Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 5 (2016), Article ID 235950, 10 pages
doi:10.4303/jdar/235950

Research Article

Endotoxin Enhancement of Morphine-Induced Conditioned Place Preference

Sulie L. Chang,[1,3] Sraboni Sarkar,[1] Michael Vigorito,[1,2] and Xin Mao[1]

[1] *Institute of Neuroimmune Pharmacology, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, USA*
[2] *Department of Psychology, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, USA*
[3] *Department of Biological Sciences, Seton Hall University, 400 South Orange Avenue, South Orange, NJ 07079, USA*
Address correspondence to Sulie L. Chang, sulie.chang@shu.edu

Received 1 October 2015; Revised 15 November 2015; Accepted 16 November 2015

Abstract Morphine, used clinically for pain management for its potent analgesic effects, has a high abuse potential. In this study, we utilized the conditioned place preference (CPP) paradigm and tail-flick latency to examine the effects of the endotoxin lipopolysaccharide (LPS) on sensitivity to morphine. Rats were pretreated with either LPS (250 μg/kg) or saline, followed by either a higher dose of LPS (2 mg/kg) or saline. Rats were then given either morphine (3.5 mg/kg) or saline every other day for 6 d starting 72 h after the last dose of LPS or saline as part of the conditioning phase. Drug preference was assessed during the postconditioning phase. LPS-treated rats displayed a stronger and prolonged morphine preference during the 20 d of extinction testing. A parallel analysis revealed that tail-flick latencies were significantly longer in LPS-treated rats. Together, our data indicate that systemic infections can increase sensitivity to morphine both physiologically and behaviorally.

Keywords systemic infection; LPS; morphine

1. Introduction

Morphine's potent analgesic effects make it optimal for use in clinical pain management. However, it's euphoric neurochemical effects cause a high potential for abuse and addiction [1]. In addition to its addictive properties, chronic morphine exposure can lead to immunosuppression, thereby increasing the susceptibility to bacterial infection [2,3,4].

Systemic infection can affect behavior, leading to cognitive complications and depression [5,6]. Many studies have reported that prolonged neuroinflammation can also interfere with cognition and initiate depression [7,8,9, 10]. In addition, neuropsychological complications, such as the development of depressive disorders, can occur after exposure to proinflammatory cytokines and bacterial endotoxins in both humans and animals [11].

Lipopolysaccharide (LPS) is a bacterial endotoxin that is widely used in research to investigate pathogen-induced inflammatory responses [12]. In the rodent model, LPS induces sickness behavior, which is characterized by depression-like behavior such as loss of appetite, subdued motor activity, and decreased cognitive function [13,14]. Repeated exposure to an endotoxin can lead to endotoxin tolerance, where the balance between proinflammatory and anti-inflammatory cytokines is lost and the immune cells become unresponsive to an additional endotoxin challenge. This can induce immunosuppression, which can lead to septic shock because of the immune cells' inability to produce sepsis-protective proinflammatory cytokines [12].

Conditioned place preference (CPP) procedures are widely used in animal studies to assess the rewarding effects of substances with the potential for abuse [15]. Morphine can induce CPP in the rodent model, particularly in Fischer 344 (F344) rats used in our study [16,17,18].

We hypothesized that systemic exposure to bacterial infection may increase the animal's sensitivity to morphine and, thus, increase the potential for morphine abuse and dependence. In this study, we examined both physiological (pain sensitivity) and behavioral (CPP) parameters in F344 rats to evaluate their sensitivity to morphine following LPS treatment.

2. Materials and methods

2.1. Animals

Adult male Fischer 344 (F344) rats, 10 weeks old, were purchased from Harlan Laboratories (Indianapolis, IN, USA). The rats were group-housed in ventilated cages (4/cage) immediately upon arrival and remained in cages throughout the experiment. The rats were kept in a temperature and humidity controlled room (21–22 °C, 50–60% humidity) with a 12 h light-dark cycle. Food and water were provided ad libitum throughout the experiment. The rats were acclimatized for two weeks and handled for 1–2 min on three consecutive days before the beginning of testing. The animals were three months old when the experiments were conducted. All experiments were performed in the light phase (8 AM–2 PM) of the light-dark cycle. All

animals were treated according to national and institutional standards, and the experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at Seton Hall University, South Orange, NJ, USA

2.2. Tail-flick latency in response to morphine after LPS treatment

To evaluate the antinociceptive effects of morphine following treatment with LPS (2 mg/kg) or saline (control), tail-flick latency tests were performed on the adult F344 rats, and the tail-flick latency measurements were recorded. The rats were then injected subcutaneously (SC) with 0.3 mg/kg of morphine, and tail-flick latencies were again measured after 30 min. The dose of morphine injected was subsequently increased to 0.56, 1, 1.75, 3, 5.6, and 10 mg/kg, and tail-flick latencies were recorded 30 min after each injection until there was no further increase seen in tail-flick latency and the rats failed to show signs of pain, as reported previously [19, 20]. A dose-response curve was generated, and the ED-50 score was calculated. An ED-50 score denotes the morphine dosage at which 50% of the maximum possible antinociceptive effects are elicited [21].

2.3. Morphine-induced conditioned place preference (CPP)

2.3.1. Animal handling

The animals were handled for 1–2 min on three consecutive days (days 1–3) before the beginning of testing.

2.3.2. Body temperature and body weight

Body temperature was recorded using a digital thermometer (Fisher Scientific, Suwanee, GA, USA) on days 4–7. To obtain baseline temperatures, each rat was monitored the day before the test session. On the test day, temperatures were recorded immediately before ($T = 0$) and at 10 h and 24 h ($T = 10$ and $T = 24$, resp.) after injection of either LPS (day 5: 250 μg/kg or day 6: 2 mg/kg) or saline.

On test days, body weight (BW) was measured before ($T = 0$) and at 24 h ($T = 24$) after injection of either LPS (day 5: 250 μg/kg or day 6: 2 mg/kg) or saline.

2.3.3. Drug administration

E. coli LPS (L6511, Sigma St. Louis, MO, USA) was dissolved in sterile saline (0.9% NaCl) and injected SC. Morphine sulfate (Sigma, St. Louis, MO, USA) was administered at a dosage of 3.5 mg/kg. Control animals were injected with saline only.

F344 rats ($n = 5$–8 per group, 12 weeks old) were randomly assigned to the following groups as per their treatment protocol.

Control group

Group 1 (SS+S)+S. Two injections of saline (SS) were given on day 5, followed by one injection of saline (S) on day 6. From days 9–14 (conditioning phase), saline (S) injections were given every day.

Experimental groups

Group 2 (SS+S)+M. Two injections of saline (SS) were given on day 5, followed by one injection of saline (S) on day 6. From days 9–14 (conditioning phase), morphine (M) injections (3.5 mg/kg) were given every other day, alternating with saline.

Group 3 (SS+L)+M. Two injections of saline (SS) were given on day 5, followed by one LPS (L) injection (2 mg/kg) on day 6. From days 9–14 (conditioning phase), morphine (M) injections (3.5 mg/kg) were given every other day, alternating with saline.

Group 4 (LL+L)+M. Two LPS (LL) injections (250 μg/kg) were given on day 5, followed by one LPS (L) injection (2 mg/kg) on day 6. From days 9–14 (conditioning phase), morphine (M) injections (3.5 mg/kg) were given every other day, alternating with saline.

2.3.4. CPP and extinction procedures

The CPP apparatus (MED Associates, East Fairfield, VT, USA) consists of an 18 cm $\times$ 20 cm white chamber with a grid floor and an 18 cm $\times$ 20 cm black chamber with a rail floor. The apparatus has guillotine doors in the middle of the two chambers that can be closed to isolate each chamber. Animal movement and time spent in each chamber was recorded by an infrared camera, and ANY-maze software (Stoelting ANY-maze, Wood Dale, IL, USA) was used to analyze the video footage and measure the time spent in each chamber.

The experiment was divided into five phases (Table 1). During the preconditioning phase, the rats were placed in the center of the apparatus with the guillotine doors open to allow them to explore the entire box for 15 min. Preconditioning 1 (P1) was done on day 4 following handling, and preconditioning 2 (P2) was performed on day 8, one day before the start of the conditioning phase. The chamber preference during the 15 min P1 phase indicated that the CPP apparatus used in this study was biased in that the rats tended to prefer one chamber over the other. Thus, a biased procedure was used for the study, meaning that the morphine-paired chamber was determined based on the chamber in which each animal spent the least amount of time (the non-preferred chamber).

The conditioning phase lasted six days. During that phase, the rats were injected with either 3.5 mg/kg of morphine or the equivalent volume of saline every other day and placed in the preassigned morphine-paired chamber. On alternating days, the rats were injected with the equivalent volume of saline and placed in the saline-paired side, with the chamber door closed for 30 min.

Table 1: Time-line of the conditioned place preference (CPP) test.

Groups	**Handling**	**Preconditioning**	**Three-day treatment**			**Behavior analysis**				
Group 1	Handling	Preconditioning 1	Two injections	SS+S	NT	CPP			Extinction	
(SS+S+S)		BW, BT	(saline)	One injection	BW, BT	Preconditioning 2	**SS+S+S**	Postconditioning	Extinction 1	Extinction 2
			BW, BT	(saline)		Blood collection	Conditioning: saline, daily	Blood collection	Daily	Alternate day
				BW, BT						
Group 2	Handling	Preconditioning 1	Two injections	SS+S	NT		**CPP**			**Extinction**
(SS+S+M)		BW, BT	(saline)	One injection	BW, BT	Preconditioning 2	**SS+S+M**	Postconditioning	Extinction 1	Extinction 2
			BW, BT	(saline)		Blood collection	Conditioning: morphine or saline, alternate days	Blood collection	Daily	Alternate day
				BW, BT						
Group 3	Handling	Preconditioning 1	Two injections	SS+L	NT		**CPP**			**Extinction**
(SS+L+M)		BW, BT	(saline)	One injection	BW, BT	Preconditioning 2	**SS+L+M**	Postconditioning	Extinction 1	Extinction 2
			BW, BT	(LPS 2 mg/kg)		Blood collection	Conditioning: morphine or saline, alternate days	Blood collection	Daily	Alternate day
				BW, BT						
Group 4	Handling	Preconditioning 1	Two injections	LL+L	NT		**CPP**			**Extinction**
(LL+L+M)		BW, BT	(LPS 250 μg/kg)	One injection	BW, BT	Preconditioning 2	**LL+L+M**	Postconditioning	Extinction 1	Extinction 2
			BW, BT	(LPS 2 mg/kg)		Blood collection	Conditioning: morphine or saline, alternate days	Blood collection	Daily	Alternate day
				BW, BT						
Days	1–3	4	5–7			8–15			16–27	28–44

BT-body temperature; BW-body weight.

In the postconditioning phase (1 d), the rats received neither a drug nor saline. Each rat was placed in the center of the apparatus and allowed free access to the entire box for 15 min.

For the first 12 d of the extinction phase (extinction 1), the rats were put in the side chamber and given free access to the box for 30 min. After extinction 1 testing, there was no evidence of extinction, so the extinction procedure was altered to test every other day, which was continued from extinction day 13 through day 28 (extinction 2). Two weeks after extinction 2, another extinction test (extinction 3) was conducted.

2.4. Measurement of inflammatory cytokines

Tail vein blood was collected immediately after preconditioning (day 8) and postconditioning (day 16). Serum was isolated from the whole blood and stored at $-80\,^{\circ}$C. Protein levels of IL-1β, TNF-α, IL-4, IL-5, IL-13, KC/GRO, and IFN-γ in the serum were determined using a 96-well inflammatory cytokine kit [7-plex MSD assay kit, MesoScale Discovery (MSD), Gaithersburg, MD, USA]. Measurement of electrochemiluminescent signal intensity was determined on the SECTOR 2400 instrument (MesoScale Discovery, Gaithersburg, MD, USA).

2.5. Statistical analysis

Statistical analysis was performed using the Graphpad Prism 5.0. Differences between and within treatment groups were analyzed by a one- or two-way ANOVA, followed by a Newman-Keul's or Bonferroni's post hoc test. ANOVA was also used to study the interaction between phases (preconditioning, postconditioning, and extinctions 1 and 2) and drugs (morphine-paired, saline-paired). The statistical mean and standard deviation of the absolute time spent in each chamber were calculated in all phases of the experiment. A value of $P \leq .05$ was considered statistically significant.

3. Results

3.1. Effect of LPS on response to morphine in the tail-flick latency test

Tail-flick latency was measured to evaluate the antinociceptive effects of morphine on F344 rats treated with LPS (Figure 1). Tail-flick latencies were significantly greater ($^{*}P < .05$) in the rats treated with 2 mg/kg LPS in response to increased dosages of morphine (0.56, 1.0, 1.75, 3, and 5.6 mg/kg) compared to the saline-treated animals. The ED-50 for the LPS-treated rats was less (0.78 ± 0.09 mg/kg, $n = 5$) than that of the saline-treated animals (2.64 ± 0.29 mg/kg, $n = 5$).

3.2. Effect of LPS on body weight

Body weight of each rat was measured on days 4–10 (Figure 2, Table 2). Body weight was significantly reduced within 24 h after injection with LPS, both at dosages of 250 μg/kg and 2 mg/kg. Rats in the LL+L group lost 20.2 ± 5.1 g of body weight ($^{***}P < .001$, $n = 8$, Table 1) 24 h after pretreatment with 250 μg/kg LPS on day 5, and another 10.8 ± 5.3 g ($^{**}P < .01$, $n = 8$) 24 h after the 2 mg/kg LPS injection on day 6.

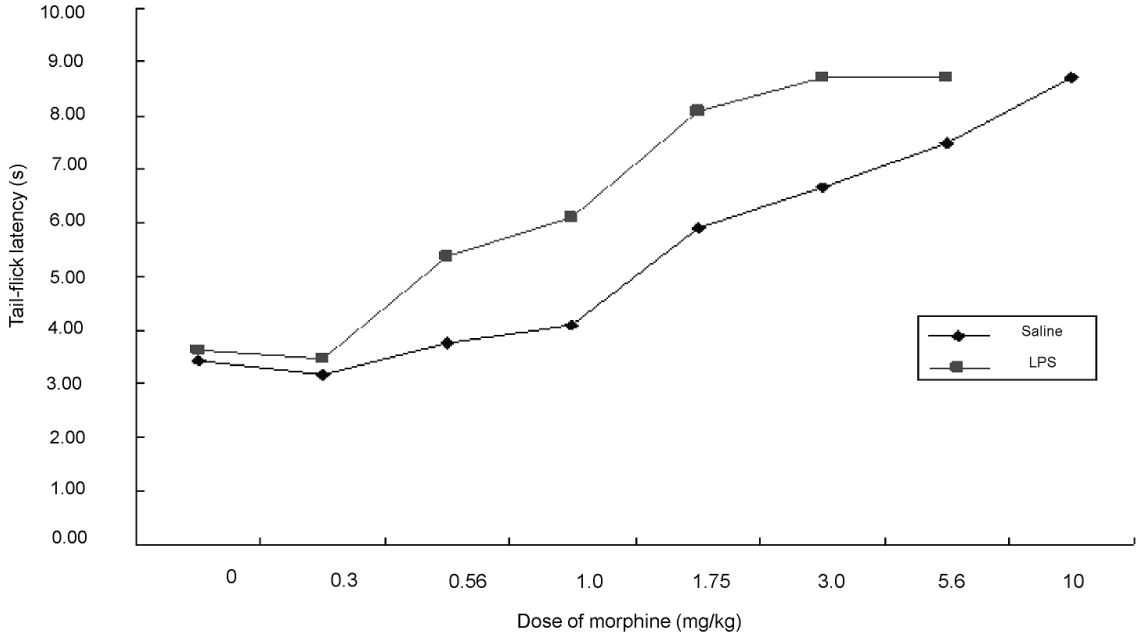

Figure 1: *Effect of LPS on response to morphine in the tail-flick latency test.* Tail-flick latencies (s) were determined in adult F344 rats treated with 0, 0.3, 0.56, 1.0, 1.75, 3.0, 5.6 or 10 mg/kg morphine, followed by either 2 mg/kg LPS ($n = 5$) or saline ($n = 5$); $^{*}P < .05$.

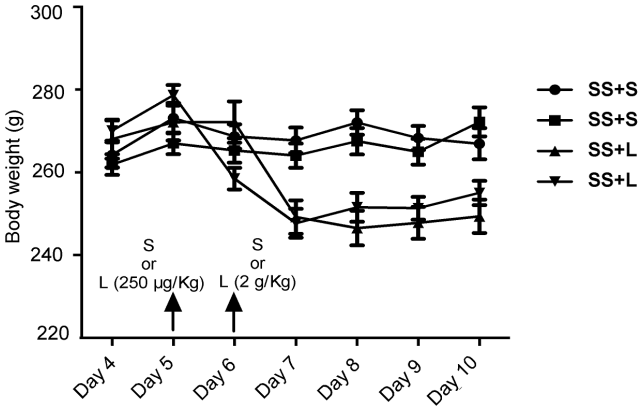

Figure 2: *Effect of LPS on body weight.* Adult F344 rats were randomly divided into four groups (SS+S+S; SS+S+M; SS+L+M; LL+L+M) and pretreated with either saline or nonpyrogenic LPS (250 μg/kg), followed by either saline or 2 mg/kg LPS during the three-day treatment. Body weight was measured before and after LPS administration. The data represent the mean $\pm$SEM, $n = 5$–8 rats per group; $^{*}P < .5$; $^{**}P < .01$; $^{***}P < .001$; $^{****}P < .0001$.

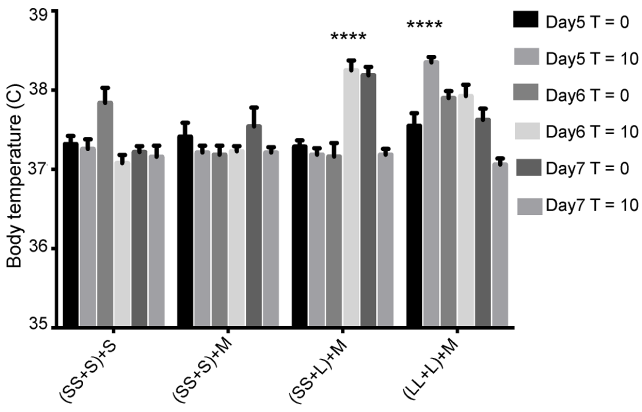

Figure 3: *Effect of LPS on body temperature.* Adult F344 rats were randomly divided into four groups (SS+S+S; SS+S+M; SS+L+M; LL+L+M) and pretreated with either saline or nonpyrogenic LPS (250 μg/kg), followed by either saline or 2 mg/kg LPS during the three-day treatment. Body temperature was measured before and after LPS administration. The data represent the mean $\pm$SEM, $n = 5$–8 rats per group; $^{****}P < .0001$.

Rats who were pretreated with saline (SS+L) lost 23.0 $\pm$ 7.8 g ($^{**}P < .005$, $n = 8$, Table 1) of body weight 24 h after the subsequent 2 mg/kg LPS injection on day 6, and another 2.7 $\pm$ 2.4 g on day 7. There was no significant reduction in body weight in the saline treated control rats (SS+S) (see Table 2).

3.3. Effect of LPS on body temperature

Body temperature was measured before ($T = 0$) and 10 h after ($T = 10$) LPS injection (day 5: 250 μg/kg or day 6: 2 mg/kg) on days 5–7 (Figure 3). The body temperatures of the rats in the LL+L group increased by an average of 0.8 °C ($^{****}P < .0001$, $n = 8$) 10 h after the pretreatment injection

Table 2: The effect of LPS on body weight (g).*

Group	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
SS+S	264.2 ± 6.9	268.7 ± 6.6	267.7 ± 6.9	272.06 ± 6.6	268.3 ± 6.4	266.9 ± 8.4	258.1 ± 10.6
SS+S	261.9 ± 6.6	265.3 ± 7.8	264.1 ± 7.7	267.5 ± 8.5	265.0 ± 8.2	272.2 ± 9.3	259.7 ± 8.1
SS+L	268.1 ± 13.4	272.2 ± 14.1	249.2 ± 11.5**	246.5 ± 11.8	247.8 ± 11.0	249.4 ± 11.4	246.5 ± 8.9
LL+L	270.1 ± 7.0	258.5 ± 7.4***	247.7 ± 9.8**	251.5 ± 9.9	251.3 ± 7.8	255.0 ± 8.3	251.2 ± 7.9

*Mean ± SEM ($n = 5$–8 rats per group). $^{**}P < .01$; $^{***}P < .001$.

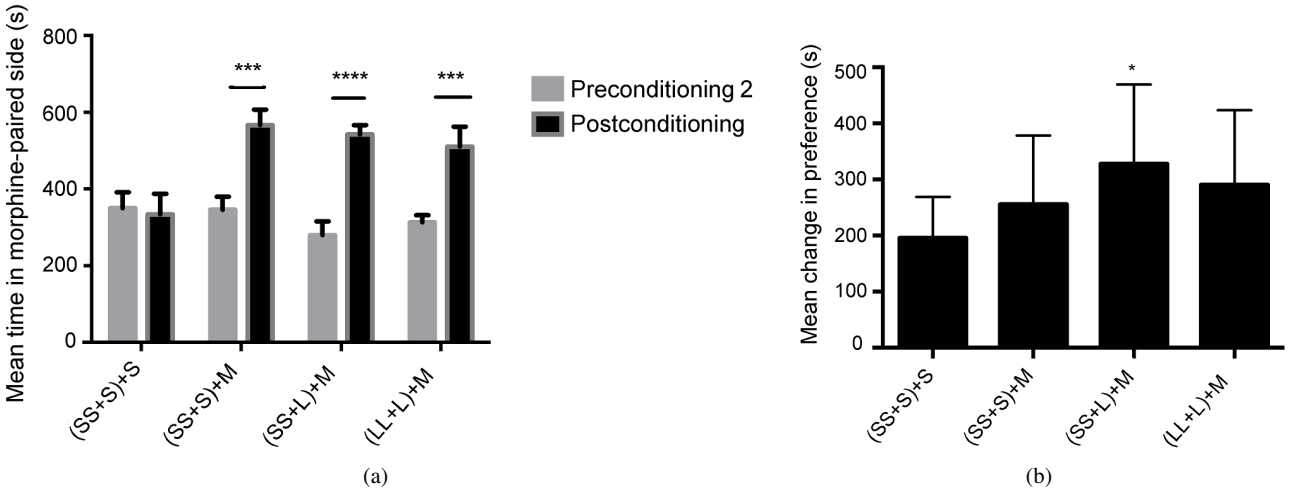

Figure 4: *Morphine preference during the CPP experiment.* Adult F344 rats were randomly divided into four groups (SS+S+S; SS+S+M; SS+L+M; LL+L+M) and pretreated with either saline or nonpyrogenic LPS (250 μg/kg), followed by either saline or 2 mg/kg LPS during the three-day treatment. Preconditioning 2 (P2) was performed 48 h after LPS treatment. During the conditioning phase, 72 h after LPS treatment, the animals were given either saline or 3.5 mg/kg morphine on alternate days. (a) Estimated total time (s) spent in the morphine-paired chamber during P2 compared to CPP. (b) Mean change in preference (s) from P2 to CPP. The data represent the mean ±SEM, $n = 5$–8 rats per group; $^{*}P < .5$; $^{***}P < .001$; $^{****}P < .0001$.

of LPS (250 μg/kg) on day 5. No significant change was observed after the 2 mg/kg LPS injection on day 6. However, the body temperatures of the rats in the SS+L group increased by an average of 1.09 °C ($^{****}P < .0001$, $n = 8$) 10 h after the 2 mg/kg LPS injection on day 6. By 48 h after the 2 mg/kg LPS injection, the body temperatures of the rats in both the SS+L and LL+L groups had returned to normal (37.4 °C).

3.4. Morphine-induced conditioned-place preference (CPP)

A biased CPP procedure was used for this study meaning that rats showed a preference for one chamber over the other in preconditioning testing. Morphine was paired with the nonpreferred side for the rats that showed a preference during preconditioning. One day following the end of six sessions of conditioning with 3.5 mg/kg morphine, CPP was established in the rats, with and without LPS treatment (Figure 4(a)). In the postconditioning phase, after CPP was achieved, all rats in the morphine treatment groups spent significantly more time in the morphine-paired chamber (SS+S+M: 566.1 s, $^{***}P < .001$, $n = 7$; SS+L+M: 542 s, $^{****}P < .0001$, $n = 8$; LL+L+M: 510 s, $^{***}P < .001$, $n = 8$) than in the saline-paired chamber compared to the preconditioning 2 (P2) phase (SS+S+M: 345.7 s, $n = 7$; SS+L+M: 275.2 s, $n = 8$; LL+L+M: 313.15 s, $n = 8$). There was no difference in the time spent in the morphine-paired chamber before (349.7 s) or after (333.4 s) CPP in the control group (SS+S+S, $n = 5$).

The change in preference for the rats that received LPS and morphine injections (Figure 4(b)) was greater (SS+L+M: 327.9 s, $n = 8$; LL+L+M: 290.7 s, $n = 8$) for the drug-paired chamber compared to the group that did not receive LPS treatment (SS+S+M: 255.5 s, $n = 7$). In particular, the group that received only the 2 mg/kg LPS injection (SS+L+M: 327.9 s, $^{*}P < .05$, $n = 8$) showed a greater change in preference compared to the saline-treated control (SS+S+S: 195.8 s, $n = 5$), and also compared to the endotoxin-tolerant (LL+L+M) group (290.7 s, $n = 8$).

The extinction phase, which lasted for more than 20 d, was longer for the rats in the morphine treatment groups. In extinction 1 (Figures 5(a), 5(c)), the total time spent (12 d) in the drug-paired chamber was the greatest for the

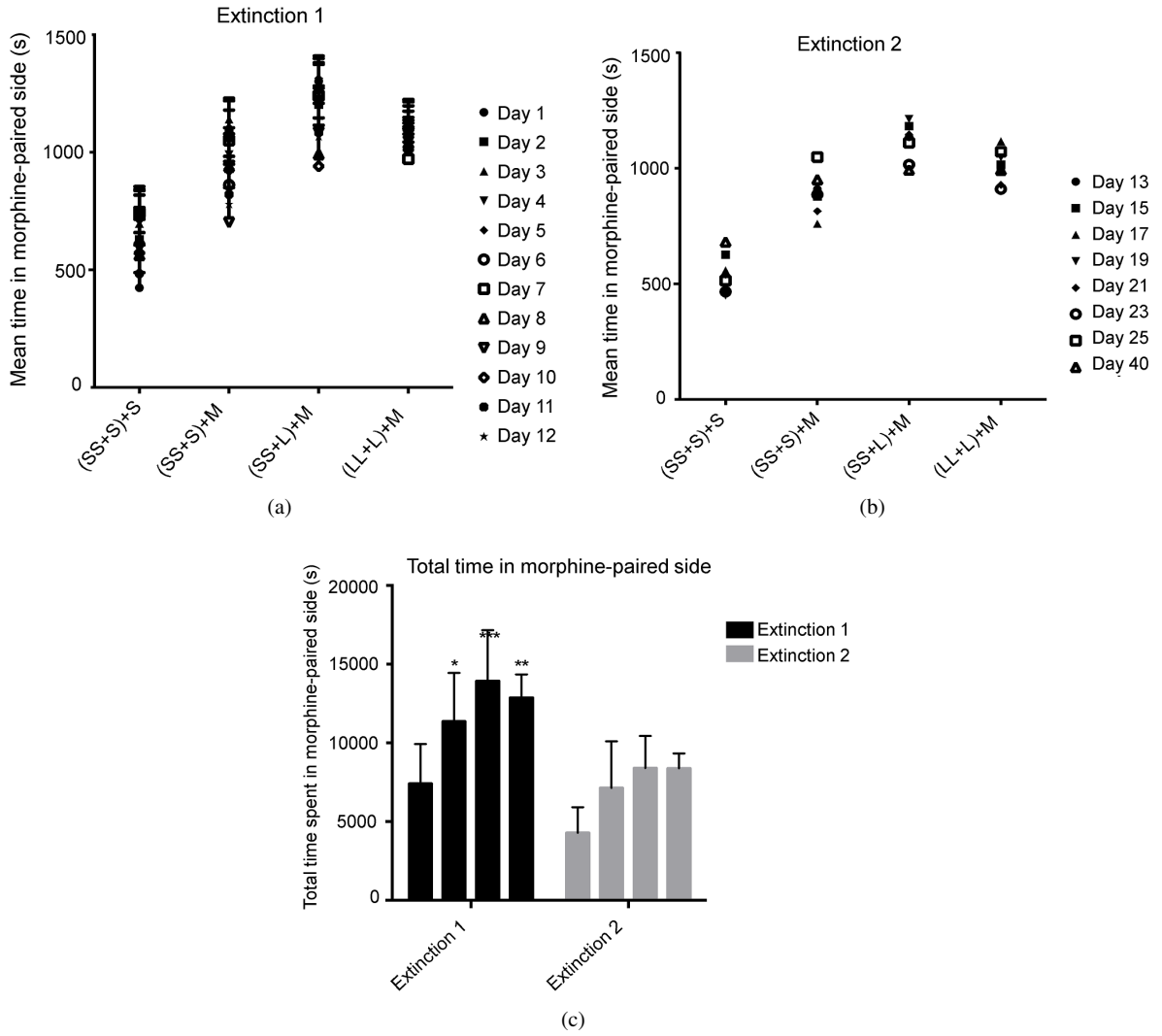

Figure 5: *Extinction phase.* Adult F344 rats were randomly divided into four groups (SS+S+S; SS+S+M; SS+L+M; LL+L+M) and pretreated with either saline or nonpyrogenic LPS (250 μg/kg), followed by either saline or 2 mg/kg LPS during the three-day treatment. During the conditioning phase, 72 h after LPS treatment, the rats were given either saline or 3.5 mg/kg morphine on alternate days. To test for extinction, the rats were given free access to the CPP apparatus for 30 min every day (extinction 1) until day 12, and then on alternate days (extinction 2) until day 28. Mean time (s) per day spent in the morphine-paired chamber during (a) extinction 1 and (b) extinction 2, and (c) the total time (s) during extinctions 1 and 2 were compared. The data represent the mean $\pm$SEM, $n = 5$–8 rats per group; $^{*}P < .5$; $^{**}P < .01$; $^{***}P < .001$.

SS+L+M group (13934.2 s, $^{***}P < .001$, $n = 8$), followed by the LL+L+M group (12864 s, $^{**}P < .01$, $n = 8$), then the SS+S+M group (11361.2 s, $^{*}P < .05$, $n = 7$) compared to the control (SS+S+S: 7416 s, $n = 5$). During extinction 2 (days 13–40, Figures 5(b), 5(c)), there was no significant difference observed between any of the groups.

3.5. Serum cytokine analysis

LPS-induced cytokine production was examined in the serum of the rats after preconditioning 2 (P2) and postconditioning (Figure 6). The serum TNF-α levels were significantly decreased in the postconditioning phase (SS+S+S: 32.6 pg/mL, $^{**}P < .01$, $n = 5$; SS+S+M: 23.1 pg/mL, $^{**}P < .01$, $n = 7$; SS+L+M: 36.6 pg/mL, $^{**}P < .01$, $n = 8$; LL+L+M: 40.8 pg/mL, $^{**}P < .01$, $n = 8$) compared to the P2 phase (SS+S+S: 18.9 pg/mL, $n = 5$; SS+S+M: 18.1 pg/mL, $n = 7$; SS+L+M: 13.7 pg/mL, $n = 8$; LL+L+M: 9.9 pg/mL, $n = 8$) in all groups. IL-5 and IFN-γ levels were not significantly different in the postconditioning phase compared to that in the P2 phase in all groups. IL-1β, IL-4, and KC/GRO levels were not significantly different after either the P2 or postconditioning phases. IL-13 cytokine levels were only detected in the LL+L+M group, and were significantly higher in the

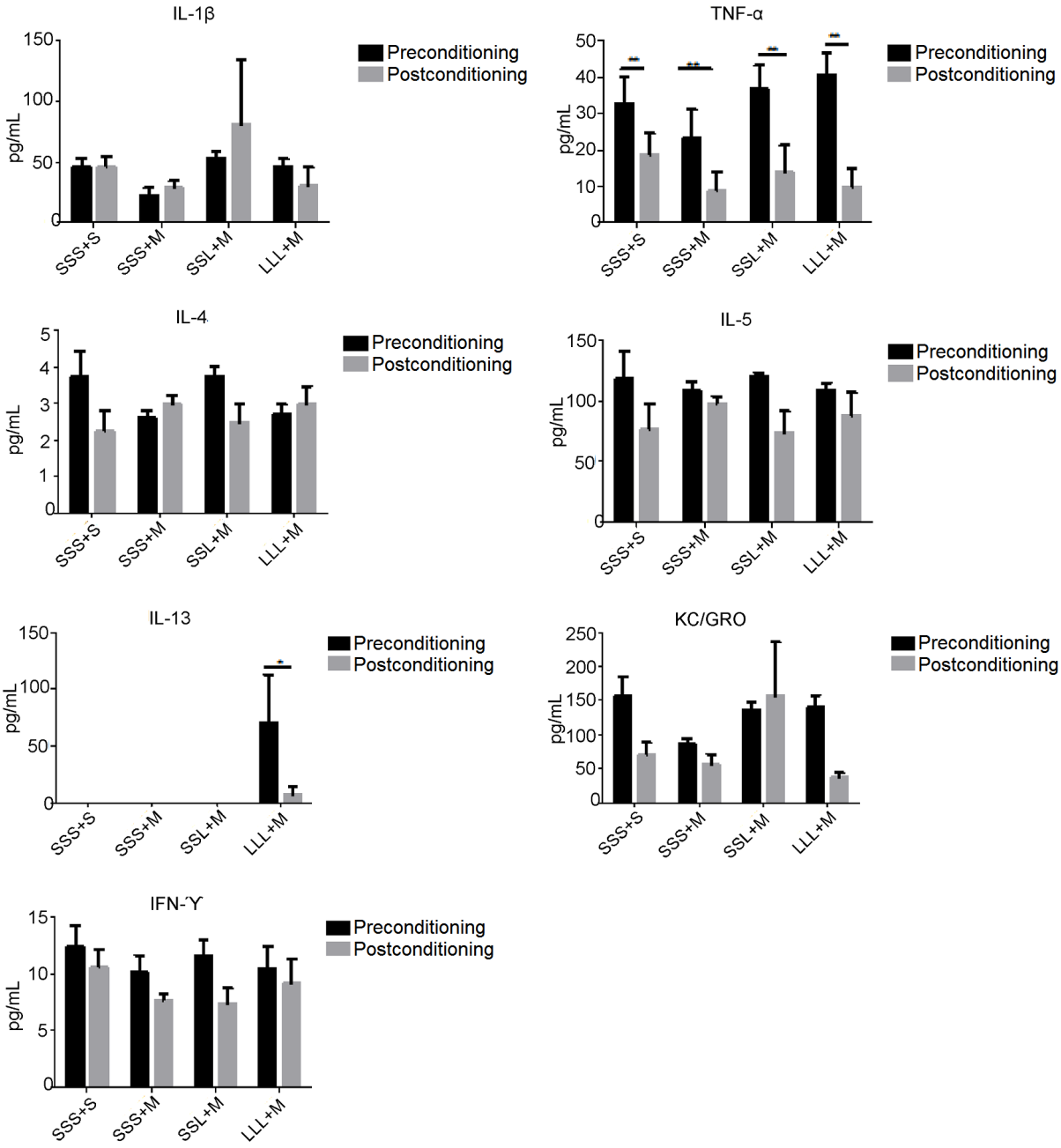

Figure 6: *Comparison of serum cytokine levels within groups after preconditioning 2 (P2) and postconditioning.* The protein levels of the cytokines, IL-1β, TNF-α, IL-4, IL-5, IL-13, KC/GRO, and IFN-γ following P2 and postconditioning were compared for each group using an electrochemiluminescent (MSD) assay. Data are represented as the mean $\pm$SEM, $n = 5$–8 rats per group; $^{*}P < .05$; $^{**}P < .01$.

postconditioning phase (LL+L+M: 70.2 pg/mL, $^{*}P < .05$, $n = 8$) compared to that in the P2 phase (LL+L+M: 7.1 pg/mL, $n = 8$).

Between groups (Figure 7), IL-1β serum levels were significantly higher in the SS+L+M (53 pg/mL, $^{**}P < .01$, $n = 8$) and LL+L+M (46.1 pg/mL, $^{**}P < .05$, $n = 8$) groups compared to the SS+S+M (20 pg/mL, $n = 7$) group after P2. However, after CPP, there was no significant difference in serum IL-1β levels between groups. KC/GRO serum levels were significantly higher in the LL+L+M group (147.8 pg/mL, $^{*}P < .05$, $n = 8$) compared to the SS+S+M (87.2 pg/mL, $n = 7$) group after P2; there was no significant difference between groups after CPP. There was no significant difference in TNF-α, IL-4, IL-5, IL-13 or IFN-γ serum concentrations between groups after P2 or CPP.

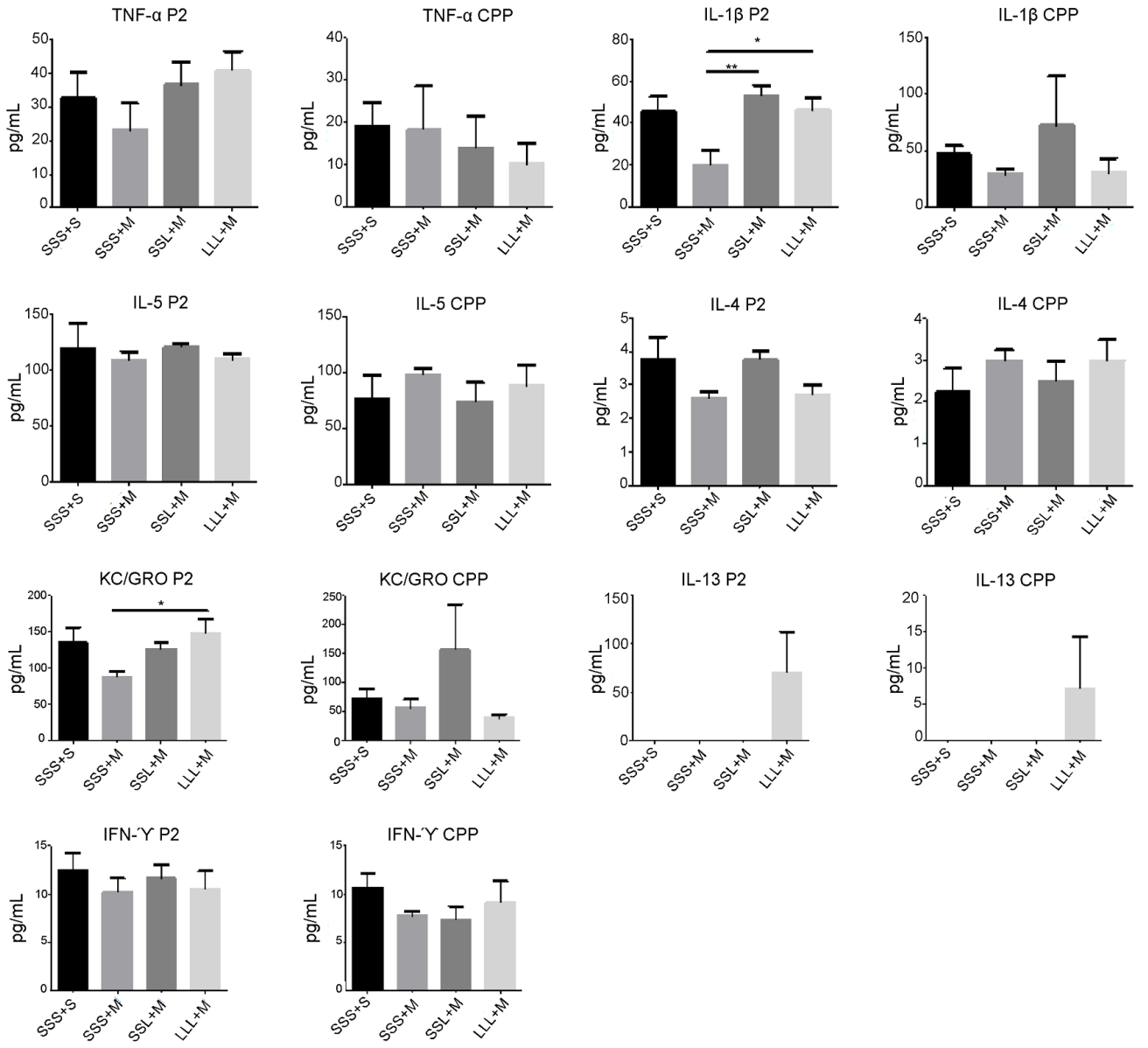

Figure 7: *Comparison of serum cytokine levels between groups after preconditioning 2 (P2) and postconditioning.* The protein levels of the cytokines, IL-1β, TNF-α, IL-4, IL-5, IL-13, KC/GRO, and IFN-γ following P2 and postconditioning were compared between the four groups (SS+S+S, SS+S+M, SS+L+M, LL+L+M) using an electrochemiluminescent (MSD) assay. Data are represented as the mean $\pm$SEM, $n = 5$–8 rats per group; $^{*}P < .05$; $^{**}P < .01$.

4. Discussion

Extensive research indicates that there are converging pathways between the nervous, endocrine, and immune systems [8]. Activation of an innate immune response induces the secretion of inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor-α (TNF-α) which, in turn, affect the complex neurochemical circuit of the central nervous system (CNS), and, consequently, modulate behavioral responses. An increased inflammatory response in the brain can trigger a plethora of behavioral dysfunctions, such as cognitive decline and sickness-related depression [7,8,9,10]. Consistent with these findings, our study demonstrates that an endotoxin challenge can contribute to behavioral disturbances.

Because of morphine's potent analgesic effects, it is widely used in clinical pain management, particularly in terminal diseases [1]. One of the ways to evaluate morphine's effects in rats is to examine its antinociceptive properties using a tail-flick latency test. The longer the tail-flick latency, the more profound is the analgesic effect of morphine. In the present study, tail-flick latency was significantly greater for rats pretreated with LPS compared

to the saline-treated group. The rats given LPS had a stronger response to morphine than those given saline, suggesting that LPS exposure alters the responsiveness to morphine, thereby prolonging its antinociceptive effects.

Before the start of the conditioning phase, body weights and body temperatures of the rats did not show any basal differences pre- and post-LPS treatment. The body weights of the rats pretreated with LPS (LL) dropped significantly after the first injection of a nonpyrogenic dose of LPS, but did not drop further after the challenge with the higher LPS dosage (LL+L). Similarly, body temperature in the rats increased after the first LPS pretreatment injection (LL), but not after the injection with the higher dose of LPS (LL+L). The lack of response to the higher challenge dose of LPS in terms of body weight and body temperature confirm that endotoxin tolerance was achieved in the LPS pretreated group.

Serum cytokine levels were measured after the P2 phase and after the completion of CPP. The cytokine levels, particularly TNF-α, were significantly higher after P2 in comparison to after CPP. This was expected since LPS treatment likely induced sickness behavior, which is often observed during increased secretion of TNF-α [22]. However, TNF-α levels were also higher after P2 in the groups that were not given LPS. This may be due to increased TNF-α production in response to the physical stress from handling, the body temperature procedures, and the preconditioning handling [23]. As expected, IL-1β serum concentrations were significantly higher in the groups given LPS (SS+L and LL+L) compared to groups without LPS (SS+S) after P2, confirming that LPS was able to induce an inflammatory response [12]. However, after CPP, there was no significant difference observed in any of the cytokine levels in the serum, suggesting that the inflammatory response after the LPS injections had diminished and reached basal levels.

In the present study, morphine-induced CPP was successfully established after six conditioning sessions. In the groups given the higher challenge dosage of LPS (SS+L and LL+L), the change in preference for morphine from P2 to CPP was greater than in the group that was not given LPS (SS+S). Moreover, the groups given the higher LPS dosage demonstrated a sustained response to the morphine-paired chamber as evidenced by greater than 20 days of extinction and a significantly longer time spent in the morphine-paired chamber during extinction. Collectively, these findings indicate that LPS enhances the sensitivity of morphine-induced CPP and that an inflammatory response that occurs during morphine use may result in more extensive behavioral changes than previously thought.

There is an imbalance between proinflammatory and anti-inflammatory cytokines during endotoxin tolerance, and the immune cells become desensitized to an additional endotoxin challenge [12]. In this study, an endotoxin tolerance effect was observed in the group of rats pretreated with low dose LPS (LL). The LPS pretreated group spent less time in the morphine-paired chamber compared to the group that did not receive the low dose LPS pretreatment. These data suggest that the inflammatory response that would normally be elicited in response to a high dose of LPS is essential for enhanced morphine-induced CPP.

Morphine's effects are elicited, in part, through opioid receptors, in particular, the mu-opioid receptor (MOR). Our previous studies showed that abuse of opioids such as morphine can alter neuronal and immune pathways via modulation of MOR gene expression [24,25]. We have recently shown that, in vitro, MOR expression is significantly increased in human astrocytic U87MG cells with and without morphine-induced desensitization after treatment with the inflammatory cytokine, IL-1β [26]. In addition, conditioned medium from LPS-stimulated macrophage cells increases MOR expression in neuroblastoma cells via inflammatory cytokines [27]. These findings suggest that the MOR signaling pathway may be a convergent point for the inflammatory response to opioids and bacterial endotoxins such as LPS [28].

The bidirectional interaction between the immune system and the CNS is essential for proper physiological and behavioral responses. Overstimulation or dysregulation of either system can detrimentally affect the other. Currently, there is increasing interest in uncovering the details of the role of the immune system in behavioral disorders. The results of our study suggest that, in the presence of systemic infection, the sensitivity to the rewarding effects of morphine is enhanced, both physiologically and behaviorally, thereby increasing the potential for morphine abuse and addiction.

Acknowledgments Funding for this study was provided, in part, by National Institutes of Health (NIH)/National Institute on Drug Abuse (NIDA) grants, R01 DA007058 and K02 DA016149, to SLC. The authors thank Dr. Louaine L. Spriggs for her editorial assistance.

Conflict of interest The authors declare that they have no conflict of interest.

References

[1] M. H. Ossipov, J. Lai, T. King, T. W. Vanderah, T. P. Malan Jr., V. J. Hruby, et al., *Antinociceptive and nociceptive actions of opioids*, J Neurobiol, 61 (2004), 126–148.

[2] M. J. Kreek, D. A. Nielsen, E. R. Butelman, and K. S. LaForge, *Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction*, Nat Neurosci, 8 (2005), 1450–1457.

[3] S. Roy, J. Wang, J. Kelschenbach, L. Koodie, and J. Martin, *Modulation of immune function by morphine: implications for susceptibility to infection*, J Neuroimmune Pharmacol, 1 (2006), 77–89.

[4] M. E. Hilburger, M. W. Adler, A. L. Truant, J. J. Meissler Jr., V. Satishchandran, T. J. Rogers, et al., *Morphine induces sepsis in mice*, J Infect Dis, 176 (1997), 183–188.

[5] B. W. Penninx, S. B. Kritchevsky, K. Yaffe, A. B. Newman, E. M. Simonsick, S. Rubin, et al., *Inflammatory markers and depressed mood in older persons: results from the Health, Aging and Body Composition study*, Biol Psychiatry, 54 (2003), 566–572.

[6] D. L. Evans, D. S. Charney, L. Lewis, R. N. Golden, J. M. Gorman, K. R. Krishnan, et al., *Mood disorders in the medically ill: scientific review and recommendations*, Biol Psychiatry, 58 (2005), 175–189.

[7] R. M. Barrientos, E. A. Higgins, J. C. Biedenkapp, D. B. Sprunger, K. J. Wright-Hardesty, L. R. Watkins, et al., *Peripheral infection and aging interact to impair hippocampal memory consolidation*, Neurobiol Aging, 27 (2006), 723–732.

[8] C. J. Henry, Y. Huang, A. Wynne, M. Hanke, J. Himler, M. T. Bailey, et al., *Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia*, J Neuroinflammation, 5 (2008), 15.

[9] J. Chen, J. B. Buchanan, N. L. Sparkman, J. P. Godbout, G. G. Freund, and R. W. Johnson, *Neuroinflammation and disruption in working memory in aged mice after acute stimulation of the peripheral innate immune system*, Brain Behav Immun, 22 (2008), 301–311.

[10] J. P. Godbout and R. W. Johnson, *Age and neuroinflammation: a lifetime of psychoneuroimmune consequences*, Neurol Clin, 24 (2006), 521–538.

[11] R. De La Garza 2nd, *Endotoxin- or pro-inflammatory cytokine-induced sickness behavior as an animal model of depression: focus on anhedonia*, Neurosci Biobehav Rev, 29 (2005), 761–770.

[12] N. F. Homji, X. Mao, E. F. Langsdorf, and S. L. Chang, *Endotoxin-induced cytokine and chemokine expression in the HIV-1 transgenic rat*, J Neuroinflammation, 9 (2012), 3.

[13] A. H. Swiergiel and A. J. Dunn, *Effects of interleukin-1β and lipopolysaccharide on behavior of mice in the elevated plus-maze and open field tests*, Pharmacol Biochem Behav, 86 (2007), 651–659.

[14] G. Liebsch, A. C. Linthorst, I. D. Neumann, J. M. Reul, F. Holsboer, and R. Landgraf, *Behavioral, physiological, and neuroendocrine stress responses and differential sensitivity to diazepam in two Wistar rat lines selectively bred for high- and low-anxiety-related behavior*, Neuropsychopharmacology, 19 (1998), 381–396.

[15] N. F. Homji, M. Vigorito, and S. L. Chang, *Morphine-induced conditioned place preference and associated behavioural plasticity in HIV-1 transgenic rats*, Int J Clin Exp Med, 5 (2012), 105–123.

[16] C. M. Davis, P. G. Roma, J. M. Dominguez, and A. L. Riley, *Morphine-induced place conditioning in Fischer and Lewis rats: acquisition and dose-response in a fully biased procedure*, Pharmacol Biochem Behav, 86 (2007), 516–523.

[17] I. Grakalic, C. W. Schindler, M. H. Baumann, K. C. Rice, and A. L. Riley, *Effects of stress modulation on morphine-induced conditioned place preferences and plasma corticosterone levels in Fischer, Lewis, and Sprague-Dawley rat strains*, Psychopharmacology (Berl), 189 (2006), 277–286.

[18] S. L. Chang and M. Vigorito, *Role of HIV-1 infection in addictive behavior: A study of an HIV-1 transgenic rat model*, Am J Infect Dis, 2 (2006), 98–106.

[19] S. L. Chang and K. P. Connaghan, *Behavioral and molecular evidence for a feedback interaction between morphine and HIV-1 viral proteins*, J Neuroimmune Pharmacol, 7 (2012), 332–340.

[20] R. Gagin, N. Kook, E. Cohen, and Y. Shavit, *Prenatal morphine enhances morphine-conditioned place preference in adult rats*, Pharmacol Biochem Behav, 58 (1997), 525–528.

[21] C. A. Paronis and S. G. Holtzman, *Increased analgesic potency of mu agonists after continuous naloxone infusion in rats*, J Pharmacol Exp Ther, 259 (1991), 582–589.

[22] R. M. Locksley, N. Killeen, and M. J. Lenardo, *The TNF and TNF receptor superfamilies: integrating mammalian biology*, Cell, 104 (2001), 487–501.

[23] S. Dimitrov, F. Shaikh, C. Pruitt, M. Green, K. Wilson, N. Beg, et al., *Differential TNF production by monocyte subsets under physical stress: Blunted mobilization of proinflammatory monocytes in prehypertensive individuals*, Brain Behav Immun, 27 (2013), 101–108.

[24] S. L. Chang, R. L. Moldow, S. D. House, and J. E. Zadina, *Morphine affects the brain-immune axis by modulating an interleukin-1 beta dependent pathway*, in AIDS, Drugs of Abuse, and the Neuroimmune Axis, H. Friedman, T. K. Eisenstein, J. Madden, and B. M. Sharp, eds., vol. 402 of Advances in Experimental Medicine and Biology, Springer-Verlag, New York, 1996, 35–42.

[25] S. D. House, X. Mao, G. Wu, D. Espinelli, W. X. Li, and S. L. Chang, *Chronic morphine potentiates the inflammatory response by disrupting interleukin-1β modulation of the hypothalamic-pituitary-adrenal axis*, J Neuroimmunol, 118 (2001), 277–285.

[26] L. S. Byrne, J. Peng, S. Sarkar, and S. L. Chang, *Interleukin-1 beta-induced up-regulation of opioid receptors in the untreated and morphine-desensitized U87 MG human astrocytoma cells*, J Neuroinflammation, 9 (2012), 252.

[27] E. F. Langsdorf and S. L. Chang, *Methamphetamine-mediated modulation of MOR expression in the SH-SY5Y neuroblastoma cell line*, Synapse, 65 (2011), 858–865.

[28] S. L. Chang and K. P. Connaghan, *Behavioral and molecular evidence for a feedback interaction between morphine and HIV-1 viral proteins*, J Neuroimmune Pharmacol, 7 (2012), 332–340.