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# Sex-Specific Effects of Minocycline on Contextual Fear Memory

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**Title: Sex-Specific Effects of Minocycline on Contextual Fear Memory**

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**Conflict of Interest:**

The authors declare no conflicts of interest.

**Abstract**

***Rational:***

Recent studies have highlighted the crucial role of microglia in fear learning and memory. This supports the potential therapeutic value of the anti-inflammatory agent minocycline, which regulates microglial activity, in psychiatric disorders involving dysregulated emotional memory.

***Objectives:***

To assess minocycline's impact during a tractable intervention window, we examined its acute systemic effects on the reconsolidation, extinction, and forgetting of contextual fear memory (CFM) in rats

***Methods:***

Male and female rats underwent contextual fear conditioning, with minocycline administered acutely before or in the absence of memory retrieval. Recent and remote CFM were assessed, with conditioned freezing used as the primary measure of fear expression.

***Results*:**

In males, minocycline administered before retrieval enhanced extinction of recently formed CFM, reducing subsequent fear expression without disrupting the original fear memory. In contrast, administration without retrieval attenuated forgetting and persistently increased fear expression. For remote CFM, minocycline given before retrieval increased fear expression, consistent with enhanced reconsolidation. In females, minocycline increased fear expression for recent memories regardless of retrieval, with no evidence of enhanced extinction or reduced forgetting.

***Conclusions:***

Acute minocycline has context-, memory age-, and sex-specific effects on CFM. In males, it can enhance extinction of recent memories but strengthen or preserve fear memories under other conditions, whereas in females it predominantly increased fear expression. These findings suggest that minocycline's effects may depend on sex-specific and memory-state-dependent mechanisms, highlighting the need to consider sex, memory age, and retrieval conditions when evaluating its therapeutic potential as an adjunct to exposure-based treatments.

## Introduction

Dysregulated fear memory formation and processing is a key feature of many psychiatric disorders (Izquiredo et al. 2016; Pittenger 2013), including post-traumatic stress disorder (PTSD) (Ressler et al. 2022), anxiety disorders (Britton et al. 2011; Lissek 2012), depression (Dibbets et al., 2015; Wurst et al., 2021), and schizophrenia (Holt et al., 2009; Tuominen et al., 2021, 2022). Modifying fear memory therefore represents an important target for developing novel psychiatric therapies.

Rodent studies suggest that microglia contribute to hippocampal-dependent fear learning and memory. Microglia support synaptic maturation and plasticity, processes underlying learning and memory in both developing and adult brains (Salter & Stevens, 2017; Wolf et al., 2017). Mice lacking the microglia-specific CX3CR1 receptor show impairments in hippocampal plasticity, learning, and neurogenesis (Bachstetter et al., 2011; Rogers et al., 2011), while microglial-derived BDNF has also been implicated in hippocampal plasticity and learning (Chaaya et al., 2019; Parkhurst et al., 2013). Increasing evidence further implicates microglia in psychiatric disorders (Dietz et al., 2020; Mondelli et al., 2017; Rahimian et al., 2021; Tay et al., 2018). Adolescents treated with minocycline, an anti-inflammatory and immune-modulatory tetracycline derivative (Clemens et al., 2018; Garrido-Mesa et al., 2013; Long et al., 2023; Plane et al., 2010; Seabrook et al., 2006; Tikka et al., 2001), showed reduced risk of later psychosis (Sellgren et al., 2019). Minocycline has also been reported to reduce PTSD symptoms (Gerst et al., 2021). However, negative findings from trials of minocycline in psychosis and depression highlight the need to better understand when microglial-targeting drugs may be therapeutically effective (Deakin et al., 2018; Inta et al., 2017; Li et al., 2026; Miyaoka et al., 2007; Panizzutti et al., 2023).

Microglia may have a particularly important role in post-learning modification of established fear memories—the learned association between a neutral conditioning stimulus (CS) and a fearful noxious stimulus (US). Depletion or inhibition of microglia with minocycline prevents the passive forgetting of fear memories over time (C. Wang et al., 2020), while minocycline can also facilitate fear extinction (Yu et al., 2017). Extinction is a new learning process in which repeated CS exposure reduces fear through competition between the new CS–no US memory and the original CS–US memory (Bouton, 1993; Pavlov, 1927). Brief CS reexposure can instead destabilize the CS–US memory, rendering it susceptible to disruption before reconsolidation, a process thought to update established memories with new information (Eisenberg et al., 2003; Nader, Schafe and Le Doux, 2000; Pedreira and Maldonado, 2003). Existing studies therefore suggest

contrasting roles for microglia: facilitating passive forgetting in the absence of retrieval but potentially inhibiting extinction following active retrieval. However, their role in reconsolidation is unknown, and the effects of minocycline on post-consolidation fear memory processes remain poorly characterised.

Recent evidence that acute minocycline attenuates fear memory consolidation during learning in humans (Xia et al., 2024) supports a role for microglia in human fear memory processing. Microglial involvement in post-learning modification of fear memories may therefore provide a therapeutic opportunity for strengthening or weakening pathological fear. Preclinical findings suggest that the effects of compounds such as minocycline may depend on the conditions under which memory is retrieved and whether this induces reconsolidation (updating and strengthening memory) or extinction (weakening fear expression) (Vaverková et al., 2020).

Here, we examine the effects of systemic minocycline on reconsolidation, extinction, and forgetting (passive memory loss without retrieval) of contextual fear memory (CFM) in rats. Given established sex differences in fear-related psychiatric conditions (Bangasser and Valentino, 2014; Kayrouz et al., 2025) and potential sex-specific effects of minocycline (Lombardo et al., 2022), we compare its effects in male and female rats, an issue not previously investigated.

## Materials and Methods

### Animals

At the onset of the experiment, rats were approximately 3 months of age. A total of 152 male (358.4 +/- 3.3g) and 88 female (237.1 +/- 2.4g) Lister Hooded rats (Envigo, UK) were used. The rats were held in light/dark reversal holding room maintained at 21$^{o}$C, lights out from 10 a.m. to 8 p.m. Animals were housed in same-sex groups of two to four per cage, using large rat home cages, all within a single holding room. Each holding cage was enriched with an envirotube, nestlet and chewstick. Behavioural training began at approximately 10.30 a.m. on each day. Food and water were available *ad lib* throughout the entire experiment. All procedures were conducted in accordance with local Cardiff University Ethical Committee approval and the United Kingdom 1986 Animals (Scientific Procedures) Act (Project license P0EA855DA).

### Apparatus

All sessions were conducted in standard modular test chambers for rats (interior dimensions: 30.5 × 24.1 × 21.0 cm) housed within sound-attenuating chambers (55.9 × 55.9 × 35.6 cm; Med Associates Inc., Vermont, USA). The test chambers were equipped with 19 equally spaced metal bars positioned 1.6 cm above the floor, through which scrambled footshocks could be delivered using a stand-alone aversive stimulator/scrambler (Med Associates Inc., Vermont, USA). Behaviour was digitally recorded (Numeroscope, Viewpoint, France) using infrared cameras (JSP Electronics Ltd, China) positioned centrally above the chambers for later offline analysis. For each trial, animals were individually transferred between their home cages and the testing room using a large transport box, which was covered with a lid to maintain darkness during transit.

**Behavioural paradigms**

*Contextual fear conditioning*

A paradigm of a single footshock exposure in a novel context was used (Fanselow et al 1990). During a 3-min contextual fear conditioning (CFC) training trial, each animal received a single scrambled footshock unconditioned stimulus (US) for 2s after being placed in one of two randomly assigned conditioning contexts (conditioned stimulus, CS) for 2 min and left in the conditioning chamber for a further 1 min before being returned to their home cage (Kirtley & Thomas, 2010; Lee, Everitt & Thomas, 2004). The statistical analyses of animals' responding before and post footshock were reported in supplementary table 1.

**Supplementary Table 1**

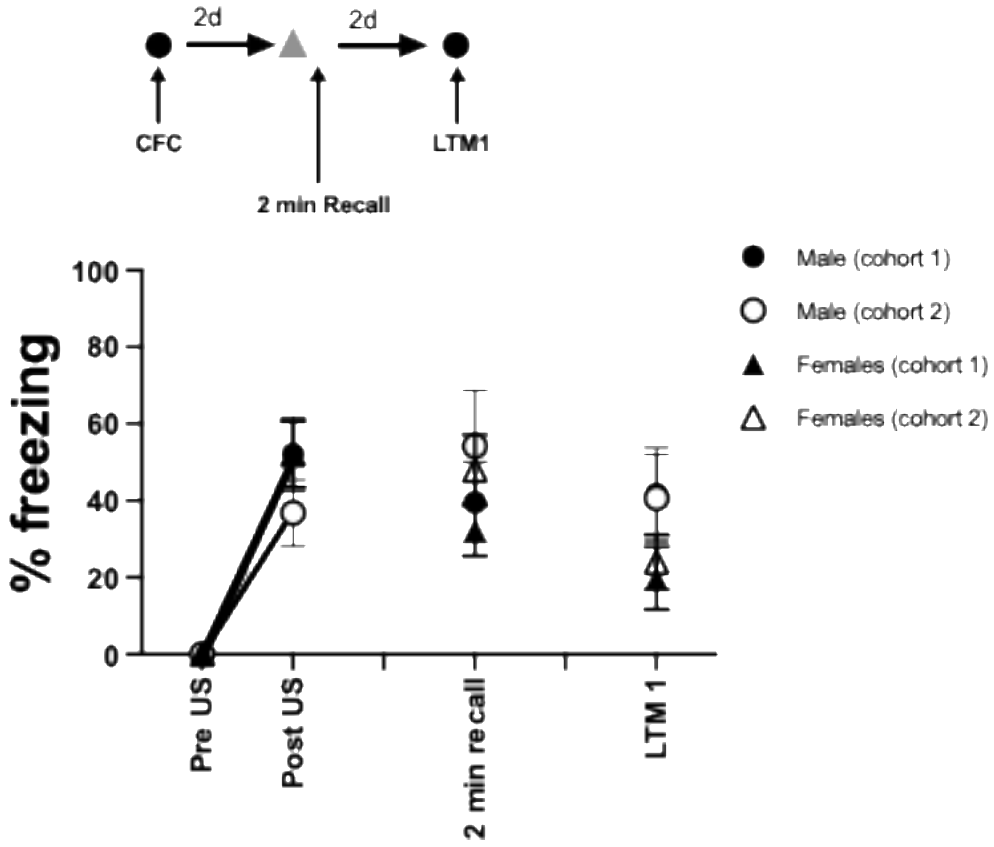
ANOVA reports of the freezing behaviour prior to and post US (footshock) during CFC for each experiment reported in Figs. 1-4. Footshock increased freezing responses in rats during CFC training and there were no group differences in freezing behaviour prior to subsequent systemic i.p. injections or behavioural manipulations.

| | Pre vs Post-US | | | Mino | | | Interaction | | |
|---|---|---|---|---|---|---|---|---|---|
| | F value | p value | Estimates of effect size | F value | p value | Estimates of effect size | F value | p value | Estimates of effect size |
| Fig. 1B | $F_{(1, 30)}$ =109.659 | $p < 0.001$ | $\eta_p^2 = 0.785$ | $F_{(1, 30)}$ =0.010 | $p = 0.920$ | $\eta_p^2 = 0.919$ | $F_{(1, 30)}$ =0.010 | $p = 0.919$ | $\eta_p^2 = 0.000$ |
| Fig. 1C | $F_{(1, 30)}$ =93.744 | $p < .001$ | $\eta_p^2 = 0.758$ | $F_{((1, 30)}$ =0.015 | $p = 0.904$ | $\eta_p^2$ =0.000 | $F_{(1, 30)}$ =0.015 | $p = 0.904$ | $\eta_p^2 = 0.000$ |
| Fig. 1D | $F_{(1, 30)}$ =231.906 | $p < 0.001$ | $\eta_p^2 = 0.885$ | $F_{(1, 30)}$ = 0.143 | $p = 0.708$ | $\eta_p^2$ = 0 .005 | $F_{(1, 30)}$ = 0.143 | $p = 0.708$ | $\eta_p^2$ = 0.005 |
| Fig. 1E | $F_{(1, 14)}$ =275.560 | $p < 0.001$ | $\eta_p^2 = 0.952$ | $F_{(1, 14)}$ =0.044 | $p = 0.837$ | $\eta_p^2 = 0.003$ | $F_{(1, 14)}$ = 0.360 | $p = 0.558$ | $\eta_p^2 = 0.025$ |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fig. 1F | $F_{(1, 14)}$ =297.190 | $P < 0.001$ | $\eta_p^2$ = 0.955 | $F_{(1, 14)}$ =0.039 | $p = 0.846$ | $\eta_p^2$ = 0.003 | $F_{(1, 14)}$ = 0.048 | $p = 0.830$ | $\eta_p^2$ = 0.003 |
| Fig. 1G | $F_{(1, 14)}$ =511.118 | $p < 0.001$ | $\eta_p^2$ = 0.973 | $F_{(1, 14)}$ =0.020 | $p = 0.890$ | $\eta_p^2$ = 0.001 | $F_{(1, 14)}$ =0.177 | $p = 0.680$ | $\eta_p^2$ = 0.013 |
| Fig. 2A | $F_{(1, 14)}$ = 241.549 | $p < 0.001$ | $\eta_p^2$ = 0.945 | $F_{(1, 14)}$ =0.000 | $p = 1.000$ | $\eta_p^2$ =0.000 | $F_{(1, 14)}$ =0.000 | $p = 1.000$ | $\eta_p^2$ =0.000 |
| Fig. 2B | $F_{(1, 14)}$ =511.118 | $p < 0.001$ | $\eta_p^2$ = 0.973 | $F_{(1, 14)}$ =0.020 | $p = 0.890$ | $\eta_p^2$ = 0.001 | $F_{(1, 14)}$ =0.177 | $p = 0.680$ | $\eta_p^2$ = 0.013 |
| Fig. 3A | t(7) = -3.585 | $p$ = 0.009 | $d$ = -1.268, 95% CI [-2.195, -0.294] | | | | | | |
| Fig. 3B | $F_{(1, 14)}$ = 39.634 | $p < 0.001$ | $\eta_p^2$ = 0.739 | $F_{(1, 14)}$ =0.023 | $p = 0.882$ | $\eta_p^2$ = 0.002 | $F_{(1, 14)}$ =0.004 | p = 0.950 | $\eta_p^2$ = 0.000 |
| Fig. 3C | t(7) = -6.063 | $p < 0.001$ | $d$ = -2.144 95% CI [-3.423, -0.827] | | | | | | |
| Fig. 3D | $F_{(1, 14)}$ = 53.763 | $p$ < 0.001 | $\eta_p^2$ = 0.793 | $F_{(1, 14)}$ =0.256 | $p = 0.621$ | $\eta_p^2$ = 0.018 | $F_{(1, 14)}$ =0.256 | p = 0.621 | $\eta_p^2$ = 0.018 |
| Fig. 4A | $F_{(1, 14)}$ = 99.780 | $p$ < 0.001 | $\eta_p^2$ = 0.877 | $F_{(1, 14)}$ = 2.966 | $p = 0.107$ | $\eta_p^2$ =0.175 | $F_{(1, 14)}$ = 2.966 | $p = 0.107$ | $\eta_p^2$ = 0.175 |
| Fig. 4B | $F_{(1, 14)}$ = 502.149 | $p$ < 0.001 | $\eta_p^2$ =0.973 | $F_{(1, 14)}$ = 0.080 | $p = 0.781$ | $\eta_p^2$ = 0.006 | $F_{(1, 14)}$ = 0.080 | p = 0.781 | $\eta_p^2$ = 0.006 |

We used different intensities of footshock (0.35 mA, 0.5mA or 0.7 mA for 2s) for CFC during the study. Our observations showed female rats tended to display lower levels of conditioned freezing behaviour, representative of the CS–US CFM association, than the males when they were subjected to the same intensity of US during conditioning (Supplementary Fig. 1). This was concordant with previous observations (Fanselow & Bolles, 1979; Torres-Rodriguez et al., 2023). In addition, when using a 0.5mA US the behavioural expression of CFM was short-lived in females (Figure 3C, D). Therefore, we used a 2 s 0.5 mA in males and a 2s 0.7mA footshock in females to establish comparable, reliable and enduring conditioned freezing behaviour to be able to index the effect of minocycline on reconsolidation and extinction of CFM in both sexes. In one experiment, male and female rats were conditioned with a stronger 2s 0.7mA footshock, to further probe minocycline's effect on extinction in males because higher footshock US's produce a stronger CFM (Fanselow, 1980; Fanselow & Bolles, 1979; Maren et al., 1994) that are more resistant to extinction (Giustino et al., 2020). For investigations of forgetting, rats were conditioned with a weaker US, a 2s 0.35 mA or 2s 0.5mA in males and females, respectively. Individual rats only underwent one training and testing manipulation. Males and female cohorts were run separately.

**Supplementary Figure 1**




**Supplementary Figure 1. Sex-specific freezing behaviour established at 0.5mA footshock** Top: Schematic of the CFC and CFM behavioural protocol. CFC was established using a single 2s scrambled footshock US (2s, 0.5 mA) given 2min into a 3 min exposure to a novel context. Arrowhead shows the administration of PBS 30 min prior to 2min CFM recall trial. ANOVA with factors of stages, sex and cohort showed a significant interaction between stage and sex ($F_{(3, 87)} = 3.027$, $p = 0.034$, $\eta_p^2 = 0.110$ ). In males, Bonferroni-adjusted pairwise comparison showed that footshock evoked freezing behaviour and the behaviour was maintained throughout at later LTM retrieval tests. In the female rats, footshock also evoked freezing behaviour which decreased over subsequent LTM tests. Results are the mean ± SEM. Males (n = 16) and females (n = 16). **p < 0.01.

*Contextual fear memory and reconsolidation*

CFM was assessed upon return to the conditioned chamber for 2 min without reinforcement (long term

memory test, LTM, or 2 min Recall). We have previously shown that a 2 min re-exposure to the conditioning context 1-2 d after CFC is also sufficient to induce the reconsolidation of CFM under these experimental conditions (Lee et al 2004). Thus, re-exposure to the training context can act as both a probe for fear memory and initiate reconsolidation. The timing of the tests in each experiment are reported in the figures and within the results section.

*Extinction and reminder*

Extinction was initiated by a prolonged 10 min re-exposure to the training context animals 48 hrs and/or 26 days after CFC (10 min Extinction). We have previously shown extinction of fully consolidated CFM to be initiated using 10 min re-exposure to the training context using this CFC procedure (Kirtley and Thomas, 2010). A reminder session was given to evidence extinction (Myers and Davis, 2007). Rats were returned to the conditioning chamber for 2 min and a single 2 s 0.25mA scrambled foosthock was delivered at the end of the session. This low intensity unconditioned stimulus (US) is below the intensity required to produce de novo conditioning (Cai et al, 2006, Blundell et al 2008). Increased expression (reinstatement) of fear memory at a later LTM test is evidence of extinction in this behavioural procedure (Trent et al 2015).

*Behavioural assessment*

Freezing behaviour was used as a measure of fear memory. One unit of freezing response was defined as the continuous absence of movement other than respiratory motion in 1 s sampled every 10s during each trial by an observer blind to the experimental group (Fanslow, 1990; Kirtley & Thomas, 2010). Freezing behaviour was expressed as the percentage of time spent freezing per 2 min epoch, except for the 1min post US period during CFC.

**Minocycline injection**

Minocycline hydrochloride (Merck, UK, Cat#: M9511) was dissolved in sterile 0.01M PBS and 1M NaOH prior use. The pH of vehicle also adjusted to 5.5 to equate the pH value of the two solutions. Both solutions were freshly made upon the day of injection. A single intra-peritoneal injection of minocycline (40 mg/kg, i.p.) or vehicle was given 30 min prior to either 2 min or 10 min Recall retrieval sessions to initiate reconsolidation or extinction of CFM, respectively. Injections in the absence of retrieval were made at the same equivalent times post CFC for No Recall groups. These groups were included to assess the effect of minocycline on the passive

forgetting of memory. Rats showed transient, mild discomfort for a few seconds at the site of injection before resuming normal behavioural responses to environmental stimuli and handling. The dose and timing of injection of minocycline was that shown to be behavioural effective in rats in cognitive tasks (Naderi, Sabetkasaei, Parvardeh & Zanjani, 2017; Zhang, Shirayama, Iyo & Hashimoto, 2007).

**Statistical analysis**

Group sizes were defined by prior power analysis based on estimates based on our previously published data using similar behavioural paradigms. Results were analyzed with two-way ANOVA with repeated measures with factors of treatment (PBS vs. minocycline), Test (LTM tests or between-session extinction test), Extinction (within-session extinction test) or Reminder (before vs after receiving reminder US) sessions, and independent samples t-test. Experiments on males and females were not run concurrently; therefore, sex was not included as a factor in formal analysis and analysis was performed on males and females separately. For repeated measures analysis, Mauchly's Test of Sphericity was applied. If the sphericity assumption was not met, the Greenhouse–Geisser correction was applied. All data from all experimental rats used in the study were included for analysis. Statistical analysis was performed using SPSS version 27. Statistical significance was set at $p < 0.05$.

**Results**

**Minocycline affects the expression of contextual fear memory in a retrieval and sex-specific manner**

We investigated the effects of minocycline on the reconsolidation, extinction, and forgetting of CFM in male and female rats. Two days after CFC, rats received a single systemic injection of minocycline or vehicle 30 min before re-exposure to the conditioned context for either 2 min (2-min Recall) or 10 min (10-min Extinction) to initiate reconsolidation and extinction of CFM respectively. (Fig. 1A). A third No Recall group received minocycline or vehicle but was returned directly to the home cage, allowing us to assess effects on the forgetting of established CFM as indexed by conditioned freezing in the absence of memory reactivation. All animals were subsequently re-exposed to the conditioned chamber 5 days (LTM1) and 26 days (LTM2) after CFC to assess the effects of minocycline on CFM persistence. Because females showed lower levels of freezing behaviour than males when using the same US during CFC (see Materials and Methods), we used a 2s 0.5mA footshock in males and 2s 0.7mA US in females to better match freezing behaviour to assess minocycline's effects on CFM.

The results showed that a single acute administration of minocycline modulated the expression of CFM in both sexes. In male rats, minocycline treatment given in the absence of fear memory retrieval (No Recall group) increased freezing behaviour measured at subsequent memory tests, LTM1 and LTM2 despite lower freezing levels at the remote LTM2 test [LTM tests (Test): $F_{(1, 30)} = 18.713$, $p < 0.001$, $\eta_p^2 = 0.384$; Mino: $F_{(1, 30)} = 6.518$, $p = 0.016$, $\eta_p^2 = 0.178$; Test x Mino: $F_{(1, 30)} = 1.050$, $p = 0.314$, $\eta_p^2 = 0.034$) (Fig. 1B). However, there was no increase in conditioned freezing measured at LTM1 and LTM2 when minocycline was given either prior to a retrieval session that might promote the reconsolidation (2 min Recall, Test: $F_{(1, 30)} = 1.136$, $p = 0.295$, $\eta_p^2 = 0.036$; Mino: $F_{(1, 29)} = 0.689$, $p = 0.413$, $\eta_p^2 = 0.022$; Test x Mino: $F_{(1, 30)} = 0.045$, $p = 0.833$, $\eta_p^2 = 0.002$, Fig. 1C), or extinction of conditioned freezing responses (10min Extinction, Test: $F_{(1, 30)} = 1.676$, $p = 0.205$, $\eta_p^2 = 0.053$; Mino: $F_{(1, 30)} = 0.206$, $p = 0.653$, $\eta_p^2 = 0.007$; Test x Mino: $F_{(1, 30)} = 0.052$, $p = 0.821$, $\eta_p^2 = 0.002$, Fig. 1D). There was no acute effect of minocycline on the freezing behaviour during the 2 min CFM retrieval session ($t(30) = 1.527$, $p = 0.137$, $d = 0.540$, 95% CI [-0.170, 1.242], Fig 1C) or the diminution of freezing behaviour during a 10 min retrieval session (within-session extinction, Extinction: $F_{(2.871, 86.123)} = 9.785$, $p < 0.001$ $\eta_p^2 = 0.246$; Mino: $F_{(1, 30)} = 0.640$, $p = 0.430$, $\eta_p^2 = 0.021$; Extinction x Mino: $F_{(2.871, 86.123)} = 0.542$, $p = 0.705$, $\eta_p^2 = 0.018$, Fig. 1D). In addition, minocycline did not affect the loss of conditioned freezing behaviour between the 1 st min of extinction training and LTM tests (between-session extinction, Test: $F_{(2, 60)} = 85.360$ $p < 0.001$, $\eta_p^2 = 0.740$; Mino: $F_{(1, 30)} = 0.139$, $p = 0.712$ $\eta_p^2 = 0.005$; Test x Mino: $F_{(2, 60)} = 0.075$, $p = 0.928$, $\eta_p^2 = 0.002$, Fig 1D). Thus, in males, minocycline exerted a differential effect on long-term fear memory expression depending upon whether memory was retrieved or not after administration, increasing conditioned freezing responses compared to controls only when the CFM was not recalled.

In females, minocycline increased freezing behaviour at LTM1 and LTM2 when administered in the absence of retrieval despite a significant reduction of freezing behaviour over the two LTM tests (No Recall, Test: $F_{(1, 14)} = 10.714$, $p = 0.006$, $\eta_p^2 = 0.434$; Mino: $F_{(1, 14)} = 9.409$, $p = 0.008$, $\eta_p^2 = 0.402$, $\eta_p^2 = 0.434$; Test x Mino $F_{(1, 14)} = 1.190$, $p = 0.294$, $\eta_p^2 = 0.002$, Fig. 1E) as well as before of a brief (2min Recall, Test: $F_{(1, 14)} = 0.273$, $p = 0.610$, $\eta_p^2 = 0.019$; Mino: $F_{(1, 14)} = 6.559$, $p = 0\ .023$, $\eta_p^2 = 0.319$; Test x Mino : $F_{(1, 14)} = 0.273$, $p = 0.610$, $\eta_p^2 = 0.019$, Fig. 1F) or prolonged re-exposure to the training context which induced extinction (10 min Recall, Test: $F_{(1, 14)} = 0.008$, $p = 0.930$, $\eta_p^2 = 0.001$; Mino: $F_{(1, 14)} = 6.367$, $p = 0.024$, $\eta_p^2 = 0.002$, $\eta_p^2 = 0.313$; Test x Mino: $F_{(1, 14)} = 0.071$, $p = 0.793$, $\eta_p^2 = 0.005$, Fig. 1G). Minocycline did not affect the ability of females to undergo extinction with a 10 min context re-exposure as indexed by a reduction in conditioned freezing responses despite showing higher responses (within-session extinction, Extinction: $F_{(4, 56)} = 16.835$, $p < 0.001$, $\eta_p^2 = 0.546$; Mino: $F_{(1, 14)} =$

17.362, $\eta_p^2$ = 0.554, p < 0.001; Extinction x Mino: $F_{(4, 56)}$ =0.907, p = 0.466, $\eta_p^2$ = 0.061), and between session-extinction, (Test: $F_{(2, 28)}$ = 6.454, p < 0.005, $\eta_p^2$ = 0.315, Mino: $F_{(1, 14)}$ = 8.143, p = 0.013, $\eta_p^2$ = 0.368 ; Extinction x Mino: $F_{(2, 28)}$ = 0.097, p = 0.908, $\eta_p^2$ = 0.007, Fig. 1G). Therefore, in females, minocycline resulted in a general and persistent increase in freezing behaviour independent of whether memory was not retrieved, retrieved or retrieved and extinguished after administration.

**Figure 1**

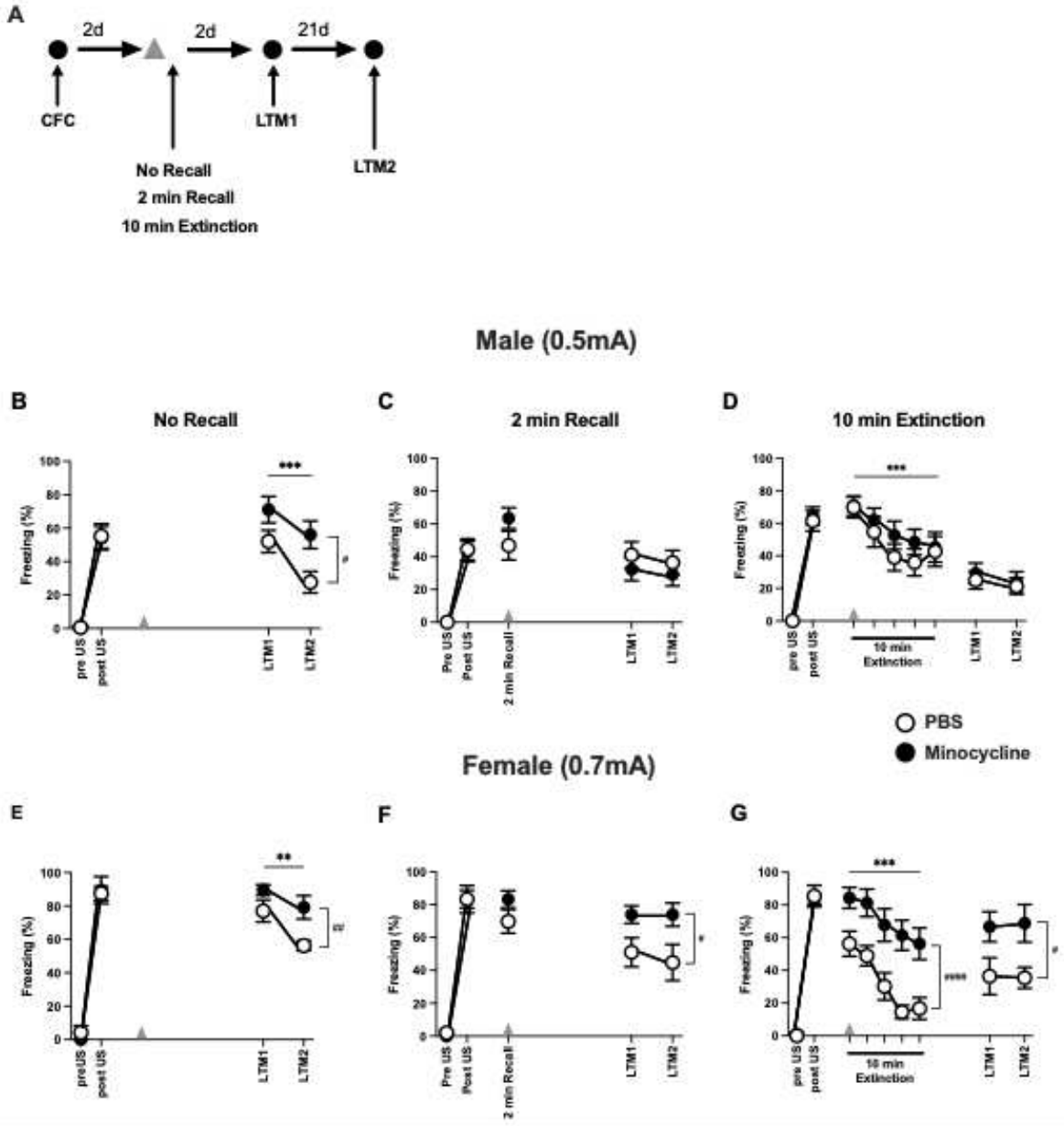


**Fig. 1. Minocycline affects the expression of contextual fear memory in a sex-specific manner.** (**A)** Schematic of the CFC (contextual fear conditioning) and contextual fear memory (CFM) behavioural protocol. CFC was established using a single 2s scrambled footshock US (males 0.5 mA, females 0.7 mA) given 2min into a 3 min exposure to a novel context. Arrowhead shows the administration of minocycline (40mg/kg, i.p.) or PBS 30 min prior to either a 2min CFM recall trial (2 min Recall), a 10 min Extinction trial (10 min Extinction, 5 x 2min epochs), or at an equivalent time without recall (No Recall). Rats were exposed to the conditioning context for 2 min 2d (long-term memory test 1, LTM1) and 23d (LTM2). (**B-F)** The effect of minocycline on conditioned freezing behaviour in male (**B-D**) and female rats (**E-G**) under conditions No recall (**B, E**), 2min Recall (**C, F**) and 10min Extinction (**D, G**). Results are the Mean ± SEM. Per group, males, n = 16, females, n = 8. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, between-or within-session comparisons; # $p < 0.05$, ## $p < 0.01$ between-group comparisons.

The enhancement of conditioned freezing by minocycline at recent (2 or 4 days after CFC, 2 min Recall or LTM1) or more remote CFM tests (26 days after CFC, LTM2) cannot be explained by a systematic difference in CFM acquisition during conditioning because prior to administration, PBS and minocycline groups showed equivalent levels of responding to the US during the CFC training (Supplementary Table 1). These results suggest that minocycline modulates the expression of an established CFM in a retrieval and sex-specific manner: 1) Without CFM retrieval, acute minocycline enhances the expression of fear memory in both sexes; 2) when given prior to CFM retrieval, minocycline enhances the expression of CFM in female rats only.

**Minocycline enhances extinction of recently acquired contextual fear memory in males**

It is possible that the effect of minocycline we observed to enhance the expression of CFM in females but not males when given prior to recall may simply reflect using different foot shock intensities for CFC in the previous experiment. To test the hypothesis, we trained males and females using the higher intensity 0.7 mA footshock which produces a stronger CFM (Fanselow, 1980; Fanselow & Bolles, 1979; Maren et al., 1994), known to be more resistant to extinction (Giustino et al., 2020), and challenged both sexes to two extinction training sessions to explore retrieved CFM expression and extinction more fully. Two days after the CFC, rats received either minocycline or PBS 30 min prior to the first 10 min extinction training (Extinction 1). Both males and females received a second 10-minute extinction session (Extinction 2) at least 21 days later. LTM tests were

interposed between extinction sessions to investigate spontaneous recovery of conditioned freezing with time and were given after a 2min Reminder session terminating with a 2s, 0.25 mA footshock to examine reinstatement of the conditioned freezing response (Myers & Davis, 2007). LTM tests after the second extinction training session were omitted for the females because conditioned freezing behaviour was already at floor level in the control PBS group (Fig. 2).

**Figure 2**

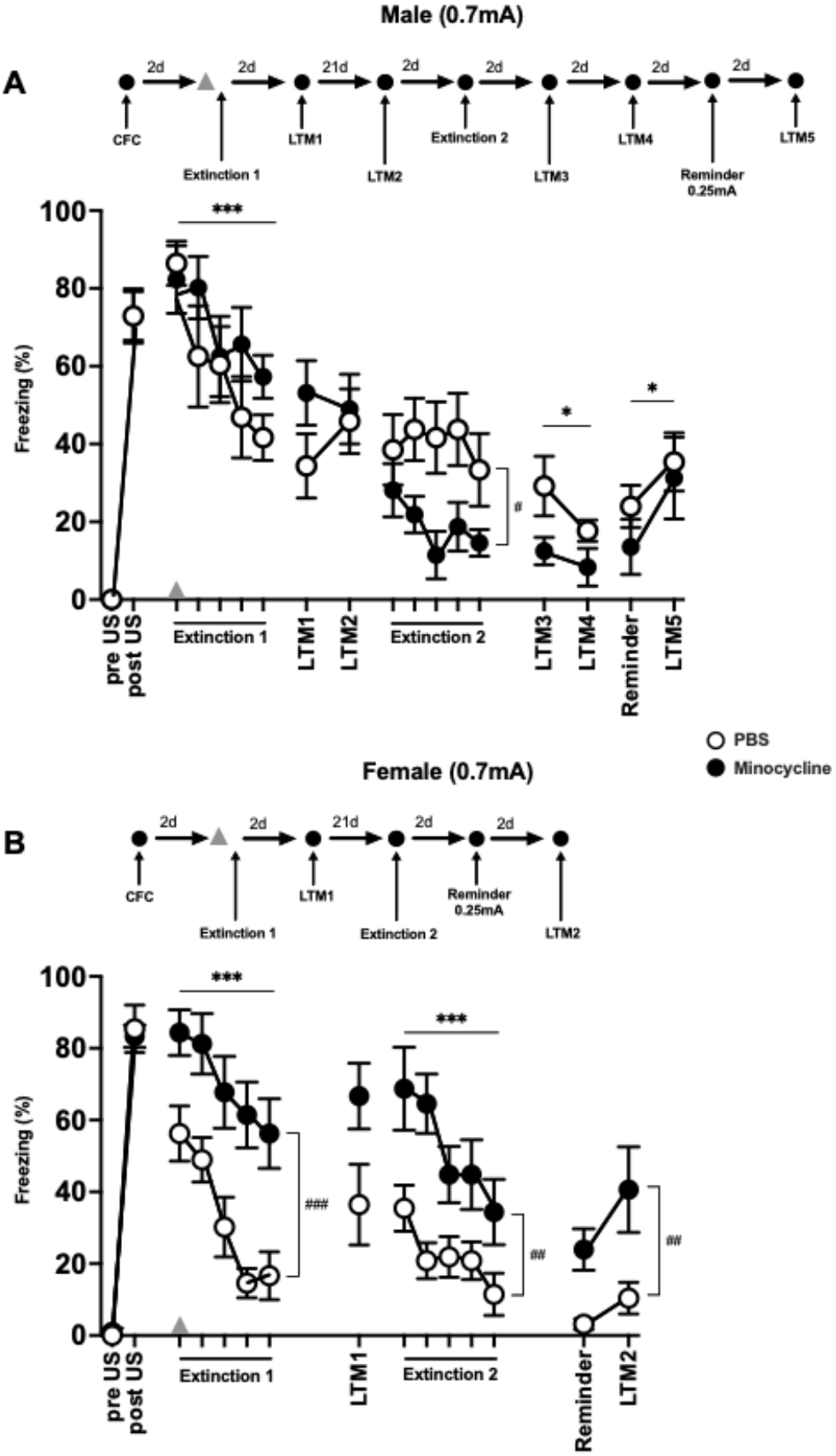

Male (0.7mA)
A
2d
2d
21d
2d
2d
2d
2d
2d
CFC
Extinction 1
LTM1
LTM2
Extinction 2
LTM3
LTM4
Reminder 0.25mA
LTM5
Freezing (%)
100
80
60
40
20
0
***
*
*
#
pre US
post US
Extinction 1
LTM1
LTM2
Extinction 2
LTM3
LTM4
Reminder
LTM5
PBS
Minocycline
Female (0.7mA)
B
2d
2d
21d
2d
2d
CFC
Extinction 1
LTM1
Extinction 2
Reminder 0.25mA
LTM2
Freezing (%)
100
80
60
40
20
0
***
***
###
##
##
pre US
post US
Extinction 1
LTM1
Extinction 2
Reminder
LTM2

**Fig. 2. Minocycline enhances extinction in males only. (A) The effect of minocycline on extinction of robust CFM with two extended trials in male rats**. Top: Schematic of the CFC and CFM behavioural protocol. Males were conditioned using a 2s, 0.7mA US. Minocycline (40mg/kg, i.p.) or PBS (arrowhead) was given 30 min prior the first of two 10 min extinction trials (Extinction 1 and 2, 5 x 2min epochs). LTM denotes 2 min CFM tests. Reminder: a 2 s 0.25mA US was given at the end a 2 min LTM test to evoke recovery of freezing behaviour after extinction. Conditioned freezing behaviour was used as a measure of CFM **(B) The effect of minocycline on extinction of robust CFM with two extended trials in female rats**: Top: schematic of behavioural protocol. Female rats were CFC using a 2s, 0.7mV US. Results are the Mean ± SEM. n = 8 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ within-session comparisons; # < 0.05, ## $p < 0.01$ between-group comparisons.

Male rats given minocycline or PBS showed similar reductions in freezing behaviour during the first extinction session (Extinction 1: $F_{(4, 56)} = 8.959$, $p < 0.001$, $\eta_p^2 = 0.390$; Mino: $F_{(1, 14)} = 0.961$, $p = 0.344$, $\eta_p^2 = 0.064$; Extinction 1 x Mino: $F_{(4, 56)} = 1.331$, $p = 0.270$, $\eta_p^2 = 0.087$), and also at LTM1 and LTM2 with no evidence of spontaneous recovery of the freezing response at the second LTM2 test 21 days later (Test: $F_{(1, 14)} = 0.565$, $p = 0.465$, , $\eta_p^2 = 0.039$; Mino: $F_{(1, 14)} = 0.996$, $p = 0.335$, $\eta_p^2 = 0.066$; Test x Mino: $F_{(1, 14)} = 2.595$, $p = 0.130$, $\eta_p^2 = 0.156$; Fig. 2A). In marked contrast, conditioned behavioural responses were significantly lower during the second extinction session (Extinction 2: $F_{(4, 56)} = 0.862$, $p = 0.492$, $\eta_p^2 = 0.058$; Mino: $F_{(1, 14)} = 9.244$, $p = 0.009$, $\eta_p^2 = 0.398$; Extinction 2 x Mino: $F_{(4, 56)} = 0.692$, $p = 0.601$, $\eta_p^2 = 0.047$) and after the second extinction session (LTM3 and LTM4, Test: $F_{(1, 14)} = 4.937$, $p = 0.043$, $\eta_p^2 = 0.261$, Mino: $F_{(1, 14)} = 4.345$, $p = 0.056$, $\eta_p^2 = 0.237$; Test x Mino: $F_{(1, 14)} = 1.075$, $p = 0.317$, $\eta_p^2 = 0.071$) in minocycline injected males. The reminder US increased conditioned freezing at LTM5 in both groups commensurate with minocycline modulating extinction rather than the original CFM (Reminder: $F_{(1, 14)} = 7.240$, $p = 0.018$, $\eta_p^2 = 0.341$; Mino: $F_{(1, 14)} = 0.563$, $p = 0.466$, $\eta_p^2 = 0.039$; Reminder x Mino: $F_{(1, 14)} = 0.332$, $p = 0.573$ $\eta_p^2 = 0.023$).

As similarly seen previously, despite showing higher levels of conditioned freezing behaviour than PBS-treated rats, female rats administered minocycline prior to extinction training, showed both within-session reductions in freezing behaviour during the first extinction session (Extinction 1: $F_{(4, 56)} = 16.835$, $p < 0.001$, $\eta_p^2 = 0.546$; Mino: $F_{(1, 14)} = 17.362$, $p < 0.001$, $\eta_p^2 = 0.554$; Extinction x Mino: $F_{(4, 56)} = 0.907$, $p = 0.466$, $\eta_p^2 = 0.061$; Fig. 2B), and marginal evidence of extinction being maintained at LTM1 ($t(14) = 2.081$, $p = 0.056$, $d = 1.041$, 95%

CI [-0.027, 2.076]). There was no evidence of spontaneous recovery freezing responses in the first 2 min of the second extinction session 21 days later Both groups also showed further extinction of conditioned fear responses with the second 10 min extinction training session, again with minocycline-treated females showing higher responses than their PBS counterparts Extinction 2: $F_{(4, 56)}$ =7.387, p < 0.001, $\eta_p^2$ = 0.345; Mino: $F_{(1, 14)}$ =12.825, p = 0.003, $\eta_p^2$ = 0.478; Extinction 2 x Mino: $F_{(4, 56)}$ = 1.265, p = 0.295, $\eta_p^2$ = 0.083). The difference in freezing behaviour between the two groups was maintained during the reminder and subsequent test session (LTM2) with a trend for the US reinstatement of freezing behaviour at LTM2 (Reminder: $F_{(1, 14)}$ = 4.101, p = 0.062, $\eta_p^2$ = 0.227; Mino: $F_{(1, 14)}$ = 10.229, p = 0.006, $\eta_p^2$ = 0.422, Reminder x Mino: $F_{(1, 14)}$ = 0.628, p = 0.44, $\eta_p^2$ = 0.043). This effect was most marked in the minocycline group as evidence of intact extinction.

The results show that minocycline given prior to extinction training selectively augments the extinction of CFM in males. Furthermore, conditioned freezing behaviour is higher in minocycline compared to vehicle treated females throughout extinction training and testing.

**Minocycline in the absence of retrieval strengthens weak contextual fear memory in males.**

Given that both male and female rats showed increased conditioned fear responses in the No Recall group, we hypothesized that acute systemic minocycline administration without the retrieval of CFM may strengthen an established fear memory. We further tested this hypothesis under conditions of a weak CFM. Rats were conditioned using a lower intensity footshock prior to receiving minocycline or PBS injections 2 days later in the absence of CFM retrieval (Fig. 3). We used a 0.35mA footshock to establish weak CFC in the male rats. CFM established with this level of footshock was weak and declined over 4 days ($t(7)$ = 3.643, p = 0.008, d = 1.288, 95% CI [0.308, 2.223]) (Fig 3A). In a separate cohort, when minocycline was given 2 days after CFC training in the absence of CFM retrieval, minocycline-treated males showed a significantly greater level of conditioned freezing than PBS-treated animals when CFM was probed remotely 21 days later ($t(14)$ = 3.575, p = 0.003, d = 1.787, 95% CI [0.586, 2.944]) (Fig. 3B).

**Figure 3**

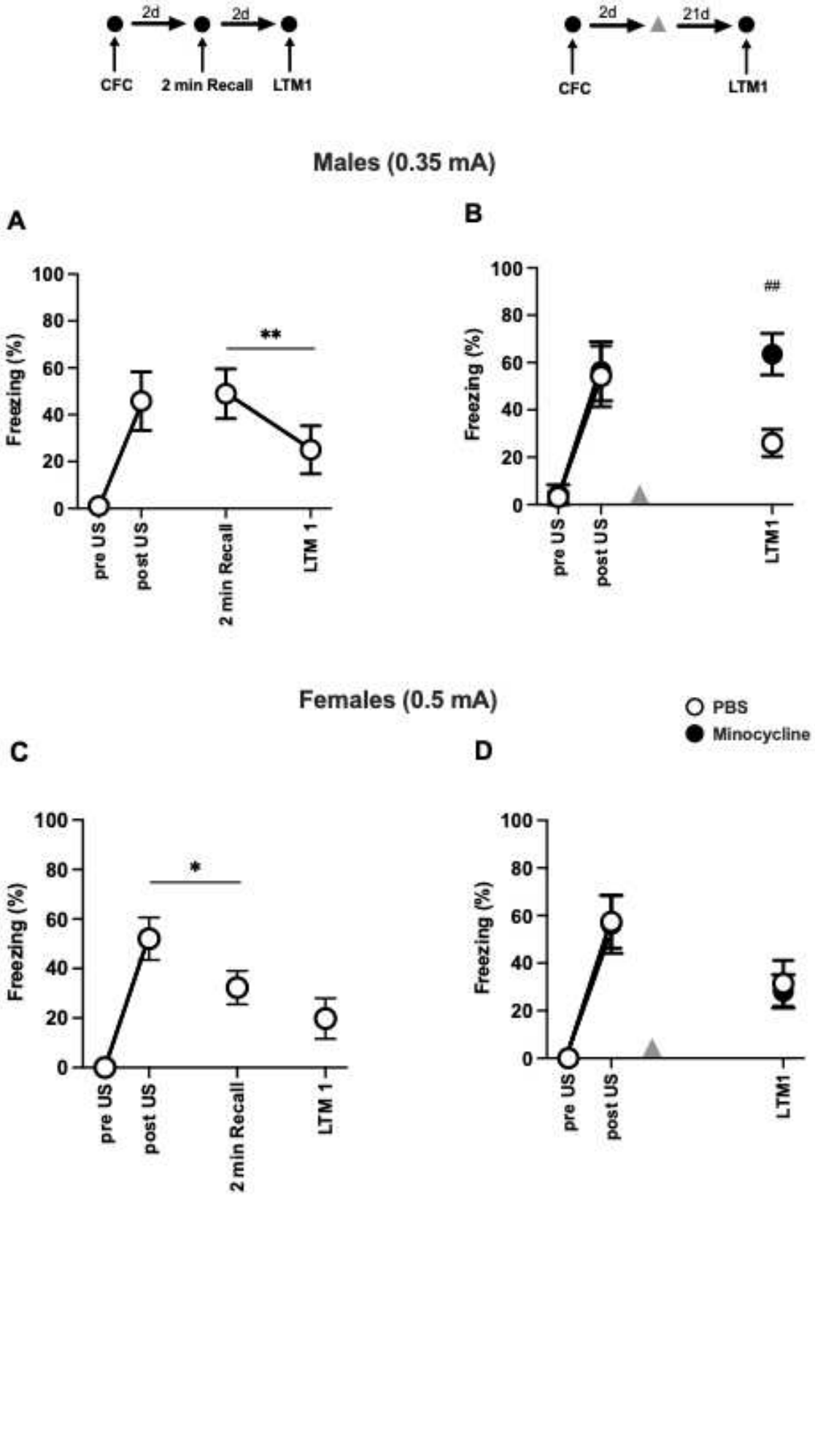
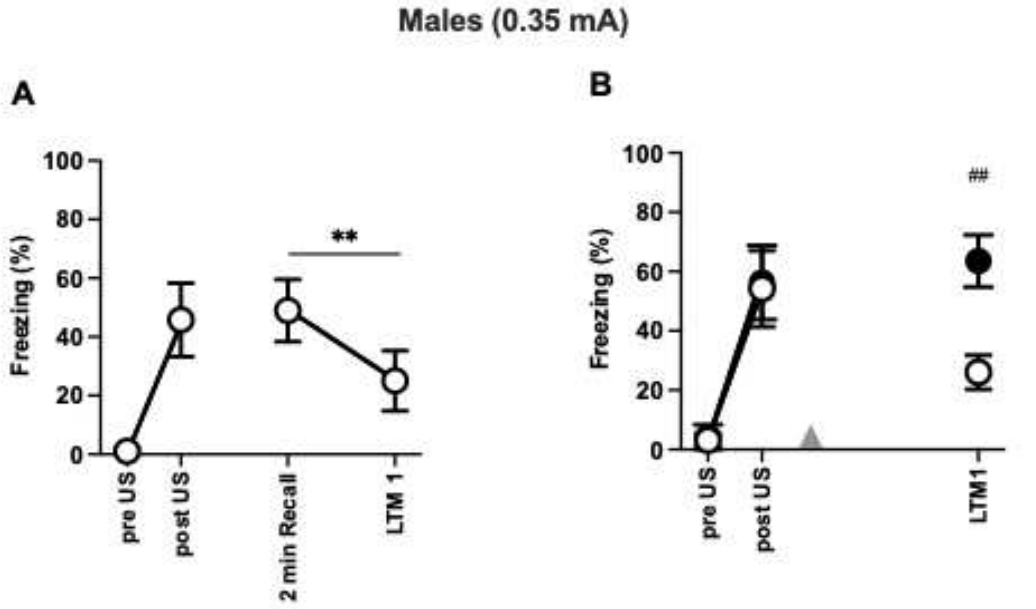

2d
2d
CFC
2 min Recall
LTM1
2d
21d
CFC
LTM1
Males (0.35 mA)
A
B
Freezing (%)
100
80
60
40
20
0
**
pre US
post US
2 min Recall
LTM 1
##
LTM1
Females (0.5 mA)
PBS
Minocycline
C
D
*

**Fig. 3. Minocycline without memory retrieval strengthens weak fear memory in males only**. Tops: Schematics of CFC and CFM (2 min Recall, LTM1) behavioural protocols. CFC was established with a 2s 0.35mA or 2s, 0.5mA US in males and females, respectively. Arrowhead: Intraperitoneal injection of minocycline (40mg/kg) or phosphate buffer saline (PBS) 2d after CFC with no recall. **(A, B) Males: (A)** CFM established with a 0.35mA US was weak and declined over 4 days. **(B)** Conditioned freezing behaviour measured remotely 21d after 2min recall in minocycline and vehicle-administered rats. **(C, D) Females**: CFM established with 0.5mA US declined quickly between the post-US epoch of CFC and the 2 min recall test, with low conditioned freezing behaviour maintained at LTM1. **(D)** Rats administered minocycline 2d after CFC in the absence of CFM recall showed equivalent low levels of conditioned fear behaviour to PBS-administered females 23 days after CFC. Results are the mean ± SEM. n = 8 per group. * $p < 0.05$, ** $p < 0.01$ between-session comparisons; ## $p < 0.01$ between-group comparison.


2d
2d
CFC
2 min Recall
LTM1
2d
21d
CFC
LTM1


Males (0.35 mA)
A
B
Freezing (%)
100
80
60
40
20
0
**
##
pre US
post US
2 min Recall
LTM 1
LTM1

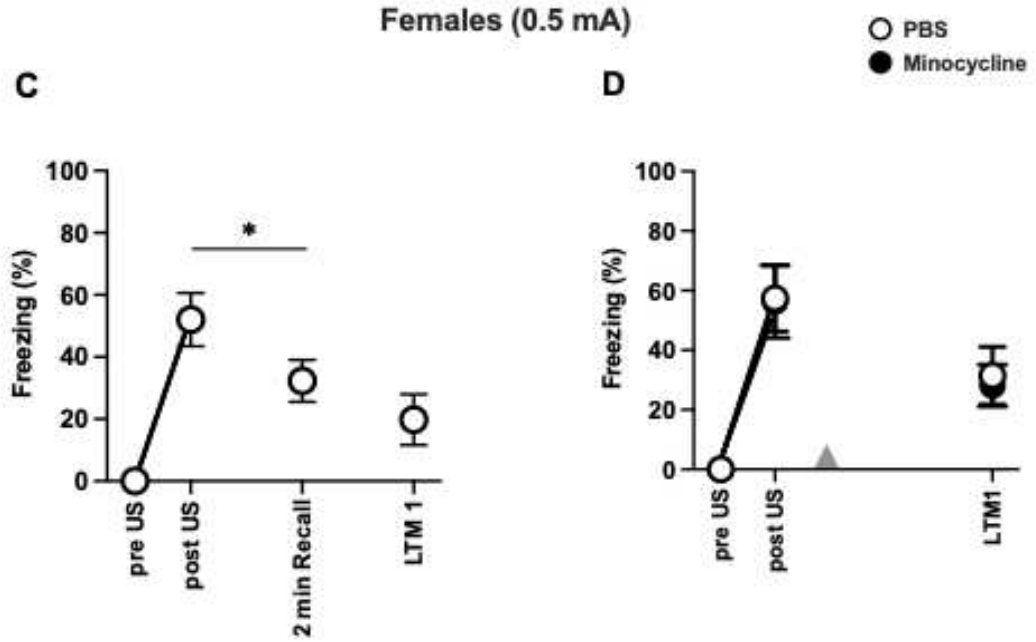

Females (0.5 mA)
PBS
Minocycline
C
D
Freezing (%)
100
80
60
40
20
0
*
pre US
post US
2 min Recall
LTM 1
LTM1

**Fig. 3. Minocycline without memory retrieval strengthens weak fear memory in males only**. Tops: Schematics of CFC and CFM (2 min Recall, LTM1) behavioural protocols. CFC was established with a 2s 0.35mA or 2s, 0.5mA US in males and females, respectively. Arrowhead: Intraperitoneal injection of minocycline (40mg/kg) or phosphate buffer saline (PBS) 2d after CFC with no recall. **(A, B) Males: (A)** CFM established with a 0.35mA US was weak and declined over 4 days. **(B)** Conditioned freezing behaviour measured remotely 21d after 2min recall in minocycline and vehicle-administered rats. **(C, D) Females**: CFM established with 0.5mA US declined quickly between the post-US epoch of CFC and the 2 min recall test, with low conditioned freezing behaviour maintained at LTM1. **(D)** Rats administered minocycline 2d after CFC in the absence of CFM recall showed equivalent low levels of conditioned fear behaviour to PBS-administered females 23 days after CFC. Results are the mean ± SEM. n = 8 per group. * $p < 0.05$, ** $p < 0.01$ between-session comparisons; ## $p < 0.01$ between-group comparison.

In female rats, CFM established with 0.5mA US declined quickly from Post-US to 2 min Recall test ($t(7) = 2.569$, $p = 0.037$, d = 0.908, 95% CI [0.051, 1.722]) and low conditioned freezing behaviour was maintained at LTM1 ($t(7) = 1.122$, $p = 0.299$, d = 0.397, 95% CI [-0.338, 1.106]) (Fig. 3C). Female rats administered minocycline after CFC in the absence of CFM recall showed equivalent low levels of conditioned freezing behaviour to PBS administered females 23 days after CFC ($t(14) = 0.610$, p = 0.552, d = -0.305, 95%CI [-1.286, 0.687) (Fig. 3D).

Thus, while CFC using a 0.35mA or 0.5mA US in males and females respectively produced a weak and short-lived CFM as measured 4-23 days later, administering minocycline without CFM retrieval strengthened weak CFM in males but not in females.

**Minocycline strengthens remotely recalled memories in males**

To investigate the effects of minocycline on the reconsolidation and extinction of a long-established (remote) fear memory, rats were given minocycline or PBS injections 30 min prior to 2 min recall 23 days after CFC training and subsequent extinction training and testing (Fig. 4A).

**Figure 4**

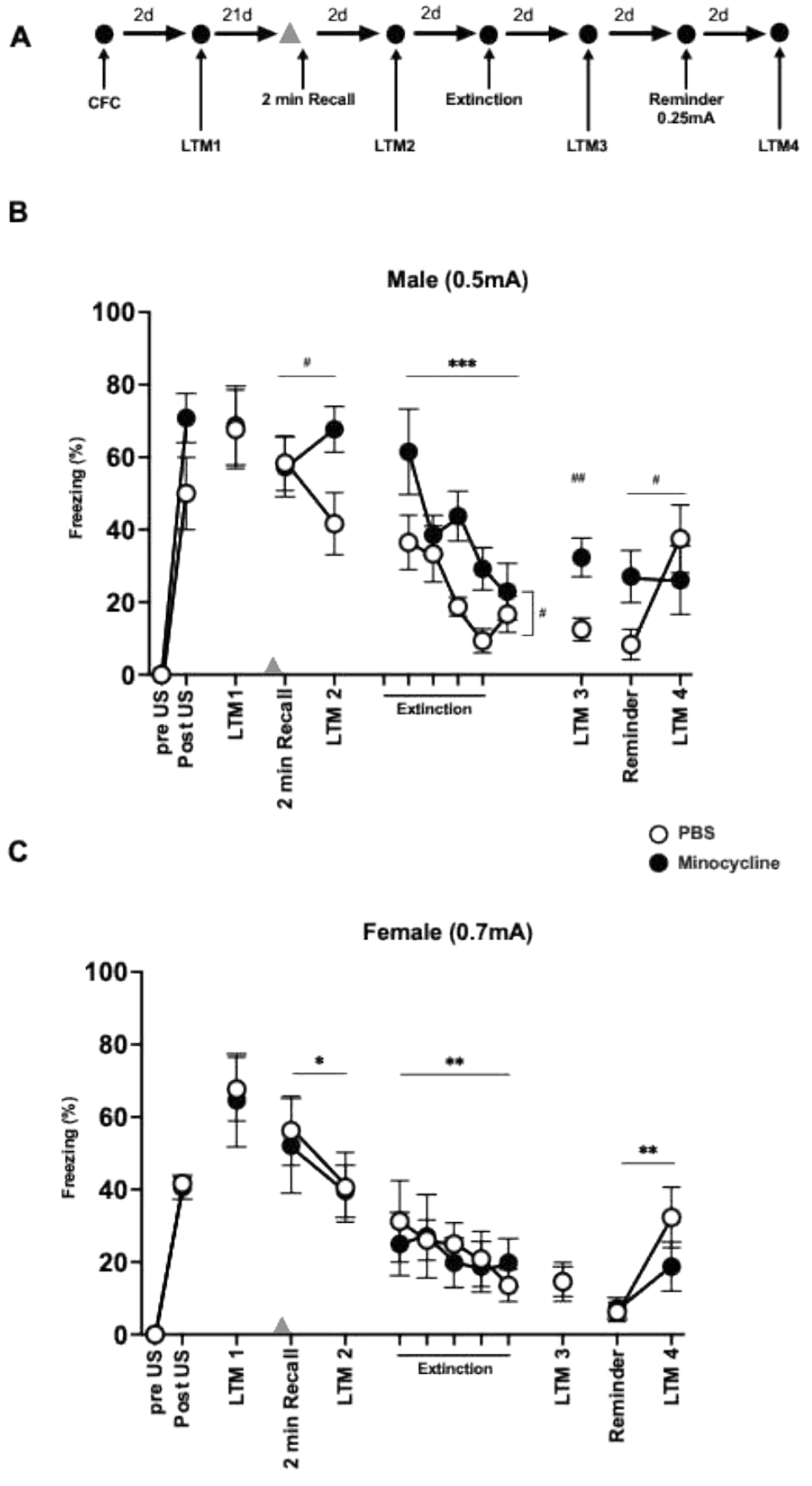

A
2d
21d
2d
2d
2d
2d
2d
CFC
LTM1
2 min Recall
LTM2
Extinction
LTM3
Reminder
0.25mA
LTM4
B
Male (0.5mA)
Freezing (%)
100
80
60
40
20
0
pre US
Post US
LTM1
2 min Recall
LTM 2
Extinction
LTM 3
Reminder
LTM 4
PBS
Minocycline
C
Female (0.7mA)
Freezing (%)
100
80
60
40
20
0
pre US
Post US
LTM 1
2 min Recall
LTM 2
Extinction
LTM 3
Reminder
LTM 4

**Fig. 4. Effect of minocycline on remote fear memory.**

**(A)** Schematic of the CFC, recall, extinction and CFM (LTM, reminder tests) behavioural protocol. Males and females were conditioned using a 2s, 0.5mA or 2, 0.7mA US respectively. Minocycline (40mg/kg, i.p.) or PBS (arrowhead) was given 30 min prior a 2min Recall session 21 days after the LTM1 test 2 days following CFC. Extinction: a 10 min non-reinforced re-exposure to the CFC context, 5 x 2min epochs). Reminder: 2 s 0.25mA US given at the end a 2 min LTM test to evoke recovery of freezing behaviour after extinction. **(B) Males:** Conditioned freezing responses. **(C) Females:** Conditioned freezing responses. Results are the mean ± SEM. n = 8 per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ between-session comparisons; # < 0.05, ## $p < 0.01$ between-group comparisons.

In males, the standard 0.5mA US produced equivalent levels of conditioned freezing behaviour measured 2 days later at LTM1 establishing that both minocycline and vehicle treatment groups showed similar levels of CFM after CFC (Fig. 4B). Minocycline injection 21 d later did not affect the immediate expression of CFM at 2 min Recall. However, the minocycline treated group showed significantly *greater* levels of freezing responses at a retrieval test 2 days later (LTM2) (Test: $F_{(1,14)} = 0.453$, $p = 0.512$, $\eta_p^2 = 0.031$; Mino: $F_{(1, 14)} = 1.585$, $p = 0.229$, $\eta_p^2 = 0.102$; Test x Mino: $F_{(1, 14)} = 8.511$, $p = 0.011$, $\eta_p^2 = 0.378$), during extinction training, despite showing within session decreases in freezing behaviour (Extinction: $F_{(4,56)} = 8.774$, $p < 0.001$, $\eta_p^2 = 0.385$, Mino: $F_{(1, 14)} = 6.882$, $p = 0.020$, $\eta_p^2 = 0.330$, Extinction x Mino: $F_{(4, 56)} = 1.393$, $p = 0.248$, $\eta_p^2 = 0.091$), and during a subsequent recall test 2 days later (LTM3) ($t(14) = 3.199$, $p = 0.006$, $d = 1.599$, 95% CI [0.436, 2.721]). Since the levels of conditioned freezing were already very low for the PBS treated animals at LTM3, precluding the value of a further extinction training session, we gave a low 0.25mA US reminder to probe CFM and extinction. The reminder was effective in evoking an increase of conditioned fear behaviour measured at LTM4 in the PBS group but not the minocycline group (Reminder: $F_{(1, 14)} = 5.458$, $p = 0.035$, $\eta_p^2 = 0.280$, Mino: $F_{(1, 14)} = 0.153$, $p = 0.701$, $\eta_p^2 = 0.011$, Reminder x Mino: $F_{(1, 14)} = 6.296$, $p = 0.025$, $\eta_p^2 = 0.310$). The failure (to reinstate freezing behaviour by the reminder indicates that minocycline targeted the long-established CFM rather than extinction processes that might be engaged following retrieval at 2 min Recall.

In female rats CFC using a 0.7mA produced similarly robust conditioned freezing responses in minocycline and PBS-treated groups measured 2 day later (LTM1, Fig. 4C). Minocycline administration 21 days later had no effect on freezing behaviour at 2 min Recall and LTM2 (Test: $F_{(1,14)} = 5.191$, $p = 0.039$, $\eta_p^2 = 0.271$, Mino: $F_{(1, 14)} = 0.041$, $p = 0.842$, $\eta_p^2 = 0.003$, Test x Mino: $F_{(1, 14)} = 0.064$, $p = 0.804$, $\eta_p^2 = 0.005$), subsequent

extinction (Within-session: Extinction: $F_{(4,56)} = 1.298$, p = 0.282, $\eta_p^2 = 0.085$, Mino: $F_{(1, 14)} = 0.023$, p = 0.882, $\eta_p^2 = 0.002$, Extinction x Mino: $F_{(1, 14)} = 0.374$, p = 0.826, $\eta_p^2 = 0.026$; Between–session LTM2, LTM3: Test: $F_{(1, 14)} = 21.248$, p < 0.001, $\eta_p^2 = 0.603$, Mino: $F_{(1, 14)} = 0.004$, p = 0.949, $\eta_p^2 = 0.000$, Test x Mino: $F_{(1, 14)} = 0.009$, p = 0.926, $\eta_p^2 = 0.001$) or reinstatement of freezing responses by a reminder US after extinction of remote CFM (Reminder: $F_{(1, 14)} = 11.484$, p = 0.004, $\eta_p^2 = 0.451$, Mino: $F_{(1, 14)} = 1.098$, p = 0.312, $\eta_p^2 = 0.073$, Reminder x Mino: $F_{(1, 14)} = 1.737$, p = 0.209, $\eta_p^2 = 0.110$) as evidenced by no differences in freezing behaviour between the PBS or minocycline groups at any point during the experiment (Fig. 4C).

These data show that a 10 min exposure to the conditioning context can extinguish remote memory established for 25 days in both male and female rats. There was however a differential effect of minocycline treatment in males and females exemplified by higher conditioned freezing responses compared to controls in males notwithstanding evidence of extinction, suggesting when given before a brief recall session minocycline enhances the expression of remote CFM in males but not females.

## Discussion

We show that acute administration of minocycline profoundly affects the expression of hippocampal-dependent CFM in rats, with effects influenced by sex, memory age, and memory-recall conditions. In males, minocycline given 48 hours CFC, without memory retrieval, increased fear expression for up to 23 days. However, when administered before retrieval, this effect was lost and extinction was enhanced, resulting in weaker fear-memory expression. The enhancement of extinction was limited to recently formed memories. In contrast, administering minocycline before retrieval of a well-established 28-day-old memory persistently increased fear responses. Females trained under conditions that produced similar levels of conditioned freezing behaviour to males, minocycline increased the expression of recent fear memories regardless of whether the memory was retrieved or not, with no apparent effect on extinction. These findings suggest that minocycline's potential to reduce fear memories associated with psychiatric disorders may be limited by sex, extinction training, and memory age.

### Acute minocycline enhances the extinction of recent and the reconsolidation of remote CFM in males.

In males, a single dose of minocycline administered two days after CFC and before retrieval enhanced the extinction of CFM. This effect was most evident following stronger CFC and repeated, prolonged (10-

minute) context re-exposures, with minocycline-treated rats showing reduced freezing at later time points. The recovery of freezing following a reminder US in both treatment groups suggests that minocycline enhanced extinction (context–no US learning) rather than disrupting reconsolidation or erasing the original context–US memory, which would be evidenced by a failure in the reinstatement of conditioned fear responses. Minocycline did not appear to affect reconsolidation of recently acquired CFM, as administration before a brief 2-minute retrieval session produced no long-term difference in fear expression compared with controls, even up to 23 days later. Thus, in males, minocycline appears to preferentially modulate extinction rather than reconsolidation of recent fear memories. These findings broadly align with previous reports on the effects of sub-chronic minocycline on fear memory extinction (Bi et al., 2016; Yu et al., 2017), while extending them by demonstrating an effect following a single dose administered outside the fear-memory consolidation window and immediately before extinction training. Our observations are also consistent with evidence that extinction is facilitated by stronger memories and greater mismatch between training and retrieval conditions (Bui & Milton, 2023).

The effects of acute minocycline administration were strongly dependent on fear-memory age. For males, in contrast to recent CFM, delayed administration before a short 2min retrieval trial persistently enhanced remote CFM expression despite extinction training. The additional absence of fear reinstatement following a reminder US suggests that, for remote memories, minocycline may strengthen the original CS–US association through reconsolidation. These opposing effects may reflect age-dependent changes in the retrieval conditions that engage extinction versus reconsolidation ('boundary' conditions; Bui & Milton, 2023). Overall, minocycline's effects on fear memory depend on memory age in males, promoting extinction and reducing fear expression for recent memories but enhancing reconsolidation and fear expression for older memories.

**Minocycline attenuates forgetting in males**

Minocycline produced opposing effects on fear memory in male rats depending on whether memory retrieval occurred. When administered before retrieval, it enhanced extinction of recent CFM, whereas administration without retrieval strengthened established fear memories, suggesting reduced natural forgetting (Davis & Zhong, 2017; Ryan & Frankland, 2022). A single dose enhanced both strong and weak CFM for over 23 days, consistent with evidence that minocycline prevents forgetting in male mice (C. Wang et al., 2020). These findings suggest that minocycline may enhance extinction when memory is retrieved but preserve the original context–US association when it is not.

**Acute minocycline persistently increases recent and remote fear memory responses in females**

In females, minocycline consistently increased conditioned fear responses regardless of whether memory was retrieved or not, with no evidence of effects on extinction or forgetting. The increase freezing behaviour is unlikely to be simply due to enhanced reconsolidation after retrieval because enhanced conditioned responses were seen immediately following administration, and before reconsolidation processes are complete (Nader et al, 2000). Thus, increases in conditioned freezing responses in females may reflect a nonspecific enhancement of fear- or anxiety-like behaviour rather than modulation of memory processes by the drug.

Our strategic approach used different footshock intensities for males and females to examine minocycline's effects on CFM, as females showed less freezing than males and may differ in the conditions required for contextual fear learning (Keiser et al., 2017; Tronson, 2018; Trott et al., 2022). However, sex differences in fear and anxiety expression, rather than CFM itself, may also contribute to these behavioural differences (Gruene et al., 2015; Pellman et al., 2017; Russo & Parsons, 2021; Shanazz et al., 2022). Future studies would benefit from additionally using multiple measures of fear when comparing sexes.

**Potential mechanisms of action of minocycline in modulating contextual fear memory expression**

Although this study did not directly investigate the mechanisms underlying minocycline's effects on fear memory, its anti-inflammatory and neuroprotective actions, particularly its modulation of microglia, provide a potential mechanism (Clemens et al., 2018; Garrido-Mesa et al., 2013; Kobayashi et al., 2013; Long et al., 2023). Microglia dynamically regulate neuronal activity and synaptic remodelling during learning and memory (Bourgognon & Cavanagh, 2020; Butovsky & Weiner, 2018; Green & Rowe, 2024; Paolicelli et al., 2022). Consistent with this, minocycline reduces complement-dependent synaptic elimination implicated in contextual fear-memory forgetting (C. Wang et al., 2020) and inhibits microglial TNF-α to enhance extinction (Yu et al., 2017). Evidence further suggests that dynamic, context-dependent microglial inflammatory signalling, including TNF-α, may contribute to variability in stress-related behavioural responses (Chen et al., 2024). Thus, minocycline's opposing effects on forgetting and extinction in males may reflect differential modulation of microglial function with memory retrieval. Recent evidence suggests that microglia differentially regulate competing fear and extinction engrams. Microglial inhibition can both constrain the excitability fear engrams (CS-US) during extinction training (Liu et al., 2026), and can facilitate extinction-ensemble (CS-no US) excitability (Wang et al., 2026), consistent with our finding that inhibition enhanced extinction without altering the original

fear-memory engram. This suggests that microglial modulation of fear memory and extinction-related ensembles may contribute to minocycline-induced enhancement of extinction.

Sex differences in fear learning, memory, extinction, and retention involve distinct molecular and neural mechanisms (Bauer, 2023; Gall et al., 2023; Tronson & Keiser, 2019; Velasco et al., 2019). Microglia may contribute to these differences, given sex-dependent variation in their density, distribution, molecular profiles, and stress responses (Colombo et al., 2022; Guneykaya et al., 2018; Nelson & Lenz, 2017; Schwarz et al., 2012; Villa et al., 2018). For example, microglial activation impairs fear extinction following chronic stress in males but not females (Torres-Rodriguez et al., 2023), while minocycline may reduce anxiety-like behaviour through sex-specific cellular mechanisms (Rooney et al., 2020; Ugursu et al., 2024). Given links between stress-related anxiety and neuroinflammatory status (Munshi et al., 2020; Iglesia et al., 2024; Ryan & Frankland, 2022), our data provide behavioural evidence to examine whether sex-specific microglial states and stress responses contribute to the differential effects of minocycline, including different doses of the drug,  on fear- and anxiety-related behaviours.

In summary, our findings show that the effects of acute systemic minocycline on fear memory and anxiety-like behaviour depend on memory activation, memory age, and sex. In males, minocycline enhanced extinction of recent fear memories when administered before retrieval but increased fear expression when given without recall, by reducing passive forgetting. For older memories, retrieval instead appeared to enable minocycline to strengthen fear memory through reconsolidation. In females, minocycline persistently increased fear expression of recently acquired CFM regardless of retrieval, without affecting extinction or forgetting, potentially reflecting enhanced anxiety-like behaviour. These findings suggest that minocycline's effects may depend on sex- and context-specific microglial states. Clinically, this highlights its potentially restricted and condition-dependent therapeutic value, while emphasising the need to consider sex, memory age, and retrieval conditions when evaluating minocycline as an adjunct to exposure therapy. Such factors may also contribute to variability seen in clinical trial outcomes.

**Acknowledgements**

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**Conflict of Interest:**

The authors declare no conflicts of interest relevant to the current work.

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