

**Original article:**

**CHEMICAL STIMULATION OF THE LATERAL HYPOTHALAMUS  
POTENTIATED THE SENSITIZATION TO MORPHINE IN RATS:  
INVOLVEMENT OF OREXIN-1 RECEPTOR IN THE VENTRAL  
TEGMENTAL AREA**

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**ABSTRACT**

Orexin plays a crucial role in drug-seeking behavior. The lateral hypothalamus (LH) is a central region that produces orexin, and its projections to the ventral tegmental area (VTA) play an important role in reward and addiction-related behaviors. In this study, we investigated the role of LH stimulation and the involvement of the orexin-1 receptor (Ox1r) in the VTA in relation to morphine sensitization. In all animals, cannulae were implanted unilaterally into the LH and VTA to inject different doses of carbachol (62.5, 125 and 250 nmol/0.5 µl saline) as a cholinergic agonist and SB334867 (1, 10 and 20 nmol/0.3 µl DMSO) as a selective Ox1r antagonist for three consecutive days (sensitization period) respectively. These drugs were injected five minutes before administration of an ineffective dose of morphine (0.5 mg/kg; sc) during the sensitization period. In all groups, the sensitization period occurred in a separate room from which the conditioning occurred. After this period, all groups exceeded five days under the conditioned place preference (CPP) paradigm without any treatment. For evaluation of morphine sensitization, place preference was induced by ineffective dose of morphine (0.5 mg/kg) and the CPP score was represented by the difference in time spent in drug- and saline-paired compartments. The results revealed that concurrent intra-LH administration of carbachol (125 nmol/0.5 µl saline) and an ineffective dose of morphine (0.5 mg/kg) significantly induce CPP. Additionally, the blockade of Ox1r in the VTA by SB334867 can attenuate the conditioning score induced by concurrent administration of carbachol and an ineffective dose of morphine. Our findings suggest that LH stimulation potentiates the effect of an ineffective dose of morphine, and induces morphine sensitization. It seems that the chemical stimulation of LH potentiates sensitization to morphine through the orexinergic system in the VTA in rats.

**Keywords:** Morphine sensitization, orexin, lateral hypothalamus, ventral tegmental are, carbachol, SB334867

## INTRODUCTION

In 1998, orexin A and B were recognized as neurotransmitters produced in the hypothalamic neurons. Since then considerable research has been undertaken to characterize this neurotransmitter system. Orexin-1 receptor (Ox1r) and orexin-2 receptor (Ox2r) were discovered shortly afterwards (Sakurai et al., 1998) while Sakurai also showed orexins to be involved in reward processing. It appears that orexin have a prominent role in the conditioned response to stimuli associated with food and drug rewards (Aston-Jones et al., 2009) and in opiate withdrawal (Georgescu et al., 2003). They are also involved in drug-seeking behavior elicited by context association with drugs. The lateral hypothalamus (LH) is a main region for producing orexins, and these neurons and their projections to the ventral tegmental area (VTA) play a crucial role in reward processing, drug abuse and addiction-related behaviors (Stephen et al., 2011). Stimulation of LH orexin neurons, or microinjection of orexin into the VTA, has been shown to reinstate an extinguished morphine preference.

Modulation of forebrain dopamine neurotransmission is one of the key means of leading VTA orexin to increase conditioned reward seeking, which is tied to reward seeking and approach effort (Berridge and Robinson, 1998; Cheer et al., 2007; Fiorillo et al., 2003). Orexin can activate mesolimbic dopamine neurons, especially those located in the VTA (Korotkova et al., 2003; Narita et al., 2007). This activation results in dopamine release in the nucleus accumbens (NAc) as a main cause of reward. Fos activation in VTA-projecting LH orexin neurons correlates with the intensity of reward during protracted abstinence (Harris and Aston-Jones, 2003). Orexin input to the VTA may contribute to the propensity of relapse during protracted abstinence in addicts (Aston-Jones et al., 2010).

The enhanced response to a stimulus, after repeated exposure to that stimulus, is termed sensitization (Robinson and Becker, 1986; Kalivas and Stewart, 1991). Sensitiza-

tion is suggested to play an effective role in the psychopathology of drug abuse (Robinson and Becker, 1986; Shippenberg et al., 1996). Repeated concomitant morphine administration causes sensitization to its rewarding effects (Carlezon et al., 1997). Morphine-induced sensitization is a major problem of morphine dependence and involves opioid drug abuse liability (Robinson and Becker, 1986). This enhanced response appears to be related to changes in the mesolimbic dopamine (DA) pathway, which arises in the VTA and projects mainly to the NAc (Kalivas and Duffy, 1993). Behavioral sensitization has been shown to be involved in the locomotor stimulant, rewarding and discriminative effects of morphine (Kuribara, 1997). Some behavioral studies have demonstrated that orexin injection into the VTA reinstates an extinct morphine and cocaine preference (Harris et al., 2005; Wang et al., 2009), and blockade of Ox1r in the VTA weakens cue-induced cocaine reinstatement (James et al., 2011) but not stress-induced cocaine reinstatement (Wang et al., 2009). The LH has a central role in morphine antinociception and pain relief effects, and also synthesizes orexin selectively (Georgescu et al., 2003). Furthermore, orexin-A has been reported to produce an analgesic effect in pain models, which was blocked by the orexin-1 receptor antagonist SB-334867, but not naloxone. Our previous study showed that morphine's analgesic effect in the formalin test might also be associated with the orexin-1 receptor (Azhdari-Zarmehri et al., 2013). In this study, we tried to evaluate the effect of LH stimulation and the orexinergic system within the VTA on sensitization to morphine in rats.

## MATERIALS AND METHODS

### *Animals*

One hundred and nine adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 210–280 g were used in this experiment. Animals were housed in groups of three per cage in a 12/12 h light/dark cycle (light on between 7:00 a.m. and 7:00 p.m.)

with free access to chow and tap water. The animals were randomly allocated to different experimental groups. Each animal was used only once. Rats were habituated to their new environment and handled for 1 week before the experimental procedure was started. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

### **Drugs**

In the present study the following drugs were used: Carbachol, as a cholinergic agonist (Sigma-Aldrich, USA) was dissolved in normal saline. SB-334867, as an Ox1r antagonist (Tocris Bioscience, Bristol, UK) was dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, Germany) as a vehicle and morphine sulfate (Temad, Iran) dissolved in sterile saline (0.9 %).

### **Stereotaxic surgery**

Rats were anesthetized by intraperitoneal injection of Xylazine (10 mg/kg) and Ketamine (100 mg/kg), and placed into stereotaxic device (Stoelting, USA). An incision was made along the midline, the scalp retracted, and the area surrounding bregma was cleaned and dried. In addition, lidocaine with epinephrine (0.2 ml) was injected in several locations around the incision. Stainless steel guide (23-gauge) cannulae 11 mm in length were aimed to the LH and/or VTA unilaterally stereotaxic coordinates: incisor bar -3.3 mm, -3 mm posterior to the bregma,  $\pm 1.6$  mm lateral to the sagittal suture and 8.8 mm down from top of the skull for LH, and for VTA, we use this coordination AP = -4.8 mm caudal to bregma, Lat =  $\pm 0.9$  mm lateral to midline, DV = 8.3 mm ventral from the skull surface. Cannulae were secured with jewelers' screws and dental acrylic cement. After the cement was completely dried and hardened, two stainless steel stylets were used to occlude the guide cannulae during

recovery period. Penicillin-G200000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) were administered immediately after surgery. Animals were individually housed and allowed to recover for 5 days before experiments.

### **Drug administration**

Microinjections were performed through the 30-gauge injector cannulae (1 mm below the tip of the guide cannulae). Polyethylene tubing (PE-20) was used to attach injector cannula to the 1- $\mu$ l Hamilton syringe. For drug microinjection, the animals were gently restrained by hand; the stylets were removed from the guide cannulae and replaced by 30-gauge injector cannulae. Carbachol administered slowly in a total volume of 0.5  $\mu$ l/rat over a period of 60 s and SB334867 solutions (with 10 % DMSO as a vehicle) were administered in a total volume of 0.3  $\mu$ l/rat over a period of 60 s into the VTA. Injection needles were left in place for an additional 60 s to facilitate drug diffusion, and then the stylets were reinserted into the guide cannulae.

### **Induction of morphine sensitization**

For basic induction of morphine sensitization, effective dose of morphine (5 mg/kg; sc) was injected once daily for three days as the sensitization period followed by five days free from morphine treatment. However, in the present study, for induction of morphine sensitization, we concurrently injected an ineffective dose of morphine and intra-LH carbachol during sensitization period. After sensitization period and 5-day free drug period, the CPP paradigm was done. In this CPP protocol we used ineffective dose of morphine during conditioning phase for evaluating the occurrence of sensitization that will be explained in the following section.

### **Conditioned place preference paradigm**

A three-compartment conditioned place preference (CPP) apparatus (30 cm  $\times$  30 cm  $\times$  40 cm) was used in these experiments

(Haghparast et al., 2009). Place conditioning was conducted using an unbiased procedure. The apparatus was made of Plexiglas (Borj Sanat, Iran) and divided into two equal-sized compartments by means of a removable white wall and shading (both were white), but distinguishable by texture. To provide the tactile difference between the compartments, one of the compartments had a smooth floor while the other compartment had a net-like floor. Two preference compartments were differently striped black and white on their walls. The null compartment was a tunnel (30 cm × 15 cm × 40 cm) connecting the two preference compartments. In this apparatus, rats showed no consistent preference for either compartment, which supports our unbiased CPP paradigm. This paradigm took place in five consecutive days, which consisted of three distinct phases: pre-conditioning, conditioning and post-conditioning. For all phases, animals were tested during the same time period each day.

#### ***Pre-conditioning phase***

On day 1, each rat was placed separately into the apparatus for 10 min, with free access to all compartments. The time spent in each compartment and the rats' movements were recorded (pre-test day). Animals were then randomly assigned to one of the two compartments for place conditioning.

#### ***Conditioning phase***

This phase started 1 day after pre-conditioning phase. It consisted of six, 30 min sessions (three saline and three drug pairing) in a 3-day schedule. These sessions were conducted twice each day (from day 2 to day 4) with a 6 h interval. On each day, separate groups of animals received a conditioning session with drug and another with saline. During sessions, the animals were confined to one compartment by closing the removable wall. Treatment compartment and order of presentation of drug/saline were counterbalanced for either group.

#### ***Post-conditioning or testing phase***

This phase was carried out on day 5 (the preference test day), 1 day after the last conditioning session, in a drug free state. Each animal was tested only once. The removable wall was removed for testing and rats were allowed to access the entire apparatus for 10 min. For each rat, the time spent in each compartment during a 10 min period was recorded by a 3CCD camera (Panasonic Inc., Japan) and analyzed using the Ethovision software (XT, Version 7), a video tracking system for automation of behavioral experiments (Noldus Information Technology, the Netherlands). The conditioning score, as a preference index, was calculated as the time spent in the drug-paired compartment minus the time spent in saline-paired compartment. Total distance traveled by each animal was also recorded in all control and experimental groups.

#### ***Conditioning score measurement***

Conditioning scores (CPP score) represent the time spent in drug-paired compartment minus the time spent in the saline-paired compartment (Azizi et al., 2009) during 10 min (600 s) post-conditioning test. In this study, CPP score is used for evaluating the effect of drugs on morphine-induced place preference in morphine-sensitized and saline-treated non-sensitized rats.

#### ***Experimental design***

To evaluate the sensitization to morphine, moderate chemical stimulation of the LH by carbachol and ineffective dose of morphine were applied for three consecutive days (sensitization period) in a room distinct from which the conditioning occurred. Five days later, the CPP paradigm was induced by ineffective doses of morphine (0.5 mg/kg; sc).

#### ***Morphine dose-response on place conditioning paradigm in sensitized rats***

To choose the ineffective dose of morphine for the next experiments, the conditioned place preference was done by graded

ineffective doses of morphine (0.25 and 0.5 mg/kg) in animals, which had previously received once daily morphine (5 mg/kg; sc) for 3 consecutive days (sensitization period). Place conditioning commenced 5 days later. Control animals received saline (1 ml/kg; sc) instead of morphine during sensitization period. Conditioning score and distance traveled were recorded and analyzed during 10 min, on the pre- and post-conditioning phases.

#### ***Effect of concurrent administration of morphine and intra-LH carbachol on development of morphine sensitization in CPP paradigm***

In this set of experiments, two super groups described and each group contained three groups. In both super groups different doses of carbachol (62.5, 125 and 250 nmol/0.5 µl saline; n = 5-7 in each group) microinjected unilaterally into the LH for stimulating this region for three consecutive days (Taslimi et al., 2012). Also, these super groups concurrently received saline or ineffective dose of morphine during the sensitization period. After the 5-day free of drug injections, the CPP paradigm was done for evaluating the morphine sensitization in this protocol. In conditioning phase, morphine was applied at the ineffective dose selected from the previous mentioned experiments. On the post-conditioning test, conditioning score and distance traveled were calculated for each rat in all morphine- and saline-treated groups.

#### ***The effects of Ox1r antagonist SB334867 microinjected into the VTA on LH stimulation-induced sensitization in rats***

To find out the role of Ox1r in the VTA in morphine sensitization induced by chemical stimulation of LH, different doses of SB334867 (1, 10 and 20 nmol/0.3 µl DMSO; n = 6 in each group) were microinjected just before the concurrent administration of intra-LH carbachol and systemic morphine (0.5 mg/kg, sc) during the sensitization period. In respective control groups, animals re-

ceived saline instead of carbachol and morphine. As described in previous section, after the 5-day free of drug injections, the CPP paradigm was done for evaluating the morphine sensitization in this protocol. In conditioning phase, morphine was applied at the ineffective dose. Then, conditioning score and distance traveled were calculated for each rat in all morphine- and saline-treated groups on the test day.

#### ***Locomotor activity measurement***

To evaluate the effect of drugs on locomotor activity in morphine- and saline-treated animals, total distance traveled (cm) during a 10 min period, on the pre- and post-conditioning phases was measured.

#### ***Histology***

After performing the test, the animals were deeply anesthetized with Ketamine and Xylazine. Then, they were transcardially perfused with 0.9 % saline and 10 % formalin solution. The brains were removed, blocked and cut coronally in 50 µm sections through the cannulae placements. The neuroanatomical location of cannulae tips placements, were confirmed using Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007). Only the animals with correct cannulae placements were included in the data analysis.

#### ***Statistics***

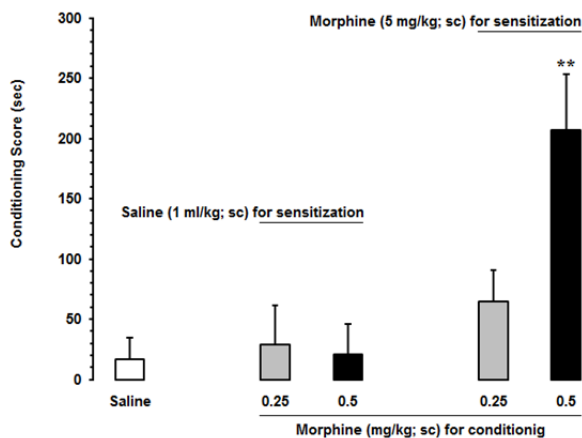
Conditioning score represents the differences between the time spent in drug-paired compartment and the time spent in the saline-paired compartment, and is expressed as mean ± SEM (standard error of mean). Data were processed by commercially available software GraphPad Prism® 5.0. In order to compare the conditioning scores and distance traveled obtained in all groups (vehicle and experimental groups), one-way analysis of variance (ANOVA) and randomized blocks model followed by post-hoc analysis (Dunnett or Newman-Keuls test) were used, as appropriated. P-values less than 0.05

( $P < 0.05$ ) were considered to be statistically significant.

## RESULTS

### *Morphine dose-response on place conditioning paradigm in sensitized rats*

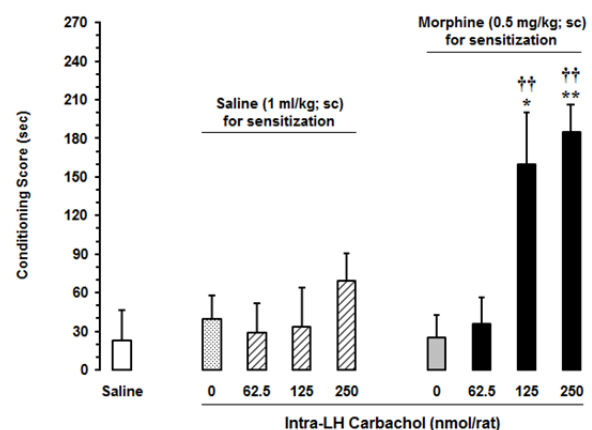
Figure 1 shows the conditioned place preference produced by graded doses of morphine (0.25 and 0.5 mg/kg) in rats, which had previously received morphine (5 mg/kg; sc) once daily for three consecutive days. Place conditioning commenced five days later. In animals with a previous history of morphine administration, an enhanced response to morphine was observed. The maximum response was observed at the morphine dose of 0.5 mg/kg [ $F(6,48) = 8.063$ ,  $P < 0.0001$ ; Figure 1]. Therefore, the dose of 0.5 mg/kg of morphine was selected as the appropriate dose for the rest of the experiments in the CPP paradigm in morphine-sensitized rats. Injection of saline (1 ml/kg; sc) instead of morphine (5 mg/kg) during the sensitization days did not produce any sensitization in the animals.



**Figure 1:** Effects of repeated concomitant morphine administration on the animal sensitivity to low doses of morphine (i.e. sensitization). Animals received three morphine (5 mg/kg; sc) injections in three consecutive days following 5 days of resting. After this period, these animals were conditioned to ineffective doses of morphine (0.25 and 0.5 mg/kg; sc). As indicated in this figure, animals with previous history of morphine showed prominent response to low doses of morphine compared with saline-treated rats. Each point shows the mean  $\pm$  SEM for 5 rats. \*\*  $P < 0.01$  different from the saline control group

### *Effect of concurrent administration of morphine and intra-LH carbachol on development of morphine sensitization in the CPP paradigm*

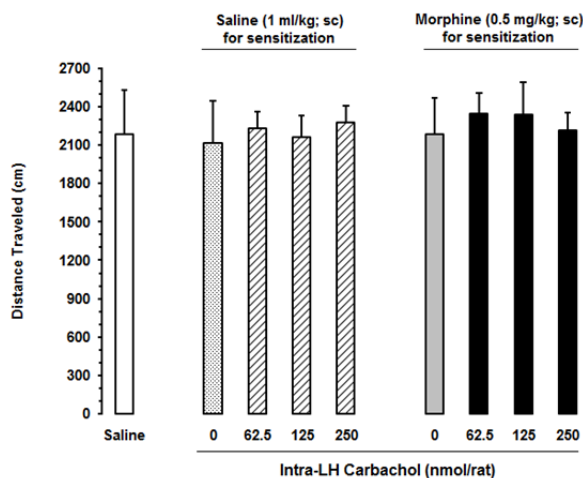
To determine the effects of LH stimulation for induction of morphine sensitization in the CPP paradigm, carbachol (62.5, 125 and 250 nmol/0.5  $\mu$ l saline;  $n = 6-7$  in each group) was unilaterally administered into the LH during the sensitization period of experiments. One-way ANOVA followed by Newman-Keuls multiple comparison test indicated that using carbachol alone (62.5, 125 and 250 nmol/ 0.5  $\mu$ l saline) for stimulating LH could not induce morphine sensitization and had no effect on morphine-induced CPP in the animals [ $F(4,24) = 0.5757$ ,  $P = 0.6835$ ; left panel Figure 2].



**Figure 2:** Effects of unilateral administration of different doses of carbachol into the lateral hypothalamus (LH) during sensitization period on conditioned place preference in saline (left panel) and morphine (right panel) treated animals that received saline (1 mg/kg; sc) or ineffective morphine (0.5 mg/kg; sc) during sensitization period respectively. Each point shows the mean  $\pm$  SEM for 5-7 rats. \*  $P < 0.05$  and \*\*  $P < 0.01$  different from the saline control group ††  $P < 0.01$  different from the respective vehicle group

In the right panel in Figure 2, the effect of concurrent administration of morphine and carbachol during the sensitization period of the CPP paradigm is shown. In this set of experiments, different doses of carbachol (62.5, 125 and 250 nmol/ 0.5  $\mu$ l saline) were injected into the LH during the sensitization period, five minutes prior to each ineffective

dose of morphine (0.5 mg/kg; sc). One-way ANOVA indicated that simultaneous administration of carbachol (125 and 250 nmol) and ineffective dose of morphine (0.5 mg/kg) significantly induced CPP compared to saline (0.5 µl/side) during the induction of sensitization [ $F(4,27) = 7.552$ ,  $P = 0.0005$ ; Figure 2] in a dose-dependent manner. On the other hand, one-way ANOVA indicated that on the test day, none of the groups showed significant differences in locomotor activity (Figure 3).

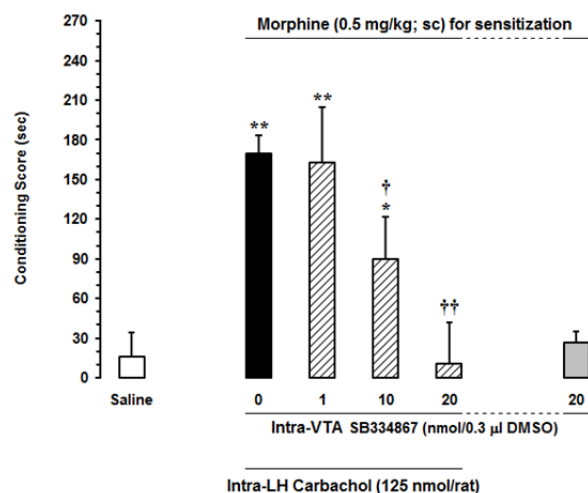


**Figure 3:** Effects of concurrent administration of different doses of carbachol into the lateral hypothalamus (LH) and saline/morphine during sensitization period on locomotor activity saline (left panel) and morphine (right panel) treated animals. Each point shows the mean  $\pm$  SEM for 5-7 rats.

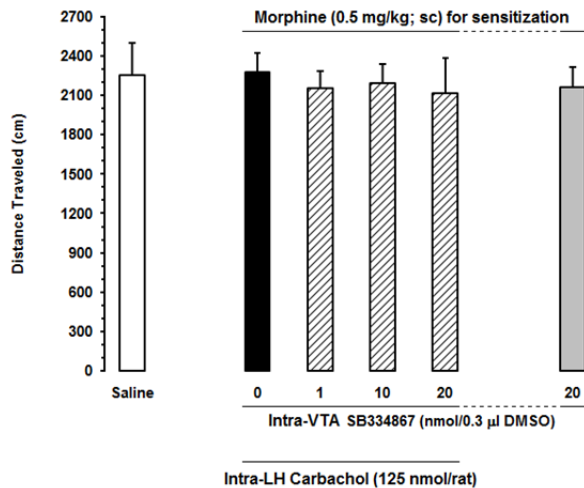
### ***The effects of Ox1r antagonist SB334867 microinjected into the VTA on LH stimulation-induced sensitization in rats***

In this set of experiments, to establish the role of orexin-1 receptor in the VTA by LH stimulation-induced sensitization to morphine, intra-VTA orexin receptors were blocked with different doses of SB334867 (1, 10 and 20 nmol/0.3 µl DMSO) during the sensitization period, five minutes before concurrent administration of intra-LH carbachol (125 nmol/ 0.5 µl saline) and morphine (0.5 mg/kg; sc). As shown in Figure 4, one-way ANOVA followed by the Newman-Keuls multiple comparison test indicated the

blockade of orexin-1 receptor in the VTA to dependently attenuate conditioning scores compared with the DMSO control group, in which animals received only DMSO instead of SB334867 [ $F(5,35) = 9.297$ ,  $P < 0.0001$ ; Figure 4]. Additionally, microinjection of the maximal dose of SB334867 into the VTA (20 nmol) alone during the sensitization period did not affect the conditioning score (left bar, Figure 4). On the other hand, one-way ANOVA indicated that none of the groups showed significant differences in locomotor activity on the test day. Thus, intra-VTA administration of different doses of SB334867 did not affect the conditioning scores due to changes in locomotor activity [ $F(5,35) = 0.1731$ ,  $P = 0.9706$ ; Figure 5].



**Figure 4:** Effects of orexin-1 receptor antagonist SB334867 (1, 10 and 20 nmol/0.3 µl DMSO) microinjected into the ventral tegmental area (VTA) prior concurrent administration of carbachol (125 nmol/rat) into the lateral hypothalamus (LH) and morphine (0.5 mg/kg) during sensitization period on conditioning score in conditioned place preference paradigm. Each point shows the mean  $\pm$  SEM for 6 rats. \*  $P < 0.05$  and \*\*  $P < 0.01$  different from the saline control group †  $P < 0.05$  and ††  $P < 0.01$  different from the respective vehicle group



**Figure 5:** Effects of orexin-1 receptor antagonist SB334867 (1, 10 and 20 nmol/0.3  $\mu$ l DMSO) microinjected into the ventral tegmental area (VTA) prior concurrent administration of carbachol (125 nmol/rat) into the lateral hypothalamus (LH) and morphine (0.5 mg/kg) during sensitization period on locomotor activity in conditioned place preference paradigm. Each point shows the mean  $\pm$  SEM for 6 rats.

## DISCUSSION

The aim of this study was to investigate the role of orexinergic outputs from the lateral hypothalamus to the ventral tegmental area in morphine sensitization in rats. This study indicates that (i) concurrent administration of intra-LH carbachol and ineffective dose of systemic morphine during the sensitization period can potentiate the rewarding effect of morphine (morphine sensitization) while (ii) injection of different doses of carbachol alone in the LH during the sensitization period cannot affect the acquisition of morphine-induced CPP and (iii) the blockade of the orexin-1 receptor in the VTA significantly reduces the potentiating effect of LH stimulation on morphine sensitization. On the other hand, the drugs do not affect locomotor activity in any groups.

The development of behavioral sensitization can be separated into two phases: initiation and expression. The initiation phase marks the immediate neural events that induce behavioral sensitization, and is commonly linked to the VTA. The expression phase is the long-term consequence of this initiation and is linked to the NAc (Kalivas

and Weber, 1988). The NAc serves as an output pathway to motor circuits. In the case of sensitization, the NAc is modulated by dopamine input from the VTA that either directly innervates this target or does so indirectly via the paraventricular nucleus (PVN) and basolateral amygdala (BLA). Previous studies have shown that orexin-1 and -2 receptors exist on dopaminergic and GABAergic neurons in the VTA and NAc (Sharf et al., 2008), so the activation of orexin receptors in the VTA after the stimulation of LH results in the activation of dopaminergic neurons in the cell bodies and dendrites of the mesolimbic pathway (Narita et al., 2006). Additionally, the intra-VTA injection of orexin may cause an increase in dopamine in the NAc (Pontieri et al., 1995): stimulation of the orexinergic receptor in the VTA may cause an increase in the activity of dopaminergic neurons followed by an increase in the amount of dopamine in the NAc. Although the ineffective dose of morphine could not release sufficient dopamine levels in the VTA and NAc, orexin was shown to compensate by increasing the release of dopamine in these regions. Our study shows that to develop the initiation phase of sensitization, collaboration between the opioid reward system and orexinergic system is necessary. Our findings also reveal that orexin release in the VTA is necessary but not sufficient for inducing morphine sensitization.

The NAc receives indirect glutamate projections via the VTA (Steketee and Kalivas, 2011). Electrophysiological studies have shown that a drug-induced change in the VTA is consistent with enhancement of glutamate transmission mediating the development of behavioral sensitization. Moreover, other neurotransmitters in the VTA, such as orexin, modulate glutamatergic transmission in the VTA and regulate drug self-administration (Borgland et al., 2006). It is documented that stimulation of D1 dopamine receptors in the VTA is necessary for sensitization to systemic psychostimulant administration (Vezina, 1996). By the mid-1990s it was proposed that stimulation of D1 recep-



tors is linked to the release of glutamate in the VTA as a necessary sequence of synaptic events to induce sensitization (Kalivas and Duffy, 1995), and in a recent study using measurements of N-Methyl-D-aspartate (NMDA) currents it has been shown that D1-mediated increases in NMDA currents on dopamine neurons result from a direct post-synaptic action to increase surface expression of NMDA receptors (Schilstrom et al., 2006). These authors speculated that the rise in pre-synaptic glutamate release might result from increased activation of dopamine cells via an increase in NMDA receptor activity relating to the prefrontal glutamatergic input to the VTA.

Ox1r activation in dopaminergic neurons in the VTA is required for the development of behavioral sensitization as well as for the induction of synaptic plasticity at excitatory synapses associated with sensitization. The orexin system is involved in the reward process and addiction because it has a connection with reward-related areas such as the VTA and NAc. Previous studies have demonstrated that orexin administration into the VTA can increase CPP and dopamine release in the NAc (Narita et al., 2006, 2007; Taslimi et al., 2011). We have shown that chemical activation of LH orexin neurons can be rewarding for animals not receiving drugs as reward inducers. This effect is mediated at least in part by VTA synapses, as it can be blocked by pharmacological inactivation of Ox1r in the VTA (Taslimi et al., 2011). We have also revealed that despite the rewarding effect of lone chemical stimulation of LH by carbachol (Taslimi et al., 2011), the same LH stimulation during the sensitization period does not affect the acquisition of an ineffective dose of morphine-induced CPP, while chemical stimulation of LH and an ineffective dose of morphine administration simultaneously can increase the conditioning response induced by morphine, and blockade of the VTA orexin-1 receptor can significantly decrease the potentiating effect of LH stimulation on morphine sensitization. To develop the initiation phase of

sensitization, therefore, collaboration between the opioid reward system and orexinergic system is necessary, while orexin release in the VTA is necessary but not sufficient to induce morphine sensitization in rats.

#### **Acknowledgements**

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#### **Conflict of interest**

The authors declare that they have no conflict of interest.

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