Propranolol Remediates Secondary Trauma Induced by Immediate Extinction

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Abstract

Impaired fear extinction capability is one of the hallmarks of posttraumatic stress disorder (PTSD). Previous studies have shown that extinction training conducted shortly after fear acquisition cannot form long-term extinction memory compared to delayed extinction, a phenomenon known as immediate extinction deficit. However, it is currently unclear whether this deficit is a one-time occurrence or whether it continues to affect subsequent re-extinction. In Experiment 1, rats began extinction training at 1 hour (immediate extinction) or 24 hours (delayed extinction) after fear acquisition, underwent re-extinction 24 hours later, and were tested for extinction memory after another 24 hours. The results showed that, compared to delayed extinction, immediate extinction was deficient in efficacy and caused the re-extinction on the following day to also exhibit efficacy deficits. In Experiment 2, rats were administered saline or the β-adrenergic receptor blocker propranolol (10 mg/kg, i.p.) immediately after fear acquisition, and the effects of immediate extinction and re-extinction were then tested. The results showed that although propranolol did not prevent the deficit in immediate extinction, it prevented the occurrence of deficits in re-extinction. In summary, immediate extinction after severe trauma not only fails to effectively suppress fear responses but may also cause secondary trauma, impairing fear extinction capability, whereas propranolol can repair the re-extinction deficits caused by immediate extinction. These results will help deepen our understanding of the pathological mechanisms of PTSD and the consequences of early intervention.

Full Text

Propranolol Rescues Secondary Trauma Induced by Immediate Extinction

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Abstract

Impaired fear extinction capacity represents a hallmark of posttraumatic stress disorder (PTSD). Previous research has demonstrated that extinction training conducted shortly after fear acquisition fails to form long-term extinction memory compared to delayed extinction, a phenomenon known as the immediate extinction deficit (IED). However, it remains unclear whether this deficit is a one-time occurrence or continues to impair subsequent re-extinction. In Experiment 1, rats underwent extinction training either 1 hour (immediate extinction) or 24 hours (delayed extinction) after fear acquisition, followed by re-extinction 24 hours later and an extinction memory test after another 24 hours. The results showed that compared to delayed extinction, immediate extinction not only was ineffective but also caused a deficit in re-extinction the following day.

In Experiment 2, rats received either saline or the β-adrenergic receptor antagonist propranolol (10 mg/kg, i.p.) immediately after fear acquisition, followed by tests of immediate extinction and re-extinction. The results revealed that although propranolol did not prevent the immediate extinction deficit, it prevented the deficit in re-extinction. In summary, immediate extinction after severe trauma not only fails to effectively suppress fear responses but may also constitute a secondary trauma that impairs fear extinction capacity, while propranolol can repair the re-extinction deficit caused by immediate extinction. These findings advance our understanding of PTSD pathological mechanisms and the consequences of early intervention.

Keywords: conditioned fear, immediate extinction deficit, re-extinction, β-adrenergic receptor antagonist, propranolol

1 Introduction

Posttraumatic stress disorder (PTSD) is a psychiatric condition that emerges after individuals experience life-threatening events, typically manifesting as fear, anxiety, trauma-related intrusive memories, nightmares, and other symptoms (American Psychiatric Association, 2013). The COVID-19 pandemic in 2020 threatened the lives of tens of thousands of people in China, some of whom may develop PTSD as a result. Trauma-related fear memories constitute a critical pathological basis for PTSD (Careaga et al., 2016). Fear conditioning represents

a crucial paradigm for investigating fear acquisition, expression, extinction, and relapse (Vervliet et al., 2013). In this paradigm, a neutral stimulus (e.g., a tone, conditioned stimulus, CS) is repeatedly paired with an aversive stimulus (e.g., electric shock, unconditioned stimulus, US), enabling the organism to learn that the CS predicts the US, thereby forming a CS-US fear memory. Subsequently, the CS alone can elicit fear responses, known as conditioned fear. If the CS is presented repeatedly without the US, the organism' s fear response gradually decreases—a process called extinction. In clinical practice, exposure therapy based on extinction principles constitutes a primary treatment method for PTSD (Vervliet et al., 2013). However, following extinction training, fear responses can recover under various conditions, such as after the passage of time (spontaneous recovery), when the CS appears in a context different from the extinction context (renewal), or after exposure to stressful events (reinstatement) (Vervliet et al., 2013). This occurs because extinction training only teaches the organism that the CS no longer predicts the US, forming a CS-noUS extinction memory that competes with, rather than erases, the original fear memory. These fear relapse phenomena indicate that fear memories are strong, persistent, and easily retrieved, whereas extinction memories are weak, context-dependent, and difficult to retrieve under stress. Therefore, disrupting fear memories or enhancing extinction memories would help prevent fear relapse.

Memories are unstable immediately after acquisition and gradually stabilize through a consolidation process. During this consolidation window, memories are vulnerable to disruption by behavioral or pharmacological interventions (McGaugh, 2000). Some scholars have suggested that conducting extinction learning during the fear memory consolidation window might permanently replace the original fear memory with a new, safe memory (Maren & Chang, 2006). However, studies in humans and rodents have shown that although both immediate extinction groups (extinction conducted 15 minutes to 6 hours post-trauma) and delayed extinction groups (extinction conducted more than 24 hours post-trauma) exhibit gradual decreases in fear responses during extinction training, the immediate extinction groups show fear response recovery 24 hours (Merz et al., 2016; Singh et al., 2018; Stafford et al., 2013) or 48 hours (Fitzgerald et al., 2015; Giustino et al., 2017; Maren & Chang, 2006; Totty et al., 2019) later, with significantly higher fear responses than the delayed extinction groups. This indicates that the fear suppression effect produced by immediate extinction is less durable than that produced by delayed extinction, a phenomenon termed the immediate extinction deficit (IED) (Maren, 2014). During immediate extinction, individuals show gradually decreasing fear responses during the extinction training session, and fear responses remain low in a test conducted 15 minutes after extinction (Chang & Maren, 2009). However, fear responses recover after longer intervals, such as 24 hours later (Huff et al., 2009; Merz et al., 2016; Stafford et al., 2013; Woods & Bouton, 2008), suggesting that extinction memory consolidation is impaired. The current consensus is that IED results from high stress and emotional arousal levels triggered by the fear acquisition process (using strong aversive stimuli such as electric shocks) that persist during imme-

diate extinction, which impairs extinction memory consolidation. Over time, fear-induced stress and emotional arousal gradually decrease, reaching lower levels by the time of delayed extinction, leaving extinction memory unaffected (Giustino et al., 2017; Maren, 2014; Maren & Chang, 2006; Totty et al., 2019).

Although previous studies have identified the immediate extinction deficit phenomenon under high stress levels, they have not further examined whether this deficit continues to affect subsequent re-extinction under low stress levels. Given that immediate extinction may be applied as an early intervention after trauma, and early intervention is widely used in clinical practice and considered an effective strategy for limiting subsequent psychopathological development (Rothbaum et al., 2014), it is necessary to investigate whether immediate extinction after severe trauma merely represents a one-time memory consolidation failure due to stress, or whether immediate extinction itself constitutes a secondary traumatic event that continually impairs extinction learning capacity. Since extinction deficits are associated with PTSD symptoms and considered a robust clinical endophenotype of PTSD (Giustino et al., 2016; Wicking et al., 2016), this question holds special significance in the current "Research Domain Criteria (RDoC)" era (Singewald & Holmes, 2019). Therefore, we conducted Experiment 1 to test this hypothesis. The results showed that immediate extinction after severe trauma produced a deficit, and re-extinction the following day also showed a deficit and failed to effectively reduce fear responses. However, if immediate extinction had not occurred previously, the extinction session on the second day (i.e., the first extinction session in the delayed extinction group) significantly reduced fear responses, suggesting that the immediate extinction procedure itself may be a secondary traumatic event that damages the capacity for fear extinction, leading to poor re-extinction outcomes.

Stress (such as electric shocks) activates the locus coeruleus-norepinephrine (LC-NE) system, causing massive NE release (Borodovitsyna et al., 2018; McCall et al., 2015). NE can dynamically modulate conditioned fear and extinction learning, either promoting or impairing these processes depending on NE levels. Under high NE levels (such as shortly after fear conditioning), the LC enhances amygdala function to promote fear learning while simultaneously impairing prefrontal cortex function involved in extinction learning. Under low NE levels, the LC enhances prefrontal inhibition of the amygdala, promoting fear extinction (Giustino & Maren, 2018). Studies have found that injecting the β-adrenergic receptor antagonist propranolol immediately after strong shock fear conditioning, either systemically (Fitzgerald et al., 2015) or into the basolateral amygdala (BLA) (Giustino et al., 2017), can reduce pre-extinction stress levels and rescue IED. Activating the LC using chemogenetic methods before weak shock conditioning induces IED, while injecting propranolol into the BLA blocks this effect (Giustino et al., 2020). Building on Experiment 1, we conducted Experiment 2 to investigate whether propranolol administered before immediate extinction could rescue IED while simultaneously preventing immediate extinction from acting as a secondary trauma that continually damages extinction capacity. We found that propranolol could prevent the damaging effects of immediate ex-

tinction on extinction capacity. These results will enhance our understanding of PTSD pathological mechanisms and early intervention outcomes, and help us apply interventions appropriately and effectively after trauma exposure to avoid secondary trauma caused by the intervention itself.

2 Methods

2.1 Experimental Animals

Male Sprague-Dawley (SD) rats (purchased from Beijing Vital River Laboratory Animal Technology) with initial body weights of 240–260 g were group-housed in stainless steel cages (28.5 cm × 22.5 cm × 50 cm) at four rats per cage with ad libitum access to water and food. The light cycle was 7:00–19:00. Laboratory temperature was maintained at 20–22°C with relative humidity of 40%–70%. After seven days of acclimation, during which animals were regularly handled to adapt to experimenter manipulation and exclude non-specific stress factors, all experimental procedures were approved by the Experimental Animal Ethics Committee of Henan Key Lab of Biological Psychiatry.

2.2 Experimental Apparatus

The rat fear conditioning system and experimental data collection and analysis system (FreezeFrame Video-Based Conditioned Fear System, Coulbourn) consisted of a conditioning chamber (30.5 cm × 25.5 cm × 30.5 cm) with a speaker mounted on one side wall, a floor composed of 18 stainless steel rods through which electric current could be delivered, and a camera mounted on top to record animal behavior. Both the speaker and shock generator were computer-controlled. Two contexts were used: Context A was an unmodified fear conditioning chamber used for fear acquisition training, while Context B was modified in visual, olfactory, and tactile aspects to be completely different from the original training chamber and used for fear extinction. Four conditioning chambers were used simultaneously in experiments.

2.3 Experimental Procedures

Experiment 1 randomly divided rats into four groups (n = 8 per group): Immediate Extinction (tone exposure in Context B 1 hour after fear acquisition), Immediate No-Extinction (no tone exposure in Context B 1 hour after fear acquisition), Delayed Extinction (tone exposure in Context B 24 hours after fear acquisition), and Delayed No-Extinction (no tone exposure in Context B 24 hours after fear acquisition). The timing of immediate and delayed extinction followed Chang and Maren (2009). All groups underwent three stages: fear acquisition, extinction, and post-extinction testing. The specific experimental procedure is shown in Figure 1 [Figure 1: see original paper]A.

Fear Acquisition: Rats were placed in the fear conditioning chamber (Context A) and allowed to acclimate for 3 minutes, followed by five tone (30 s, 80 dB, 2

kHz)-shock (1 s, 1.0 mA) pairing trials with inter-trial intervals of 1–3 minutes (average 2 minutes). Animals were returned to their home cages 2 minutes after the final shock.

Fear Extinction: Rats were placed in Context B and allowed to acclimate for 2 minutes, followed by 30 tone CS trials (30 s each) with 1-minute inter-trial intervals. Animals were returned to their home cages 1 minute after the final tone. Pilot studies showed that 30 CS trials significantly reduced fear responses.

Post-Extinction Test: Rats were placed in the test environment, allowed to acclimate for 2 minutes, and then presented with four continuous CS tones (80 dB, 2 kHz). Animals were returned to their home cages 1 minute after tone offset.

Experiment 2 randomly divided rats into two groups (n = 10 per group): Immediate Extinction Saline and Immediate Extinction Propranolol. Except for receiving an intraperitoneal injection of 10 mg/kg saline or propranolol immediately after fear acquisition, these groups underwent the same fear acquisition, extinction, and testing procedures as Experiment 1. The specific experimental procedure is shown in Figure 2 [Figure 2: see original paper]A. The dosage was selected based on previous behavioral studies using a single dose of 10 mg/kg propranolol (Fan et al., 2011; Fitzgerald et al., 2015; Khan et al., 2018; Muravieva & Alberini, 2010; Przybyslawski et al., 1999; Robinson & Franklin, 2010; Rodriguez-Romaguera et al., 2009; Taherian et al., 2014), which demonstrated that this dose significantly reduces fear levels without affecting extinction acquisition, consolidation, or retrieval (Rodriguez-Romaguera et al., 2009). Propranolol was administered immediately after fear acquisition and 1 hour before immediate extinction (Chang & Maren, 2009, 2011) because previous studies reported inconsistent effects of propranolol on extinction memory depending on timing. For instance, systemic propranolol injection (10 mg/kg) 20 minutes before delayed extinction reduced fear during extinction without impairing extinction memory acquisition, consolidation, or retrieval (Rodriguez-Romaguera et al., 2009), whereas Cain et al. (2004) reported that subcutaneous propranolol (10 mg/kg) 20 minutes before delayed extinction increased fear during extinction, and Fitzgerald et al. (2015) found that systemic propranolol (10 mg/kg) 30 minutes before immediate extinction reduced fear and promoted extinction, while the same injection before delayed extinction increased fear and impaired next-day extinction retrieval. The present study aimed to reduce elevated NE levels induced by fear acquisition training without affecting the immediate extinction process itself, so propranolol was administered immediately after fear acquisition (Fitzgerald et al., 2015) but with a longer 1-hour interval before immediate extinction (Chang & Maren, 2009, 2011).

2.4 Measurement Indices

The fear conditioning experimental data collection and analysis system automatically analyzed fear behavior. Rat fear behavior was manifested as "freezing" —

the absence of all movement except breathing, recorded when lasting more than 1 second. The fear level index was calculated as the percentage of freezing time relative to CS presentation time. All data were analyzed using SPSS 24.0. Repeated measures ANOVA was performed on freezing behavior across all stages (fear acquisition, fear extinction, fear testing), with Bonferroni post-hoc tests or independent and paired samples t-tests used to compare freezing differences between stages.

3 Results

3.1 Experiment 1: Immediate Extinction Induced Re-Extinction Deficit

Fear Acquisition Stage (see Figure 1B): Repeated measures ANOVA showed no significant main effect of interval (1 h vs. 24 h) ($p = 0.68$), no significant main effect of treatment (extinction vs. no extinction) ($p = 0.64$), and no significant interval × treatment interaction ($p = 0.34$). The trial effect was significant, $F(4, 112) = 52.09$, $p < 0.001$, partial $^2 = 0.65$, with fear levels increasing across training trials in all groups. The trial × interval interaction was not significant ($p = 0.94$). The trial × treatment interaction was significant, $F(4, 112) = 3.11$, $p = 0.02$, partial $^2 = 0.10$, while the trial × interval × treatment interaction was not significant ($p = 0.14$). One-way ANOVA revealed no differences between groups in the final acquisition trial ($p = 0.97$) or in the mean of trials 2-5 ($p = 0.72$), indicating equivalent fear acquisition across groups.

Because the extinction chamber was small and monotonous, the extinction stage lasted 47 minutes (30 trials) and was conducted during the daytime, some rats showed reduced exploration and transitioned to resting or even sleep behavior as they became familiar with the extinction environment. According to control rats that did not receive extinction, resting immobility was already substantial by the 10th extinction trial (after 17 minutes in Context B) (see Figure 1B). However, the computer analysis software cannot distinguish between freezing and sleep behavior (both involve immobility). Therefore, we present only the first 10 trials of each extinction session and compare only the mean fear levels across the first four CS trials of extinction 1, extinction 2, and testing (Figure 1D) to exclude interference from resting or sleep behavior. The mean of the first four CS trials of extinction 1 reflects fear expression after fear conditioning, the mean of the first four CS trials of extinction 2 reflects retention of extinction 1, and the mean of the four CS trials during testing reflects retention of extinction 2.

Fear Extinction 1 Stage (see Figure 1B): Repeated measures ANOVA showed a marginally significant main effect of interval (1 h vs. 24 h), $F(1, 28) = 3.91$, $p = 0.06$, partial $^2 = 0.12$, a significant main effect of treatment (extinction vs. no extinction), $F(1, 28) = 23.01$, $p < 0.001$, partial $^2 = 0.45$, and no significant interval × treatment interaction ($p = 0.74$). The trial effect was significant, $F(9, 252) = 1.97$, $p = 0.04$, partial $^2 = 0.07$. The trial × interval interaction was not

significant (p = 0.79). The trial × treatment interaction was significant, F(9, 252) = 3.36, p = 0.001, partial ^{2} = 0.11, while the trial × interval × treatment interaction was not significant (p = 0.99).

Fear Extinction 2 Stage (see Figure 1B middle): Repeated measures ANOVA showed no significant main effect of interval (1 h vs. 24 h) (p = 0.34), a significant main effect of treatment (extinction vs. no extinction), F(1, 28) = 8.06, p = 0.01, partial ^{2} = 0.22, and a significant interval × treatment interaction, F(1, 28) = 4.22, p = 0.049, partial ^{2} = 0.13. The trial effect was not significant (p = 0.16). The trial × interval interaction was significant, F(9, 252) = 2.05, p = 0.03, partial ^{2} = 0.07. The trial × treatment interaction was significant, F(9, 252) = 4.55, p < 0.001, partial ^{2} = 0.14, while the trial × interval × treatment interaction was not significant (p = 0.48).

Fear Testing Stage (see Figure 1B): Repeated measures ANOVA showed a significant main effect of interval (1 h vs. 24 h), F(1, 28) = 5.55, p = 0.03, partial ^{2} = 0.17, a significant main effect of treatment (extinction vs. no extinction), F(1, 28) = 7.36, p = 0.01, partial ^{2} = 0.21, and no significant interval × treatment interaction (p = 0.19). The trial main effect was significant, F(3, 84) = 4.78, p = 0.004, partial ^{2} = 0.15. The trial × interval interaction was not significant (p = 0.12). The trial × treatment interaction was not significant (p = 0.57). The trial × interval × treatment interaction was not significant (p = 0.20).

Fear Testing Stage (see Figure 1C): Two-way ANOVA showed a significant main effect of interval (1 h vs. 24 h), F(1, 28) = 5.55, p = 0.03, partial ^{2} = 0.17, a significant main effect of treatment (extinction vs. no extinction), F(1, 28) = 7.37, p = 0.01, partial ^{2} = 0.21, and no significant interval × treatment interaction (p = 0.19). Independent samples t-tests showed no significant difference between the immediate extinction and immediate no-extinction groups (p = 0.42), but the immediate extinction group showed significantly higher fear than the delayed extinction group, t(14) = 3.08, p = 0.01, Cohen' s d = 1.54, indicating the IED phenomenon. No significant difference was found between the immediate no-extinction and delayed no-extinction groups (p = 0.54). The delayed extinction group showed significantly lower fear than the delayed no-extinction group, t(14) = 3.54, p = 0.01, Cohen' s d = 1.77, indicating effective delayed extinction.

Figure 1D shows the mean freezing across the first four CS trials of extinction 1, extinction 2, and testing for immediate and delayed extinction groups. Repeated measures ANOVA revealed a significant main effect of interval (1 h vs. 24 h), F(1, 14) = 6.29, p = 0.03, partial ^{2} = 0.31, a significant main effect of stage (extinction 1, extinction 2, and testing), F(2, 28) = 15.50, p < 0.001, partial ^{2} = 0.53, and no significant interval × stage interaction (p = 0.10). One-way repeated measures ANOVA showed no significant stage effect in the immediate extinction group (p = 0.19), indicating no extinction effect and suggesting that re-extinction under low stress was also impaired. The delayed extinction group showed a significant stage effect, F(2, 14) = 29.61, p < 0.001, partial ^{2} = 0.81, with extinction 1 significantly higher than extinction 2 (p = 0.006) and testing

($p = 0.001$), and a marginally significant difference between extinction 2 and testing ($p = 0.06$), indicating significant re-extinction effects.

3.2 Experiment 2: Propranolol Rescued Re-Extinction Deficit Induced by Immediate Extinction

Fear Acquisition Stage (see Figure 2B): Repeated measures ANOVA showed no significant main effect of drug (saline vs. propranolol) ($p = 0.96$). The trial main effect was significant, $F(4, 72) = 28.26$, $p < 0.001$, partial $^2 = 0.61$, with fear levels increasing across training trials in both groups. The drug × trial interaction was not significant ($p = 0.81$).

Fear Extinction 1 Stage (see Figure 2B): Repeated measures ANOVA showed no significant main effect of drug (saline vs. propranolol) ($p = 0.45$). The trial main effect was significant, $F(9, 162) = 6.80$, $p < 0.001$, partial $^2 = 0.27$, with fear levels gradually decreasing across extinction trials in both groups. The drug × trial interaction was not significant ($p = 0.99$).

Fear Extinction 2 Stage (see Figure 2B): Repeated measures ANOVA showed no significant main effect of drug (saline vs. propranolol) ($p = 0.29$). The trial main effect was significant, $F(9, 162) = 8.23$, $p < 0.001$, partial $^2 = 0.31$, with fear levels gradually decreasing across extinction trials in both groups. The drug × trial interaction was not significant ($p = 0.24$).

Fear Testing Stage (see Figure 2B): Repeated measures ANOVA showed a significant main effect of drug (saline vs. propranolol), $F(1, 18) = 24.64$, $p < 0.001$, partial $^2 = 0.58$. The trial main effect was not significant ($p = 0.54$). The drug × trial interaction was not significant ($p = 0.22$).

Figure 2C shows the mean freezing across the first four CS trials of extinction 1, extinction 2, and testing for saline and propranolol groups. Repeated measures ANOVA revealed a marginally significant main effect of drug (saline vs. propranolol), $F(1, 18) = 4.00$, $p = 0.06$, partial $^2 = 0.18$, a significant main effect of stage, $F(2, 36) = 20.48$, $p < 0.001$, partial $^2 = 0.53$, and a significant drug × stage interaction, $F(2, 36) = 3.59$, $p = 0.04$, partial $^2 = 0.17$. Simple effects analysis showed no significant differences across stages in the saline group, consistent with Experiment 1 results. In the propranolol group, extinction 1 and extinction 2 were significantly higher than testing ($p < 0.001$ and $p = 0.001$, respectively), while extinction 1 and 2 did not differ ($p = 0.11$). No differences were found between propranolol and saline groups during extinction 1 ($p = 0.45$) or extinction 2 ($p = 0.27$). During testing, the propranolol group showed significantly lower fear than the saline group, $F(1, 18) = 11.67$, $p = 0.003$, partial $^2 = 0.39$. These results indicate that propranolol did not prevent the immediate extinction deficit but prevented impairment of re-extinction under low stress conditions the following day.

4 Discussion

This study investigated whether poor immediate extinction outcomes after severe trauma represent a one-time occurrence or whether immediate extinction itself acts as a secondary traumatic event that damages extinction learning capacity, causing deficits in subsequent re-extinction. We found that each extinction session in the delayed extinction group significantly reduced fear responses, whereas the immediate extinction group failed to form long-term extinction memory, and re-extinction effectiveness was also impaired. Systemic propranolol injection immediately after fear acquisition did not prevent the immediate extinction deficit (IED) but prevented deficits in re-extinction.

The results of Experiments 1 and 2 (Figures 1D and 2D) showed that immediate extinction produced IED compared to delayed extinction, consistent with previous findings (Fitzgerald et al., 2015; Giustino et al., 2017; Maren & Chang, 2006; Merz et al., 2016; Singh et al., 2018; Stafford et al., 2013; Totty et al., 2019). Research has shown that the infralimbic (IL) region of the medial prefrontal cortex (mPFC), corresponding to the human ventromedial prefrontal cortex, plays a central role in extinction memory consolidation and retrieval (Giustino et al., 2019; Siddiqui et al., 2017; Sierra-Mercado et al., 2011). The BLA partners with the mPFC during fear memory acquisition and extinction, with reciprocal projections between them. The BLA receives both excitatory and inhibitory projections from the mPFC, and whether fear can be extinguished depends on the activity levels of IL-BLA and BLA-IL pathways, which have an inverse relationship. When the BLA is excited, especially hyperexcited, BLA-IL projections reduce IL activity, thereby impairing extinction memory. Strong shock fear conditioning causes rapid and persistent reductions in IL neuron firing rates, with the greatest impairment in IL neuron spontaneous firing occurring within the first 10 minutes after training (Fitzgerald et al., 2015). Recently, Giustino and colleagues (2020) found that strong shock fear conditioning causes immediate and dramatic increases in spontaneous firing of BLA neurons, a phenomenon that persists for 1 hour after fear training, with some neurons showing even longer-lasting increases. Immediate extinction occurs during this time window, which may underlie IED.

If immediate extinction merely represents a memory consolidation impairment caused by stress rather than simultaneously acting as a traumatic event that affects subsequent re-extinction, then extinction 2 on the second day should not show deficits, as stress responses triggered by fear conditioning would have decreased to low levels by the next day. However, our study showed that after immediate extinction, re-extinction the following day still exhibited deficits, whereas extinction on the second day without prior immediate extinction (i.e., the first extinction session in the delayed extinction group) significantly reduced fear levels 24 hours later. This indicates that immediate extinction impairs rats' capacity for fear extinction, independent of stress effects, suggesting that immediate extinction may be a secondary traumatic event that exacerbates stress-induced adverse effects. Previous research has found that after acute stress, the

amygdala is activated, alertness is enhanced, and threat sensitivity is heightened, with neutral stimuli activating the amygdala to a degree comparable to threat stimuli (van Marle et al., 2009). Because immediate extinction occurs during BLA hyperexcitability, presenting the CS during this period may cause BLA hypersensitization to the CS, forming stronger CS-US associations (Kyriazi et al., 2018), and causing more persistent BLA excitation and IL inhibition, all of which may render CS-elicited fear responses more resistant to extinction, creating a maladaptive mechanism (Sah, 2017; Sharp, 2017).

Administering propranolol immediately after fear acquisition and conducting extinction 1 one hour later produced no difference between propranolol and saline groups during extinction 1, indicating that propranolol did not affect fear expression. The mean of the first four CS trials of extinction 2 did not differ significantly from that of extinction 1 (Figure 2C), with both showing high fear levels, indicating that propranolol also did not affect fear memory consolidation and retrieval, consistent with previous research. Meta-analytic studies have shown that propranolol administered after traumatic events cannot disrupt fear memory consolidation or reduce PTSD incidence or symptoms (Argolo et al., 2015; Astill Wright et al., 2019). Therefore, the reduction in fear responses during the post-extinction test cannot be explained by propranolol impairing fear memory consolidation and retrieval.

Previous studies have found that systemic propranolol injection (10 mg/kg) 20 minutes before extinction on the day after fear acquisition significantly reduces fear during extinction without affecting extinction memory acquisition, consolidation, or retrieval, and does not prevent subsequent fear reinstatement (Rodriguez-Romaguera et al., 2009). This suggests that pre-extinction propranolol only acutely reduces fear levels during extinction (perhaps temporarily inhibiting fear memory retrieval) without permanently affecting fear memory or extinction memory processes. This conclusion is supported by human studies (Chalkia et al., 2019). The propranolol in our study did not significantly reduce fear levels during extinction 1, possibly because both the timing of administration and the interval to extinction training differed from previous studies. We administered propranolol immediately after fear acquisition, with extinction training occurring 1 hour later.

Notably, the finding that post-stress propranolol did not rescue IED in Experiment 2 differs from Fitzgerald et al. (2015). In their study, propranolol (also 10 mg/kg, i.p.) administered immediately after fear conditioning rescued IED without impairing fear memory consolidation. Potential reasons for this discrepancy include: (1) Different rat strains—Fitzgerald et al. used Long-Evans rats, whereas we used Sprague-Dawley rats, which show strain differences in task-related cognitive abilities (Gökçek-Saraç et al., 2015) and drug sensitivity (Hölscher, 2002). (2) Different experimental parameters—Fitzgerald et al. used a 30-minute drug-to-extinction interval and 45 extinction trials, whereas our study used a 1-hour interval and only 30 extinction trials. Therefore, even if propranolol reduced stress levels, fewer extinction trials resulted in weaker ex-

tinction memory encoding that could not compete effectively with fear memory, leading to poor extinction outcomes. The same 30 extinction trials were effective under low-stress delayed extinction conditions, possibly because immediate extinction occurs temporally close to fear acquisition, causing fear and extinction memory consolidation processes to be too close in time, with extinction memory consolidation subject to proactive interference from fear memory, as storing fear memory has greater survival value. Additionally, although fear acquisition and extinction training are temporally close, they occur in different environments with an event boundary between them. According to Dunsmoor et al. (2018), event boundaries guide selective consolidation, prioritizing emotional information (fear memory) at the expense of consolidating related but conflicting information (extinction memory) experienced shortly thereafter. Although propranolol did not rescue the impaired consolidation process of immediate extinction, the significantly lower fear levels in the post-extinction test compared to extinction 2 and the saline group indicate that propranolol rescued the re-extinction deficit caused by immediate extinction. The mechanism may involve propranolol stabilizing mPFC neural activity, preventing further mPFC functional impairment by immediate extinction (Fitzgerald et al., 2015), blocking stress-induced increases in BLA neuron firing (Giustino et al., 2020), and preventing BLA hypersensitization to the CS even when it is presented, thereby allowing re-extinction-generated extinction memory to be normally acquired and consolidated, resulting in reduced fear responses during the post-extinction test.

In summary, our study confirms that immediate extinction after severe trauma is a secondary traumatic event that impairs extinction learning capacity, causing deficits in subsequent re-extinction under low stress conditions. Systemic injection of 10 mg/kg propranolol after fear acquisition does not affect fear memory consolidation and retrieval, nor does it rescue the impaired consolidation of immediate extinction, but it can prevent immediate extinction under high stress from causing secondary trauma, thereby allowing extinction memory generated during subsequent re-extinction to be normally consolidated and retrieved. These findings reaffirm that behaviorally-based extinction interventions are not suitable for immediate implementation after severe stress. However, this study has several limitations: First, only one dose of propranolol was administered at a single time point, without investigating whether shortening the interval between drug administration and extinction training or increasing the dose could prevent IED. Second, we only investigated whether high-stress immediate extinction causes secondary trauma, but not whether high-stress delayed extinction also causes secondary trauma and impairs extinction capacity, as one study reported that a single unsignaled shock before delayed extinction also impaired extinction effectiveness (Maren & Chang, 2006). Third, we only examined re-extinction after a 24-hour interval, without investigating re-extinction after longer intervals to determine whether time can repair secondary trauma caused by immediate extinction. Fourth, we did not investigate whether propranolol administered before re-extinction could also enable normal acquisition, consolidation, and retrieval of extinction memory when extinction capacity is

already impaired after immediate extinction. We will explore these questions in future studies.

Argolo, F. C., Cavalcanti-Ribeiro, P., Netto, L. R., & Quarantini, L. C. (2015). Prevention of posttraumatic stress disorder with propranolol: A meta-analytic review. Journal of Psychosomatic Research, 79(2), 89–93.

American Psychiatric Association. (2013). Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub.

Astill Wright, L., Sijbrandij, M., Sinnerton, R., Lewis, C., Roberts, N. P., & Bisson, J. I. (2019). Pharmacological prevention and early treatment of post-traumatic stress disorder and acute stress disorder: A systematic review and meta-analysis. Translational Psychiatry, 9(1), 334.

Borodovitsyna, O., Joshi, N., & Chandler, D. (2018). Persistent Stress-Induced Neuroplastic Changes in the Locus Coeruleus/Norepinephrine System. Neural Plasticity, 2018, 1892570.

Cain, C. K., Blouin, A. M., & Barad, M. (2004). Adrenergic transmission facilitates extinction of conditional fear in mice. Learning & Memory, 11(2), 179–187.

Careaga, M. B. L., Girardi, C. E. N., & Suchecki, D. (2016). Understanding posttraumatic stress disorder through fear conditioning, extinction and reconsolidation. Neuroscience & Biobehavioral Reviews, 71, 48–57.

Chalkia, A., Weermeijer, J., Van Oudenhove, L., & Beckers, T. (2019). Acute but not permanent effects of propranolol on fear memory expression in humans. Frontiers in Human Neuroscience, 13, 51.

Chang, C. H., & Maren, S. (2009). Early extinction after fear conditioning yields a context-independent and short-term suppression of conditional freezing in rats. Learning & Memory, 16(1), 62–68.

Chang, C. H., & Maren, S. (2011). Medial prefrontal cortex activation facilitates re-extinction of fear in rats. Learning & Memory, 18(4), 221–225.

Cho, J. H., Deisseroth, K., & Bolshakov, V. Y. (2013). Synaptic encoding of fear extinction in mPFC-amygdala circuits. Neuron, 80(6), 1491–1507.

Dunsmoor, J. E., Kroes, M. C. W., Moscatelli, C. M., Evans, M. D., Davachi, L., & Phelps, E. A. (2018). Event segmentation protects emotional memories from competing experiences encoded close in time. Nature Human Behaviour, 2(4), 291–299.

Fan, S. J., Jiang, H., Yang, L. J., Liu, X., Song, J., & Pan, F. (2011). Effects of adrenergic agents on stress-induced brain microstructural and immunochemical changes in adult male Wistar rats. Annals of Anatomy-Anatomischer Anzeiger, 193(5), 418–424.

Fitzgerald, P. J., Giustino, T. F., Seemann, J. R., & Maren, S. (2015). Noradrenergic blockade stabilizes prefrontal activity and enables fear extinction under stress. Proceedings of the National Academy of Sciences of the United States of America, 112(28), E3729–3737.

Giustino, T. F., Fitzgerald, P. J., & Maren, S. (2016). Revisiting propranolol and PTSD: Memory erasure or extinction enhancement? Neurobiology of Learning and Memory, 130, 26–33.

Giustino, T. F., Fitzgerald, P. J., Ressler, R. L., & Maren, S. (2019). Locus coeruleus toggles reciprocal prefrontal firing to reinstate fear. Proceedings of the National Academy of Sciences of the United States of America, 116(17), 8570–8575.

Giustino, T. F., & Maren, S. (2018). Noradrenergic modulation of fear conditioning and extinction. Frontiers in Behavioral Neuroscience, 12, 43.

Giustino, T. F., Ramanathan, K. R., Totty, M. S., Miles, O. W., & Maren, S. (2020). Locus coeruleus norepinephrine drives stress-induced increases in basolateral amygdala firing and impairs extinction learning. Journal of Neuroscience, 40(4), 907–916.

Giustino, T. F., Seemann, J. R., Acca, G. M., Goode, T. D., Fitzgerald, P. J., & Maren, S. (2017). Beta-adrenoceptor blockade in the basolateral amygdala, but not the medial prefrontal cortex, rescues the immediate extinction deficit. Neuropsychopharmacology, 42(13), 2537–2544.

Gökçek-Saraç, Ç., Wesierska, M., & Jakubowska-Doğru, E. (2015). Comparison of spatial learning in the partially baited radial-arm maze task between commonly used rat strains: Wistar, Sprague-Dawley, Long-Evans, and outcrossed Wistar/Sprague-Dawley. Learning & Behavior, 43(1), 83–94.

Hölscher, C. (2002). Different strains of rats show different sensitivity to block of long-term potentiation by nitric oxide synthase inhibitors. European Journal of Pharmacology, 457(2-3), 99–106.

Huff, N. C., Hernandez, J. A., Blanding, N. Q., & LaBar, K. S. (2009). Delayed extinction attenuates conditioned fear renewal and spontaneous recovery in humans. Behavioral Neuroscience, 123(4), 834–843.

Khan, V., Sharma, S., Bhandari, U., Ali, S. M., & Haque, S. E. (2018). Raspberry ketone protects against isoproterenol-induced myocardial infarction in rats. Life Sciences, 194, 205–212.

Kyriazi, P., Headley, D. B., & Paré, D. (2018). Multi-dimensional coding by basolateral amygdala neurons. Neuron, 99(6), 1315–1328.e1315.

Maren, S. (2014). Nature and causes of the immediate extinction deficit: A brief review. Neurobiology of Learning and Memory, 113, 19–24.

Maren, S., & Chang, C. H. (2006). Recent fear is resistant to extinction. Proceedings of the National Academy of Sciences of the United States of America,

103(47), 18020–18025.

McCall, J. G., Al-Hasani, R., Siuda, E. R., Hong, D. Y., Norris, A. J., Ford, C. P., & Bruchas, M. R. (2015). CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. Neuron, 87(3), 605–620.

McGaugh, J. L. (2000). Memory—a century of consolidation. Science, 287(5451), 248–251.

Merz, C. J., Hamacher-Dang, T. C., & Wolf, O. T. (2016). Immediate extinction promotes the return of fear. Neurobiology of Learning and Memory, 131, 109–116.

Muravieva, E. V., & Alberini, C. M. (2010). Limited efficacy of propranolol on the reconsolidation of fear memories. Learning & Memory, 17(6), 306–313.

Przybyslawski, J., Roullet, P., & Sara, S. J. (1999). Attenuation of emotional and nonemotional memories after their reactivation: Role of beta adrenergic receptors. Journal of Neuroscience, 19(15), 6623–6628.

Robinson, M. J., & Franklin, K. B. (2010). Reconsolidation of a morphine place preference: Impact of the strength and age of memory on disruption by propranolol and midazolam. Behavioural Brain Research, 213(2), 201–207.

Rodriguez-Romaguera, J., Sotres-Bayon, F., Mueller, D., & Quirk, G. J. (2009). Systemic propranolol acts centrally to reduce conditioned fear in rats without impairing extinction. Biological Psychiatry, 65(10), 887–892.

Rothbaum, B. O., Kearns, M. C., Reiser, E., Davis, J. S., Kerley, K. A., Rothbaum, A. O., ···Ressler, K. J. (2014). Early intervention following trauma may mitigate genetic risk for PTSD in civilians: A pilot prospective emergency department study. Journal of Clinical Psychiatry, 75(12), 1380–1387.

Sah, P. (2017). Fear, anxiety, and the amygdala. Neuron, 96(1), 1–2.

Sharp, B. M. (2017). Basolateral amygdala and stress-induced hyperexcitability affect motivated behaviors and addiction. Translational Psychiatry, 7(8), e1194.

Siddiqui, S. A., Singh, S., Ranjan, V., Ugale, R., Saha, S., & Prakash, A. (2017). Enhanced histone acetylation in the infralimbic prefrontal cortex is associated with fear extinction. Cellular and Molecular Neurobiology, 37(7), 1287–1301.

Sierra-Mercado, D., Padilla-Coreano, N., & Quirk, G. J. (2011). Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology, 36(2), 529–538.

Singewald, N., & Holmes, A. (2019). Rodent models of impaired fear extinction. Psychopharmacology, 236(1), 21–40.

Singh, S., Siddiqui, S. A., Tripathy, S., Kumar, S., Saha, S., Ugale, R., ··· Prakash, A. (2018). Decreased level of histone acetylation in the infralimbic

prefrontal cortex following immediate extinction may result in deficit of extinction memory. Brain Research Bulletin, 140, 355–364.

Stafford, J. M., Maughan, D. K., Ilioi, E. C., & Lattal, K. M. (2013). Exposure to a fearful context during periods of memory plasticity impairs extinction via hyperactivation of frontal-amygdalar circuits. Learning & Memory, 20(3), 156–163.

Taherian, F., Vafaei, A. A., Vaezi, G. H., Eskandarian, S., Kashef, A., & Rashidy-Pour, A. (2014). Propranolol-induced impairment of contextual fear memory reconsolidation in rats: A similar effect on weak and strong recent and remote memories. Basic & Clinical Neuroscience, 5(3), 231–239.

Totty, M. S., Payne, M. R., & Maren, S. (2019). Event boundaries do not cause the immediate extinction deficit after Pavlovian fear conditioning in rats. Scientific Reports, 9(1), 9459.

van Marle, H. J., Hermans, E. J., Qin, S., & Fernández, G. (2009). From specificity to sensitivity: How acute stress affects amygdala processing of biologically salient stimuli. Biological Psychiatry, 66(7), 649–655.

Vervliet, B., Craske, M. G., & Hermans, D. (2013). Fear extinction and relapse: State of the art. Annual Review of Clinical Psychology, 9, 215–248.

Wicking, M., Steiger, F., Nees, F., Diener, S. J., Grimm, O., Ruttorf, M., ···Flor, H. (2016). Deficient fear extinction memory in posttraumatic stress disorder. Neurobiology of Learning and Memory, 136, 116–126.

Woods, A. M., & Bouton, M. E. (2008). Immediate extinction causes a less durable loss of performance than delayed extinction following either fear or appetitive conditioning. Learning & Memory, 15(12), 909–920.

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