Original Article

# Effect of repeated morphine withdrawal on spatial learning, memory and serum cortisol level in mice

Mahdieh Matinfar, Mahsa Masjedi Esfahani, Neda Aslany, Seyyed Hamid Reza Davoodi, Pouya Parsaei[1], Ghasem Zarei[2], Parham Reisi[2,3]

Applied Physiology Research Center, [2]Department of Physiology, School of Medicine, [3]Biosensor Research Center, Isfahan University of Medical Sciences, Isfahan, [1]Young Researchers Club, Shahrekord Branch Islamic Azad University, Shahrekord, Iran

## Abstract

**Background:** One of the serious problems that opioid addicted people are facing is repeated withdrawal syndrome that is accompanying with a significant stress load for addicts. Therefore, the aim of this study was to evaluate the effects of repeated withdrawal on spatial learning, memory and serum cortisol levels in morphine-dependent mice.

**Materials and Methods:** Male NMRI mice received morphine as daily increasing doses for 3 days. After that, the mice underwent one time or repeated spontaneous or pharmacologic (naloxone-precipitated) withdrawal. Then spatial learning and memory were investigated by morris water maze test, and at the end trunk blood samples were collected for measurement of serum cortisol levels.

**Results:** The results showed that only repeated spontaneous withdrawal significantly increases escape latency ($P < 0.05$), and in other models of withdrawal, spatial learning and memory were intact. The results of probe trial were intact in all groups. Radioimmunoassay showed that serum cortisol levels were increased significantly in all models of withdrawal ($P < 0.05$ and $P < 0.01$) except the repeated spontaneous withdrawal.

**Conclusion:** The results showed that short periods of withdrawal syndrome can increase serum cortisol levels; however they do not affect spatial learning and memory. Nevertheless, repeated spontaneous withdrawal can make learning slow.

**Key Words:** Cortisol, morphine, naloxone, spatial learning and memory, withdrawal syndrome

**Address for correspondence:**
Dr. Parham Reisi, Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: p_reisi@med.mui.ac.ir
**Received:** 31.07.2012, **Accepted:** 01.09.2012

## INTRODUCTION

Morphine is a potent analgesic drug, which is obtained from the dark poppy and cause severe dependence in human central nervous system. Addiction to morphine and its derivatives is regarded as one of the most serious problems of today's society, which imposes a high psycho-socioeconomic burden not only on its sufferers but also on basic structures of the society.[1] In addition, morphine addiction can be considered a chronic disease associated with significant physiological and psychological changes.[1]

One of the serious problems that opioid addicted people are facing is repeated withdrawal syndrome.

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.advbiores.net |
| | DOI: 10.4103/2277-9175.120868 |



**How to cite this article:** Matinfar M, Esfahani MM, Aslany N, Davoodi SH, Parsaei P, Zarei G, *et al.* Effect of repeated morphine withdrawal on spatial learning, memory and serum cortisol level in mice. Adv Biomed Res 2013;2:80.

Since, most of the drug addicts cannot get sufficient dosage at the right time because of financial problems, therefore, they frequently suffer from withdrawal syndrome. Also, the misconception which is pretty popular among the concerned family of the addicts is imprisoning the addicted person frequently in order to force him to quit; this can also cause repeated withdrawal syndrome conditions for the addict. It has been demonstrated that these sudden repeated cessations are accompanying with a significant stress load for addicts.[2] Stress-induced changes results in the activation of the hypothalamo-pituitary-adrenal (HPA) axis. This activation leads to a sequence of events from enhanced secretion of corticotropin-releasing hormone (CRH) from the hypothalamus into the portal vessels, through increased splicing of adrenocorticotropin (ACTH) from pro-opiomelanocortin (POMC) precursor in the pituitary, to elevated synthesis and release of glucocorticoids.[3] Studies indicated that increase in brain cortisol concentration may play an important role in memory impairment following morphine withdrawal.[4,5]

Given that studies have shown that repeated morphine withdrawals cause a chronic and constant condition of stress[6] and also it has been suggested that HPA axis activity in the first-time addiction is significantly different comparing with repeated addictions.[7] Therefore, changes in learning and memory in these conditions may also vary.

Due to the fact that the possibility of returning patients to use morphine and repeated withdrawal is high[8] and also because learning and memory has not been studied yet in this situation, so the aim of this study was to evaluate the effects of short periods of one time and repeated spontaneous and pharmacologic (naloxone-precipitated) withdrawal on learning process, memory performance and serum cortisol levels (as the major stress hormone) in morphine-dependent mice. Notably, this study can be helpful to find a correct understanding of the principles of addiction treatment and associated complications.

## MATERIALS AND METHODS

### Subjects

Male NMRI mice (3 to 4 months old), were purchased from the breeding colony of the Pasteur institute (Tehran, Iran). Animals were housed in transparent cages and maintained on a 12:12-h light/dark cycle and constant temperature (20 ± 1°C). Tap water and standard mouse chow were available *ad libitum*. The Ethic Committee for Animal Experiments at Isfahan University approved the study and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996.

To create a dependence on morphine in mice, morphine was injected daily with increasing doses (10-20-40 mg/kg, 3 times per day for 3 days; subcutaneous injection; Darou pakhsh co., Iran).[6] Animals in the control group received equal volume of saline at the same time.

This study was conducted during four different sections. Each section contains two parts: A behavioral study and measurement of serum cortical levels.

Section one: Once naloxone precipitated withdrawal (one time pharmacologic withdrawal).

Experimental groups were, the saline, the saline + naloxone and the morphine + naloxane (in this group after dependence they only receive single injection of morphine and an injection of naloxone). After development of dependence during the first 3 days, the animals received 40 mg/kg morphine at the fourth day of the experiment and 3 h after the morphine injection they received a single injection of naloxone (10 mg/kg; intraperitoneal injection; Tolid Daru Co., Iran) as described above. The saline groups had treated with saline at the same times. Behavioral study begun 21 h after naloxone injection (24 h after morphine injection) and the mice, which were candidates for serum cortisol measurements were decapitated 20 min after naloxone injection.[5,6]

Section two: Repeated naloxone precipitated withdrawal (repeated pharmacologic withdrawal).

Experimental groups were, the saline, the saline + naloxone and the morphine + naloxane (in this group after dependence they received several injections of morphine and naloxone). After development of dependence during the first 3 days, the animals received 40 mg/kg morphine injection during fourth to fourteen days of the experiment and 3 h after each morphine injection, they received an injection of naloxone. The saline groups had treated with saline at the same times. Behavioral study begun 21 h after the last naloxone injection (24 h after the last morphine injection) and the mice, which were candidates for serum cortisol measurements were decapitated 20 min after the last naloxone injection.

Section three: Once spontaneous withdrawal.

Experimental groups were the saline and the

morphine + spontaneous withdrawal (Morphine + SW) (the animals in this group only experienced one episode of withdrawal after dependence). After development of dependence during the first 3 days, the animals received 40 mg/kg morphine injection at the fourth day of the experiment. The saline groups had treated with saline at the same times. Behavioral study begun 72 h after morphine injection and the mice, which were candidates for serum cortisol measurements were decapitated 4 h after morphine injection.

Section four: Repeated spontaneous withdrawal.

Experimental groups were, the saline and the morphine + spontaneous withdrawal (Morphine + SW) (the animals in this group experienced several episodes of withdrawal after dependence). After development of dependence during the first 3 days, the animals received 40 mg/kg morphine injection at the fourth, nineth and twelfth days of the experiment. The saline group had treated with saline at the same times. Behavioral study begun 72 h after the last morphine injection and the mice, which were candidates for serum cortisol measurements were decapitated 4 h after the last morphine injection.

### Blood sampling and cortisol assay

Mice were lightly anesthetized with diethyl ether inhalation and then rapidly decapitated; trunk blood samples were collected. The elapsed time between the start of ether exposure and decapitation was almost 3-4 min. After centrifugation of blood samples, serum was extracted and transferred to small-capped vials and stored frozen (−20°C) until analyzed.

Serum cortisol level was measured by radioimmunoassay (RIA). The IMMUNOTECH CORTISOL assay kit (Immunotech, France) was used for the quantitative determination of cortisol.

### Morris water maze test (MWM)

The circular tank (100 cm in diameter) was filled with water (21 ± 1°C) made opaque and was surrounded by a variety of extra-maze cues. The tank was divided into four quadrants, and four start positions were located at the interactions of the quadrants. Data were recorded using custom software (Radiab1). In the spatial acquisition phase, the mice learned to find a submerged platform using extra-maze cues. A transparent lucid platform (10 × 10 cm) was submerged 2 cm underneath the water in north-east quadrant of the tank, where it remained for all spatial trials. Each mouse participated in 16 trials, which were organized into daily block of 4 trials (1 trial/start position within a block) for 4 consecutive days. For each trial, the mouse was given a maximum time of 60 seconds to locate the platform, after which it remained there for 30 seconds; If the mouse did not locate the platform within 60 seconds, the experimenter guided the mouse toward its location. The next trial started immediately after removal from the platform. Escape latencies (s) was recorded.

In the retention phase, 1 day after the spatial acquisition phase, 60-s probe trial was conducted to examine how well the rats had learned the exact location of the platform. During this trial, the platform was removed from the tank. The quadrant time (percent time spent in the training quadrant) was recorded during the probe trial. To test possible deficits in sensory-motor processes, mice were tested in the water maze with a visible platform on a new location on the final day of training.[9]

### Statistical analysis

Data were analyzed using the SPSS 16 for Windows. In Morris water maze test, escape latencies were analyzed statistically by two way repeated measures ANOVA followed by LSD test for between-subjects differences and within effects across the blocks. The probe trial data for percentage of time spent in each of the 4 zones were analyzed by multivariate ANOVA followed by LSD test. The serum cortical levels were analyzed by *t*-test and one-way analysis of variance (ANOVA) and tukey test used for the post-test. The significant level was set at $P < 0.05$. Results are given as mean ± S.E.M.

## RESULTS

Phase 1: All mice showed a reduction in escape latencies (BLOCK effect, $F(3,90) = 16.57$, $P < 0.001$; Figure 1a) across blocks of trials, indicating spatial acquisition. For escape latencies, there was not any difference between the groups ($F(3, 30) = 2.05$, $P = 0.15$; Figure 1a).

For the results of probe trial as measured by the mean percentage (%) time spent in each of the 4 zones, between group comparison indicated that all groups spent same time ($P = 0.155$) in zone 1, where the platform was previously located [Figure 1b].

The serum cortisol level at the end of experiments was significantly increased in the morphine + naloxone group comparing to the saline ($P < 0.01$) and saline + naloxone ($P < 0.01$) groups. However, there were no differences between the saline and saline + naloxone groups [Table 1].

Phase 2: All mice showed a reduction in escape

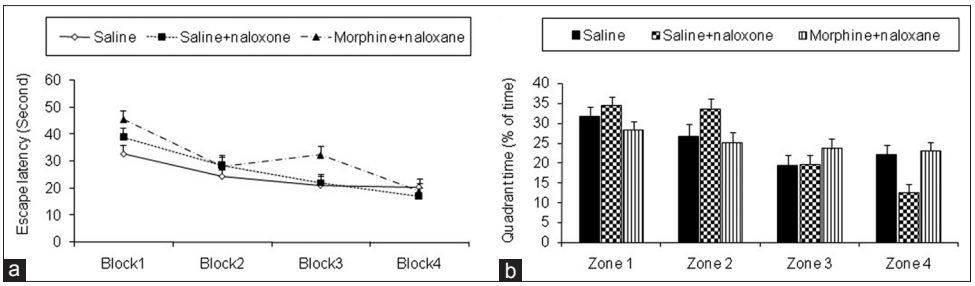


**Figure 1:** Effects of one time pharmacologic withdrawal on performance during the spatial acquisition and the probe trial of morris water maze test. The escape latency (a) Each point that represents the block is mean±SEM of 4 swims, and the quadrant time, as measured by mean percentage (%) time spent in each of the 4 zones, 1 day after spatial acquisition phase (b) For latency, lower numbers indicate better performance (*n*=11)

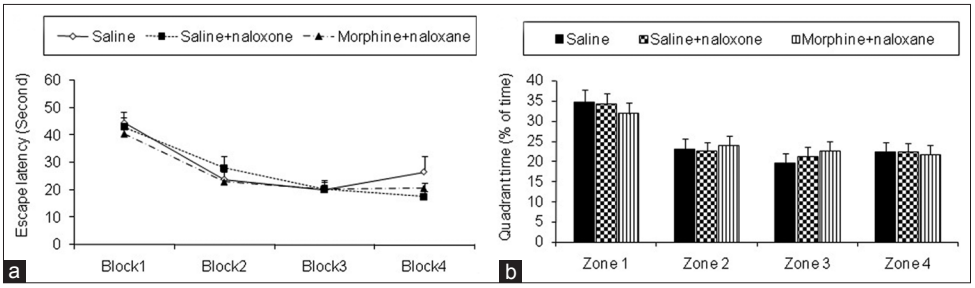


**Figure 2:** Effects of repeated pharmacologic withdrawal on performance during the spatial acquisition and the probe trial of morris water maze test. The escape latency (a) Each point that represents the block is mean ± SEM of 4 swims, and the quadrant time, as measured by mean percentage (%) time spent in each of the 4 zones, 1 day after spatial acquisition phase (b) For latency, lower numbers indicate better performance (*n*=10-11)

**Table 1: Effects of one time and repeated pharmacologic and spontaneous withdrawal on serum cortisol levels in addicted mice (**P*<0.05 and ***P*<0.01 with respect to the saline group of that part, ††*P*<0.001 with respect to the saline+naloxone group, *n*=7) (SW, Spontaneous withdrawal)**

| | Pharmacologic withdrawal | | | Spontaneous withdrawal | |
|---|---|---|---|---|---|
| | Saline | Saline+ naloxone | Morphine+ naloxone | Saline | Morphine+ SW |
| One time | 3.96±0.84 | 5.38±1.25 | 8.76±1**†† | 2.97±0.41 | 4.05±0.39* |
| Repeated | 2.37±0.29 | 2.47±0.33 | 5.08±0.69**†† | 1.78±0.32 | 2.33±0.5 |

latencies (BLOCK effect, F (3,87) = 21.77, $P < 0.001$; Figure 2a) across blocks of trials, indicating spatial acquisition. For escape latencies, there was not any difference between the groups (F (2,29) = 1.95, $P = 0.16$; Figure 2a).

For the results of probe trial as measured by the mean percentage (%) time spent in each of the four zones, between group comparison indicated that all groups spent same time ($P = 0.74$) in zone 1, where the platform was previously located [Figure 2b].

The serum cortisol levels at the end of experiments was significantly increased in the morphine + naloxone group comparing to the saline ($P < 0.01$) and saline + naloxone ($P < 0.01$) groups. However, there were no differences between the saline and saline + naloxone groups [Table 1].

Phase 3: All mice showed a reduction in escape latencies (BLOCK effect, F (3,60) = 9.05, $P < 0.001$; Figure 3a) across blocks of trials, indicating spatial acquisition. For escape latencies, there was not any difference between the groups (F (1,20) = 1.27, $P = 0.273$; Figure 3a).

For the results of probe trial as measured by the mean percentage (%) time spent in each of the 4 zones, between group comparison indicated that all groups spent same time ($P = 0.2$) in zone 1, where the platform was previously located [Figure 3b].

The serum cortisol levels at the end of experiments was significantly increased in the morphine + SW group comparing to the saline ($P < 0.05$) [Table 1].

Phase 4: All mice showed a reduction in escape latencies (BLOCK effect, F (3,57) = 23.73, $P < 0.001$; Figure 4a) across blocks of trials, indicating spatial acquisition. However, the morphine + SW group found the platform slower than the saline group (31.88 ± 1.88 s and 26.1 ± 1.8 s, respectively; F (1,19)= 4.97, $P < 0.05$; Figure 4a).

For the results of probe trial as measured by the mean percentage (%) time spent in each of the 4 zones, between group comparison indicated that all groups spent same time ($P = 0.51$) in zone 1, where the platform was previously located [Figure 4b].

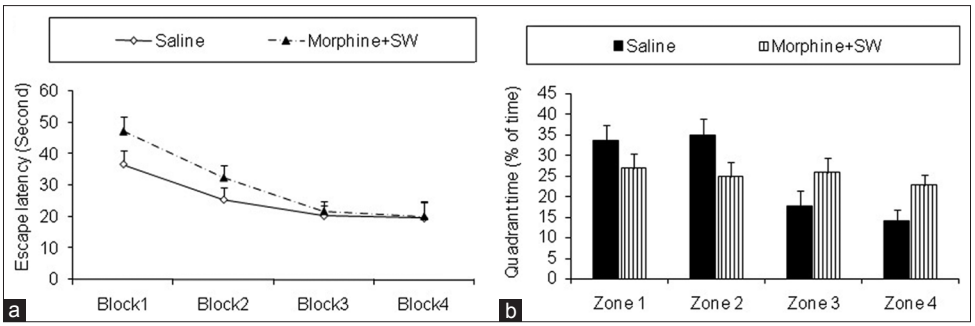


**Figure 3:** Effects of one time spontaneous withdrawal on performance during the spatial acquisition and the probe trial of morris water maze test. The escape latency (a) Each point that represents the block is mean ± SEM of 4 swims, and the quadrant time, as measured by mean percentage (%) time spent in each of the 4 zones, 1 day after spatial acquisition phase (b) For latency, lower numbers indicate better performance (*n*=11)

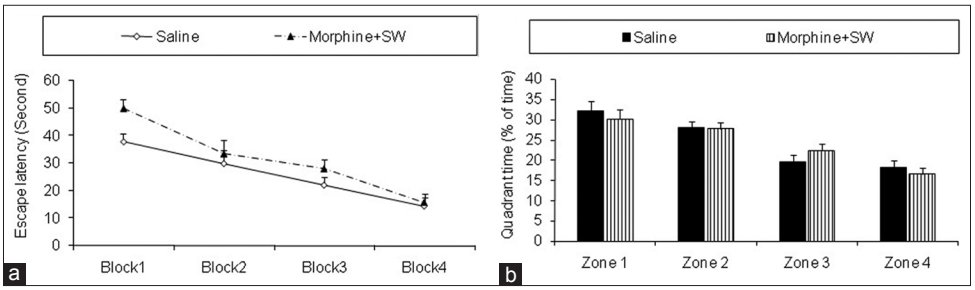


**Figure 4:** Effects of repeated spontaneous withdrawal on performance during the spatial acquisition and the probe trial of morris water maze test. The escape latency (a) Each point that represents the block is mean ± SEM of 4 swims, and the quadrant time, as measured by mean percentage (%) time spent in each of the 4 zones, 1 day after spatial acquisition phase (b) For latency, lower numbers indicate better performance (*n*=10-11)

The serum cortisol levels had no significant differences between the groups at the end of experiments ($P = 0.1$) [Table 1].

## DISCUSSION

Although, numerous studies has been done on the role of opiate system in tasks aimed at measurement of cognitive behavior, but the role of morphine dependence and withdrawal is still remained controversial.[10-12] A lot of these studies have been concentrated on the effects of chronic morphine usage on cognitive functions;[11] but as we know one of the most serious and common challenges, which the majority of drug addicts dealt with these days is repeated short or long periods of drug withdrawal due to financial problems or forced withdrawal. It has been demonstrated that these sudden repeated cessations are accompanying with a significant stress load for addicts.[2] According to what we said and as the review of existing evidences shows that prolonged exposure to stress exerts destructive effects on learning and memory,[13,14] the present study evaluated the effect of repeated short periods of morphine withdrawal on learning process, memory performance and serum cortisol concentrations (as the major stress hormone) in mice.

Just as previous studies has shown, one time and repeated naloxone-precipitated withdrawal and also one time spontaneous withdrawal were associated with significant increase in serum glucocorticoids concentrations compared in controls.[5,6,15] Since the elapsed time between ether exposure and decapitation was below 5 min, this increase in cortisol level was considered to be independent from anesthetic effects of ether.[16] As it has been expected and in agreement with previous studies, changes observed in the serum cortisol concentrations of naloxone precipitated withdrawal groups were more pronounced than spontaneous withdrawal ones.[5,6]

Our results showed that learning performance and reference memory of the first three phases have not shown any significant difference compared to their control groups. But the data from the fourth phase of the study was in part different from the others: Repeated spontaneous withdrawal in morphine dependent mice was associated with slowed acquisition of water maze task, yet all the mice finally learnt and though the eventual memory remained intact. According to Rabbani *et al.* learning and memory impairment after morphine withdrawal is in part due to increased corticosterone concentration.[5]

## CONCLUSION

In our study, although, we have the least increase in serum cortisol levels in repeated spontaneous

withdrawal group, but the most significant cognitive dysfunctions appeared in this group. This discrepancy can be justified by several explanations.

First, different methods (i.e., route of morphine and the amount used, time, learning evaluation methods, etc) have been used in this study compared with the previous ones and this can be responsible for the results. It has been shown that different methods of evaluation and circumstances are important in how stress affects learning and memory.[13] Besides the last studies evaluated the passive or recognition and not reference memory.[5,12,17]

Second, several evidences becomes available, which shows that chronic morphine exposure is associated with tolerance development to its ACTH and glucocorticoids elevating effects.[7,18-20] According to Zenela *et al.*, none of the different types of morphine addiction regimens and withdrawal methods were associated with serum corticosterone levels significantly other than naloxane-percipitated withdrawal. Repeated spontaneous withdrawal (just treated like our withdrawal group in phase four) failed to cause a significant increase in serum corticosterone.[6]

Third, we just seared the serum cortisol level of the repeated spontaneous group 4 h after the last morphine injection, but we had no control groups to evaluate cortisol during each time of withdrawal. It may be possible that serum cortisol became elevated after second or third time of withdrawal to the amounts even higher than of the naloxone-precipitated group. Researchers have revealed that glucocorticoids exert their actions via intracellular receptors, which regulate the transcription of target genes and this makes them slow in onset of biological actions, despite their persistent and long lasting actions.[21] Thus, we can see their effects even though they had already resolved from the body. Furthermore, the mechanisms that corticosterones use to exert their effects on learning and memory are very complex. Okuda *et al.* have shown that glucocorticoids impaired short term memory performance while enhance 24 h retention performance in rats.[22]

Additionally, glucocorticoids have different receptors. Type I receptors are involved in the agonistic interaction between corticosteroid secretion and opioid mediated behaviors, while type II receptors are related with the inhibitory effects of glucocorticoids on some behavioral effects of morphine such as analgesic and cataleptic effects.[23-25] As we have already said repeated spontaneous morphine withdrawal leads to resistance and tolerance to glucocorticoids and ACTH elevating effects of morphine[7,18-20] and though resulted in a stable and pretty low serum glucocorticoids levels, which may induce morphine analgesic and cataleptic effects by removing the inhibition exerted by glucocorticoids.

The other fact is that morphine affects learning and memory in different manners other than its glucocorticoids mediated effects. Changing neuron plasticity in various regions of brain including hippocampus, ventral tegmental area, nucleus accumbens and prefrontal cortex,[13,26-29] and interactions on $\alpha_2$ adrenoreceptors[30] and inhibition in central cholinergic activity[31] are some of the recognized mechanisms of morphine action on cognitive functions. On the other hand, the researches have shown that these morphine effects can be reversed by naloxone.[10,17,31,32]

If proven by further researches, these results can be used to introduce a true method of drug withdrawal accompanying with the least possible impairment on learning and memory of the addict patients. It can also be a motivation for further researches of course with more detailed methods using complementary histological and molecular studies in this arena, which finally can help us to find the exact mechanism through which morphine and its withdrawal exert their destructive effects on learning and memory.

## ACKNOWLEDGMENTS

This research was supported by Applied Physiology Research Center of Isfahan University of Medical Sciences, Isfahan, Iran.

## REFERENCES

1. Martin WR, Fraser HF. A comparative study of physiological and subjective effects of heroin and morphine administered intravenously in postaddicts. J Pharmacol Exp Ther 1961;133:388-99.
2. Deroche V, Piazza PV, Casolini P, Le MM, Simon H. Sensitization to the psychomotor effects of amphetamine and morphine induced by food restriction depends on corticosterone secretion. Brain Res 1993;611:352-6.
3. Morley JE. The endocrinology of the opiates and opioid peptides. Metabolism 1981;30:195-209.
4. Castellano C, Pavone F. Dose-and strain-dependent effects of dermorphin and [D-Ala2-D-Leu5] enkephalin on passive avoidance behavior in mice. Behav Neurosci 1985;99:1120-7.
5. Rabbani M, Hajhashemi V, Mesripour A. Increase in brain corticosterone concentration and recognition memory impairment following morphine withdrawal in mice. Stress 2009;12:451-6.
6. Zelena D, Barna I, Mlynarik M, Gupta OP, Jezova D, Makara GB. Stress symptoms induced by repeated morphine withdrawal in comparison to other chronic stress models in mice. Neuroendocrinology 2005;81:205-15.
7. Buckingham JC, Cooper TA. Differences in hypothalamo-pituitary-adrenocortical activity in the rat after acute and prolonged treatment with morphine. Neuroendocrinology 1984;38:411-7.
8. Schwartz AS, Marchok PL. Persistence of relapse of morphine-seeking behavior in rats: The relative role of certain biological variables. Psychopharmacologia 1976;47:149-52.
9. Han RW, Yin XQ, Chang M, Peng YL, Li W, Wang R. Neuropeptide S

facilitates spatial memory and mitigates spatial memory impairment induced by N-methyl-D-aspartate receptor antagonist in mice. Neurosci Lett 2009;455:74-7.
10. McNamara RK, Skelton RW. Pretraining morphine impairs acquisition and performance in the Morris water maze: Motivation reduction rather than amnesia. Psychobiology 1991;19:313-22.
11. Miladi GH, Rashidy-Pour A, Fathollahi Y. Effects of morphine dependence on the performance of rats in reference and working versions of the water maze. Physiol Behav 2008;93:622-7.
12. Pourmotabbed A, Tahmasian M, Shahi M, Karami Darabkhani H, Fathollahi Y. Facilitating effects of morphine dependence on spatial learning and memory in rat. DARU 2007;15:156-61.
13. Conrad CD. A critical review of chronic stress effects on spatial learning and memory. Prog Neuropsychopharmacol Biol Psychiatry 2010;34:742-55.
14. McEwen BS, Sapolsky RM. Stress and cognitive function. Curr Opin Neurobiol 1995;5:205-16.
15. Houshyar H, Manalo S, Dallman MF. Time-dependent alterations in mRNA expression of brain neuropeptides regulating energy balance and hypothalamo-pituitary-adrenal activity after withdrawal from intermittent morphine treatment. J Neurosci 2004;24:9414-24.
16. Glowa JR. Behavioral and neuroendocrine effects of diethyl ether exposure in the mouse. Neurotoxicol Teratol 1993;15:215-21.
17. Castellano C, Pavone F, Puglisi AS. Morphine and memory in DBA/2 mice: Effects of stress and of prior experience. Behav Brain Res 1984;11:3-10.
18. Alcaraz C, Vargas ML, Milanes MV. Chronic naloxone-induced supersensitivity affects neither tolerance to nor physical dependence on morphine at hypothalamus-pituitary-adrenocortical axis. Neuropeptides 1996;30:29-36.
19. Ignar DM, Kuhn CM. Effects of specific mu and kappa opiate tolerance and abstinence on hypothalamo-pituitary-adrenal axis secretion in the rat. J Pharmacol Exp Ther 1990;255:1287-95.
20. Kuki M. Hypothalamo-pituitary-adrenocortical (HPA) function in the morphine dependent rat (author's transl). Nihon Yakurigaku Zasshi 1979;75:215-25.
21. Buckingham JC. Glucocorticoids: Exemplars of multi-tasking. Br J Pharmacol 2006;147:S258-68.
22. Okuda S, Roozendaal B, McGaugh JL. Glucocorticoid effects on object recognition memory require training-associated emotional arousal. Proc Natl Acad Sci U S A 2004;101:853-8.
23. Holaday JW, Law PY, Loh HH, Li CH. Adrenal steroids indirectly modulate morphine and beta-endorphin effects. J Pharmacol Exp Ther 1979;208:176-83.
24. MacLennan AJ, Drugan RC, Hyson RL, Maier SF, Madden J, Barchas JD. Corticosterone: A critical factor in an opioid form of stress-induced analgesia. Science 1982;215:1530-2.
25. Ratka A, Veldhuis HD, De Kloet ER. Corticosteroid effects on morphine-induced antinociception as a function of two types of corticosteroid receptors in brain. Neuropharmacology 1988;27:15-21.
26. Lu G, Zhou QX, Kang S, Li QL, Zhao LC, Chen JD, et al. Chronic morphine treatment impaired hippocampal long-term potentiation and spatial memory via accumulation of extracellular adenosine acting on adenosine A1 receptors. J Neurosci 2010;30:5058-70.
27. Nestler EJ. Neurobiology. Total recall-the memory of addiction. Science 2001;292:2266-7.
28. Robbins TW, Everitt BJ. Drug addiction: Bad habits add up. Nature 1999;398:567-70.
29. Williams JT, Christie MJ, Manzoni O. Cellular and synaptic adaptations mediating opioid dependence. Physiol Rev 2001;81:299-343.
30. Homayoun H, Khavandgar S, Zarrindast MR. Morphine state-dependent learning: Interactions with alpha2-adrenoceptors and acute stress. Behav Pharmacol 2003;14:41-8.
31. Li Z, Wu CF, Pei G, Xu NJ. Reversal of morphine-induced memory impairment in mice by withdrawal in Morris water maze: Possible involvement of cholinergic system. Pharmacol Biochem Behav 2001;68:507-13.
32. Mauk MD, Warren JT, Thompson RF. Selective, naloxone-reversible morphine depression of learned behavioral and hippocampal responses. Science 1982;216:434-6.

**Source of Support:** Nil, **Conflict of Interest:** None declared.