Title: Atorvastatin Attenuates Stress-Induced Reinstatement of Morphine Seeking in a Rat Self-Administration Model

Authors: Niloofar Aghajani[1], Hossein Azizi[1,2], Saeed Semnanian[1,2,*]

1. *Institute for Brain and Cognition, Tarbiat Modares University, Tehran, Iran.*
2. *Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.*

***Corresponding Author**: Saeed Semnanian, Institute for Brain and Cognition, Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. Email addresses: ssemnan@modares.ac.ir

To appear in: **Basic and Clinical Neuroscience**

Received date: 2026/04/12

Revised date: 2026/06/13

Accepted date: 2026/06/15

Please cite this article as:

Aghajani, N., Azizi, H., Semnanian, S. (In Press). Atorvastatin Attenuates Stress-Induced Reinstatement of Morphine Seeking in a Rat Self-Administration Model. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2026. Doi: http://dx.doi.org/10.32598/bcn.2026.6125.3

DOI: http://dx.doi.org/10.32598/bcn.2026.6125.3

Abstract

Background: Opioid use disorder (OUD) is a chronic relapsing condition characterized by high rates of stress-induced relapse even after prolonged abstinence. Converging evidence implicates neuroimmune, glutamatergic transmitters and stress-related mechanisms in relapse vulnerability. Statins exert pleiotropic anti-inflammatory and neuroprotective effects and have recently emerged as potential modulators of relapse-related processes.

Methods: Adult male Wistar rats were trained to self-administer intravenous morphine (0.5 mg/kg/infusion) for 14 days under an FR1 schedule (fixed ratio). Following acquisition, animals underwent 14 days of extinction during which they received daily intraperitoneal injections of saline or atorvastatin (1 mg/kg). Stress-induced reinstatement was triggered by intermittent foot-shock exposure on day 29. Active lever responding served as the primary index of morphine-seeking behavior.

Results: Rats acquired stable morphine self-administration across the acquisition phase. During late extinction, atorvastatin-treated animals exhibited significantly reduced active lever responding compared with saline-treated controls ($p = 0.0022$). Intermittent foot-shock stress robustly reinstated morphine seeking in saline-treated rats, whereas reinstatement responding was significantly attenuated in the atorvastatin group ($p = 0.0022$). Quantification of relapse magnitude relative to extinction baseline revealed a significantly smaller increase in active responding in atorvastatin-treated rats (Δ analysis, $p = 0.0022$). Inactive lever responding remained low across conditions.

Conclusions: Chronic atorvastatin treatment reduced morphine-seeking behavior during extinction and attenuated stress-induced reinstatement in an operant self-administration model. These findings support the potential of statins as adjunctive agents targeting relapse-related neurobiological mechanisms in OUD.

Keywords: Morphine; Atorvastatin; Reinstatement; Extinction, Self-Administration.

Introduction

Opioid use disorder (OUD) is a chronic relapsing condition associated with high global prevalence and substantial morbidity and mortality (1). Although opioid agonist maintenance therapies are effective in suppressing withdrawal symptoms and reducing overdose risk, relapse remains the most persistent clinical challenge (2). Importantly, relapse frequently occurs after prolonged abstinence, when acute withdrawal has subsided, indicating the involvement of enduring neurobiological adaptations that extend beyond physical dependence. Stressful life events, drug-associated cues, and pharmacological priming reliably reinstate opioid-seeking behavior in humans as well as in preclinical models (3,4), underscoring the need for adjunctive pharmacological strategies that specifically target relapse vulnerability rather than withdrawal alone (2).

A growing body of preclinical evidence indicates that dysregulation of stress-responsive and neuroimmune signaling pathways plays a central role in relapse susceptibility. Opioids activate toll-like receptor 4 (TLR4) signaling in microglia, leading to increased production of pro-inflammatory cytokines and disruption of synaptic plasticity within corticolimbic circuits critical for motivated behavior, including the nucleus accumbens (NAc) and prefrontal cortex (5,6). In parallel, chronic opioid exposure alters hypothalamic–pituitary–adrenal axis function, resulting in exaggerated corticosterone release and enhanced corticotropin-releasing factor (CRF) signaling during stress, which robustly potentiates relapse-like behavior (3,7).

Disruption of glutamate homeostasis within corticostriatal circuitry represents another core neurobiological mechanism underlying relapse vulnerability (8). Repeated drug exposure downregulates the astrocytic glutamate transporter GLT-1, impairing regulation of extracellular glutamate levels in the NAc. This dysregulation amplifies glutamatergic signaling during stress or cue exposure and facilitates reinstatement of drug-seeking behavior (9). Conversely, pharmacological restoration of GLT-1 function normalizes extracellular glutamate tone and reliably attenuates relapse-like responding, identifying glutamate homeostasis as a central substrate of relapse-related behavior (8–10).

Beyond their lipid-lowering properties, statins have emerged as potential modulators of relapse-related neurobiological processes. Statins exert pleiotropic effects within the central nervous system, including anti-inflammatory, antioxidant, and synaptic regulatory actions that are mechanistically relevant to addiction-related circuitry (11,12). In a seminal study, Chauvet and colleagues demonstrated that brain-penetrant statins such as simvastatin and atorvastatin—but not the hydrophilic statin pravastatin—reduce relapse-like drug seeking and attenuate stress-induced reinstatement in rodent models of cocaine and nicotine self-administration (13).

On this basis, it has been hypothesized that statins might modulate intracellular signaling pathways that overlap with those activated by environmental enrichment—a non-pharmacological intervention known to reduce relapse vulnerability—thereby stabilizing stress-responsive and plasticity-related neural circuits (11,12). Despite the well-documented pleiotropic effects of statins, clinical data regarding their efficacy in substance use disorders remain notably scarce. However, statins have demonstrated high tolerability and therapeutic potential as adjunctive treatments in other psychiatric conditions, including major depressive disorder (13) and schizophrenia (14). This established clinical safety profile, combined with robust preclinical evidence demonstrating their ability to prevent drug-seeking behaviors (11) and modulate neuroinflammation (15), positions brain-penetrant statins like atorvastatin as highly promising candidates for repurposing in addiction pharmacotherapy. Given the lack of clinical trials in addiction, further preclinical investigations using highly translational models, such as operant intravenous self-administration (IVSA), are imperative to bridge this translational gap.

Importantly, emerging evidence suggests that statin-mediated modulation of relapse-related processes extends to opioids. Hashemizadeh and colleagues recently reported that chronic atorvastatin treatment attenuates morphine-conditioned place preference and facilitates extinction, implicating statin-sensitive neuroimmune and glutamatergic mechanisms in opioid reward and relapse vulnerability (16). However, conditioned place preference paradigms primarily capture Pavlovian drug-context associations and do not address the instrumental motivation of drug-seeking. Crucially, a specific mechanistic gap remains regarding stress-induced relapse, which operates through distinct neurobiological circuits—including stress-responsive neuroimmune and glutamatergic pathways. Therefore, it remains unknown whether statin-mediated neuroprotection extends to mitigating stress-triggered opioid reinstatement.

Building on these converging lines of evidence, the present study employed an intravenous morphine self-administration paradigm in Wistar rats, followed by extinction and stress-induced reinstatement testing. This operant framework enables direct assessment of relapse-like behavior under stress conditions while preserving the motivational and instrumental dimensions of opioid seeking. We hypothesized that chronic atorvastatin treatment would reduce morphine-seeking behavior during extinction and attenuate stress-induced reinstatement by upregulating GLT-1 expression and reducing pro-inflammatory cytokines in the mesocorticolimbic pathway..

Materials and methods

Animals

Adult male Wistar rats (290–310 g at the start of the experiments) were used in this study. Animals were housed under controlled environmental conditions (12 h light/dark cycle; temperature 22 ± 2°C; relative humidity 50–60%) with free access to food and water, except during behavioral testing sessions. All experimental procedures were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Tarbiat Modares University (ir.modares.aec.142.034).

Experimental design

The study consisted of three consecutive phases: morphine self-administration (days 0–14), extinction training combined with pharmacological treatment (days 15–28), and stress-induced reinstatement testing (day 29).

Following stable acquisition of morphine self-administration, animals were randomly assigned to receive either saline or atorvastatin (1 mg/kg) during the extinction phase. Group allocation was performed using a predefined randomization procedure. Behavioral testing and subsequent data analysis were conducted by an experimenter blinded to treatment conditions. After completion of extinction training, rats remained undisturbed in their home cages for 24 h before reinstatement testing to ensure a stable baseline state prior to stress exposure. The primary outcome measure was active lever responding during the reinstatement session, considered an index of relapse-like morphine-seeking behavior. A schematic representation of the experimental timeline is provided in Figure 1.

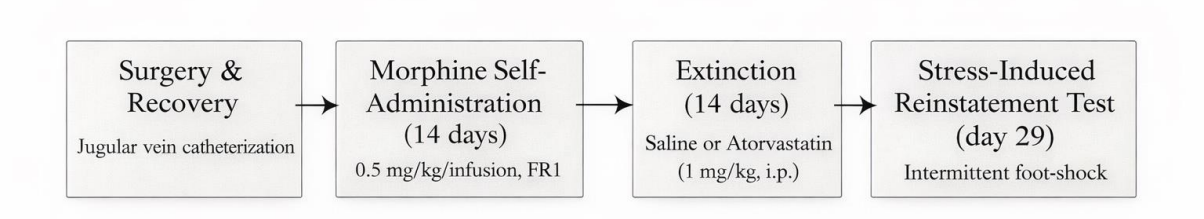


Figure 1. Experimental timeline of the study. Rats underwent jugular vein catheterization followed by a recovery period, morphine self-administration training, extinction with daily saline or atorvastatin treatment, and stress-induced reinstatement testing on day 29 following intermittent foot-shock exposure (FR1: fixed ratio).

Drugs

Morphine sulfate was dissolved in sterile 0.9% saline and delivered intravenously at a dose of 0.5 mg/kg per infusion (0.1 ml/infusion), followed by a 20 s timeout period during which additional responses had no programmed consequences. Atorvastatin was administered intraperitoneally at a dose of 1 mg/kg once daily throughout the extinction phase (days 15–28). The rationale for selecting the 1 mg/kg dose of atorvastatin was based on its established pharmacokinetic profile and prior behavioral studies in models of substance use disorders. Atorvastatin is a highly lipophilic statin that readily crosses the blood-brain barrier, allowing for effective central nervous system exposure at relatively low systemic doses. The specific dose of 1 mg/kg was chosen based on previous studies demonstrating that low-dose systemic administration of brain-penetrant statins can effectively modulate neuroinflammatory pathways and attenuate drug-seeking behaviors in rodents, without inducing locomotor deficits or overt toxicity (11,16).

Operant apparatus

Behavioral sessions were conducted in standard operant conditioning chambers equipped with two levers (active and inactive), a cue light positioned above the active lever, a tone generator, and an infusion pump connected to a swivel-tether system. Behavioral events were recorded using dedicated operant control software (Matin Shargh Co., Tehran, Iran).

Jugular vein catheterization

Rats were anesthetized with Intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Adequate anesthesia was confirmed by the absence of pedal withdrawal reflexes. Under aseptic conditions, a chronic indwelling catheter was implanted into the jugular vein and exteriorized at the dorsal scapular region. Body temperature was maintained throughout surgery using a heating pad. Animals were monitored until full recovery and allowed a 7-day recovery period before initiation of self-administration training.

Post-operative care

Post-surgical care included analgesia with ketoprofen (5 mg/kg, i.p.) for 1–2 days and prophylactic cefazolin (20 mg/kg, i.p.) for three consecutive days following catheter implantation. Catheters were flushed daily with sterile heparinized saline to maintain patency. Animals showing signs of catheter failure or postoperative complications were excluded according to predefined criteria established prior to behavioral testing.

Morphine self-administration (days 0–14)

After recovery, rats were trained to self-administer morphine during daily 2 h sessions for 14 consecutive days under a fixed ratio 1 (FR1) schedule. Each active lever press resulted in an intravenous morphine infusion paired with a discrete light/tone cue and followed by a 20 s timeout period. Inactive lever presses were recorded but had no programmed consequences.

Extinction training (days 15–28)

Extinction sessions were conducted for 2 h per day for 14 consecutive days. During extinction, active lever responses no longer resulted in morphine infusion or cue presentation. Inactive lever responses continued to have no programmed consequences. Animals received daily intraperitoneal injections of either saline or atorvastatin (1 mg/kg) during this phase. Animals received daily intraperitoneal injections of either saline or atorvastatin (1 mg/kg) 30 minutes prior to each extinction session. The selected dose of 1 mg/kg was chosen based on previous studies demonstrating its neuroprotective and anti-inflammatory efficacy in rodent models without inducing motor impairment (17,18).

Stress-induced reinstatement (day 29)

On day 29, reinstatement was induced using an intermittent foot-shock stress protocol administered outside the operant chamber. Foot shocks (1.0 mA, 0.5 s duration) were delivered over a 15 min period with variable inter-shock intervals (mean ~40 s; range 10–70 s). Immediately after stress exposure, animals were returned reinstatement session for 2 h conducted under extinction conditions (no morphine and no cue presentation). Active lever responses during this session were used as a measure of stress-induced relapse-like behavior.

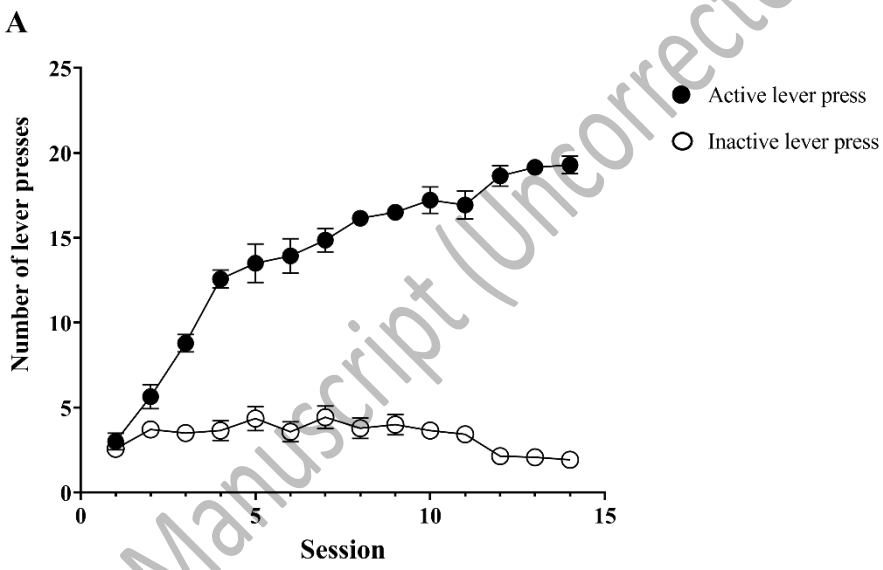
Statistical analysis

Behavioral data were analyzed using GraphPad Prism version 8.4.3 (GraphPad Software, San Diego, CA, USA). For longitudinal datasets evaluating changes across multiple time points, a repeated-measures analysis of variance (ANOVA) was employed, with treatment as a between-subjects factor and session as a within-subjects factor. For point-wise comparisons between two independent groups, considering the sample size constraints, the non-parametric Mann–Whitney U test was utilized. When ANOVA revealed significant main effects or interactions, *post hoc* analyses were performed using Sidak's multiple comparisons test to adjust for multiple testing. Statistical significance was set at $p<0.05$. Data are presented as mean ± SEM.

Results

Acquisition of morphine self-administration

Rats acquired stable intravenous morphine self-administration across the 14-day acquisition phase. Active lever responding increased progressively across sessions, whereas inactive lever responding remained low, indicating reliable lever discrimination and the establishment of goal-directed drug-taking behavior (Figure 2A). Morphine intake rose in parallel with the escalation of active responding (Figure 2B). Morphine intake during the final three acquisition sessions (sessions 12–14) did not differ significantly across days (repeated-measures one-way ANOVA: $F(1.65, 18.18) = 0.64$, $p = 0.51$; $n = 12$), confirming stabilization of drug intake prior to extinction training (Figure 2C).



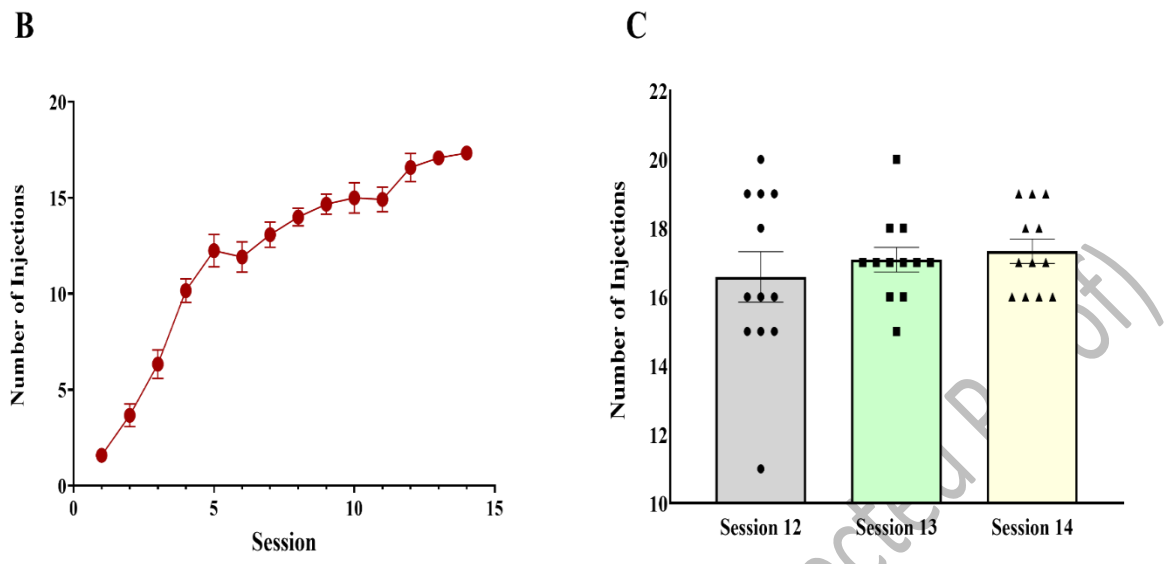


Figure 2. Acquisition and stabilization of morphine self-administration. (A) Active and inactive lever presses across 14 days of acquisition sessions. (B) Morphine intake across acquisition sessions. (C) Morphine intake during the final three acquisition sessions (days 12–14), consistent with stabilization before extinction. Data are presented as mean ± SEM (n = 12).

Extinction of morphine-seeking behavior

Active lever responding declined progressively across the 14 daily extinction sessions in both groups (Figure 3A). Two-way repeated-measures ANOVA revealed a significant main effect of session ($F(13,84) = 74.65$, $p < 0.0001$), confirming successful extinction over time. A significant main effect of treatment was also observed ($F(1,84) = 15.71$, $p < 0.0001$), indicating overall lower responding in atorvastatin-treated rats compared with saline controls. Importantly, a significant treatment × session interaction was detected ($F(13,84) = 2.50$, $p = 0.0061$), demonstrating that the rate of extinction differed between groups, with a more pronounced decline in the atorvastatin-treated animals.

To specifically assess late extinction performance, active lever presses were averaged across the final three extinction sessions (sessions 12–14) for each rat (Figure 3B). Atorvastatin-treated rats exhibited significantly lower active lever responding than saline-treated controls (Mann–Whitney U test: $U = 0$, $p = 0.0022$; $n = 6$ per group), indicating reduced morphine-seeking behavior during late extinction.

Inactive lever responding during late extinction was uniformly zero in both groups, supporting preserved response discrimination and excluding nonspecific suppression of operant behavior.

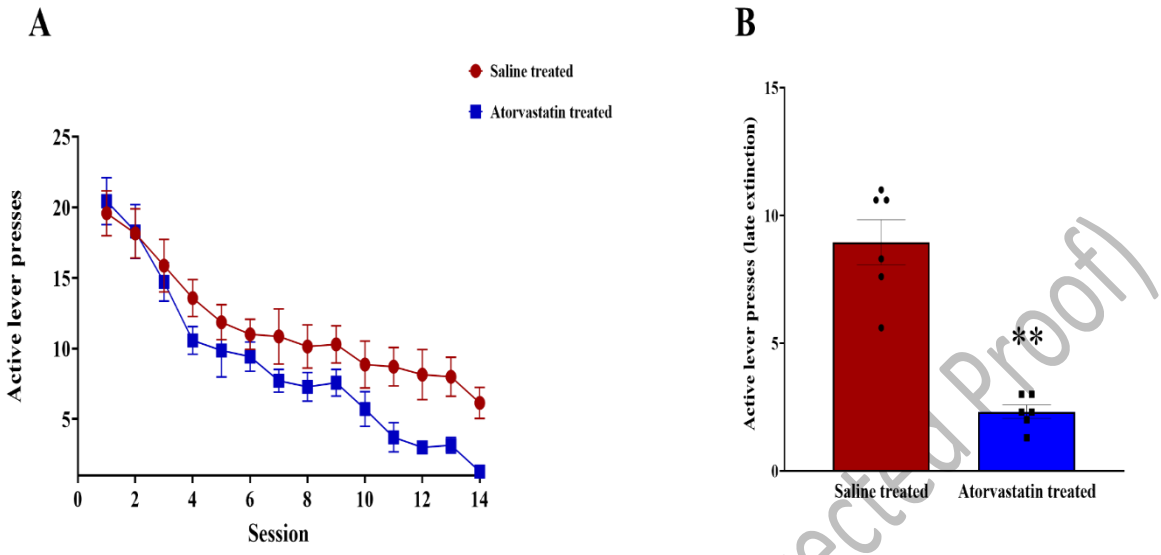


Figure 3. Atorvastatin accelerates extinction of morphine-seeking behavior and reduces late extinction responding. (A) Active lever presses across 14 daily extinction sessions in saline- and atorvastatin-treated rats. Two-way repeated-measures ANOVA revealed significant effects of session, treatment, and a treatment × session interaction (see Results for statistics). (B) Mean active lever presses averaged across the final three extinction sessions (sessions 12–14). Atorvastatin-treated rats exhibited significantly lower responding compared with saline-treated controls (Mann–Whitney U test, U = 0, p = 0.0022; r=0.83; n = 6 per group). Data are presented as mean ± SEM.

Stress-induced reinstatement of morphine seeking

Intermittent foot-shock stress elicited reinstatement-like morphine seeking. Active lever responding during the reinstatement test session was significantly lower in atorvastatin-treated rats compared with saline controls (Mann–Whitney U test: U = 0, p = 0.0022; r = 0.83; n = 6 per group; Figure 4A).

Inactive lever responding during the reinstatement session remained low overall but was modestly reduced in the atorvastatin group (Mann–Whitney U test: p = 0.0130; r = 0.74; n = 6 per group; Figure 4B).

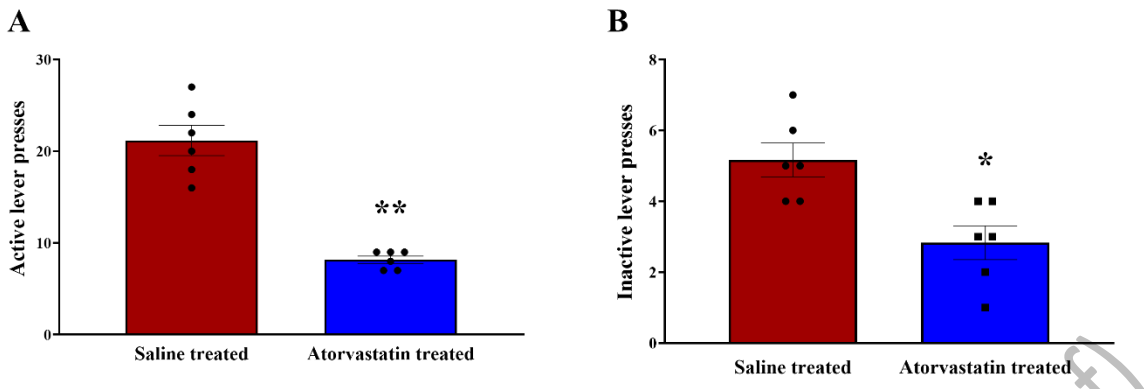


Figure 4. Stress-induced reinstatement of morphine seeking. (A) Active lever presses during the reinstatement test session following intermittent foot-shock stress. Atorvastatin-treated rats showed lower active responding than saline-treated controls (Mann–Whitney U test: U = 0, p = 0.0022; r = 0.83; n = 6 per group). (B) Inactive lever presses during the same session remained low overall but were significantly reduced in the atorvastatin-treated group compared with saline controls. (Mann–Whitney U test: p = 0.0130; r = 0.74; n = 6 per group). Data are presented as mean ± SEM

To quantify the magnitude of stress-induced reinstatement relative to extinction baseline, Δ active responding was calculated for each animal as (reinstatement − baseline), with baseline defined as the mean of the final three extinction sessions. The increase in active lever responding was significantly smaller in atorvastatin-treated rats than saline controls (Mann–Whitney U test: U = 0, p = 0.0022; r = 0.83; n = 6 per group), indicating attenuation of stress-induced relapse-like behavior (Figure 5).

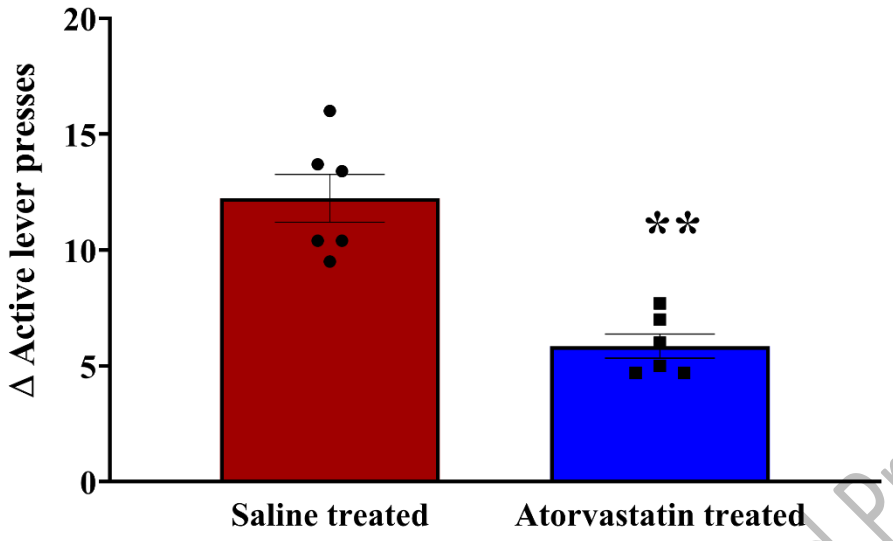


Figure 5. Attenuation of stress-induced reinstatement magnitude by atorvastatin. Δ active lever responding was calculated for each animal as the difference between active lever presses during the reinstatement session and extinction baseline (mean of extinction sessions 12–14). Atorvastatin-treated rats exhibited a significantly lower active lever responding compared with saline controls. Data are presented as mean ± SEM. (Mann–Whitney U test: U = 0, p = 0.0022; r = 0.83, n = 6 per group).

Discussion

The present study demonstrates that chronic atorvastatin treatment significantly attenuates stress-induced reinstatement of morphine seeking in an operant self-administration model. Atorvastatin markedly reduced active lever responding during the reinstatement session, whereas the reduction in inactive responding was comparatively smaller. This pattern indicates a preferential suppression of relapse-like drug-seeking behavior rather than generalized behavioral inhibition.

Stress is one of the most robust and reproducible triggers of relapse across drug classes (1,3,19). In rodents, intermittent foot-shock reliably reinstates heroin and morphine seeking following extinction (3,20,21). The reinstatement model has been instrumental in identifying neurobiological substrates of relapse vulnerability (19,20). Stress-induced reinstatement critically depends on activation of CRF systems within the extended amygdala and dysregulation of stress circuitry (22–24). The robust reinstatement observed in saline-treated rats confirms the sensitivity of this model, whereas the significant attenuation in atorvastatin-treated animals suggests modulation of stress-amplified relapse pathways.

The present findings extend the seminal work of Chauvet et al., who demonstrated that brain-penetrant statins reduce relapse-like behavior in cocaine and nicotine self-administration

paradigms. Importantly, pravastatin—poorly brain penetrant—was ineffective, implicating central rather than peripheral mechanisms (11). The current data generalize this effect to intravenous morphine self-administration, suggesting that statins modulate shared mesocorticolimbic relapse circuitry across drug classes (25,26). This cross-drug convergence strengthens the hypothesis that statins target core relapse mechanisms rather than drug-specific pharmacodynamics.

Our results also complement recent findings in morphine conditioned place preference (CPP) paradigms, in which atorvastatin facilitated extinction and reduced reinstatement-like behavior (16). While CPP reflects Pavlovian associative learning, operant self-administration more directly captures the instrumental and motivational components of drug seeking (20,27). The convergence of CPP (16) and operant reinstatement findings therefore enhances the translational relevance of statin-mediated relapse attenuation in opioid models.

Neuroimmune mechanisms provide a biologically plausible substrate for these effects. Opioids activate TLR4 signaling in microglia and initiate pro-inflammatory cascades within corticolimbic regions (5,28,29). Increasing evidence indicates that glial activation and neuroimmune signaling contribute to synaptic remodeling and relapse vulnerability (30,31). While we did not directly assess molecular markers in this behavioral study, previous studies suggest that statins may inhibit isoprenoid-dependent inflammatory signaling pathways and potentially suppress NF-κB activation within the central nervous system (32–34). Through attenuation of neuroimmune amplification, atorvastatin may stabilize corticostriatal circuitry during stress exposure, thereby reducing relapse susceptibility.

Glutamatergic dysregulation is another central mechanism underlying reinstatement (8,35). Repeated drug exposure reduces GLT-1 expression in the NAc, leading to exaggerated glutamate spillover during relapse triggers (36,37). Pharmacological restoration of GLT-1 function attenuates reinstatement across substances of abuse (9,37,38). Although GLT-1 expression was not directly measured in the present study, statins have been reported to influence synaptic plasticity and neurotrophic signaling under inflammatory conditions (34,39). Thus, improvement of glutamate homeostasis represents a plausible complementary mechanism contributing to the observed behavioral effects.

Stress-induced reinstatement further depends on CRF-dependent activation within the central amygdala and bed nucleus of the stria terminalis (22–24). Chronic stress enhances relapse vulnerability via feedforward interactions between stress hormones and excitatory transmission (22,23). Given evidence that neuroimmune activation can potentiate stress responsiveness (30), attenuation of inflammatory signaling by atorvastatin may indirectly dampen stress-related

neural sensitization and thereby reduce reinstatement magnitude. It is important to note that while the current literature heavily implicates neuroimmune signaling and disrupted glutamate homeostasis in stress-induced relapse, much of this evidence is derived from chronic stress paradigms (33,40). In the present study, reinstatement was triggered by an acute intermittent foot-shock stress. The neurobiological dynamics of acute stress differ significantly from chronic stress, particularly regarding the rapid temporal dynamics of the HPA-axis, non-genomic glucocorticoid actions, and the extent of microglial priming and neuroimmune activation (41,42). Therefore, extrapolating mechanistic pathways from chronic stress models to our acute stress-induced reinstatement paradigm must be done with caution. While atorvastatin may plausibly attenuate acute stress-induced neuroinflammation or rapidly modulate glutamatergic transmission, direct molecular investigations in acute stress models are required to confirm whether the pathways established in chronic conditions identically operate in this acute setting.

Several limitations of the present study should be acknowledged. First, while our behavioral findings demonstrate the attenuating effect of atorvastatin on stress- and prime-induced morphine seeking, a primary limitation is the lack of direct molecular and biochemical assessments. The proposed involvement of neuroimmune signaling (e.g., TLR4/NF-κB pathways) and glutamatergic modulation (e.g., GLT-1) is hypothesized based on the existing literature. Because we did not directly measure these markers, these mechanistic interpretations remain speculative. Future studies must incorporate molecular techniques to confirm these pathways directly.

Second, regarding the behavioral outcomes, although inactive lever responding was uniformly zero during extinction, it showed a modest but significant reduction in the atorvastatin group during reinstatement. Because this metric alone cannot fully rule out performance-related confounds, the lack of independent locomotor assessments (e.g., Open-field test) is a limitation of the present study. Future research should incorporate these tests to definitively exclude any generalized sedation, motor suppression, or altered stress responsivity.

Furthermore, there are important methodological constraints regarding generalizability. The present study exclusively utilized adult male rats to establish baseline pharmacological efficacy while minimizing confounding factors such as estrous cycle fluctuations. Given the well-documented sex differences in opioid addiction, withdrawal symptoms, and stress reactivity, the exclusion of female subjects restricts the broader translation of our findings. Additionally,

our operant self-administration paradigm was conducted with a relatively small sample size (n=6 per group). While sufficient to detect the primary main effects, this restricts overall statistical power. Finally, this study did not include a dose-response evaluation for atorvastatin. Future investigations utilizing larger cohorts, incorporating both male and female subjects, and evaluating multiple dose ranges are essential to fully validate and expand upon the current findings.

Finally, it has been hypothesized that statins might modulate intracellular signaling pathways that overlap with those engaged by environmental enrichment—an intervention known to reduce relapse vulnerability through the stabilization of plasticity-related circuits (11,43). However, it must be emphasized that this potential mechanistic overlap remains hypothetical. Because the present study was exclusively behavioral, we did not directly evaluate these intracellular pathways. Nevertheless, the current data suggest that pharmacological modulation of such pathways warrants further direct molecular investigation as a potential adjunctive strategy against stress-induced relapse in opioid use disorder.

In conclusion, chronic atorvastatin significantly attenuated stress-induced reinstatement of morphine seeking in an operant self-administration model. Together with prior findings in psychostimulant and nicotine paradigms (11) and opioid CPP models (16), the present study supports a model in which statins modulate core neuroimmune, glutamatergic, and stress-responsive mechanisms underlying relapse vulnerability. While these results suggest that statins warrant further investigation as adjunctive strategies for reducing stress-related relapse risk in opioid use disorder, this translational potential must be interpreted with caution. The current proof-of-concept study is limited by a small sample size (n=6 per group), the exclusive use of male subjects, and the lack of a dose-response evaluation. Future preclinical studies incorporating female subjects to assess sex differences, dose-response analyses, and larger cohorts are essential to validate these findings before advancing toward clinical applicability.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of Tarbiat Modares University, Tehran, Iran (Code: ir.modares.aec.142.034).

Funding

This study was supported by the Iran National Science Foundation (INSF), Cognitive Sciences and Technologies Council of Iran and Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

Authors' contribution

N.A.: Performing the experiments, Formal analysis, Writing original draft, Methodology. **H.A.**: Conceptualization, Methodology, Writing review and editing. **S.S.**: Conceptualization, Funding acquisition, Project administration, Supervision, Writing review and editing.

Conflict of interest statement

The authors report no conflict of interest related to this study.

References:

1. Volkow ND, Morales M. The Brain on Drugs: From Reward to Addiction. Cell. 2015 Aug 13;162(4):712–25. doi:10.1016/j.cell.2015.07.046 PubMed PMID: 26276628.
2. Sinha R. Chronic stress, drug use, and vulnerability to addiction. Ann N Y Acad Sci. 2008 Oct;1141:105–30. doi:10.1196/annals.1441.030 PubMed PMID: 18991954; PubMed Central PMCID: PMC2732004.
3. Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. Brain Res Brain Res Rev. 2000 Aug;33(1):13–33. doi:10.1016/s0165-0173(00)00024-2 PubMed PMID: 10967352.
4. Erb S, Shaham Y, Stewart J. Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology (Berl). 1996 Dec;128(4):408–12. doi:10.1007/s002130050150 PubMed PMID: 8986011.
5. Hutchinson MR, Northcutt AL, Hiranita T, Wang X, Lewis SS, Thomas J, et al. Opioid activation of toll-like receptor 4 contributes to drug reinforcement. J Neurosci Off J Soc Neurosci. 2012 Aug 15;32(33):11187–200. doi:10.1523/JNEUROSCI.0684-12.2012 PubMed PMID: 22895704; PubMed Central PMCID: PMC3454463.
6. Grace PM, Hutchinson MR, Maier SF, Watkins LR. Pathological pain and the neuroimmune interface. Nat Rev Immunol. 2014 Apr;14(4):217–31. doi:10.1038/nri3621 PubMed PMID: 24577438; PubMed Central PMCID: PMC5525062.
7. Mantsch JR, Baker DA, Francis DM, Katz ES, Hoks MA, Serge JP. Stressor- and corticotropin releasing factor-induced reinstatement and active stress-related behavioral responses are augmented following long-access cocaine self-administration by rats. Psychopharmacology (Berl). 2008 Jan;195(4):591–603. doi:10.1007/s00213-007-0950-5 PubMed PMID: 17899015; PubMed Central PMCID: PMC3888801.
8. Kalivas PW. The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci. 2009 Aug;10(8):561–72. doi:10.1038/nrn2515 PubMed PMID: 19571793.
9. Knackstedt LA, Moussawi K, Lalumiere R, Schwendt M, Klugmann M, Kalivas PW. Extinction Training after Cocaine Self-Administration Induces Glutamatergic Plasticity to Inhibit Cocaine Seeking. J Neurosci. 2010 Jun 9;30(23):7984–92. doi:10.1523/JNEUROSCI.1244-10.2010 PubMed PMID: 20534846.
10. Scofield MD, Kalivas PW. Astrocytic Dysfunction and Addiction: Consequences of Impaired Glutamate Homeostasis. Neurosci Rev J Bringing Neurobiol Neurol Psychiatry. 2014 Dec;20(6):610–22. doi:10.1177/1073858413520347 PubMed PMID: 24496610; PubMed Central PMCID: PMC4913887.
11. Chauvet C, Nicolas C, Lafay-Chebassier C, Jaber M, Thiriet N, Solinas M. Statins Reduce the Risks of Relapse to Addiction in Rats. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol. 2016 May;41(6):1588–97. doi:10.1038/npp.2015.317 PubMed PMID: 26466819; PubMed Central PMCID: PMC4832020.
12. Solinas M, Thiriet N, El Rawas R, Lardeux V, Jaber M. Environmental enrichment during early stages of life reduces the behavioral, neurochemical, and molecular effects of cocaine. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol. 2009 Apr;34(5):1102–11. doi:10.1038/npp.2008.51 PubMed PMID: 18463628.

13. Salagre E, Fernandes BS, Dodd S, Brownstein DJ, Berk M. Statins for the treatment of depression: A meta-analysis of randomized, double-blind, placebo-controlled trials. J Affect Disord. 2016 Aug;200:235–42. doi:10.1016/j.jad.2016.04.047 PubMed PMID: 27148902.
14. Nomura I, Kishi T, Ikuta T, Iwata N. Statin add-on therapy in the antipsychotic treatment of schizophrenia: A meta-analysis. Psychiatry Res. 2018 Feb;260:41–7. doi:10.1016/j.psychres.2017.11.033 PubMed PMID: 29172097.
15. Kohno M, Link J, Dennis LE, McCready H, Huckans M, Hoffman WF, et al. Neuroinflammation in addiction: A review of neuroimaging studies and potential immunotherapies. Pharmacol Biochem Behav. 2019 Apr 1;179:34–42. doi:10.1016/j.pbb.2019.01.007
16. Hashemizadeh S, Alaee E, Aghajani N, Azizi H, Semnanian S. Atorvastatin facilitates extinction and prevents reinstatement of morphine-induced conditioned place preference in rats. Biomed Pharmacother Biomedecine Pharmacother. 2024 Dec;181:117639. doi:10.1016/j.biopha.2024.117639 PubMed PMID: 39520913.
17. Ludka FK, Zomkowski ADE, Cunha MP, Dal-Cim T, Zeni ALB, Rodrigues ALS, et al. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the L-arginine-nitric oxide-cyclic guanosine monophosphate pathway and increases BDNF levels. Eur Neuropsychopharmacol J Eur Coll Neuropsychopharmacol. 2013 May;23(5):400–12. doi:10.1016/j.euroneuro.2012.05.005 PubMed PMID: 22682406.
18. Piermartiri TCB, Figueiredo CP, Rial D, Duarte FS, Bezerra SC, Mancini G, et al. Atorvastatin prevents hippocampal cell death, neuroinflammation and oxidative stress following amyloid-β(1-40) administration in mice: evidence for dissociation between cognitive deficits and neuronal damage. Exp Neurol. 2010 Dec;226(2):274–84. doi:10.1016/j.expneurol.2010.08.030 PubMed PMID: 20816828.
19. Shaham Y, Shalev U, Lu L, de Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl). 2003 Jul;168(1–2):3–20. doi:10.1007/s00213-002-1224-x PubMed PMID: 12402102.
20. Bossert JM, Marchant NJ, Calu DJ, Shaham Y. The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology (Berl). 2013 Oct;229(3):453–76. doi:10.1007/s00213-013-3120-y PubMed PMID: 23685858; PubMed Central PMCID: PMC3770775.
21. Bossert JM, Ghitza UE, Lu L, Epstein DH, Shaham Y. Neurobiology of relapse to heroin and cocaine seeking: an update and clinical implications. Eur J Pharmacol. 2005 Dec 5;526(1–3):36–50. doi:10.1016/j.ejphar.2005.09.030 PubMed PMID: 16289451.
22. Koob GF. A role for brain stress systems in addiction. Neuron. 2008 Jul 10;59(1):11–34. doi:10.1016/j.neuron.2008.06.012 PubMed PMID: 18614026; PubMed Central PMCID: PMC2748830.
23. Funk CK, O'Dell LE, Crawford EF, Koob GF. Corticotropin-releasing factor within the central nucleus of the amygdala mediates enhanced ethanol self-administration in withdrawn, ethanol-dependent rats. J Neurosci Off J Soc Neurosci. 2006 Nov 1;26(44):11324–32. doi:10.1523/JNEUROSCI.3096-06.2006 PubMed PMID: 17079660; PubMed Central PMCID: PMC6674550.
24. Erb S, Shaham Y, Stewart J. The role of corticotropin-releasing factor and corticosterone in stress- and cocaine-induced relapse to cocaine seeking in rats. J Neurosci Off J Soc Neurosci.

1998 Jul 15;18(14):5529–36. doi:10.1523/JNEUROSCI.18-14-05529.1998 PubMed PMID: 9651233; PubMed Central PMCID: PMC6793509.
25. Kalivas PW, McFarland K. Brain circuitry and the reinstatement of cocaine-seeking behavior. Psychopharmacology (Berl). 2003 Jul;168(1–2):44–56. doi:10.1007/s00213-003-1393-2 PubMed PMID: 12652346.
26. Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol. 2010 Jan;35(1):217–38. doi:10.1038/npp.2009.110 PubMed PMID: 19710631; PubMed Central PMCID: PMC2805560.
27. O'Brien CP. A range of research-based pharmacotherapies for addiction. Science. 1997 Oct 3;278(5335):66–70. doi:10.1126/science.278.5335.66 PubMed PMID: 9311929.
28. Al N, Mr H, X W, Mv B, T H, Ta C, et al. DAT isn't all that: cocaine reward and reinforcement require Toll-like receptor 4 signaling. Mol Psychiatry. 2015 Dec;20(12). doi:10.1038/mp.2014.177 PubMed PMID: 25644383.
29. Watkins LR, Hutchinson MR, Rice KC, Maier SF. The "toll" of opioid-induced glial activation: improving the clinical efficacy of opioids by targeting glia. Trends Pharmacol Sci. 2009 Nov;30(11):581–91. doi:10.1016/j.tips.2009.08.002 PubMed PMID: 19762094; PubMed Central PMCID: PMC2783351.
30. Crews FT, Vetreno RP. Neuroimmune basis of alcoholic brain damage. Int Rev Neurobiol. 2014;118:315–57. doi:10.1016/B978-0-12-801284-0.00010-5 PubMed PMID: 25175868; PubMed Central PMCID: PMC5765863.
31. Lacagnina MJ, Rivera PD, Bilbo SD. Glial and Neuroimmune Mechanisms as Critical Modulators of Drug Use and Abuse. Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol. 2017 Jan;42(1):156–77. doi:10.1038/npp.2016.121 PubMed PMID: 27402494; PubMed Central PMCID: PMC5143481.
32. van der Most PJ, Dolga AM, Nijholt IM, Luiten PGM, Eisel ULM. Statins: mechanisms of neuroprotection. Prog Neurobiol. 2009 May;88(1):64–75. doi:10.1016/j.pneurobio.2009.02.002 PubMed PMID: 19428962.
33. Wood WG, Mőller WE, Eckert GP. Statins and neuroprotection: basic pharmacology needed. Mol Neurobiol. 2014 Aug;50(1):214–20. doi:10.1007/s12035-014-8647-3 PubMed PMID: 24474514.
34. Liao JK, Laufs U. Pleiotropic effects of statins. Annu Rev Pharmacol Toxicol. 2005;45:89–118. doi:10.1146/annurev.pharmtox.45.120403.095748 PubMed PMID: 15822172; PubMed Central PMCID: PMC2694580.
35. Knackstedt LA, Kalivas PW. Glutamate and reinstatement. Curr Opin Pharmacol. 2009 Feb;9(1):59–64. doi:10.1016/j.coph.2008.12.003 PubMed PMID: 19157986; PubMed Central PMCID: PMC2667702.
36. Reissner KJ, Kalivas PW. Using glutamate homeostasis as a target for treating addictive disorders. Behav Pharmacol. 2010 Sep;21(5–6):514–22. doi:10.1097/FBP.0b013e32833d41b2 PubMed PMID: 20634691; PubMed Central PMCID: PMC2932669.
37. Das SC, Yamamoto BK, Hristov AM, Sari Y. Ceftriaxone attenuates ethanol drinking and restores extracellular glutamate concentration through normalization of GLT-1 in nucleus accumbens of male alcohol-preferring rats. Neuropharmacology. 2015 Oct;97:67–74. doi:10.1016/j.neuropharm.2015.05.009 PubMed PMID: 26002627; PubMed Central PMCID: PMC4537362.

38. Reissner KJ, Gipson CD, Tran PK, Knackstedt LA, Scofield MD, Kalivas PW. Glutamate transporter GLT-1 mediates N-acetylcysteine inhibition of cocaine reinstatement. Addict Biol. 2015 Mar;20(2):316–23. doi:10.1111/adb.12127 PubMed PMID: 24612076; PubMed Central PMCID: PMC4437505.
39. Vaughan CJ, Delanty N. Neuroprotective properties of statins in cerebral ischemia and stroke. Stroke. 1999 Sep;30(9):1969–73. doi:10.1161/01.str.30.9.1969 PubMed PMID: 10471452.
40. Popoli M, Yan Z, McEwen B, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci. 2011 Nov 30;13(1):22–37. doi:10.1038/nrn3138 PubMed PMID: 22127301; PubMed Central PMCID: PMC3645314.
41. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev. 2007 Jul;87(3):873–904. doi:10.1152/physrev.00041.2006 PubMed PMID: 17615391.
42. Dhabhar FS. Effects of stress on immune function: the good, the bad, and the beautiful. Immunol Res. 2014 May;58(2–3):193–210. doi:10.1007/s12026-014-8517-0 PubMed PMID: 24798553.
43. Solinas M, Thiriet N, Chauvet C, Jaber M. Prevention and treatment of drug addiction by environmental enrichment. Prog Neurobiol. 2010 Dec;92(4):572–92. doi:10.1016/j.pneurobio.2010.08.002 PubMed PMID: 20713127.