

Effects of Venlafaxine & Methadone Alone and in Combination with Spontaneous Morphine withdrawal Syndrome & Pain Sensation in Rats

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ABSTRACT

Introduction: Methadone has been used as a drug to detoxify opioid tolerance. Naloxane precipitated morphine withdrawal behaviours were attenuated by venlafaxine as an antidepressant. On the contrary, after detoxifying the opioids, spontaneous withdrawal syndrome may occur with pain sensitivity. Therefore the present study aimed to examine the effects of chronic methadone (70 mg/kg, in drinking water, 7 days), venlafaxine (80 mg/kg/day, intraperitoneally, 7 days) and their combinations with the spontaneous morphine withdrawal syndrome and pain sensitivity.

Methods: Twenty eight young male Sprague-Dawley rats were randomly divided into 4 groups: control, venlafaxine treated, methadone treated and venlafaxine + methadone treated. Morphine sulfate (10 mg/kg/day, subcutaneously, 4 days) was injected to all animals. Then primary withdrawal behaviours and tail flick test were performed. The test was then followed by methadone or its vehicle administration. Second intervention was venlafaxine or its vehicle injection. Then final withdrawal behaviors and tail flick test were performed.

Results: Combination of chronic methadone substitution and venlafaxine administration, significantly reduced freezing behavior of spontaneous morphine withdrawal syndrome ($P < 0.01$, $379 \pm 144\%$). Chronic methadone administration ($P < 0.05$, $35 \pm 8\%$ difference with venlafaxine treated group) induced hyperalgesia. A positive correlation ($P = 0.001$, $+63\%$) was observed between the animals final freezing scores and their response latencies to the painful stimulus.

Discussion: Combination of chronic methadone and venlafaxine administrations reduces freezing withdrawal behavior. Further investigations on analgesic interventions are needed to overcome this hyperalgesia.

Key Words:

Morphine Withdrawal Syndrome, Methadone, Venlafaxine, Pain, Rat

1. Introduction

O

piate addiction is a multidimensional phenomenon with sophisticated reasons and results (Lu et al., 2001). Opiates are potentially addictive and produce highly aversive states of withdrawal syndrome when they are abruptly discontinued (Bishop et al., 2011).

One of these states is pain sensitivity (Bie et al., 2005).

As a treatment for opioid addiction, slow tapering with temporary substitution of long-acting opioids, can attenuate withdrawal syndrome (Amato et al., 2013). It has been reported that the behavioral signs induced by abruptly discontinuing of the chronic methadone use are moderate; on the contrary, these behavioral signs are significantly more severe by chronic administration of morphine (Enquist et al., 2012).

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Opioid rotation might improve analgesia (Trescot et al., 2008). In Contrary, more pain sensitivity has been reported in opioid addicts who previously underwent methadone maintenance (Silverman, 2009).

Venlafaxine is an antidepressant drug that inhibits serotonin and norepinephrine reuptake. Affinity of venlafaxine to the binding sites of serotonin transporters is at least 15 times more than its affinity to those of norepinephrine transporters (Gould et al., 2006). Venlafaxine has more efficacy than serotonin reuptake inhibitors in treatment of major depression (Sir et al., 2005). It has been demonstrated that venlafaxine (10 and 20 mg/kg, i.p.) reduces naloxane-precipitated morphine withdrawal syndrome (Lu et al., 2001). Venlafaxine also has an analgesic effect (Gultekin & Ahmedov, 2006).

Serotonergic changes play an important role in the spontaneous opiate withdrawal syndrome (Sharma et al., 2006). Monoamines are also involved in depressive disorders (Krishnan & Nestler, 2008).

Monoamines and pain related biomolecule's levels may be altered by methadone and venlafaxine. Therefore we examined the effects of chronic methadone and venlafaxine alone and in combination with the spontaneous morphine withdrawal syndrome and pain sensitivity.

2. Methods

2.1. Animals

Twenty eight young male Sprague-Dawley rats weighing 180-220 g obtained from Experimental Animal House of Ahvaz Jundishapur University of Medical Sci-

ences, and were randomly divided into 4 groups (four animals in each cage). They were provided with standard pellet and water ad-lib. Rats were housed under standard conditions (12 hours light/dark cycle, lights on at 7 am, temperature 23±2 °c). All experiments lasted 27 days and were performed at 9 am-3 pm. Animals were handled 5 min/day for 7 days in the laboratory and were returned to their cages after each injection in order to decrease their stress. All interventions and behavioral experiments followed by Ahvaz Jundishapur University of Medical Sciences (AJUMS) Guide for the Care and Use of Laboratory Animals and were approved by local Institutional Ethics Committee (Ethics Code: ajums.REC.1392.327).

2.2. Drugs

Morphine Sulfate Pentahydrate and Methadone Hydrochloride (racemic mixture) obtained from Temad pharmaceutical company (Tehran, Iran). Venlafaxine Hydrochloride obtained from Tehran Chemie and Pour-sina Pharmaceutical Companies (Tehran, Iran). All drugs were in the powder form.

Fresh stocks of morphine, methadone and venlafaxine were prepared daily according to the weight of animals in the experimental design. Morphine Sulfate Pentahydrate or Venlafaxine Hydrochloride were dissolved in normal saline (NaCl 0.9 %) so that the animal receive the considered dose in 1 milliliter (Lu et al., 2001). Morphine (10 mg/kg) injected subcutaneously (s.c.) for 4 days (Tahsili-fahadan et al., 2010). Methadone Hydrochloride (70 mg/kg) was dissolved in the sucrose solution 3% (w/v) (to reduce bitter taste of methadone) in the concentration of 0.5 mg/ml. It was administered orally (in drinking water) for 7 days (Liu et al., 1978). Venla-

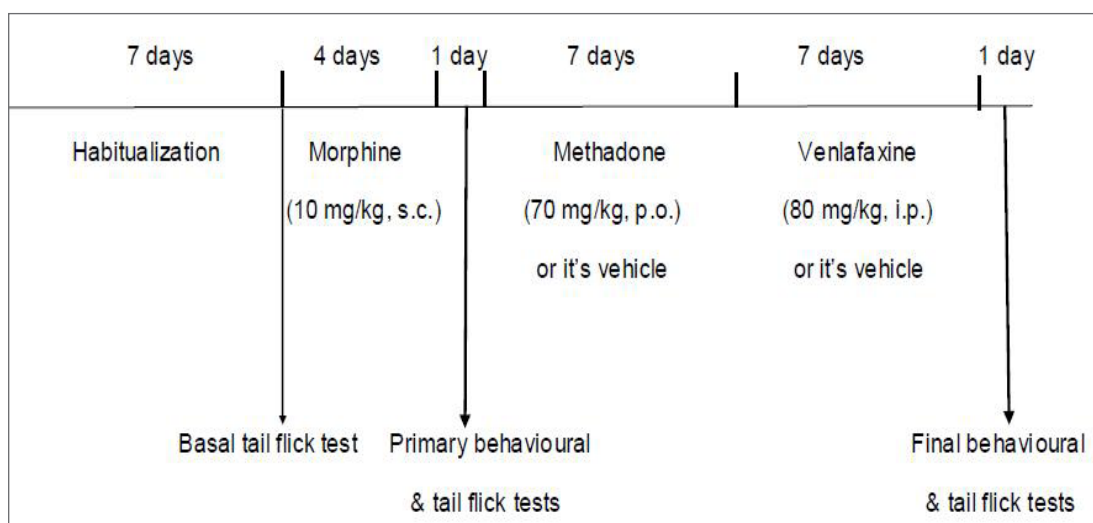


Figure 1. Experimental Design.

faxine Hydrochloride (80 mg/kg) was dissolved in the vehicle and injected intraperitoneally (i.p.) for 7 days (Capello et al., 2011).

2.3. Measures

2.3.1. Tail flick assay

Latencies to respond to heat stimulus were measured by a Tail flick analgesimeter (Borj Sanat, Tehran, Iran, 2012). Animals were placed in the plexiglass restrainers during the test. Then a light beam with a medium intensity (40% of maximum) was radiated on the rat's tail after pressing the start button. Radiation was immediately stopped with bending reflex of the tail was laid over a light sensor. The interval between the resumption of light emission and the bending reflex of the tail was recorded by the automatic timer as tail flick latency. Radiation was terminated automatically after 10 seconds (cut-off time) when the animal did not respond to the painful stimulus. Tail flick test was performed 3 times for each animal with 3 minutes interval between the two tests. Average of latencies for each rat was calculated. Average of basal latencies were lower than 3 seconds (Cavun, Goktalay & Millington, 2005).

2.3.2. Assessment of behaviors

In order to monitor withdrawal behaviors, we used a transparent plexiglass cylinder (46 cm height and 20 cm diameter, Borj sanat, Tehran, Iran). The top of the cylinder was open and a black sheet was placed beneath the cylinder to enhance the visual contrast. A digital camera was set up over the cylinder to record animal's behaviors. A thin layer of tiny wooden chaff was used as bedding.

Withdrawal signs were checked immediately after behavioral recording (checked signs) or counting at 30 minutes by observation of video films (counted behaviors). In order to avoid bias, video files were coded until final statistical analysis. Also only one observer scored and checked withdrawal symptoms.

Stretching hands forward and opening the jaw simultaneously was defined as yawning. Other behaviors and their definitions mentioned in this study, are based on the previous criteria (Punch et al., 1997) with two modifications including: 1) Every 3 seconds standing on feet was scored as rearing. 2) Every 3 seconds manipulation of the head or body with limbs was scored as grooming. Freezing (more than ten seconds immobility) time recorded using a stopwatch. Each shaking of the body or

head was scored as wet-dog shaking. If animal used the forepaws to displace the bedding, it was scored as digging. Watery defecations were named as diarrhea. Falling down the eyelids was named as ptosis. If animals made a loud voice when were placed in the cylinder, it was named as irritability. Secretion of tiny brown droplets from the corner of eyes was named as lacrimation. Secretion of a brown liquid from the nose was named as rhinorrhea. "C-shaped" body or side lying was considered as abnormal posture. Extension of the penis out was named as penile erection. Secretion of semen fluid was named as ejaculation. Defecation numbers were counted on the black floor during 30 minutes behavioral test.

2.3.3. Experimental design

After habituation, all rats were injected morphine (10 mg/kg/day, s.c.) from 8th to 11th day. Twenty-four hours after the last injection, primary withdrawal behaviors and tail flick latencies were recorded. Control group received vehicle of methadone (p.o. from 13th to 19th day) and vehicle of venlafaxine (i.p., from 20th to 26th day). Venlafaxine treated group received vehicle of methadone (p.o., from 13th day to 19th day) and venlafaxine (80 mg/kg, i.p., from 20th to 26th day). Methadone treated group received methadone (70 mg/kg, p.o., from 13th to 19th day) and vehicle of venlafaxine (i.p., from 20th to 26th day). Combination treated group received methadone (70 mg/kg, p.o.) and venlafaxine (80 mg/kg, i.p.) according to the experimental design. One day after the last venlafaxine or its vehicle injection, final behavioral and tail flick tests were performed (Figure 1). Venlafaxine or its vehicle was administered after methadone intervention in order to remodel clinical treatments. The word "Combination" in this study is representative of administration of both methadone and venlafaxine but not in the same time.

2.3.4. Statistical analysis

Data were represented as means \pm SEM. "Statistical Package for Social Sciences" (IBM SPSS version 15.00, Chicago, IL, USA) was used for data analysis and Graph-Pad Prism Software (version 4.00 for Windows, San Diego California, USA) to draw histograms. The normality of data was determined using Kolmogorov-Smirnov test. Parametric data were analyzed by one or two-way (Bonferroni test) Analysis of Variance (ANOVA) whenever was necessary. Post hoc tests were performed after one-way ANOVA. Significance was assumed at the $P < 0.05$ level. Nonparametric data were analyzed by Kruskal-Wallis, Mann-Whitney, McNemar and Friedman nonparametric tests. Percentage changes of values

between the final and primary tests were used whenever the distribution was not normal. Percentage changes of values were computed by the formula as follows: ((Final value-Primary value/Primary value)) × 100.

3. Results

3.1. Counted behaviours

3.1.1. Freezing

Rat's immobility for more than 10 seconds in sedentary situation or while the legs were extended, were sometimes associated with abnormal posture and ptosis. Percentage changes of freezing scores were recorded and analyzed. The difference of percentage changes of freezing scores between control and venlafaxine treated group; and between control and methadone treated group were not significant ($P > 0.05$). While percentage changes of freezing score in methadone and venlafaxine treated group decreased significantly ($P < 0.01$) in comparison with the control group (Figure 2).

Other counted withdrawal behaviors: No significant effect of methadone, venlafaxine and their combinations on other withdrawal behaviors were observed among groups ($P > 0.05$ for all comparisons).

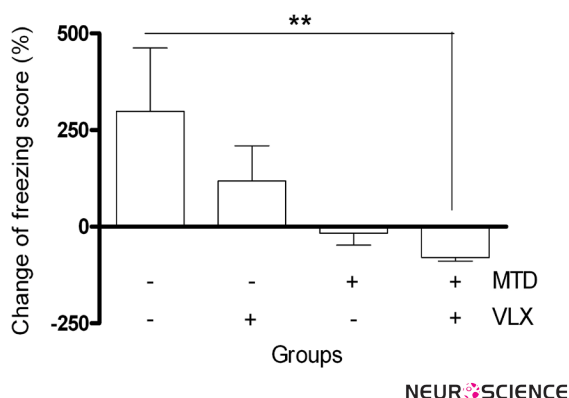


Figure 2. Effect of methadone (70 mg/kg, for 7 days, p.o.) and venlafaxine (80 mg/kg, for 7 days, i.p.) on percentage changes of freezing. MTD=Methadone, VLX=Venlafaxine. The sign of + or - is representative of received or not received the treatment. ** $P < 0.01$, $379 \pm 144\%$ difference in change of freezing score, Two-Independent Samples, Mann whitney test, $n=6-8$.

3.2. Checked signs

No significant effect of methadone, venlafaxine and their combinations on withdrawal checked signs were observed among groups ($P > 0.05$ for all comparisons).

3.3. Tail flick

Significant difference ($P < 0.05$) in percentage change of response latencies was observed between the final and basal (8th day) tail flick tests (Figure 3A). Although Change of response latencies between final (27th day) and primary (12th day) tail flick tests was not significantly different among 4 groups (Figure 3B).

3.4. Correlations between pain sensitivity and freezing withdrawal behaviour

Possible correlations between final latencies to respond to painful stimulus and final freezing scores in 30 minutes behavioral test were examined in control and drug treated animals separately (Figure 4). Final freezing scores in the control group was positively correlated with the mean response latencies to the painful stimulus in final tail-flick test ($r = +0.883$, $P < 0.01$, $df=6$, $F=21.149$). Also correlation between final response latencies and freez-

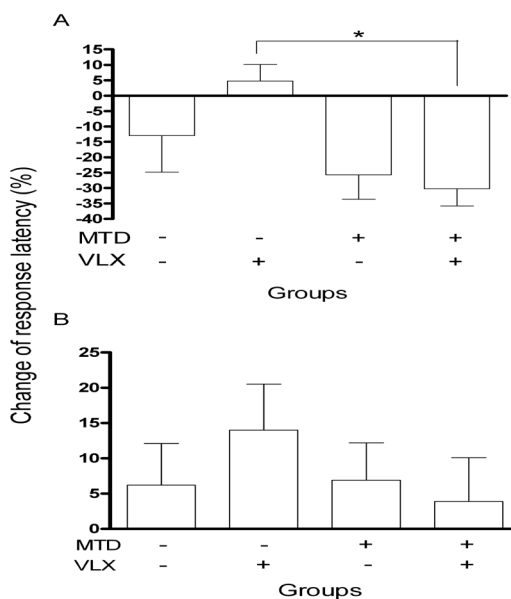
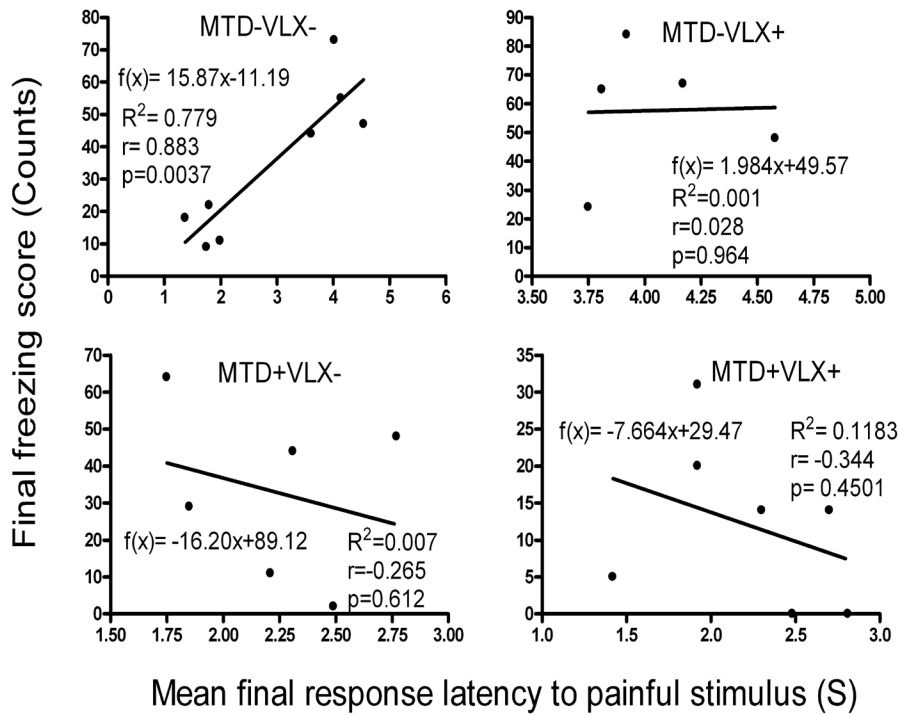


Figure 3. Effect of chronic methadone (70 mg/kg, for 7 days, p.o) and venlafaxine (80 mg/kg, for 7 days, s.c.) on percentage changes of response latency in tail flick tests. A. Between final and basal tests. B. Between final and primary tests MTD=Methadone, VLX=Venlafaxine. The sign of + or - is representative of received or not received the treatment. * $P < 0.05$, $35 \pm 8\%$ difference, One-way ANOVA, Tamhane's post hoc test, $n=6-8$.

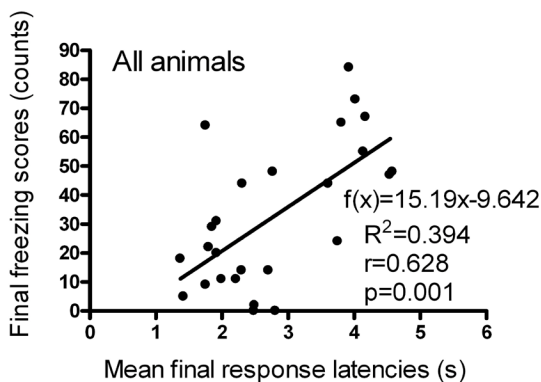


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Figure 4. Plots of the final freezing scores versus mean final latencies to respond to the painful stimulus in different groups. The line represents linear regression. MTD=Methadone, VLX=Venlafaxine. The sign of + or - is representative of received or not received the treatment.

ing scores in all animals was calculated (Figure 5). A positive correlation was observed between final freezing scores and final response latencies to the painful stimulus in all animals ($r = +0.628$, $P = 0.001$, $df = 24$, $F = 15.61$)

4. Discussion



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Figure 5. Plot of the final freezing scores of all animals versus their mean final latencies to respond to the painful stimulus in one model. The line represents linear regression.

In this study, results of withdrawal syndrome test indicate that the so-called behaviors might be classified into 3 categories: 1) Behaviors that were rare or did not appear by morphine, methadone and venlafaxine administrations including: diarrhea, irritability, rhinorrhea, penile erection, ejaculation, digging, hopping and jumping. 2) Behaviors that appeared but were not significantly affected by methadone and venlafaxine interventions, such as: Rearing, grooming, defecation, wet-dog shaking, yawning, abnormal posture, lacrimation and ptosis. 3) Behavior that was affected by combination of methadone and venlafaxine interventions was freezing.

Freezing elicits by selective stimulation of dopamine D_2 receptor expressing neurons in caudate and putamen nuclei (Kravitz et al., 2010). Also Intracerebroventricular injection of stressin1_A (an agonist of corticotrophin releasing factor type 1) promotes behavioral activation at the dose of 0.04 nmol, but it augments freezing at the dose of 1 nmol (Zhao et al., 2007); these mechanisms may explain the route by which combination of methadone and venlafaxine led to the reduction of freezing. Most studies on freezing behavior are related to the fear conditioning or open field tests (like: Sanders et al., 2013); in this study, attenuation of freezing behavior of

spontaneous morphine withdrawal syndrome by combination of methadone and venlafaxine interventions might be a novel report. Our results showed that administration of methadone solely or venlafaxine did not significantly reduce final freezing scores. However methadone and venlafaxine administration significantly decreased final freezing scores. Leri et al., (2009) have represented that chronic treatment with methadone in rats did not show decreased dopamine D_2 receptors mRNA levels in the striatum. With respect to these findings by Kravitz, Zhao and Leri and with regard to our result that methadone did not increase freezing behavior, it seems that D_2 receptor mRNA was not increased by methadone. Therefore, methadone affected on freezing behavior probably via nondopaminergic receptors such as CRF_1 receptor.

Steimer (2002) in a review reported that "freezing is a coping behavior associated with autonomic inhibition and increased glucocorticoid secretion. It is induced by stimulation of anterior and medial hypothalamus, central nucleus of amygdala, dorsolateral periaqueductal gray (PAG) matter and septohippocampal inhibition". Also it has been shown that intrahippocampal injections of an inverse agonist of gamma-amino butyric acid / benzodiazepine receptor (RY024) elicit freezing (Bailey et al., 2002). With regard to the role of PAG matter in inducing freezing behavior, correlations observed between the final freezing scores and the final latencies (to respond to the painful stimulus) in this study might be more explained by the findings of Graeff, Guimaraes and De Andrade (1996). They suggested that "dorsal raphe nucleus-periventricular pathway innervates the periventricular and PAG matter inhibits innate fight/flight responses to hindering pain".

We observed that chronic methadone administration, decreased final (27th day) response latencies (to the painful heat stimulus) compared with the basal (8th day) one's. On the contrary, it did not significantly change final response latencies compared with the primary (14th day) one's. These findings imply that chronic methadone administration augments the final sensitivity to the painful stimulus compared with the basal one. It was possible that the hyperalgesic effect of methadone might be masked between the final and "primary" tail flick tests. Furthermore, we may deduce that the "basal" analgesia was higher than the final one, except in venlafaxine treated group. In this group positive values that may be attributed to the effect of venlafaxine were shown. On the other hand, the final analgesia was higher than the "Primary" one. These findings might provide a novel comparison of pain sensitivity among normal state, withdrawal period and three treatment states. These findings

may also corroborate previous reports (Mitchell et al., 2006; Hay et al., 2009). Although hyperalgesic effect of chronic racemic methadone administration in this study differs from the analgesia observed by l-methadone (Lemberg et al., 2006). Mechanisms related to the central glutamine, spinal dynorphines, and decreased neurotransmitter reuptake have been proposed for hyperalgesia (Lee et al., 2011).

A wide range of factors might be involved in the morphine withdrawal syndrome; for example: "Orexin type 1 receptor" in diarrhea, teeth chattering, wet-dog shaking and ptosis (Azizi et al., 2010), "Orphan receptor like type 1" and "Corticosterone" in grooming (Blakley et al., 2004), "Strain" in wet-dog shaking and diarrhea (Cobuzzi & Riley., 2011), " Ca^{2+} channel" in diarrhea (Kishioka et al., 1996), "Acetyl choline" in lacrimation and rhinorrhea (Kassa & Kunesova., 2006), "cAMP System" in jumping and defecation (Aricioglu, Means, & Regunathan, 2004), "Glycyl-glutamine" in teeth chattering, ptosis and wet-dog shaking (Cavun et al., 2005), "Enkephalin" in ptosis (Ruiz et al., 1996), "Time" in teeth chattering (Ruiz et al., 1996), " D_2 Dopamine Receptor" in yawning and penile erection (Piepponen et al., 1996), and "N-methyl-D-aspartate Receptor" of glutamate in abnormal posture and ejaculation (Jhamandas et al., 1996).

Suitable expression rates and concentrations of the so-called biomolecules and neurotransmitters in different brain regions remained to be clear. Also exact mechanisms to prevent these withdrawal behaviors and the hyperalgesia remained to be clear; thus In-vivo, In-vitro and radioligand binding studies may be needed to provide new spectra about the change of receptor's levels and reduction of morphine withdrawal syndrome.

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