

1-2013

Morphine prevents the development of stress-enhanced fear learning

Jennifer L. Szczytkowski-Thomson
Messiah University, jthomson@messiah.edu

Christina Lebonville

Donald Lyslea

Follow this and additional works at: https://mosaic.messiah.edu/psych_ed



Part of the [Psychology Commons](#)

Permanent URL: https://mosaic.messiah.edu/psych_ed/35

Recommended Citation

Szczytkowski-Thomson, Jennifer L.; Lebonville, Christina; and Lyslea, Donald, "Morphine prevents the development of stress-enhanced fear learning" (2013). *Psychology Educator Scholarship*. 35.
https://mosaic.messiah.edu/psych_ed/35

Sharpening Intellect | Deepening Christian Faith | Inspiring Action

Messiah University is a Christian university of the liberal and applied arts and sciences. Our mission is to educate men and women toward maturity of intellect, character and Christian faith in preparation for lives of service, leadership and reconciliation in church and society.

Morphine prevents the development of stress-enhanced fear learning

Jennifer L. Szczytkowski-Thomson, Christina L. Lebonville, Donald T. Lyslea

Abstract

The current study investigates the pharmacotherapeutic use of morphine as a preventative treatment for stress-enhanced fear learning, an animal model that closely mimics symptoms of post-traumatic stress disorder (PTSD). PTSD is a chronic and debilitating anxiety disorder characterized by exaggerated fear and/or anxiety that may develop as a result of exposure to a traumatic event. In this model, rats are exposed to a severe stressor (15 foot shocks) in one environment (Context A) and then subsequently exposed to a milder form of the same stressor (single foot shock) in a different environment (Context B). Animals that did not receive prior shock treatment exhibit fear responsiveness to Context B in line with the severity of the single shock given in this context. Animals that had received prior shock treatment in Context A exhibit an exaggerated learned fear response to Context B. Furthermore, animals receiving a single dose of morphine immediately following the severe stressor in Context A continue to show an enhanced fear response in Context B. However, animals receiving repeated morphine administration (three injections) after exposure to the severe stressor in Context A or a single dose of morphine at 48 h after the severe stressor no longer exhibit an enhancement in fear learning to Context B. These results are consistent with clinical studies suggesting that morphine treatment following a severe stressor may be useful in preventing or reducing the severity of PTSD in at-risk populations.

Highlights

► We utilize the stress-enhanced fear learning (SEFL) model of PTSD. ► Repeated morphine administration following trauma blocks SEFL ► Single morphine injection given immediately following trauma has no effect on SEFL. ► A single morphine injection given 48-hours after the trauma block SEFL ► Morphine may be an effective preventative therapy for populations at-risk for PTSD.

[Previous article](#)

[Next article](#)

[Keywords](#)

[Morphine](#)

[Post-traumatic stress disorder](#)

[Stress-enhanced fear learning](#)

[Fear conditioning](#)

[Foot shock](#)

[1. Introduction](#)

Severe and chronic stress induces a myriad of health complications and negatively affects an individual's physiological and psychological well-being. Traumatic events, physical or psychological, impact the individual and can induce relatively permanent alterations in the way the brain processes future events. This change may take a maladaptive form that impairs the normal functioning of the individual (Christopher, 2004). One of the most severe disorders of the stress response is post-traumatic stress disorder (PTSD), a chronic and debilitating anxiety disorder characterized by exaggerated fear or anxiety that develops when the normal fear

response to a traumatic event does not subside over time and begins to interfere with the individual's daily functioning (Rosen and Schulkin, 1998). At-risk populations include active military personnel, emergency responders, cancer patients, victims of natural disasters or accidents and abused children. For example, first-responders to the attacks on the World Trade Center in 2001 reported a high prevalence of PTSD based on DSM-IV criteria (Bowler et al., 2010). In addition, a 2010 study by Thomas et al. demonstrated that soldiers having faced combat in Iraq reported rates of PTSD between 20.7% (at 3-months post-deployment) and 30.5% (12-months post-deployment). Given these astonishing statistics, it is imperative to investigate potential preventative measures against the development of PTSD and other anxiety and stress disorders of similar etiology.

While there have been a number of advancements in the treatment of PTSD, potential preventative measures remain a highly sought after clinical tool (Shalev, 2009). There is currently limited evidence to suggest the success of preventative drug therapies. However, recent new evidence has indicated that morphine treatment following a traumatic event reduces the risk of developing PTSD and the severity of symptoms in those who do develop the disorder (Bryant et al., 2009, Holbrook et al., 2010, Nixon et al., 2010, Stoddard et al., 2009). More specifically, Holbrook and colleagues recently published in the *New England Journal of Medicine*, an analysis of clinical data from the Navy–Marine Corps CTR EMED suggesting that the use of morphine in US military personnel after combat injury reduced the risk of subsequent PTSD development (Holbrook et al., 2010). Morphine has also been suggested to reduce the development of PTSD in children who experience a single traumatic event (Nixon et al., 2010). In fact, the relationship between morphine and PTSD has even been extended to very young

children, ages 1 to 4 years old, admitted to a pediatric burn center with acute burns (Stoddard et al., 2009). This study showed a correlation between morphine administration and a decrease in posttraumatic symptoms assessed 3 to 6 months after the burn event. It has been suggested from these correlational studies that the pharmacotherapeutic use of morphine following a traumatic event may impede the memory consolidation and associated fear learning that are necessary for the development of PTSD. These clinical studies suggest that one potential preventative therapy for PTSD might be the administration of opiates following exposure to the traumatic event, however, the clinical situation is complex and it is difficult to claim direct causal relationship between morphine and the behavioral outcome. In this case, animal models of PTSD provide an opportunity to investigate the hypothesis that morphine administration following a traumatic event can alter the course of the development of PTSD.

Several animal models of PTSD have been developed, including such models as single prolonged stress (Yamamoto et al., 2009), inescapable shock (van der Kolk, 1987), predator exposure and swim stress. While there is no single universally accepted animal model of PTSD, one excellent model that captures the critical aspect of enhancement in future fear learning situations is the stress-enhanced fear learning (SEFL) paradigm developed by Fanselow and colleagues (Rau et al., 2005, Rau et al., 2009). This model involves a procedure in which rats are exposed to a severe stressor (15 foot shocks) in one environment (Context A) and then subsequently exposed to a milder form of the same stressor (single foot shock) in a different environment (Context B). Animals that do not receive the initial stressor (15 foot shocks) exhibit fear learning to Context B proportional to the administration of the single shock. In these experiments, fear learning is measured as the degree of freezing behavior displayed during the

time spent in the specific context. The rats receiving the prior shocks in Context A show enhanced fear learning to Context B where the single shock was presented, i.e., these animals show freezing behavior that is greater than that which would be expected following exposure to the single shock that was received in this context. This stress-enhanced fear learning closely mimics the sensitized reactions to environmental events characteristic of individuals with PTSD. Moreover, the model shares other characteristics with PTSD such as individual differences in susceptibility, the finding that amnesia for the traumatic event does not preclude the development of SEFL, and resistance to extinction trials after the immediate trauma (Rau et al., 2005).

The present study is the first to test the hypothesis that morphine administration following a severe stressor blocks the development of stress-enhanced fear learning. Rats were exposed to 15 shocks in Context A and later received a single shock in Context B. Animals that were exposed to Context A but not shocked in that context exhibited conditioned fear to Context B proportional to the administration of the single shock. In line with previous experiments, animals receiving prior shock treatment in Context A (15 shocks) showed an exaggerated fear response to Context B. The effect of morphine was tested in animals that received three injections of morphine beginning immediately following removal from the severe stressor in Context A and again at 24 and 48 h post-Context A treatment. In a subsequent experiment, animals received a single injection of morphine either immediately or 48 h post-Context A treatment.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (250–400 g in weight, Charles-River Laboratories, Raleigh, NC) were housed individually in a colony room under a reversed day–night (12-hour) cycle. The rats received free access to food and water throughout the experiment and during at least a one-week acclimation period prior to experimentation, where the rats were handled semi-daily. All manipulations were initiated 1 h into the dark phase of the day–night cycle. All procedures were conducted in accordance with and approval by the UNC Institutional Animal Care and Use Committee.

2.2. Stress-enhanced fear conditioning procedure

The current experiments utilized the stress-enhanced fear learning (SEFL) model originally developed by Michael Fanselow and colleagues (Rau et al., 2005; Rau et al., 2009). The SEFL paradigm employs two distinct contexts, referred to as Context A and Context B. For our studies, Context A was a standard rodent chamber (BRS/LVE, Laurel, MD; $26.7 \times 24.8 \times 30.7$) housed in a metal sound attenuating cubicle (BRS/LVE, Laurel, MD) in a quiet room separate from the animal colony. The chamber has a 16 bar grid floor design (.48 cm in diameter spaced 1.8 cm apart), smooth metal side walls, and clear plastic front and rear walls, the interior lights were kept off, and cedar bedding and vanilla extract were used in the chambers to create a distinct environment from Context B and the home cages. Grid floors were wired to a BRS/LVE shock generator (SG903) and scrambler (SC922). Context B was a standard rodent chamber (Med Associates, St. Albans, VT) housed in a wooden sound attenuating cubicle (Med Associates, St. Albans, VT; $20.5 \times 23.5 \times 30.7$) in a room separate from the one housing Context A and the animal colony. White noise was provided as background sound in the room. The chamber has a 19 bar grid floor design (.48 cm in diameter spaced 1.6 cm apart), textured, beige, and rubber

lined walls with a clear plastic front wall, the interior lights were kept on, and corn cob bedding and peppermint extract were used in the chambers to create a distinct environment from Context A and the home cages. Grid floors were wired to a Med Associates shock generator and scrambler (ENV-414). Context B was set-up to record the animal's behavior using a video recording system (Sony Video Camera Model HDR-CX150) directed at the clear front wall of the chamber.

The experimental design is summarized in Table 1. On Day 1, animals were placed into Context A for 90 min and received 15 foot shocks (1 mA, 1 s) on a 6-minute variable interval schedule with the first interval beginning 5 min into the session. Non-shock control animals were exposed to Context A for an equivalent amount of time, without shocks being delivered. After the session, animals were returned to their home cages. Animals were given 6 days before the second stage of the SEFL paradigm. On Day 7, animals were placed into Context B for 30 min to allow for habituation to the novel context and to test for any generalization between Contexts A and B. On Day 8, each animal was placed into Context B for 3 min 12 s before receiving a single shock (1 mA, 1 s). Baseline level of freezing was determined during the period before the delivery of the shock in Context B. Animals were removed from Context B at 3 min 44 s and returned to their home cage. On Days 9, 10, 15 and 23 (test days 1, 2, 7, and 14 post-Context B conditioning, respectively), animals were tested in Context B for the assessment of freezing behavior, a behavioral quantification of learned fear, during an 8 min 32 s session (no shocks administered). Freezing behavior was measured as the time spent immobile, except for movement necessary for respiration, during the 8 min 32 s spent in Context B on test day (Rau et al., 2005). The complete

session recording was analyzed by raters blind to the treatment condition. The rating procedure was shown to have high inter-rater reliability.

Table 1. Experimental design.

	Day 1 (severe stressor)	Day 7 (habituation)	Day 8 (mild stressor)	Days 9, 10, 15, 23 (test days 1, 2, 7, 14)
Group 1 (severe stressor)	Context A 15 shocks (1 mA, 1 s) over 90 min	Context B 30 min	Context B 3 min 44 s with 1 shock (1 mA, 1 s) at 3 min 12 s	Context B 8 min 32 s
Group 2 (no-severe stressor)	Context A 90 min	Context B 30 min	Context B 3 min 44 s with 1 shock (1 mA, 1 s) at 3 min 12 s	Context B 8 min 32 s

2.3. Pharmacological manipulations

Morphine was obtained from the National Institute on Drug Abuse (NIDA). For the initial test, animals in separate groups were treated with morphine dissolved in saline (7.5 mg/kg, subcutaneously) or saline immediately after the 90 min session in Context A (shocked or non-shocked) and additionally 24 and 48 h after the session. In the subsequent test, morphine (15 mg/kg, subcutaneously) or saline was given as a single injection either immediately or 48 h after the 90 min session in Context A. Thus, the design of each study included groups that received shock or no shock in Context A and were treated with either morphine or saline.

2.4. Statistical analysis

Percent freezing in Context B was determined for the time period prior to the single shock experience in the context (baseline) and subsequently during each of the four test sessions. The effect of Context A treatment (shock or no shock) and morphine or saline administration on baseline freezing in Context B prior to the shock was analyzed for statistical significance using a standard 2×2 analysis of variance (ANOVA). Percent freezing during the test sessions was analyzed using a $2 \times 2 \times 4$ repeated measures ANOVA with test day (Days 1, 2, 7 and 14) as the repeated measure and Context A treatment (shock or no shock) and drug treatment (morphine or saline) as between-subject variables. For all experiments, the significance level was set at .05. Significant interactions were examined using Tukey's post-hoc comparisons.

3. Results

3.1. Stress-enhanced fear learning

Fig. 1 shows the establishment of the stress-enhanced fear learning (SEFL) model using the specified parameters and without any injections. The results show a significant main effect of shock in Context A on conditioned freezing in Context B, $F(1, 10) = 8.80, p < .05$, indicating that the prior administration of shock in Context A enhanced fear learning to a single shock experience in Context B. There was also a significant main effect across test days, $F(3, 30) = 12.36, p < .01$, with a significant linear component, $F(1, 30) = 15.53, p < .01$, indicating that conditioned fear diminished over the test days. There was no significant interaction of the prior shock experience with the test days indicating a consistent effect of prior shock in Context A across the test days. There were also no significant differences in baseline freezing in Context B

prior to the shock. Thus, there was a pronounced SEFL using the specified apparatus and parameters that remains robust across multiple test days.

Fig. 1. Shows the establishment of the stress-enhanced fear learning (SEFL) model. There were no significant differences in baseline level of freezing, determined during the period before the delivery of the shock in Context B. The animals that received shock in Context A (solid circle) exhibited a significant increase in conditioned freezing in Context B compared to the group that did not receive prior shock treatment (open circle) ($n = 6$). Thus, there was a pronounced SEFL using the specified apparatus and parameters.

3.2. Effects of multiple injections of morphine after the shock in Context A

Fig. 2 shows the effect of morphine (7.5 mg/kg) administered immediately after the Context A experience and again 24 and 48 h later. The results showed no significant effects on baseline freezing in Context B determined prior to the shock in that context. In contrast, the results showed a significant effect of shock in Context A on conditioned freezing in Context B, $F(1, 20) = 12.66, p < .01$, indicating that the prior administration of shock enhanced fear learning to a single shock experience in Context B. There was also a significant effect across test days, $F(3, 60) = 50.82, p < .01$, with a significant linear component, $F(1, 60) = 115.67, p < .01$ indicating that conditioned fear diminished over the test days. Most importantly, there was a significant interaction between the shock experience in Context A and the morphine injections, $F(1, 20) = 7.91, p < .05$. Tukey's post-hoc comparisons of the four groups showed that between the two

saline-treated groups, the group shocked in Context A ($M = 58.54$, 95% CI [39.5, 77.58]) exhibited a significantly higher percentage of freezing behavior to Context B than the group that did not receive prior shock treatment ($M = 20.12$, 95% CI [3.0, 37.16]) indicating a significant SEFL effect. Most notably, the group receiving repeated morphine treatment that had been exposed to the severe stressor (15 shocks) in Context A was not significantly different from the non-shocked, saline-treated group. Thus, there was a pronounced SEFL effect that was prevented by the series of morphine injections following shock in Context A.

Fig. 2. Shows the effect of repeated morphine (7.5 mg/kg; 0, 24 and 48 h post-shock treatment in Context A) administration on conditioned freezing behavior in Context B. There were no significant differences in baseline level of freezing, determined during the period before the delivery of the shock in Context B. Within the saline-treated groups, the animals that received the severe stressor (15 shocks) in Context A (solid circle) exhibited a significant increase in conditioned freezing in Context B compared to the animals that did not receive prior shock treatment (open circle). There was no effect of morphine on freezing in Context B for the animals that did not receive prior shock treatment (open square). Most importantly, the group shocked in Context A that then received repeated morphine treatment (closed square) did not show significant SEFL ($n = 6$). Thus, the pronounced SEFL was prevented by the series of morphine injections following the shock in Context A.

3.3. Effects of injection of a single morphine injection immediately after shock in Context A

Fig. 3A shows that morphine (15 mg/kg) administered immediately after the Context A experience had no significant effect. The analysis showed no significant effects on baseline freezing in Context B prior to the shock in that context. In contrast, the results show a significant main effect of shock in Context A on conditioned freezing in Context B, $F(1, 19) = 4.59$, $p < .05$, indicating that the prior administration of shock enhanced fear learning to the single shock experience in Context B. There was no significant main effect of drug. There was a significant main effect of test days, $F(3, 57) = 41.02$, $p < .01$, with a significant linear component, $F(1, 57) = 71.81$, $p < .01$, indicating that conditioned fear diminished over the test days. There was no significant interaction between the prior shock experience and test day. Interestingly, there was no significant interaction between the shock experience in Context A and the morphine injections. Thus, morphine administration immediately after the Context A shock experience had no significant effect on the development of SEFL.

Fig. 3. Shows the effect of morphine (15 mg/kg) administered immediately after the Context A experience (A) or 48 h later (B). For both A and B the animals that received shock in Context A followed by treatment with saline (solid circle) showed a significant increase in conditioned freezing in Context B compared to the group that received saline but did not receive prior shock treatment (open circle). Morphine administration did not alter conditioning in Context B for the group that did not receive prior shock treatment in Context A (open square). Interestingly, the group that received morphine treatment immediately after the severe stressor in Context A (closed square) continued to exhibit significant SEFL (A). In contrast, the group shocked in

Context A that received morphine treatment (closed square) 48 h later did not show significant SEFL (B). There were no significant differences in baseline level of freezing which was determined during the period before the delivery of the shock in Context B ($n = 5-6$). Thus, the pronounced SEFL was prevented by a single injection of morphine 48 h following the shock in Context A, but not when it was administered immediately after the severe stressor.

3.4. Effects of a single morphine injection 48 h after shock in Context A

Fig. 3B shows the effect of morphine (15 mg/kg) administered 48 h after the Context A experience. The results showed no significant effects on baseline freezing in Context B prior to the shock in that context. As in the previous experiments, the results showed a significant effect of shock in Context A on conditioned freezing in Context B, $F(1, 18) = 21.36, p < .01$, indicating that the prior administration of shock enhanced fear learning to a single shock experience in Context B. There was also a significant effect across test days, $F(3, 54) = 45.98, p < .01$, with a significant linear component, $F(1, 54) = 104.99, p < .01$, indicating that conditioned fear diminished over the test days but did not interact with the prior shock experience. Most importantly, there was a significant interaction between the shock experience in Context A and the morphine injection, $F(1, 18) = 4.57, p < .05$. Tukey's post-hoc comparisons of the four groups indicated that between the two saline-treated groups, the group receiving shock in Context A ($M = 42.77, 95\% \text{ CI } [22.92, 62.62]$) showed a significantly higher percentage of freezing in comparison to the group that was not shocked in Context A ($M = 6.14, 95\% \text{ CI } [1.88, 10.41]$) indicating a significant SEFL effect. No other statistically significant differences were found. Most notably, the group receiving a single dose of morphine at 48 h post-stressor (15 shocks in Context A) was not significantly different from the non-shocked, saline-treated group. Thus,

there was a pronounced SEFL that was attenuated by the injection of morphine 48 h following the shock in Context A.

4. Discussion

Recent clinical data has indicated that morphine treatment following a traumatic event may reduce the risk of developing PTSD and the severity of symptoms in those who develop the disorder (Bryant et al., 2009, Holbrook et al., 2010, Nixon et al., 2010, Stoddard et al., 2009). From these clinical correlational studies, it has been suggested that morphine administration following a trauma interrupts the memory consolidation that is necessary for the development of PTSD. In the current study, rats were subjected to the stress-enhanced fear learning (SEFL) paradigm in which they received a severe stressor (15 shocks) in Context A followed by a milder form of the same stressor in Context B (1 shock). Results from the first experiment indicate that animals that had received the severe stressor in Context A exhibit an increase in freezing behavior in Context B that is disproportionate to the single shock received there. This enhancement in fearful behavior is similar to that observed in previous studies and has been postulated to result from a sensitization to fearful stimuli and an enhancement of subsequent fear learning (Rau et al., 2005, Rau and Fanselow, 2009). This model suggests that there is an alteration in the processing of fearful stimuli following exposure to the severe stressor that manifests as an enhancement of future fear learning and indicates that SEFL closely mimics certain aspects of the human condition of PTSD.

This study investigated the effect of morphine administration on the expression of SEFL. Repeated morphine administration (7.5 mg/kg; 0, 24, 48 h post-Context A) resulted in a

significant reduction in freezing behavior and thus, an attenuation of the enhanced fear learning that is the hallmark of this animal model. Interestingly, administration of a single dose of morphine (15 mg/kg) had a time-dependent effect on the expression of SEFL. A single dose of morphine administered immediately after the severe stressor in Context A did not reduce the exaggerated fear response in Context B. However, a single dose of morphine 48 h after the severe stressor in Context A attenuated the exaggerated fear response in Context B. These results raise several interesting points. First, prior research has shown that increasing doses of the anesthetic isoflurane administered during the shock treatment in Context A will reduce SEFL behavior in Context B (Rau et al., 2009). In the present experiments, the fact that there is no effect of morphine when the drug is administered immediately following the severe stressor would likely rule out the possibility that the effect of morphine on SEFL is due simply to a decrease in the pain experience since the analgesic effects of the drug would be most potent when given immediately after the repeated shocks in Context A. Furthermore, there is no effect of morphine on fear learning to Context B in control animals that did not receive severe shock in Context A indicating that morphine itself did not alter the pain perception of the subsequent single shock in Context B.

Interestingly, the finding that a single dose of morphine reduces SEFL when administered 48 h after the severe stressor suggests that morphine's mechanism of action is related to consolidation processes that are occurring around that time point as morphine has a half-life of approximately 2–4 h. Related results have been found in other studies utilizing animal models of PTSD. For example, Kozlovsky et al. (2012) demonstrated that administration of a CRH-R1 antisense oligodeoxynucleotide into the hippocampus 48 h after exposure to a predator scent stress

paradigm results in decreased anxiety indices and increased BDNF and pERK1/2 levels. CRH-R1 antisense administration at 1 h after the stressor had no effect. These results suggest that 48 h post-stressor is a critical time point in the modulation of HPA axis activity and the HPA axis is one pathway through which morphine may exert its effects on SEFL expression. Another method by which morphine may alter SEFL is by disrupting the consolidation of fear memories.

Memories associated with traumatic experiences appear to undergo a systems consolidation process such that the memory is localized to different brain regions at different times following the event. Protein kinase M zeta PKM ζ is a protein kinase that has been shown to be involved in both long-term potentiation (LTP) and memory storage processes (Ling et al., 2002, Sacktor et al., 1993). Dorsal hippocampal (DH) inactivation of PKM ζ 1 h after exposure to predator scent stress reduced both anxiety and responsiveness to a cue of the trauma given 8 days later (Cohen et al., 2010). Conversely, PKM ζ inactivation within the insular cortex 10 days after the trauma was also able to reduce these PTSD-like symptoms. However, inactivation of DH PKM ζ at 10 days or IC PKM ζ inactivation at 1 h post-stressor had no effect suggesting that the storage of the memory was moved over time.

Prior work has shown that pharmacological amnesic agents do not induce an attenuation of SEFL (Rau et al., 2005) suggesting that memory of the traumatic event is not central to the development of SEFL. Rather, it seems that there is an alteration in the threshold for learning regarding future fearful stimuli such that mildly aversive stimuli become capable of producing exaggerated fear responses. This change in behavior may be expressed due to plasticity of the neural circuitry underlying fear learning. The hippocampus plays a critical role in mediating learning and memory, in general, and has recently been shown to be an important neural

substrate for learning regarding fearful or anxiety-provoking stimuli. It is widely recognized that damage to the human hippocampal formation results in an amnesic syndrome (e.g., Nadel and Moscovitch, 1997). Furthermore, many studies in animals have examined the effects of hippocampal lesions on learning and memory processes including contextual fear acquisition (e.g., Phillips and LeDoux, 1992). Although the effects of hippocampal lesions are complicated and the interpretation of the effects is intensely debated in recent years, there is overwhelming evidence that the hippocampal formation plays a critical role in contextual fear conditioning (e.g., Anagnostaras et al., 2001, Bannerman et al., 2004). At the heart of this debate is the postulation of unique roles of the dorsal and ventral hippocampus in fear conditioning and memory. For example, lesion studies in rats have suggested that the dorsal hippocampus is especially important in the acquisition of contextual fear (e.g., Bannerman et al., 2004), but there is some evidence suggesting it is primarily responsible for encoding spatial learning (see Bannerman et al., 2004). In contrast, the ventral hippocampus may have a preferential role in brain processes associated with anxiety related behaviors. Interestingly, mice with lesions to the dorsal hippocampus, while still capable of acquiring contextual fear learning, exhibit a rapid decline in the ability to recall fear memories at future time points (Zelikowsky et al., 2012).

Given the above findings, it is unclear as to the specific targets on which morphine might be exerting its effects; however, previous research has shown a role for opioid receptors in both fear and contextual learning paradigms. For example, deletion of the mu-opioid receptor results in impaired acquisition of fear learning in mice (Sanders et al., 2005) and an injection of morphine into either the amygdala or the hippocampus results in impairment of contextual fear conditioning (Westbrook et al., 1997). More specifically, the intercalated nucleus (ICN) of the

amygdala has recently been postulated to contribute to fear extinction via its inhibitory actions on the output capacity of the central nucleus of the amygdala (Pare and Smith, 1993). The neurons in the ICN display mu-opioid receptors, the activation of which may lead to the modulation of communication between the basolateral and central amygdalar regions and inhibition of behavioral fear responses (Poulin et al., 2006). In addition, recent studies have found that cytokines, historically viewed as immune molecules, are not only present in the brain but may play important roles in processes such as learning and memory. For example, the cytokine interleukin-1 β (IL-1 β) has been shown to be involved in learning and memory, and is altered in response to stressful stimuli. Acute stress increases IL-1 β reactivity in astroglia in the hippocampus, amygdala, hypothalamus and PAG (Sugama et al., 2011). Interestingly, evidence suggests that, under some circumstances, IL-1 β is required for the normal physiological regulation of hippocampal function and memory processing as it has been shown that the absence of IL-1 β impairs memory consolidation and the targeted deletion of IL-1 β in animal studies leads to severely impaired hippocampal dependent memory (Goshen et al., 2007, Pickering and O'Connor, 2007). Mice with targeted deletion of the IL-1 receptors display a severely impaired hippocampal dependent but normal hippocampal independent memory. Furthermore, these mice demonstrate diminished short-term plasticity and exhibit no long-term potentiation (Avital et al., 2003). There is also evidence for the role of IL-1 β in the consolidation of memories that involved contextual fear which is dependent upon hippocampal function (e.g. Pugh et al., 2001). In contrast, the injection of pathophysiological levels of IL-1 β can produce detrimental effects on memory. These detrimental effects of high levels of IL-1 β are also specific for the consolidation of memory that depend on the hippocampus, whereas hippocampal independent memories are not altered by high levels of IL-1 β (Goshen et al., 2007, Goshen and

Yirmiya, 2009, Pugh et al., 2001). Thus, although the effects are complex and may depend on factors such as the magnitude and timing of IL-1 β expression, there is now substantial evidence indicating that IL-1 β is a critical element in behavioral outcomes such as the learning of fear responses and the potential long-term consequences of that form of learning. Furthermore, morphine has been shown to reduce the expression of hippocampal IL-1 β (Patel et al., 1996) suggesting that IL-1 β may be a potential mechanism through which morphine is acting to block SEFL.

5. Conclusion

Post-traumatic stress disorder (PTSD) is commonly defined as a severe and chronic anxiety disorder that results when the normal fear response to a traumatic event does not subside over time and begins to interfere with the individual's daily functioning (Rosen and Schulkin, 1998). Aside from its psychological symptoms, PTSD patients may also display increased susceptibility to drug addictions (Barys et al., 2011), altered glucose metabolism and inflammation associated with type 2 diabetes (Nowotny et al., 2010), imbalances in T-cell distribution and proliferation indicative of compromised immune functioning (Sommershof et al., 2009) and increased risk of cardiovascular disease (Kibler, 2009). Currently, there is little knowledge about how PTSD formation can be prevented in the aftermath of a major stress or trauma, but there is emerging research focusing on the potential of opioid pharmacotherapy in the prevention of this devastating disorder. The present research provides support for the effectiveness of opioid pharmacotherapy in the suppression of SEFL and has the promise to provide critical insights into the neural mechanisms and potential new treatments for PTSD.

Acknowledgment

JLS was supported by the Seeding Postdoctoral Innovators in Research and Education (SPIRE) program funded by the National Institutes of Health, Training, Workforce Development, and Diversity division of the National Institute of General Medical Sciences (NIGMS) grant K12GM000678.

References

Anagnostaras et al., 2001

S.G. Anagnostaras, G.D. Gale, M.S. Fanselow

Hippocampus and contextual fear conditioning: recent controversies and advances

Hippocampus, 11 (2001), pp. 8-17

Avital et al., 2003

A. Avital, I. Goshen, A. Kamsler, M. Segal, K. Iverfeldt, G. Richter-Levin, et al.

Impaired interleukin-1 signaling is associated with deficits in hippocampal memory processes and neural plasticity

Hippocampus, 13 (2003), pp. 826-834

Bannerman et al., 2004

D.M. Bannerman, J.N. Rawlins, S.B. McHugh, R.M. Deacon, B.K. Yee, T. Bast, et al.

Regional dissociations within the hippocampus—memory and anxiety

Neurosci Biobehav Rev, 28 (2004), pp. 273-283

Barsy et al., 2011

B. Barsy, E. Mikics, B. Barsvari, J. Haller

The long-term impact of footshock stress on addiction-related behaviors in rats

Neuropharmacology, 60 (2011), pp. 267-273

Bowler et al., 2010

R.M. Bowler, H. Han, V. Gocheva, S. Nakagawa, H. Alper, L. DiGrande, et al.

Gender differences in probable posttraumatic stress disorder among police responders to the
2001 World Trade Center terrorist attack

Am J Ind Med, 53 (2010), pp. 1186-1196

Bryant et al., 2009

R.A. Bryant, M. Creamer, M. O'Donnell, D. Silove, A.C. McFarlane

A study of the protective function of acute morphine administration on subsequent
posttraumatic stress disorder

Biol Psychiatry, 65 (2009), pp. 438-440

Christopher, 2004

M. Christopher

A broader view of trauma: a biopsychosocial-evolutionary view of the role of the traumatic stress response in the emergence of pathology and/or growth

Clin Psychol Rev, 24 (2004), pp. 75-98

Cohen et al., 2010

H. Cohen, N. Kozlovsky, M.A. Matar, Z. Kaplan, J. Zohar

Mapping the brain pathways of traumatic memory: inactivation of protein kinase M zeta in different brain regions disrupts traumatic memory processes and attenuates traumatic stress responses in rats

Eur Neuropsychopharmacol, 20 (2010), pp. 253-271

Goshen et al., 2007

I. Goshen, T. Kreisel, H. Ounallah-Saad, P. Renbaum, Y. Zalzstein, T. Ben-Hur, et al.

A dual role for interleukin-1 in hippocampal-dependent memory processes

Psychoneuroendocrinology, 32 (2007), pp. 1106-1115

Goshen and Yirmiya, 2009

I. Goshen, R. Yirmiya

Interleukin-1 (IL-1): a central regulator of stress responses

Front Neuroendocrinol, 30 (2009), pp. 30-45

Holbrook et al., 2010

T.L. Holbrook, M.R. Galarneau, J.L. Dye, K. Quinn, A.L. Dougherty

Morphine use after combat injury in Iraq and post-traumatic stress disorder

N Engl J Med, 362 (2010), pp. 110-117

Kibler, 2009

J.L. Kibler

Posttraumatic stress and cardiovascular disease risk

J Trauma Dissociation, 10 (2009), pp. 135-150

Kozlovsky et al., 2012

N. Kozlovsky, J. Zohar, Z. Kaplan, H. Cohen

Microinfusion of a corticotrophin-releasing hormone receptor 1 antisense oligodeoxynucleotide into the dorsal hippocampus attenuates stress responses at specific times after stress exposure

J Neuroendocrinol, 24 (3) (2012), pp. 489-503

Ling et al., 2002

D.S. Ling, L.S. Benardo, P.A. Serrano, N. Blace, M.T. Kelly, J.F. Crary, et al.

Protein kinase Mzeta is necessary and sufficient for LTP maintenance

Nat Neurosci, 5 (2002), pp. 295-296

Nadel and Moscovitch, 1997

L. Nadel, M. Moscovitch

Memory consolidation, retrograde amnesia and the hippocampal complex

Curr Opin Neurobiol, 7 (1997), pp. 217-227

Nixon et al., 2010

R.D. Nixon, T.J. Nehmy, A.A. Ellis, S.A. Ball, A. Menne, A.C. McKinnon

Predictors of posttraumatic stress in children following injury: the influence of appraisals,
heart rate, and morphine use

Behav Res Ther, 48 (2010), pp. 810-815

Nowotny et al., 2010

B. Nowotny, M. Cavka, C. Herder, H. Loffler, U. Poschen, L. Joksimovic, et al.

Effects of acute psychological stress on glucose metabolism and subclinical inflammation in
patients with post-traumatic stress disorder

Horm Metab Res, 42 (2010), pp. 746-753

Pare and Smith, 1993

D. Pare, Y. Smith

The intercalated cell masses project to the central and medial nuclei of the amygdala in cats

Neuroscience, 57 (1993), pp. 1077-1090

Patel et al., 1996

N.A. Patel, A.A. Romero, J.E. Zadina, S.L. Chang

Chronic exposure to morphine attenuates expression of interleukin-1 beta in the rat
hippocampus

Brain Res, 712 (1996), pp. 340-344

Phillips and LeDoux, 1992

R.G. Phillips, J.E. LeDoux

Differential contribution of amygdala and hippocampus to cued and contextual fear
conditioning

Behav Neurosci, 106 (1992), pp. 274-285

Pickering and O'Connor, 2007

M. Pickering, J.J. O'Connor

Pro-inflammatory cytokines and their effects in the dentate gyrus

Dentate Gyrus, 163 (2007), pp. 339-354

Poulin et al., 2006

J.F. Poulin, B. Chevalier, S. Laforest, G. Drolet

Enkephalinergic afferents of the centromedial amygdala in the rat

J Comp Neurol, 496 (2006), pp. 859-876

Pugh et al., 2001

C.R. Pugh, M. Fleshner, L.R. Watkins, S.F. Maier, J.W. Rudy

The immune system and memory consolidation: a role for the cytokine IL-1 beta

Neurosci Biobehav Rev, 25 (2001), pp. 29-41

Google Scholar

Rau et al., 2005

V. Rau, J.P. DeCola, M.S. Fanselow

Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder

Neurosci Biobehav Rev, 29 (2005), pp. 1207-1223

Rau and Fanselow, 2009

V. Rau, M.S. Fanselow

Exposure to a stressor produces a long lasting enhancement of fear learning in rats

Stress, 12 (2009), pp. 125-133

Rau et al., 2009

V. Rau, I. Oh, M. Laster, E.I. Eger 2nd, M.S. Fanselow

Isoflurane suppresses stress-enhanced fear learning in a rodent model of post-traumatic stress disorder

Anesthesiology, 110 (2009), pp. 487-495

Rosen and Schulkin, 1998

J.B. Rosen, J. Schulkin

From normal fear to pathological anxiety

Psychol Rev, 105 (1998), pp. 325-350

Sacktor et al., 1993

T.C. Sacktor, P. Osten, H. Valsamis, X. Jiang, M.U. Naik, E. Sublette

Persistent activation of the zeta isoform of protein kinase C in the maintenance of long-term potentiation

Proc Natl Acad Sci U S A, 90 (1993), pp. 8342-8346

Sanders et al., 2005

M.J. Sanders, B.L. Kieffer, M.S. Fanselow

Deletion of the mu opioid receptor results in impaired acquisition of Pavlovian context fear

Neurobiol Learn Mem, 84 (2005), pp. 33-41

Shalev, 2009

A.Y. Shalev

Posttraumatic stress disorder and stress-related disorders

Psychiatr Clin North Am, 32 (2009), pp. 687-704

Sommershof et al., 2009

A. Sommershof, H. Aichinger, H. Engler, H. Adenauer, C. Catani, E.M. Boneberg, et al.

Substantial reduction of naive and regulatory T cells following traumatic stress

Brain Behav Immun, 23 (2009), pp. 1117-1124

Stoddard et al., 2009

F.J. Stoddard Jr., E.A. Sorrentino, T.A. Ceranoglu, G. Saxe, J.M. Murphy, J.E. Drake, et al.

Preliminary evidence for the effects of morphine on posttraumatic stress disorder symptoms in one- to four-year-olds with burns

J Burn Care Res, 30 (2009), pp. 836-843

Sugama et al., 2011

S. Sugama, T. Takenouchi, K. Sekiyama, H. Kitani, M. Hashimoto

Immunological responses of astroglia in the rat brain under acute stress: interleukin 1 beta co-localized in astroglia

Neuroscience, 192 (2011), pp. 429-437

van der Kolk, 1987

B.A. van der Kolk

The drug treatment of post-traumatic stress disorder

J Affect Disord, 13 (1987), pp. 203-213

Westbrook et al., 1997

R.F. Westbrook, A.J. Good, M.J. Kiernan

Microinjection of morphine into the nucleus accumbens impairs contextual learning in rats

Behav Neurosci, 111 (1997), pp. 996-1013

Yamamoto et al., 2009

S. Yamamoto, S. Morinobu, S. Takei, M. Fuchikami, A. Matsuki, S. Yamawaki, et al.

Single prolonged stress: toward an animal model of posttraumatic stress disorder

Depress Anxiety, 26 (2009), pp. 1110-1117

Zelikowsky et al., 2012

M. Zelikowsky, S. Bissiere, M.S. Fanselow

Contextual fear memories formed in the absence of the dorsal hippocampus decay across time

J Neurosci, 32 (2012), pp. 3393-3397