MK-801, an NMDAR antagonist, or saline prior to six blocks of extinction training. Animals were then tested for extinction recall the following day. Given that past work in male rats has shown that MK-801 has acute motoric effects, whereby rats demonstrate diminished freezing (Baker and Richardson 2017; Kim and Richardson 2010; Langton and Richardson 2009), MK-801-treated rats are expected to show very low levels of freezing throughout extinction training. However, if NMDAR activation is necessary for the consolidation of extinction, animals administered MK-801 should show higher levels of CS-elicited freezing when tested for extinction recall drug-free the following day, compared to animals administered saline.

Table 1 presents levels of baseline freezing across Experiments 1-4. In Experiment 1, all groups showed comparable levels of baseline freezing prior to extinction training (largest $F_{(1, 39)} = 2.51$, $p = .12$). CS-elicited freezing decreased across extinction training (see *Figure 1a*, $F_{(5, 195)} = 31.90$, $p < .001$). However, during extinction training, groups that received MK-801 showed significantly less freezing compared to those that received saline (significant main effect of drug, $F_{(1, 39)} = 45.00$, $p < .001$, significant extinction x drug interaction, $F_{(5, 39)} = 13.37$, $p < .001$). There was no significant main effect of reproductive experience, or a reproductive experience x drug interaction (largest $F_{(1,39)} = 3.13$, $p = .09$). An inspection of the data, however, indicated that CS-elicited freezing may have differed between the two saline groups during extinction training. This was confirmed through the use of post-hoc tests. Among the animals that received saline, primiparous females showed higher levels of CS-elicited freezing on blocks 3 ($p = .04$), 4 ($p = .03$) and 5 ($p < .01$), of extinction training compared to nulliparous females.

There were no significant main effects of drug, or a drug x reproductive experience interaction on levels of baseline freezing prior to extinction recall (see *Table 1*, largest $F_{(1, 39)} = 2.87$, $p = .10$). There was, however, a significant main effect of reproductive experience, where primiparous females showed slightly, but significantly, higher levels of baseline freezing compared to nulliparous females ($F_{(1, 39)} = 4.61$, $p = .04$). Baseline freezing prior to extinction recall was therefore entered as a covariate when analysing CS-elicited freezing during extinction recall. While there were no significant main effects of drug or reproductive experience at extinction recall (largest $F_{(1, 38)} = .68$, $p = .42$), there was a significant reproductive experience x drug interaction (see *Figure 1b;* $F_{(1, 38)} = 12.43$, $p < .01$). Post-hoc LSD tests revealed a significant difference between the two nulliparous groups, where those that received MK-801 exhibited higher levels of CS-elicited freezing at extinction recall compared to those that received vehicle ($p = .01$). Primiparous rats, however, exhibited similar levels of CS-elicited freezing at test, regardless of drug ($p = .21$). Moreover, primiparous females that received saline exhibited higher levels of CS-elicited freezing at extinction recall compared to nulliparous females that received saline ($p < .01$). There was no significant difference in CS-elicited freezing between the two MK-801 groups ($p = .56$).

Experiment 2

Experiment 1 found that the involvement of NMDAR activation on extinction recall may depend upon reproductive experience. Whereas blocking NMDAR activation during extinction training led to impaired extinction recall in nulliparous rats, this effect was not evident in primiparous rats. However, it is possible that the results of Experiment 1 are due to a ceiling effect given that primiparous rats administered saline showed significantly higher levels of CS-elicited freezing at test compared to nulliparous rats administered saline. Therefore, in Experiment 2, nulliparous and primiparous females were given nine blocks of extinction training, rather than six blocks, in an attempt to reduce levels of CS-elicited freezing among saline animals at extinction recall. Both nulliparous and primiparous females received additional extinction blocks to ensure that the results were not solely attributable to the increased length of extinction training.

All groups showed comparable levels of baseline freezing prior to extinction training and extinction recall (see *Table 1*, largest $F_{(1, 48)} = 2.77$, $p = .10$). CS-elicited freezing decreased across extinction training (see *Figure 2a*; $F_{(8, 384)} = 22.50$, $p < .01$). As with Experiment 1, groups that received MK-801 showed significantly less CS-elicited freezing compared to those that received saline (main effect of drug, $F_{(1, 48)} = 35.93$, $p < .01$, and extinction x drug interaction, $F_{(1, 48)} = 5.46$, $p < .01$). There was also a significant main effect of reproductive experience, where primiparous females showed higher levels of CS-elicited freezing across the

nine blocks of extinction training compared to nulliparous females, $F_{(1, 48)} = 5.07$, $p = .03$. Post-hoc LSD tests revealed that, among animals that received saline, primiparous females showed significantly higher levels of CS-elicited freezing than nulliparous females on blocks 3 ($p = .013$), 4 ($p < .01$) and 5 ($p = .04$) of extinction training.

At extinction recall, there was no significant main effect of reproductive experience, and no significant reproductive experience x drug interaction (see *Figure 2b*, largest $F_{(1, 48)} = 1.75$, $p = .19$). However, there was a significant main effect of drug, where animals administered MK-801 showed lower levels of CS-elicited freezing compared to animals administered saline ($F_{(1, 48)} = 4.09$, $p = .05$). Post-hoc LSD tests revealed that this main effect was primarily driven by differences between the two nulliparous groups. Nulliparous females administered MK-801 showed significantly higher levels of freezing during test compared to those administered saline, $p = .05$. Primiparous females, however, exhibited similar levels of CS-elicited freezing at test, regardless of drug, $p = .46$. Levels of CS-elicited freezing did not significantly differ between the two saline groups.

Experiment 3a

As with Experiment 1, in Experiment 2 the pharmacological blockade of NMDARs prior to extinction impaired extinction recall in nulliparous females, but not primiparous females, even when the amount of extinction training provided was increased. There was no significant difference in CS-elicited freezing during extinction recall between the two saline groups in Experiment 2, indicating that the results of Experiment 1 were unlikely due to a ceiling effect. However, an inspection of the data from Experiment 2 reveals that primiparous females receiving saline still showed higher levels of CS-elicited freezing compared to nulliparous females receiving saline. Moreover, a t-test revealed no significant difference in CS-elicited freezing during extinction recall between the primiparous saline groups in Experiment 1 and Experiment 2 (data not shown). As such, in Experiment 3a, a further attempt was made to reduce CS-elicited freezing during extinction recall among primiparous females by exposing rats to a weaker conditioning protocol consisting of one, rather than two, CS-US pairings during fear conditioning.

Both groups showed comparable baseline levels of freezing prior to both extinction training and extinction recall (see *Table 1*, largest $t_{(18)} = 1.19$, $p = .27$). Rats receiving MK-801 showed significantly less CS-elicited freezing compared to rats receiving saline over the nine extinction blocks (see *Figure 3a*; $F_{(1, 18)} = 68.95$, $p < .01$). While we did not find a significant decrease in CS-elicited freezing across extinction training in rats receiving saline using a repeated measures ANOVA analysis ($F_{(8, 72)} = 2.55$, $p = .09$), we did find a significant linear trend ($F_{(1, 9)} = 7.802$, $p = .02$) in CS-elicited freezing in this group. Moreover, we have previously

demonstrated that primiparous females show a significant reduction in CS-elicited freezing over six blocks of extinction training (see Experiment 1). There was no significant difference in CS-elicited freezing between the two groups at extinction recall (see *Figure 3b;* $t_{(18)} = .23$, $p = .82$).

Experiment 3b

In Experiment 3a, we did not detect a significant effect of MK-801 on extinction recall in primiparous females. However, it is possible that the failure to detect such an effect was due to the use of a single conditioning trial. To address this possibility, nulliparous females were presented with a single conditioning trial prior to undergoing extinction training in Experiment 3b. In order to prevent floor effects, rats in this experiment rats received six blocks of extinction training, rather than nine blocks, given that nulliparous females showed better within-session extinction than primiparous females in Experiments 1-2.

Both groups showed comparable baseline levels of freezing prior to extinction training, and extinction recall (see *Table 1,* largest $t_{(20)} = 1.78$, $p = .10$). Rats receiving MK-801 showed significantly less CS-elicited freezing compared to rats receiving saline over the nine extinction blocks (see *Figure 4a*, $F_{(1, 18)} = 68.95$, $p < .01$). CS-elicited freezing decreased across extinction training in rats receiving saline ($F_{(5, 50)} = 7.10$, $p < .01$). At extinction recall, the saline group showed significantly lower levels of CS-elicited freezing compared to the MK-801 group (see *Figure 4b*, $t_{(20)} = 2.10$, $p = .05$).

Experiment 4

Together, the results of Experiments 1-3 indicate that the formation of extinction memories may be NMDAR-independent in primiparous females, but not nulliparous females. Experiment 4 sought to determine whether NMDAR-independent memory formation in primiparous females is specific to extinction memories, or generalizable to other types of memories. Specifically, it examined whether the formation of conditioning memories is also NMDAR-independent in primiparous females. In Experiment 4, MK-801 was administered prior to cued fear conditioning in nulliparous and primiparous females. Animals' recall of conditioning was tested the following day.

There was no significant effect of estrous phase on levels of baseline freezing, and on levels of CS-elicited, and context-elicited freezing during test (data not reported). Therefore, data were collapsed across estrous phase in subsequent analyses. All groups showed comparable levels of baseline freezing prior to cued recall (largest $F_{(1, 71)} = 1.02$, $p = .32$). At conditioning recall, animals that received MK-801 showed lower levels

of CS-elicited freezing compared to those that received saline (see *Figure 5a;* $F_{(1, 71)} = 16.98$, $p < .01$). Post-hoc LSD tests indicated that there was a significant difference between the saline and MK-801 treated animals among both nulliparous ($p = .01$) and primiparous ($p < .01$) females. However, there was no main effect of drug on context-elicited freezing (see *Figure 5b;* $F_{(1, 71)} = .34$, $p = .56$). Primiparous females showed higher levels of CS-elicited ($F_{(1, 71)} = 4.01$, $p = .05$) and context-elicited freezing ($F_{(1, 71)} = 11.32$, $p < .01$) compared to nulliparous females. No significant reproductive experience x drug interaction on CS-elicited, or context-elicited freezing was detected (largest $F_{(1, 71)} = 1.62$, $p = .21$).

Experiment 5

In Experiment 4, systemic administration of MK-801 prior to fear conditioning reduced CS-elicited freezing the following day in both nulliparous and primiparous females, indicating that NMDAR-independent memory formation in primiparous females may be specific to extinction. However, it is of note that delay fear conditioning (in which the CS-US presentations overlap, as occurred in the present study) is more reliant on the amygdala than the hippocampus (Phillips and LeDoux 1992; Raybuck and Lattal 2011). As such, an alternative possibility is that reproductive experience causes a switch to NMDAR-independent memory formation for hippocampally-dependent tasks. Indeed, in Experiment 4, MK-801 was not found to have an impairing effect on context-elicited fear in primiparous females. While this was also the case in nulliparous females, the failure to detect an impairing effect of MK-801 in this group may have been due to a floor effect, particularly given that we were still able to detect a main effect of reproductive experience in the analysis of these results. In Experiment 5, we investigated whether NMDAR-independent memory formation is specific to hippocampally-dependent tasks in reproductively experienced females by examining the effect of systemic MK-801 administration prior to unsignalled contextual conditioning (i.e. when there is no discrete cue signalling the US) in nulliparous and primiparous females.

At conditioning recall, animals that received MK-801 showed significantly less context-elicited freezing compared to animals that received saline (see *Figure 6*, $F_{(1, 30)} = 14.36$, $p < .01$). Post-hoc LSD tests indicated that there was a significant difference between the saline and MK-801 treated animals among both nulliparous ($p = .01$) and primiparous ($p = .01$) females. Nulliparous and primiparous females showed comparable levels of freezing at recall ($F_{(1, 30)} = .55$, $p = .46$).

Discussion

There are three major findings from the present study. Firstly, we found that fear extinction is disrupted by MK-801 in nulliparous, but not primiparous females. This indicates that extinction is dependent on NMDARs in nulliparous, but not primiparous females (Experiments 1-3). It is unlikely that these findings were due to a ceiling effect driven by poor extinction recall in saline-treated primiparous rats, given that extending the length of extinction training (Experiment 2), and reducing the strength of conditioning (Experiment 3), which led to equivalent levels of extinction recall between saline-treated primiparous and nulliparous rats, and reduced freezing during extinction recall in primiparous rats, respectively, yielded similar results. Secondly, we found that both cued and contextual fear conditioning are disrupted by MK-801 in nulliparous and primiparous females, indicating that conditioning is dependent on NMDARs in females regardless of reproductive experience (Experiments 4-5). Thirdly, across the first four experiments, we found that primiparous females consistently showed heightened fear responses, including higher levels of CS-elicited freezing during extinction training and at extinction recall, compared to nulliparous females. Each of these findings will be discussed in turn.

The main finding from this study is that MK-801 leads to impaired extinction recall in nulliparous, but not primiparous females, suggesting that extinction may become NMDAR-independent following reproductive experience. In nulliparous females, fear extinction appears to be dependent on both estradiol (Milad et al. 2009) and NMDAR (as demonstrated by the present work). These findings are consistent with the proposal that estradiol might enhance extinction through its interactions with NMDAR. When administered onto hippocampal brain slices, estradiol increases both NMDAR binding and the amplitude of NMDA-mediated excitatory post-synaptic potentials (Foy et al. 1999; Woolley et al. 1997). Estradiol has also been shown to increase long-term potentiation (LTP) via its effect on NMDAR-mediated transmission in the hippocampus (Smith and McMahon 2005). Moreover, blocking NMDAR activity in the hippocampus inhibits estradiol-induced improvements in the object recognition task (Lewis et al. 2008). Therefore, it is possible that estradiol enhances fear extinction in females by upregulating NMDAR activity. In primiparous rats, fear extinction does not appear to be modulated by estradiol (no impact of estrous cycle; Milligan-Saville and Graham 2016), or NMDARs (as demonstrated by the present work). It is therefore possible that primiparous females rely on a different molecular signalling process for fear extinction. It would be of interest to investigate whether other neural processes that play a well-established role in fear extinction in males are similarly involved in fear extinction in nulliparous females, and whether their involvement is similarly altered in primiparous females. For instance, it may be of interest to

investigate the involvement of gamma-Aminobutyric acid (GABA) receptors in fear extinction in female rats, given that there is substantial evidence to suggest that these receptors are critically involved in fear extinction in adult male rats (see Makkar et al. 2010).

The possibility that fear extinction may be NMDAR-independent in primiparous females may be considered somewhat surprising given that the existing literature indicates that NMDARs are essential for learning and consolidating many memories, including extinction memories. Notably, however, there are some other instances of NMDAR-independent extinction. For instance, NMDARs are not required for extinction in adolescent male rats conditioned as juveniles (Bisby, Baker and Richardson 2018), and for re-extinction in adult male rats (Laurent et al. 2008). Moreover, systemic administration of MK-801 prior to extinction training does not impair extinction recall in infant male rats (Kim and Richardson 2010; Langton et al. 2007). As with primiparous females (Milligan-Saville & Graham, 2016), infant male rats also do not show renewal or reinstatement (Kim and Richardson 2007a; Kim and Richardson 2007b). Given that the features of extinction in primiparous females are characteristic of those that are present during early development (i.e., in both instances, extinction is relapse-resistant and unaffected by MK-801), one may speculate that reproductive experience causes a switch to infant-like extinction processes. However, unlike infant male rats, primiparous females in this study showed poor extinction recall. To determine whether reproductive experience does, in fact, result in infant-like extinction processes, it would be useful to investigate other molecular features of fear extinction in primiparous females. For instance, in addition to being NMDAR-independent, fear extinction is also independent of GABA receptor activation and does not involve the infralimbic cortex in infant male rats (Kim et al. 2009; Kim and Richardson 2007b). As such, future studies should investigate whether these signals and neural circuits are involved in fear extinction in reproductively experienced females.

The second main finding of this study was that the blockade of NMDARs prior to conditioning led to reduced fear responses in both nulliparous and primiparous females the following day, indicating that the activation of NMDARs is required for the formation of fear conditioning memories in females, irrespective of reproductive status. This was the case for both cued and contextual fear conditioning, indicating that NMDAR-independent memory formation amongst primiparous rats may be specific to fear extinction, rather than generalizable to other hippocampally-mediated tasks. However, it is also of note that while fear conditioning involves the development of fear, fear extinction involves the inhibition of fear. Therefore, an alternative possibility is that reproductive experience causes NMDAR-independent memory formation in other paradigms involving fear inhibition, such as AX+/BX- discrimination learning (Myers and Davis 2004) and latent

inhibition (i.e. the reduction in learning that occurs when a non-reinforced CS presentation is given to subjects prior to conditioning; Lubow 1973). There is evidence to suggest that the blockade of NMDARs using systemic or central administration of MK-801 disrupts latent inhibition in adult male rodents (Lewis and Gould 2004; Schauz and Koch 2000; Traverso et al. 2012). As such, it would be of interest to determine whether NMDARs are required for latent inhibition in nulliparous and primiparous female rats. Doing so may allow us to determine whether NMDAR-independent memory formation is generalizable to other tasks involving fear inhibition in primiparous females.

The third main finding of this study was that primiparous females consistently showed heightened fear responses relative to nulliparous females during extinction training and extinction recall. It is possible that these heightened fear response are due to an impaired ability to acquire fear extinction in primiparous females. Alternatively, it is possible that these heightened fear responses are due to stronger conditioning in primiparous females. Indeed, in Experiment 4, primiparous females showed stronger fear conditioning memories (both to a conditioned cue and context) compared to nulliparous females. Notably, however, unlike in Experiment 4, nulliparous and primiparous females showed comparable levels of context-elicited fear in Experiment 5. Conditioning procedures between Experiment 4 and Experiment 5 differed in a number of ways. Firstly, while rats were trained to fear an explicit cue in Experiment 4, this was not the case in Experiment 5. Moreover, rats were pre-exposed to the conditioning context in Experiment 4, but not in Experiment 5. Such procedural differences may have reduced the predictive value of the context in Experiment 4. Therefore, one possibility is that differences in contextual fear conditioning between nulliparous and primiparous females are only detectable under circumstances in which these females are given sub-optimal conditioning. It is also of note that nulliparous and primiparous females showed comparable levels of freezing on the first block of extinction training across Experiments 1 and 2, suggesting that primiparous females may not show stronger conditionability relative to nulliparous females. However, it is possible that the failure to observe a difference between these groups was due to a ceiling effect. Future research is therefore required to determine whether primiparous females show heightened fear responses during extinction training and extinction recall as a result of stronger conditioning or impaired extinction acquisition. One way of doing so involves giving nulliparous and primiparous females weaker conditioning (e.g. reduced shock intensity) to determine whether there is an effect of reproductive experience on freezing on the first block of extinction training the following day.

The heightened fear responses across extinction training and extinction recall observed in primiparous females in Experiments 1-4 may be attributable to the molecular and hormonal changes that occur over

pregnancy and the postpartum period. For instance, pregnancy is associated with chronically elevated levels of estradiol and progesterone (see Workman et al. 2012). Exposure to such high levels of these hormones over a prolonged period may cause structural changes in the brain that affect fear conditioning and extinction. Moreover, compared to nulliparous females, reproductively experienced females have higher levels of BDNF – a protein that is involved in fear conditioning and extinction (Choi et al. 2010; Liu et al. 2004; Macbeth et al. 2008, Rosas-Vidal et al. 2014). While this protein has been shown to facilitate fear extinction (Rosas-Vidal et al. 2014), it is possible that its effects on strengthening fear conditioning override its potential effects on fear extinction in primiparous females, thereby resulting in heightened fear responses during extinction training and extinction recall. Notably, the heightened fear responses among primiparous females found in the current study were not found in Milligan-Saville and Graham's (2016) study. However, Milligan-Saville and Graham (2016) did find a higher level of variability in extinction recall among primiparous females compared to nulliparous females. While extinction in primiparous females was, at times, comparable to virgin proestrus rats (which showed good extinction recall), it was, at other times, comparable to virgin metestrus rats (which showed poor extinction recall). Such variability may be due to individual differences in the experience of pregnancy and motherhood and the effects of these on fear extinction. For instance, differences in litter gender composition and levels of maternal behaviour (e.g. licking/grooming in rats) may have contributed to different fear responses. Indeed, such factors have previously been shown to affect protein expression and/or behaviour in parous females (Francis et al. 2000; Hao et al. 2011), and may therefore play a role in altering a female's ability to acquire and extinguish learned fear responses. As such, the association between such factors, and fear expression and extinction in mothers warrants further investigation.

In the current study, primiparous females were tested approximately two weeks after weaning. It is therefore uncertain whether it is pregnancy, motherhood, or a combination of both experiences that alters fear extinction and expression in primiparous females. It would be useful to disentangle how each of these experiences alters fear extinction, particularly given that pregnancy and motherhood are associated with distinct neuroendocrine changes. Pregnancy is associated with chronically elevated levels of estradiol and progesterone in both rats and humans, while oxytocin, prolactin and glucocorticoid levels are elevated following parturition (Leuner et al. 2010). Moreover, previous studies have shown that pregnancy and motherhood can have distinct effects on learning and memory. For example, while female rats tested during the late phase of their pregnancy show impaired eyeblink conditioning following exposure to stress, postpartum females and virgins that been exposed to pups show enhanced eyeblink conditioning following acute stress (Leuner and Shors 2006). Such

findings indicate that mothering alone can cause stress to enhance subsequent learning. Mothering experiences, however, are not sufficient in enhancing spatial reference and working memory. Primiparous females that have experienced both pregnancy and motherhood make significantly fewer working and reference memory errors in the radial arm maze relative to nulliparous females that have been exposed to pups (Pawluski et al. 2006). To determine whether pregnancy and motherhood have different effects on fear extinction in females, future studies can examine fear extinction in nulliparous females that have fostered pups, and primiparous females that have been separated from their pups immediately after parturition.

Given the prevalence of anxiety disorders among women, it is essential that we find ways of optimising treatment outcomes in this population. Moreover, given the prevalence of post-partum anxiety in women (see Ross et al. 2006), it is particularly important to ensure that treatments are suitable for women during different reproductive stages. Since the hormonal, behavioural and molecular features of fear extinction appear to be altered by reproductive experience, it is possible that reproductive status might be an important consideration when delivering anxiety treatments to women. For instance, the heightened fear responses in primiparous females observed in the present study may indicate that mothers could require a greater number of exposure therapy sessions to achieve the same outcomes as non-mothers. Moreover, changes in the signalling processes underlying fear extinction resulting from reproductive experience may indicate that the effectiveness of pharmacological adjuncts to psychological therapy differ between mothers and non-mothers. For instance, D-cycloserine (DCS; an NMDAR agonist) has recently been proposed as a pharmacological adjunct to exposure therapy given that it enhances extinction recall in adult male rats and humans (see Singewald et al. 2015). However, given that primiparous females may not rely on NMDARs for fear extinction, it is possible that DCS may not be as effective in augmenting exposure therapy outcomes in mothers. These potential clinical implications are purely speculative at this stage. However, the current and past findings indicating that motherhood alters the behavioural, hormonal and molecular features of fear extinction suggest that further investigations of fear extinction in females that consider reproductive experience are warranted. Such studies may allow for the development of more tailored and effective treatments for anxiety disorders among women.

References

Baker JD, Azorlosa JL (1996) The NMDA antagonist MK-801 blocks the extinction of Pavlovian fear conditioning. Behav Neurosc. 110:618

Baker KD, Richardson R (2017) Pharmacological evidence that a failure to recruit NMDA receptors contributes to impaired fear extinction retention in adolescent rats. Neurobiol Learn Mem 143:18-26. https://doi:10.1016/j.nlm.2016.10.014

Barha CK, Dalton GL, Galea LAM (2010) Low doses of 17 alpha-estradiol and 17 bold italic beta-estradiol facilitate, whereas higher doses of estrone and 17 alpha-and 17 bold italic beta-estradiol impair, contextual Fear conditioning in adult female rats. Neuropsychopharmacology 35:547-559. https://doi:10.1038/npp.2009.161

Bisby MA, Baker, KD, Richardson R (2018). Elucidating the mechanisms of fear extinction in developing animals: a special case of NMDA receptor-independent extinction in adolescent rats. Learn Mem 25:158-164

Bridges RS, Byrnes EM (2006) Reproductive experience reduces circulating 17β-estradiol and prolactin levels during proestrus and alters estrogen sensitivity in female rats. Endocrinol 147:2575-2582. https://doi:10.1210/en.2005-0917

Burgos-Robles A, Vidal-Gonzalez I, Santini E, Quirk GJ (2007) Consolidation of fear extinction requires NMDA receptor-dependent bursting in the ventromedial prefrontal cortex. Neuron 53:871-880. https://doi:10.1016/j.neuron.2007.02.021

Chang YJ, Yang CH, Liang YC, Yeh CM, Huang CC, Hsu KS (2009) Estrogen modulates sexually dimorphic contextual fear extinction in rats through estrogen receptor β. Hippocampus 19:1142-1150

Choi DC, Maguschak KA, Ye K, Jang S-W, Myers KM, Ressler KJ (2010) Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. Proc Natl Acad Sci U.S.A. 107:2675-2680

Fanselow MS (1980) Conditional and unconditional components of post-shock freezing. Pavlovian J Biol Sci 15:177-182

Foa EB, McLean CP (2016) The efficacy of exposure therapy for anxiety-related disorders and its underlying mechanisms: the case of OCD and PTSD. Annu Rev Clin Psychol 12:1-28

Foy M, Xu J, Xie X, Brinton R, Thompson R, Berger T (1999) 17β-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. J Neurophysiol 81:925-929

Francis DD, Champagne FC, Meaney MJ (2000) Variations in maternal behaviour are associated with differences in oxytocin receptor levels in the rat. J Neuroendocrinol 12:1145-1148

Graham BM, Daher M (2016) Estradiol and progesterone have opposing roles in the regulation of fear extinction in female rats. Neuropsychopharmacology 41:774

Graham BM, Milad MR (2011) The study of fear extinction: implications for anxiety disorders. Am J Psychiatry 168:1255-1265

Graham BM, Milad MR (2013) Blockade of estrogen by hormonal contraceptives impairs fear extinction in female rats and women. Biol Psychiatry 73:371-378

Graham BM, Scott E (2018) Effects of systemic estradiol on fear extinction in female rats are dependent on interactions between dose, estrous phase, and endogenous estradiol levels. Horm Behav 97:67-74

Gruene TM, Roberts E, Thomas V, Ronzio A, Shansky RM (2015) Sex-specific neuroanatomical correlates of fear expression in prefrontal-amygdala circuits. Biol Psychiatry 78:186-193. https://doi:10.1016/j.biopsych.2014.11.014

Gupta RR, Sen S, Diepenhorst LL, Rudick CN, Maren S (2001) Estrogen modulates sexually dimorphic contextual fear conditioning and hippocampal long-term potentiation (LTP) in rats. Brain Res 888:356-365. https://doi:10.1016/S0006-8993(00)03116-4

Hao Y, Huang W, Nielsen DA, Kosten TA (2011) Litter gender composition and sex affect maternal behavior and DNA methylation levels of the oprm1 gene in rat offspring. Front Psychiatry 2:21 https://doi:10.3389/fpsyt.2011.00021

Kim JH, Hamlin AS, Richardson R (2009) Fear extinction across development: the involvement of the medial prefrontal cortex as assessed by temporary inactivation and immunohistochemistry. J Neurosci 29:10802-10808. https://doi:10.1523/JNEUROSCI.0596-09.2009

Kim JH, Richardson R (2007a) A developmental dissociation in reinstatement of an extinguished fear response in rats. Neurobiol Learn Mem 88:48-57. https://doi:10.1016/j.nlm.2007.03.004

Kim JH, Richardson R (2007b) A developmental dissociation of context and GABA effects on extinguished fear in rats. Behav Neurosci 121:131-139. https://doi:10.1037/0735-7044.121.1.131

Kim JH, Richardson R (2010) Extinction in preweanling rats does not involve NMDA receptors. Neurobiol Learn Mem 94:176-182. https://doi:10.1016/j.nlm.2010.05.004

Kinsley CH et al. (1999) Motherhood improves learning and memory. Nature 402:137-138. https://doi:10.1038/45957

Kinsley CH et al. (2006) Motherhood and the hormones of pregnancy modify concentrations of hippocampal neuronal dendritic spines. Horm Behav 49:131-142. https://doi:10.1016/j.yhbeh.2005.05.017

Langton JM, Kim JH, Nicholas J, Richardson R (2007) The effect of the NMDA receptor antagonist MK-801 on the acquisition and extinction of learned fear in the developing rat. Learn Mem 14:665-668. https://doi:10.1101/lm.692407

Langton JM, Richardson R (2009) The role of context in the re-extinction of learned fear. Neurobiol Learn Mem 92:496-503

Laurent V, Marchand AR, Westbrook RF (2008) The basolateral amygdala is necessary for learning but not relearning extinction of context conditioned fear. Learn Mem 15:304-314. https://doi:10.1101/lm.928208

Ledgerwood L, Richardson R, Cranney J (2003) Effects of D-cycloserine on extinction of conditioned freezing. Behav Neurosci 117:341. https://doi:10.1037/0735-7044.117.2.341

Lemaire V et al. (2006) Motherhood-induced memory improvement persists across lifespan in rats but is abolished by a gestational stress. Eur J Neurosci 23:3368-3374. https://doi:10.1111/j.1460-9568.2006.04870.x

Leuner B, Glasper ER, Gould E (2010) Parenting and plasticity. Trends Neurosci 33:465-473 doi:https://doi.org/10.1016/j.tins.2010.07.003

Leuner B, Gould E (2010) Dendritic growth in medial prefrontal cortex and cognitive flexibility are enhanced during the postpartum period. J Neurosci 30:13499-13503. https://doi:10.1523/JNEUROSCI.3388-10.2010

Leuner B, Shors TJ (2006) Learning during motherhood: A resistance to stress. Horm Behav 50:38-51. https://doi:10.1016/j.yhbeh.2006.01.002

Lewis MC, Gould TJ (2004) Latent inhibition of cued fear conditioning: an NMDA receptor-dependent process that can be established in the presence of anisomycin. Eur J Neurosci 20:818-826

Lewis MC, Kerr KM, Orr PT, Frick KM (2008) Estradiol-induced enhancement of object memory consolidation involves NMDA receptors and protein kinase A in the dorsal hippocampus of female C57BL/6 mice. Behav Neurosci 122:716-721. https://doi:10.1037/0735-7044.122.3.716

Li S, Graham BM (2016) Estradiol is associated with altered cognitive and physiological responses during fear conditioning and extinction in healthy and spider phobic women. Behav Neurosci 130:614-623. https://doi:10.1037/bne0000166

Li SH, Graham BM (2017) Why are women so vulnerable to anxiety, trauma-related and stress-related disorders? The potential role of sex hormones. Lancet Psychiatry 4:73-82. https://doi.org/10.1016/S2215-0366(16)30358-3

Liu IYC, Lyons WE, Mamounas LA, Thompson RF (2004) Brain-derived neurotrophic factor plays a critical role in contextual fear conditioning J Neurosci 24:7958-7963. https://doi:10.1037/h0042199

Liu JL et al. (2009) A NMDA receptor antagonist, MK-801 impairs consolidating extinction of auditory conditioned fear responses in a Pavlovian model. PLoS One 4:e7548. https://doi:10.1371/journal.pone.0007548

Love G et al. (2005) Maternal experience produces long-lasting behavioral modifications in the rat Behav Neurosci 119:1084-1096. https://doi:10.1037/0735-7044.119.4.1084

Lubow RE (1973) Latent inhibition. Psychol Bull 79:398-407

Macbeth AH, Scharfman HE, MacLusky NJ, Gautreaux C, Luine VN (2008) Effects of multiparity on recognition memory, monoaminergic neurotransmitters, and brain-derived neurotrophic factor (BDNF). Horm Behav 54:7-17

Makkar SR, Zhang SQ, Cranney J (2010) Behavioral and neural analysis of GABA in the acquisition, consolidation, reconsolidation, and extinction of fear memory. Neuropsychopharmacology 35:1625

Markus EJ, Zecevic M (1997) Sex differences and estrous cycle changes in hippocampus-dependent fear conditioning. Psychobiol 25:246-252

McLean CP, Asnaani A, Litz BT, Hofmann SG (2011) Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. J Psychiatr Res 45:1027-1035

Milad MR, Igoe SA, Lebron-Milad K, Novales JE (2009) Estrous cycle phase and gonadal hormones influence conditioned fear extinction. Neuroscience 164:887-895. https://doi:10.1016/j.neuroscience.2009.09.011

Milad MR, Zeidan MA, Contero A, Pitman RK, Klibanski A, Rauch SL, Goldstein JM (2010) The influence of gonadal hormones on conditioned fear extinction in healthy humans. Neuroscience 168:652-658

Milligan-Saville J, Graham BG (2016) Mothers do it differently: reproductive experience alters fear extinction in female rats and women. Transl Psychiatry 6: e928

Monte LM, Ellis RR (2014) Fertility of Women in the United States: 2012. Econ. 24:1071-1100

Myers KM, Davis M (2004) AX+, BX-discrimination learning in the fear-potentiated startle paradigm: possible relevance to inhibitory fear learning in extinction. Learn Mem 11:464-475

Myers KM, Davis M (2007) Mechanisms of fear extinction. Mol Psychiatry 12:120

Paris JJ, Frye CA (2008) Estrous cycle, pregnancy, and parity enhance performance of rats in object recognition or object placement tasks. Reproduction 136:105-115. https://doi:10.1530/REP-07-0512

Pawluski JL, Galea LA (2006) Hippocampal morphology is differentially affected by reproductive experience in the mother. J Neurobiol 66:71-81. https://doi:10.1002/neu.20194

Pawluski JL, Vanderbyl BL, Ragan K, Galea LA (2006) First reproductive experience persistently affects spatial reference and working memory in the mother and these effects are not due to pregnancy or 'mothering' alone. Behav Brain Res 175:157-165. https://doi:10.1016/j.bbr.2006.08.017

Phillips R, LeDoux J (1992) Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274

Pineles SL et al. (2016) Extinction retention and the menstrual cycle: Different associations for women with posttraumatic stress disorder. J Abnorm Psychol 125:349

Quirk GJ, Mueller D (2008) Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacology 33:56

Rasia-Filho AA, Fabian C, Rigoti KM, Achaval M (2004) Influence of sex, estrous cycle and motherhood on dendritic spine density in the rat medial amygdala revealed by the Golgi method. Neuroscience 126:839-847. https://doi:10.1016/j.neuroscience.2004.04.009

Raybuck JD, Lattal KM (2011) Double dissociation of amygdala and hippocampal contributions to trace and delay fear conditioning. PloS One 6:e15982

Rey CD, Lipps J, Shansky RM (2014) Dopamine d1 receptor activation rescues extinction impairments in low-estrogen female rats and induces cortical layer-specific activation changes in prefrontal–amygdala circuits. Neuropsychopharmacology 39:1282

Rosas-Vidal LE, Do-Monte FH, Sotres-Bayon F, Quirk GJ (2014) Hippocampal–prefrontal BDNF and memory for fear extinction. Neuropsychopharmacology 39:2161-2169

Ross LE, McLean LM (2006) Anxiety disorders during pregnancy and the postpartum period: a systematic review. J Clin Psychiatry 67:1285-1298.

Schauz C, Koch M (2000) Blockade of NMDA receptors in the amygdala prevents latent inhibition of fear-conditioning. Learn Mem 7:393-399

Singewald N, Schmuckermair C, Whittle N, Holmes A, Ressler K (2015) Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. Pharmacol Ther 149:150-190

Smith CC, McMahon LL (2005) Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. J Neurosci 25:7780-7791. https://doi:10.1523/JNEUROSCI.0762-05.2005

Sotres-Bayon F, Bush DE, LeDoux JE (2007) Acquisition of fear extinction requires activation of NR2B-containing NMDA receptors in the lateral amygdala. Neuropsychopharmacology 32:1929-1940. https://doi:10.1038/sj.npp.1301316

Traverso LM, Ruiz G, De la Casa LG (2012) Effect of the NMDA antagonist MK-801 on latent inhibition of fear conditioning. Pharmacol Biochem Behav 102:488-494. https://doi.org/10.1016/j.pbb.2012.06.014

Walker DL, Ressler KJ, Lu K-T, Davis M (2002) Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. J Neurosci 22:2343-2351

Wegerer M, Kerschbaum H, Blechert J, Wilhelm FH (2014) Low levels of estradiol are associated with elevated conditioned responding during fear extinction and with intrusive memories in daily life. Neurobiol Learn Mem 116:145-154

White EC, Graham BM (2016) Estradiol levels in women predict skin conductance response but not valence and expectancy ratings in conditioned fear extinction. Neurobiol Learn Mem 134:339-348

Woolley CS, Weiland NG, McEwen BS, Schwartzkroin PA (1997) Estradiol increases the sensitivity of hippocampal CA1 pyramidal cells to NMDA receptor-mediated synaptic input: correlation with dendritic spine density. J. Neurosci. 17:1848-1859

Workman JL, Barha CK, Galea LAM (2012) Endocrine substrates of cognitive and affective changes during pregnancy and postpartum. Behav. Neurosci. 126:54

Zeidan MA et al. (2011) Estradiol modulates medial prefrontal cortex and amygdala activity during fear extinction in women and female rats. Biol Psychiatry 70:920-927

Table 1

	NP Saline	NP MK-801	PP Saline	PP MK-801
Experiment 1				
Extinction	4.29 (2.10)	0.94 (0.68)	7.25 (3.22)	3.50 (2.01)
Recall	0.00 (0.00)	0.00 (0.00)	1.00 (1.00)	8.50 (4.66)
Experiment 2				
Extinction	11.46 (5.63)	1.14 (0.62)	5.83 (3.02)	7.50 (3.18)
Recall	0.83 (0.83)	1.82 (1.02)	0.33 (0.33)	3.21 (2.85)
Experiment 3a				
Extinction	2.71 (1.91)	0.00 (0.00)	-	-
Recall	0.00 (0.00)	6.04 (3.39)	-	-
Experiment 3b				
Extinction	-	-	3.25 (2.74)	0.00 (0.00)
Recall	-	-	0.00 (0.00)	1.00 (1.00)
Experiment 4				
Recall (cue)	3.00 (2.25)	8.68 (4.26)	1.05 (1.05)	3.42 (1.67)

Note: NP = nulliparous, PP = primiparous

Mean levels of baseline freezing (±SEM), measured immediately prior to the first block of extinction training and/or recall, in nulliparous and primiparous groups across Experiments 1-4.

Figure captions

Fig. 1 In Experiment 1, nulliparous and primiparous rats were conditioned with 2 CS-US pairings on Day 1. Rats were administered saline or MK-801 prior to 30 blocks of extinction training on Day 2, and tested for extinction recall on Day 3. **(a)** Mean (±SEM) levels of CS-elicited freezing for groups Nulliparous-saline (n = 12), Nulliparous-MK-801 (n = 11), Primiparous-saline (n = 10) and Primiparous-MK-801 (n = 10) during extinction training in Experiment 1. * MK-801 < Saline ($p < .05$). # Primiparous-saline > Nulliparous-saline ($p < .05$). **(b)** Mean (±SEM) levels of CS-elicited freezing during extinction recall in Experiment 1. * Nulliparous-saline < Primiparous-saline ($p < .05$). ** Nulliparous-saline < Nulliparous-MK-801 ($p < .05$)

Fig. 2 In Experiment 2, nulliparous and primiparous rats were conditioned with 2 CS-US pairings on Day 1. Rats were administered saline or MK-801 prior to 45 blocks of extinction training on Day 2, and tested for extinction recall on Day 3. **(a)** Mean (±SEM) levels of CS-elicited freezing for groups Nulliparous-saline (n = 12), Nulliparous-MK-801 (n = 11), Primiparous-saline (n = 15) and Primiparous-MK-801 (n = 13) during extinction training in Experiment 2. * MK-801 < Saline ($p < .05$). # Primiparous-saline > Nulliparous-saline ($p < .05$). **(b)** Mean (±SEM) levels of CS-elicited freezing during extinction recall in Experiment 2. * Nulliparous-saline < Nulliparous-MK-801 ($p < .05$).

Fig. 3 In Experiment 3a, primiparous rats were conditioned with 1 CS-US pairing on Day 1. Rats were administered saline or MK-801 prior to 45 blocks of extinction training on Day 2, and tested for extinction recall on Day 3. (a) Mean (±SEM) levels of CS-elicited freezing in primiparous females administered saline (n = 10) and primiparous administered MK-801 (n = 10) during extinction training in Experiment 3a. * MK-801 < Saline. (b) Mean (±SEM) levels of CS-elicited freezing during extinction recall in Experiment 3a.

Fig. 4 In Experiment 3b, nulliparous rats were conditioned with 1 CS-US pairing on Day 1. Rats were administered saline or MK-801 prior to 30 blocks of extinction training on Day 2, and tested for extinction recall on Day 3. (a) Mean (±SEM) levels of CS-elicited freezing in nulliparous females administered saline (n = 11) and nulliparous females administered MK-801 (n = 11) during extinction training in Experiment 3b. * MK-801 < Saline (b) Mean (±SEM) levels of CS-elicited freezing during extinction recall in Experiment 3b. * Saline < MK-801 ($p < .05$)

Fig. 5 In Experiment 5, nulliparous and primiparous rats were administered saline or MK-801 prior to conditioning with 2 CS-US pairings on Day 1. Rats were tested for freezing to the CS and the conditioning context on Day 2. (a) Mean (±SEM) levels of CS-elicited freezing for groups Nulliparous-saline (n = 19),

Nulliparous-MK-801 (n = 18), Primiparous-saline (n = 19) and Primiparous-MK-801 (n = 19) during conditioning recall (cue) in Experiment 4. *Nulliparous-saline > Nulliparous-MK-801 ($p < .05$). **Primiparous-Saline > Primiparous-MK-801 ($p < .05$). ***Primiparous > Nulliparous ($p = .05$). (b) Mean (±SEM) levels of context-elicited freezing during conditioning recall in Experiment 4. *Primiparous > Nulliparous ($p < .05$).

Fig. 6 In Experiment 5, nulliparous and primiparous rats were administered saline or MK-801 prior to contextual conditioning with 2 US presentations on Day 1. Rats were tested for freezing to the conditioning context on Day 2. Mean (±SEM) levels of context-elicited freezing for groups Nulliparous-saline (n = 8), Nulliparous-MK-801 (n = 8), Primiparous-saline (n = 9) and Primiparous-MK-801 (n = 9) during conditioning recall (context) in Experiment 5. * Nulliparous-saline > Nulliparous-MK-801 ($p < .05$). **Primiparous-saline > Primiparous-MK-801.