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Candesartan Ameliorates Impaired Fear Extinction Induced by Innate Immune Activation

María M. Quiñones^a, Lizette Maldonado^b, Bethzaly Velazquez^c, and James T. Porter^c

^aDept. of Clinical Psychology, Ponce Health Sciences University

^bDept of Chemistry, Pontifical Catholic University of Puerto Rico, Ponce, Puerto Rico

^cDept. of Basic Sciences, Ponce Health Sciences University-Medical School/Ponce Research Institute, Ponce, Puerto Rico

Abstract

Patients with post-traumatic stress disorder (PTSD) tend to show signs of a relatively increased inflammatory state suggesting that activation of the immune system may contribute to the development of PTSD. In the present study, we tested whether activation of the innate immune system can disrupt acquisition or recall of auditory fear extinction using an animal model of PTSD. Male adolescent rats received auditory fear conditioning in context A. The next day, an intraperitoneal injection of lipopolysaccharide (LPS; 100 µg/kg) prior to auditory fear extinction in context B impaired acquisition and recall of extinction. LPS (100 µg/kg) given after extinction training did not impair extinction recall suggesting that LPS did not affect consolidation of extinction. In contrast to cued fear extinction, contextual fear extinction was not affected by prior injection of LPS (100 µg/kg). Although LPS also reduced locomotion, we could dissociate the effects of LPS on extinction and locomotion by using a lower dose of LPS (50 µg/kg) which impaired locomotion without affecting extinction. In addition, 15 hrs after an injection of 250 µg/kg LPS in adult rats, extinction learning and recall were impaired without affecting locomotion. A sub-chronic treatment with candesartan, an angiotensin II type 1 receptor blocker, prevented the LPS-induced impairment of extinction in adult rats. Our results demonstrate that activation of the innate immune system can disrupt auditory fear extinction in adolescent and adult animals. These findings also provide direction for clinical studies of novel treatments that modulate the innate immune system for stress-related disorders like PTSD.

Keywords

amygdala; medial prefrontal cortex; angiotensin; extinction; lipopolysaccharide

Correspondence should be addressed to: James T. Porter, Ph.D., Department of Basic Sciences, Ponce Health Sciences University-School of Medicine/Ponce Research Institute, PO Box 7004 Ponce, PR 00732-7004, Tel: (787) 844-3620, Fax: (787) 844-1980, jporter@psm.edu.

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a severe stress disorder that can develop after experiencing significant physical and/or psychological trauma (Jakovljević et al., 2012). Current treatments for PTSD produce inconsistent results (Asnis et al., 2004; Cohen et al., 2011; Rosen et al., 2013). Therefore, the search for novel and effective pharmacological treatment is of major interest. Patients with PTSD show lower levels of peripheral cortisol (Bierer et al., 2006), increased glucocorticoid sensitivity (Rohleder et al., 2004), and altered diurnal rhythms of cortisol levels (de Kloet et al., 2006; Yehuda et al., 2005) leading to a clinical pattern of low HPA axis activity.

By activating glucocorticoid receptors in immune cells, cortisol modulates gene transcription to decrease inflammation (Adcock et al., 2004). Since cortisol has anti-inflammatory effects, the reduced cortisol levels in PTSD could lead to a relatively increased inflammatory state in these patients. Consistent with this possibility, previous studies found that patients with PTSD also have increased expression of the pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α (Gill et al., 2008; Guo et al., 2012; Lindqvist et al., 2014; Rohleder et al., 2004; Sánchez-Lemus et al., 2009; van Zuiden et al., 2012). It has been hypothesized that increased inflammatory cytokines are involved in the pathogenesis of PTSD symptoms such as re-experiencing the traumatic event (Jones and Thomsen, 2013). In support of this hypothesis, the levels of IL-6 and TNF- α positively correlate with the severity of PTSD symptoms (Gola et al., 2013). However, it is not clear whether an inflammatory state leads to PTSD or whether PTSD causes an inflammatory state.

One possibility is that the inflammatory state contributes to the impaired fear extinction seen in patients with PTSD (Milad et al., 2009; Schelling et al., 2006). In this paper, we directly tested whether activating the innate immune response with the endotoxin lipopolysaccharide (LPS) affects acquisition or recall of auditory or contextual fear extinction in rats. LPS induces macrophages to produce inflammatory mediators, including TNF- α , IL-1 β , and IL-6 (Dantzer et al., 2008; Fujihara et al., 2003). After peripheral injection of LPS, increases in these inflammatory cytokines occur in fear-related brain structures such as the amygdala (Prager et al., 2013), the prefrontal cortex (PFC) (Yang et al., 2013), and the hippocampus (Kranjac et al., 2012).

The renin-angiotensin pathway promotes inflammation (Phillips and Kagiya, 2002) through angiotensin II stimulation of the angiotensin II type 1 (AT₁) receptor (Nishimura et al., 2000; Savoia and Schiffrin, 2007). A recent study showed that the AT₁ receptor blocker (ARB) candesartan reduced the acute brain inflammation induced by LPS (Benicky et al., 2011). In the present study, we examined whether activation of the innate immune system with LPS affects fear extinction and whether candesartan could reverse the effects. We found that intraperitoneal injection of LPS impairs auditory fear extinction in adolescent and adult male rats and that sub-chronic treatment with candesartan prevents the LPS-induced impairment.

MATERIALS AND METHODS

Subjects

The procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Ponce Health Sciences University in compliance with NIH guidelines for the care and use of laboratory animals. Male Sprague-Dawley rats (28–30 days or 60–70 days postnatal) were transported from the Ponce Health Sciences University colony to a satellite facility nearby where they were housed in transparent polyethylene cages inside a negative-pressure Biobubble (Colorado Clean Room, Ft. Collins, CO). Rats were maintained on a 12/12 hr light/dark schedule with free access to food (standard laboratory rat chow) and water.

Behavioral apparatus

Rats were fear conditioned in a chamber of 25 × 29 × 28 cm (Context A) with aluminum and Plexiglas walls (Coulbourn Inst., Allentown, PA). The floor consisted of stainless steel bars that could be electrified to deliver a mild shock and illumination was provided by a single overhead light. Rats were extinguished and tested in a hexagonal chamber (Context B). A citric scent filled the chamber and the illumination color was changed to accentuate a different context. The chambers had speakers mounted on the outside wall and were situated inside a sound-attenuating box (Med Associates, Burlington, VT) with a ventilating fan, which produced an ambient noise level of 60 dB. The conditioned stimulus (CS) was a 4 kHz tone with duration of 10 seconds and an intensity of 80 dB. The unconditioned stimulus (US) was a 0.44 mA scrambled footshock, 0.5 sec in duration, which co-terminated with the tone during the conditioning phase. Between sessions, floor trays and shock bars were cleaned with soapy water and the chamber walls were wiped with a damp cloth. Behavior was recorded with digital video cameras (Micro Video Products, Ontario, Canada).

Fear conditioning and extinction and drugs

A Pavlovian fear conditioning paradigm was used to model PTSD behavior in rats. For auditory fear conditioning, 30 day-old adolescent rats received 3 tone-shock pairings in context A (Conditioning phase) on day 1. After matching for equivalent levels of freezing, conditioned rats were divided into a saline group and a LPS group. On day 2, rats received an intraperitoneal (i.p.) injection of LPS (*Escherichia coli* serotype 055:B5; Sigma- Aldrich, St Louis, MO; 50 or 100 µg/kg) or saline 1.5 or 3 hours prior to fear extinction. Fear extinction training consisted of 30 tone-alone trials of 10 seconds each in a novel chamber designated as context B. Twenty-four hours later, all rats were tested for fear expression with a 2 minute tone-alone presentation in context B.

To test the effect of LPS on contextual fear extinction, adolescent rats received 3 tone-shock pairings (conditioning phase) on day 1. The following day they received an i.p. injection of LPS (100 µg/kg) or saline 3 hours prior to fear extinction. During fear extinction training rats were exposed to the original conditioning chamber for 5 min without tones. On day 3 all rats were exposed to the same context again for 5 min to measure recall of fear extinction.

To avoid the locomotor effects of the LPS injections from interfering with quantification of fear, the above protocols were modified in the experiments done with young adult P60 rats. On day 1, the P60 rats received auditory-cue fear conditioning as described above. On day 2, the P60 rats received an i.p. injection of LPS (250 µg/kg) or saline. This dose has been previously shown to impair memory in adult rodents even when tested 24 hrs later (Haba et al., 2012). The P60 rats underwent extinction training as described above 15 hrs after the LPS or saline injection and were tested for fear expression 24 hrs later.

Two different groups of rats received the same fear conditioning on day 1. On day 2, rats received an i.p. injection of LPS (250 µg/kg) or saline. On day 3, 15 hrs after the LPS/Saline injection, rats were exposed to the same conditioning context for 5 min to induce contextual fear extinction. On day 4, rats were re-exposed to the same context again for 5 min to test for extinction memory.

To evaluate whether an ARB could modulate the effects of LPS on extinction memory, adult rats were randomly assigned to either the candesartan or vehicle groups. On day 1, the rats received the designated daily i.p. injection of vehicle or the AT₁ receptor antagonist candesartan (Tocris, Bristol, UK) at a dose of 1 mg/kg in 99.9% dimethyl sulfoxide and saline. On day 2, rats received auditory fear conditioning 2 hours after their daily injection of candesartan or vehicle. On day 3, rats were treated with candesartan or vehicle and given an i.p. injection of LPS 250 µg/kg two hours later. 15 hours later on day 4, rats underwent extinction training in context B two hours after receiving their daily injection of vehicle or candesartan. Extinction memory recall was tested on the fifth day in context B. To evaluate whether candesartan could modulate extinction in the absence of inflammation, the protocol was repeated and the rats received an injection of saline instead of LPS on day 3.

Open field

Ten minutes before rats began extinction training, they were put in an open field test for 5 min to evaluate LPS effects on locomotion (velocity) and anxiety (time in center). Movement was measured by photosensors and analyzed using a computerized video tracking system (Ethovision XT 8.5, Noldus Information Technology, Wageningen, Netherlands).

Statistical analysis

The percent of time spent freezing (Blanchard and Blanchard, 1972) was used as a measure of conditioned fear. Freezing is the cessation of all movements except respiration. The total time spent freezing during the 10 sec tone was analyzed using commercial software (FreezeScan; Clever Systems). Student's *t*-test and repeated measures ANOVA (STATISTICA, Statsoft, Tulsa, OK) were used to analyze the behavioral data. Following a significant main effect, post-hoc tests were performed with Tukey tests. Values are reported as the mean ± the standard error of the mean (S.E.M.).

RESULTS

To begin to understand the effects of inflammation on fear extinction, we first determined whether activation of the innate immune response by peripheral administration of LPS

affects auditory fear extinction. Since fear extinction memory in adolescent rats is less robust than in adult rats (McCallum et al., 2010; Pattwell et al., 2012), extinction during adolescence could be particularly sensitive to the effects of inflammation. Therefore, the first sets of experiments were performed with adolescent rats. On day 1, rats received 3 tone-shock pairings and were separated into two groups matched for similar freezing to the third conditioning tone (LPS 76% freezing, Saline 78% freezing, $t(48) = 0.34$, $p = 0.74$). The following day, rats received an i.p. injection of saline or LPS (100 $\mu\text{g/kg}$) 1.5 or 3 hours prior extinction training (30 tones), since increases in inflammatory cytokines are first seen in the amygdala two hours after i.p. injection of LPS (Engler et al., 2011). LPS-injected rats showed impaired acquisition of fear extinction (Figure 1A). A repeated-measures ANOVA (Trial $\times$ Drug) across the extinction phase on day 2 revealed a main effect of drug ($F_{(1,48)} = 4.34$, $p = 0.04$) and a significant Trial $\times$ Drug interaction ($F_{(29,1392)} = 1.79$, $p = 0.01$). *Post-hoc* comparisons indicated that LPS-injected rats froze significantly more than saline-injected rats during trial 4 ($p = 0.04$), trials 19 to 22 ($p < 0.02$), and from trials 25 to 28 ($p < 0.04$) of extinction suggesting that LPS impaired acquisition of extinction. As shown in Figure 1B, the LPS-injected group also showed more freezing behavior during the recall of extinction on day 3 ($t(48) = -2.43$, $p = 0.02$) suggesting that LPS impaired extinction memory.

LPS can induce sickness behavior including reduced locomotion (Cunningham and Sanderson, 2008) which could confound our analysis of fear. Therefore, we also assessed the effects of LPS on locomotion and anxiety-like behavior in an open field test 10 mins before extinction training on day 2. As indicated in Figure 1C, the LPS-injected group moved slower than their saline-injected counterparts (Saline 12 ± 0.7 , LPS 8 ± 0.7 , $t(41) = 3.57$, $p = 0.001$) indicating that the increase in freezing during extinction in the LPS-injected rats could be due to reduced locomotion rather than fear of the tone. In contrast, both groups spent an equal amount of time exploring the center of the open field ($t(41) = 0.11$, $p = 0.91$) indicating that LPS did not affect the anxiety levels (Figure 1C). Since we also saw more freezing in the LPS-injected animals during extinction recall, we tested whether LPS affects locomotion or anxiety 24 hrs later. As shown in Figure 1D, 100 $\mu\text{g/kg}$ LPS did not affect locomotion ($t(10) = -0.45$, $p = 0.66$) or time spent in the center of the open field ($t(10) = 0.69$, $p = 0.50$) 24 hrs later. Thus the increased freezing of the LPS-injected animals during extinction recall was not due to lingering effects on locomotion.

To further assess whether the effects on locomotion and extinction could be dissociated, we used a lower dose of LPS (Figure 2). As in the previous experiment, on day 1, rats received 3 tone-shock pairings and were separated into two groups matched for similar freezing to the third conditioning tone (Figure 2A; LPS 77% freezing, Saline 80% freezing, $t(13) = 0.34$, $p = 0.74$). The following day, rats were injected with saline or LPS (50 $\mu\text{g/kg}$) 3 hours prior extinction training. A repeated-measures ANOVA across the extinction phase on day 2 discerned no main effect between groups ($F_{(1,15)} = 0.01$, $p = 0.93$) and no interaction between trial and drug ($F_{(29,435)} = 0.89$, $p = 0.64$). Also, both groups showed similar freezing during extinction recall on day 3 (Figure 2B; $t(13) = -0.68$, $p = 0.51$). In addition, we examined the effect of this dose of LPS on locomotion 10 mins prior to extinction training on day 2 (Figure 2C). Although 50 $\mu\text{g/kg}$ LPS did not affect fear extinction, this dose did reduce

locomotion (Saline 12.4 ± 0.9 , LPS 9.0 ± 0.6 , $t(12) = 3.07$, $p = 0.01$) indicating that the effects of LPS on fear extinction and locomotion are dissociable. Furthermore, the 50 and 100 $\mu\text{g/kg}$ doses of LPS produced similar effects on locomotion (LPS 50, 9.0 cm/s; LPS 100, 8.4 cm/s; $t(25) = 0.45$, $p = 0.66$) indicating that the 100 $\mu\text{g/kg}$ dose did not further impair locomotion. As with the 100 $\mu\text{g/kg}$ dose, 50 $\mu\text{g/kg}$ LPS did not affect anxiety-like behavior (Figure 2C, $t(12) = 1.64$, $p = 0.13$).

To test whether LPS impairs consolidation of auditory fear extinction memory, rats were injected with either LPS 100 $\mu\text{g/kg}$ or saline immediately after extinction training on day 2 (Figure 3). On the first day rats received fear conditioning and were separated into two groups matched for similar freezing to the third conditioning tone. Rats showed no significant difference in fear acquisition between groups (Figure 3A; LPS 73% freezing, Saline 74% freezing, $t(22) = 0.08$, $p = 0.94$). On day 2 rats underwent extinction training and were immediately injected with LPS 100 $\mu\text{g/kg}$ or saline at the end of the training session. A repeated-measures ANOVA across the extinction phase on day 2 revealed no significant main effect of group ($F_{(1,22)} = 0.04$, $p = 0.85$) and no interaction between trial and group ($F_{(29,638)} = 0.84$, $p = 0.71$) showing that both groups acquired fear extinction. As shown in Figure 3B, both groups showed similar recall of extinction on day 3 suggesting that LPS does not disrupt consolidation of fear extinction ($t(22) = 0.27$, $p = 0.79$).

Since our experiments up to this point were done with adolescent rats and adolescent rats show less robust fear extinction (Kim et al., 2011), we next tested whether LPS also disrupts fear extinction in adult rats. To enhance the dissociation between LPS effects on locomotion and extinction, a different behavioral protocol was employed with a higher dose of LPS. We chose this protocol based on data showing that 250 $\mu\text{g/kg}$ LPS produces behavioral effects without affecting locomotion between 10 and 24 hrs after LPS injection in mice (Haba et al., 2012). On the first day adult rats received cued fear conditioning and were separated into two groups matched for similar freezing to the third conditioning tone (Figure 4A; LPS 82% freezing, Saline 84% freezing, $t(32) = 0.42$, $p = 0.68$). The following day, rats were injected with saline or LPS (250 $\mu\text{g/kg}$) 15 hours prior to extinction training. As shown in Figure 4A, LPS-injected rats showed impaired acquisition of fear extinction. A repeated-measures ANOVA (Trial $\times$ Drug) across the extinction phase on day 3 revealed a main group difference ($F_{(1,34)} = 6.63$, $p = 0.01$). *Post-hoc* comparisons indicated that LPS-injected rats froze significantly more than saline-injected rats during trials 14–15, 17–21, 23, 25, 27 and 30 ($p < 0.04$) suggesting that LPS impaired acquisition of extinction. As shown in Figure 4B, the LPS-injected group also showed more freezing behavior during the recall of extinction on day 4 ($t(32) = -2.57$, $p = 0.02$) indicating that extinction memory was impaired. This dose of LPS did not affect locomotion ($t(32) = 1.13$, $p = 0.27$) or time spent in the center of the open field ($t(32) = -0.31$, $p = 0.76$) measured 10 min prior to extinction training on day 3 (Figure 4C).

Taken together our results suggest that LPS disrupts acquisition of cued fear extinction. Next we tested whether peripheral administration of LPS also disrupts hippocampal-based contextual fear extinction in adolescent rats. On day 1 adolescent rats received fear conditioning with 3 tone-shock pairings and were separated into two groups matched for similar freezing to the third conditioning tone (Figure 5A; LPS 69% freezing, Saline 64%

freezing, $t(34) = -0.32$, $p = 0.76$). On day 2, the groups received an i.p. injection of 100 $\mu\text{g/kg}$ LPS or saline and were placed in the original conditioning context for 5 minutes for contextual fear extinction. A repeated measures ANOVA across the extinction phase revealed no significant main effect of drug ($F_{(1,34)} = 2.48$, $p = 0.13$). The next day, both groups showed similar recall of contextual fear extinction (Figure 5B; $t(34) = 0.62$, $p = 0.54$).

To test whether LPS affects contextual fear extinction in adult rats, P60 rats received fear conditioning with 3 tone-shock pairings and were separated into two groups matched for similar freezing to the third conditioning tone (Figure 5C; LPS 57% freezing, Saline 58% freezing, $t(10) = -0.02$, $p = 0.49$). The following day, rats were injected with saline or LPS (250 $\mu\text{g/kg}$). On day 3, 15 hours later, rats were exposed to the original conditioning context for 5 minutes to induce contextual fear extinction. A repeated measures ANOVA across the extinction phase revealed no main effect of drug ($F_{(29,986)} = 1.1806$, $p = 0.24$). The next day, both groups also showed similar recall of contextual fear extinction (Figure 5D; $t(10) = 1.76$, $p = 0.11$).

Since the ARB candesartan reduces LPS-induced pro-inflammatory cytokine expression in various brain structures (Benicky et al., 2011), we tested whether sub-chronic treatment with candesartan (4 daily i.p. injections; 1 mg/kg in 50% dimethyl sulfoxide and saline) would prevent the observed impairment on extinction learning and memory. On the first day, rats received candesartan (1 mg/kg) treatment or vehicle (Figure 6A). On day 2 rats received auditory fear conditioning 2 hours after treatment with candesartan or vehicle (Figure 6A; vehicle-LPS 85% freezing, candesartan-LPS 88% freezing, $t(35) = -0.62$, $p = 0.54$). The following day, rats received an injection of candesartan or vehicle and 2 hours later were injected with saline or LPS (250 $\mu\text{g/kg}$). 15 hours later on day 4, rats underwent extinction training in context B two hours after receiving their daily injection of vehicle or candesartan. Extinction memory recall was tested on the fifth day in context B. As shown in Figure 6A, rats in the Vehicle-LPS group showed impaired acquisition of fear extinction. *Post-hoc* comparisons indicated that rats in the Vehicle-LPS group froze significantly more than rats treated with candesartan during trials 28–30 ($p < 0.05$) suggesting that candesartan prevents the LPS-induced impaired acquisition of extinction. The candesartan-LPS group also showed less freezing behavior during extinction recall on day 5 ($t(35) = 2.64$, $p = 0.01$) compared to rats in the vehicle-LPS group (Figure 6B).

Next we tested whether candesartan could enhance extinction training and recall in the absence of inflammation. On day 1, rats received candesartan treatment or vehicle (Figure 6C). The following day rats received cued conditioning 2 hours after treatment with candesartan and were then separated into two groups matched for similar freezing to the third conditioning tone (Figure 6C; vehicle-saline 81% freezing, candesartan-saline 84% freezing, $t(33) = -0.51$, $p > 0.05$). On day 3, rats received candesartan treatment or vehicle and 2 hours later were injected with saline 15 hours prior to extinction training. No significant differences were found between groups during acquisition of fear extinction on day 4 (Figure 9C, $F_{(1,29)} = 1.47$, $p > 0.05$). Also, no differences were found between both groups during the recall of extinction on day 5 (Figure 6D, $t(33) = 0.52$, $p = 0.61$).

DISCUSSION

Accumulating evidence suggests that inflammation plays a role in PTSD (Lindqvist et al. 2014; Pace and Heim, 2011; Pace et al., 2012; Smith et al., 2011; von Känel et al., 2007; Zieker et al., 2007). In the present study, we found that activating peripheral inflammation with LPS impairs acquisition and recall of auditory fear extinction in adolescent and adult rats. The lack of effect of LPS given post-extinction suggests that LPS disrupts acquisition rather than consolidation of the auditory fear extinction memory. LPS did not affect contextual extinction, suggesting the observed impairment in auditory fear extinction was not related to disruption of hippocampal function. Of clinical interest, a four day treatment with candesartan was able to prevent LPS from impairing fear extinction learning and memory.

Previous studies found that LPS disrupts fear memory consolidation (Gonzalez et al., 2013; Kranjac et al., 2012; Pugh et al., 1998). Our findings suggest that activation of the immune system impairs acquisition of fear extinction rather than consolidation of extinction memory. Our results showed that LPS-treated rats froze significantly more across several extinction training trials and showed impaired recall of extinction. Additionally, post-extinction training administration of LPS did not affect consolidation of extinction memory. This is consistent with a previous study showing that LPS impaired training and probe sessions in the Morris water maze task (Choi et al., 2012).

Although LPS induced sickness behavior which reduced locomotor activity, LPS impaired fear extinction independent of its effects on locomotion. First, locomotor activity was reduced by a dose of LPS that did not disrupt fear extinction training or recall indicating that the effects on locomotion and fear extinction can be dissociated. In addition, extinction learning was impaired 15 hours after administration of LPS when locomotion was not affected. Furthermore, LPS impaired extinction recall 24 hours later when LPS' effect on locomotion had dissipated.

Although LPS disrupts consolidation of hippocampal-dependent memory tasks such as the water maze, inhibitory avoidance, and contextual fear conditioning (Pugh et al., 1998; Wu et al., 2007; Yin et al., 2013), our findings suggest that innate immune activation impairs acquisition of auditory fear extinction through a mechanism that does not involve disruption of the hippocampus. PTSD has been associated with increased levels of pro-inflammatory cytokine such as TNF- α , IL-1 β and IL-6 in the blood (Andrews and Neises, 2012). Injection of LPS increases TNF- α , IL-1 β and IL-6 in critically relevant fear-related structures such as the amygdala and the medial prefrontal cortex (mPFC) (Benicky et al., 2011). It is possible that TNF- α and IL-1 β impair extinction learning by increasing activation of the central amygdala, since this structure increases conditioned fear (LeDoux et al., 1988; McNally et al., 2011) and shows heightened activity 24 hours after LPS injection (Haba et al., 2012).

Another potential mechanism mediating the impaired extinction induced by LPS is reduced activity in the infralimbic (IL) portion of the mPFC, which has been implicated in extinction learning and recall (Godsil et al., 2013; Quirk and Mueller, 2008; Sierra-Mercado et al., 2011). IL inhibits conditioned fear responses through projections onto inhibitory neurons in

the amygdala which leads to reduced activity in the central amygdala and less fear expression (Amano et al., 2010; Milad and Quirk, 2012; Orsini and Maren, 2012). Therefore, increased cytokine expression could disrupt fear extinction by increasing activity in the amygdala or decreasing activity in IL.

One of the most important findings in our study was that treatment with the AT₁ receptor antagonist candesartan prevented the LPS-induced impairment of acquisition and recall of extinction memory. Since ARBs modulate LPS-induced inflammation by blocking AT₁ receptors and inhibiting NF- κ B activity (Miyoshi et al., 2008), candesartan could be enhancing acquisition of extinction through reduced cytokine-induced NF- κ B activity in fear extinction related structures. Since ARBs also exhibit partial peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonistic effects and cognitive enhancement beyond blockade of AT₁ receptors (Kurtz and Klein, 2009; Min et al., 2012; Schupp et al., 2004; Tsukuda et al., 2009), candesartan could also enhance fear extinction through activation of the PPAR- γ pathway. Regardless of the mechanism, ARBs appear to be particularly useful for enhancing extinction of aversive memories and may explain why trauma-exposed patients taking ARBs had lower risk of PTSD symptoms (Khoury et al., 2012).

A previous study showed that chronic treatment with the ARB losartan enhanced extinction training and consolidation of extinction memory (Marvar et al., 2014). In the present study, extinction training and recall were not enhanced by treatment with candesartan alone. We used 1 mg/kg of candesartan because this dose reduces brain inflammation induced by LPS (Benicky et al., 2011); however, it is possible that a larger dose could facilitate extinction. Further experimentation is required to determine whether the dose of candesartan or other experimental differences account for the discrepancy.

In this study we did not directly measure cytokine levels or NF- κ B activity; however, the same dose of candesartan was previously shown to reduce LPS-induced cytokine expression in numerous brain structure (Benicky et al., 2011). Never-the-less, it remains unclear whether pro-inflammatory cytokines, NF- κ B activity, or other mechanisms are responsible for the impaired fear extinction induced by LPS.

Although freezing behavior in rats is not a perfect model of PTSD, our study raises the possibility that a pro-inflammatory state after a traumatic event could contribute to the impaired fear extinction observed in patients with PTSD (Milad et al., 2009). Future studies should elucidate the molecular mechanisms through which inflammatory changes affect extinction learning and memory. Our results suggest that the inflammatory status of the patient may affect the efficacy of integrative treatments that include behavioral interventions such as exposure therapy. These results provide direction for clinical studies of novel treatments that modulate the immune system for stress-related disorders, such as PTSD.

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We examined the effects of innate immune activation with lipopolysaccharide on fear extinction.

Activation of the innate immune system impaired auditory fear extinction in adolescent and adult animals.

A sub-chronic treatment with candesartan, an angiotensin II type 1 receptor blocker, prevented the LPS-induced impairment of extinction.

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Figure 1. An injection of LPS (100 ug/kg) before fear extinction disrupts extinction recall
 A. On day 1, rats (P30) were fear conditioned with three tone-shock pairings in context A and separated in two different groups (saline, $n = 24$; LPS, $n = 20$). On day 2, rats were given extinction training in a novel context B. Arrow indicates injection of LPS or saline 1.5 or 3 hrs before extinction. B. Freezing response to a test tone in context B on day 3. C. An open field test given just before extinction showed that LPS reduced locomotion but did not affect anxiety. D. An open field test performed 24 h after extinction training showed no significant effects on locomotion or anxiety (saline, $n = 12$; LPS, $n = 12$). Data represent means $\pm$ SEM. * $p < 0.05$

Figure 2. LPS (50 ug/kg) injected before fear extinction does not disrupt extinction recall
A. Lower dose of LPS (50 ug/kg) did not significantly affect acquisition or (B) recall of fear extinction. Arrow indicates injection of LPS or saline 3 hrs before extinction. C. This dose of LPS reduced locomotion but did not affect anxiety. Data represent means $\pm$ SEM (saline, n = 18; LPS, n = 18). *p < 0.05

Figure 3. LPS (100 ug/kg) injected after extinction does not affect extinction recall

A. Behavior of groups before injecting LPS after extinction training on day 2 (arrow). B. Post-extinction injection did not affect extinction recall on day 3. Data represent means $\pm$ SEM (saline, n = 12; LPS, n = 12).

Figure 4. An injection of LPS (250 ug/kg) 15 hrs prior extinction disrupts fear extinction recall
A. On day 1, rats (P60) were fear conditioned with three tone-shock pairings in context A and separated in two different groups (saline, n = 18; LPS, n = 18). On day 2, rats received an LPS injection (arrow). 15 hrs later on day 3, rats were given extinction training in a novel context B. B. On day 4, the LPS-injected group froze more in response to a test tone in context B. C. Open field test performed before extinction training on day 3 showed no effect of LPS on locomotion or anxiety. Data represent means $\pm$ SEM. * $p < 0.05$

Figure 5. LPS does not disrupt contextual fear extinction

A. LPS (100 ug/kg) injection 3 hrs prior to contextual fear extinction on day 2 (arrow) did not affect recall of contextual fear extinction in adolescent rats (B) (saline, $n = 18$; LPS, $n = 18$). C. LPS (250 ug/kg) injection 15 hrs prior contextual fear extinction on day 2 (arrow) did not affect recall in adult rats (D). Data represent means $\pm$ SEM (saline, $n = 6$; LPS, $n = 6$).

Figure 6. Candesartan prevents LPS-induced impairment of fear extinction

A. Pretreatment of adult rats with candesartan (blue arrows) prevented the impairment in acquisition of extinction caused by injection of LPS (green arrow; 250 ug/kg). B. Candesartan also enhanced recall of fear extinction (vehicle-LPS, n = 23; candesartan-LPS, n = 14). C–D. Candesartan by itself did not enhance extinction training nor recall of extinction (vehicle-saline, n = 19; candesartan-saline, n = 16). Data represent means $\pm$ SEM. *p < 0.05