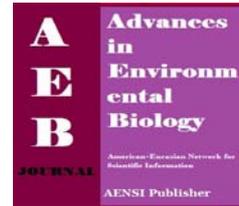




AENSI Journals

## Advances in Environmental Biology

Journal home page: <http://www.aensiweb.com/aeb.html>

### The Modulatory Role of GABA-B Receptors within the Shell Part of Nucleus Accumbens on the Expression of Morphine-Induced Conditioned Place Preference in Morphine-Tolerant Rats

<sup>1</sup>Maryam Rafiei-Rad and <sup>2</sup>Hedayat Sahraei

<sup>1</sup>Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran

<sup>2</sup>Department of Physiology and Biophysics, School of Medicine, Baqiyatallah (A.S.) University of Medical Sciences, Tehran, Iran

#### ARTICLE INFO

##### Article history:

Received 12 October 2013

Received in revised form 18

December 2013

Accepted 29 December 2013

Available online 25 February 2014

##### Key words:

Morphine, Baclofen, GABA-B, CGP35348, Expression, Tolerance, Conditioned Place Preference, Rat

#### ABSTRACT

**Introduction:** Nucleus accumbens shell is one of the most important effecting areas of morphine in terms of inducing euphoria and reward. GABAergic system, especially GABA-B receptors, has an important role in modulating euphoric effects of morphine. In this research, effect of injecting baclofen drugs (GABA-B receptor agonist) and CGP35348 (GABA-B receptor antagonist) into nucleus accumbens shell on inducing tolerance resulted from morphine was done using Conditioned Place Preference (CPP) method in Wistar rats with mean weight of  $230 \pm 20$  g. **Methodology:** Five days after the surgery and cannulation, different amounts of morphine were subcutaneously (S.C.) injected and conditioning stages were done using unbiased method. The obtained results showed that subcutaneous injection of morphine (0.5-7.5 mg/kg) could make conditioned preference place dose-dependent. In the current study, injection of baclofen and CGP35348 into nucleus accumbens on expression of conditioned place preference was studied in morphine-tolerant rats. **Results:** Injection of baclofen with doses of 1.5 and 12  $\mu$ g/rat reinforced expression of conditioned place preference in morphine-tolerant rats; but, in case of CGP35348, injection of this drug did not induce any specific effect on expression of conditioned place preference in morphine-tolerant rats. **Discussion:** The resulted findings showed probable capability of the studied receptor in this research for suppressing reward results of morphine using baclofen in response to tolerance. In general, it seems that stimulation or inhibition of GABA-B receptors in nucleus accumbens shell plays an important role in expression of conditioned place preference resulted from morphine in morphine-tolerant rats.

© 2014 AENSI Publisher All rights reserved.

**To Cite This Article:** Maryam Rafiei-Rad and Hedayat Sahraei., The Modulatory Role of GABA-B Receptors within the Shell Part of Nucleus Accumbens on the Expression of Morphine-Induced Conditioned Place Preference in Morphine-Tolerant Rats. *Adv. Environ. Biol.*, 8(1), 108-114, 2014

#### INTRODUCTION

The objective of this research was to experience mental addiction in animals using an experimental model. Thus, Conditioned Place Preference (CPP) method, which is used today as a standard method for assessing drug reward and mental addiction in animals, was selected. This method was used to study effect of one of neurotransmitter systems which has great importance in drug reward phenomenon, i.e. GABAergic system [1]. In recent years, GABA-B receptor agonists have received great attention; including in relation to alcohol dependence [2], cocaine [3] and nicotine [4] abuse and addiction to narcotics [5]. Interference of GABA-B receptor agonist in dopaminergic modulatory effects regarding narcotic abuse has been reported in previous studies [6, 7] There are many pieces of evidence that mesolimbic dopaminergic pathway has a fundamental role in intermediating reward resulting from drugs [8]. In normal conditions, activity of dopaminergic neurons in this pathway is affected by tonic inhibition of GABAergic intermediary neurons locating in ventral tegmental area (VTA) which decrease release of dopamine in nucleus accumbens and reduce amount of reward there [9]. Thus, the drugs that are used could cause increased dopamine neuron firing directly by stimulating dopaminergic neurons, indirectly by inhibiting performance of GABAergic inhibitory intermediary neurons or using both methods. Accordingly, brain reward would be increased and taking medication or tendency to drug consumption is induced [9, 10]. In the current study, morphine tolerance was investigated using baclofen drug as an agonist and CGP35348 as a selected antagonist of GABA-B receptor.

**Corresponding Author:** Maryam RafieiRad, Department of Biology, Izeh Branch, Islamic Azad University, Izeh, Iran.  
Tel & Fax: (+98692)5224202, E-mail: rafieirad.m@gmail.com, rafieirad.m@Izehiau.ac.ir

*Experimental Procedure:**Animals:*

Female Wistar rats ( $250 \pm 20$  g, Pasture Institute, Tehran, IRAN) were used throughout the study (6-8 rats for each experiment). Animals were housed in groups of 4 per cage in a 12/12 h light-cycle (lights on at 07.00 a.m.), with ad-lib food and water available. The animals were randomly allocated to different groups of the experiment. All experiments were conducted in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Committee) on the Use and Care of Animals.

*Drugs:*

The following drugs were used in these experiments: morphine sulfate (TEMAD, Iran), baclofen and CGP35348 (Novartis Basel, Switzerland), Ketamine hydrochloride and Xylazine (Alfasan Worden, Holland). The drugs were dissolved in sterile saline. Morphine was injected subcutaneously on a volume of 1 ml/kg and other drugs were given intra- nucleus accumbens on a volume of 0.5  $\mu$ l/rat and were prepared before use. The control groups received saline.

*Surgical procedures:*

Rats were anesthetized with Ketamine hydrochloride (70 mg/kg, i.p.) + Xylazine (10 mg/kg, i.p.) and two stainless steel cannulas (23-gauge) were placed stereotaxically (Stolting instruments, USA) into the NAc. Stainless steel, 23-gauge guide cannulas were implanted bilaterally 1.5 mm above the intended site of injection according to the atlas of Paxinos and Watson [11]. Stereotaxic coordinates for the NAc were: incisor bar (-3.3 mm), 1.2 mm anterior to the bregma,  $\pm 0.8$  mm lateral to the sagittal suture and 6.8 mm down from top of the skull. Cannulas were secured to jewelers' screws with dental acrylic. After completing the surgery, a dummy inner cannula was inserted into the guide cannula and left in place until injections were made. The length of the dummy cannula matched that of the guide cannula. Animals were allowed one week to recover from surgery and anesthesia. For drug infusion, the animals were gently restrained by hand; the stylets were removed from the guide cannulas and replaced by 30-gauge injection needles (0.5 mm below the tip of the guide cannula). The solutions were slowly administered in a total volume of 1  $\mu$ l/rat (0.5  $\mu$ l in each side) over a period of 60 s. Injection needles were left in place for an additional 60 s to facilitate diffusion of the drugs. Then, it was transferred to the CPP device to continue the experiment.

*Apparatus:*

A two compartment CPP apparatus (30X60X30 cm) was used in these experiments. Place conditioning was conducted using an un-Biased procedure, with minor changes to the design as previously described [12]. The apparatus was made of wood. Both compartments were identical in size (the apparatus was divided into two equal-sized compartments by means of a removable white guillotine door) and shading (both were white), but distinguishable by texture and olfactory cues. To provide the tactile difference between the compartments, one of the compartments had a smooth floor, while the other compartment had a nylon white mesh floor. A drop of menthol was placed at the right center of the compartment with a textured (nylon mesh) floor, to provide the olfactory difference between the compartments. The two compartments were differently striped black on their sides. In this apparatus, rats showed no consistent preference for either compartment, which supports our un-biased CPP paradigm.

*Behavioral testing:*

Measurement of conditioned place preference: Conditioned place preference consisted of three phases: pre-conditioning, conditioning and post conditioning. Pre-conditioning: On day 1 (pre-exposure), each rat was placed separately into the apparatus for 10 min, with free access to all compartments. Conditioning: This phase consisted of a 3-day schedule of conditioning sessions. In this phase, animals received three trials in which they experienced the effects of the drugs while confined in one compartment for 45 min and three trials in which they experienced the effects of saline while confined in the other compartment. Access to the other compartments was blocked on these days. Post conditioning phase: On the 5<sup>th</sup> day (the preference test day) the partition was removed, and the rats could access the entire apparatus. The mean time that each rat spent in either compartment during a 10 min period was determined as the preference criterion. Induction of morphine tolerance: In order to induce morphine tolerance, Morphine Sulfate (prepared by Temad Co. Iran) was used for 3 days, once per day, at a definite time with dose of 12.5 mg/kg as optimum dose in other experiments. The experimental period of conditioned place preference started immediately on the day after tolerance inducement. In this stage, expression was studied. Expression: In this stage, the animals received 0.5  $\mu$ L of the mentioned drug (GABA-B receptor agonist or antagonist) via intracannula injection (i-NAc) for 1 minute after tolerance inducement and passing CPP stages on the last day (testing day). In order to prevent drug relapse, injection cannula was kept still

for 1 min and, after this period and removing the injection cannula, the animal was placed inside the CPP device and tested as mentioned before.

#### Histology:

After the completion of testing, all animals were anesthetized and received a transcardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. The brains were removed, blocked and cut coronally in 40  $\mu$ m sections through the cannula placements. The tissues were stained with cresyl violet and were examined by light microscopy by an observer unfamiliar with the behavioral data. Only the animals with correct cannula placements were included in the data analysis (Fig. 1).



**Fig. 1:** Location of cannula tips in the nucleus accumbens of animals used in the dose-response studies and experiments involving GABAergic agents. Symbols (●) indicate where the cannula tips are placed.

#### Statistical Calculations:

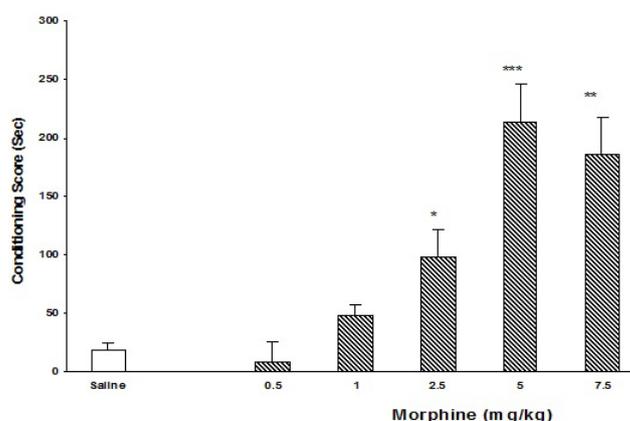
All the results were presented as mean  $\pm$  standard deviation. In each group, 6-8 animals were tested. The results were evaluated by one-way and two-way Analysis of Variance. In case F value was significant, Turkey's post-hoc test was used to determine difference between the groups. In all the cases,  $p < 0.05$  was considered boundary of significant differences. Statistical calculations were done in SPSS software and diagrams were drawn by Microsoft Excel.

## RESULTS AND DISCUSSION

### Results

#### Morphine dose-response on CPP paradigm:

Injection of morphine sulfate with 0.5, 1, 2.5, 5 and 7.5 mg/kg doses to the tested animals showed that, in the house in which morphine was received; significant conditioned place preference was observed in subcutaneous 2.5-7.5 mg/kg doses. Therefore, 5 mg/kg subcutaneous dose was used in further stages as an effective dose which could induce conditioned place preference (Figure 1) ( $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ).

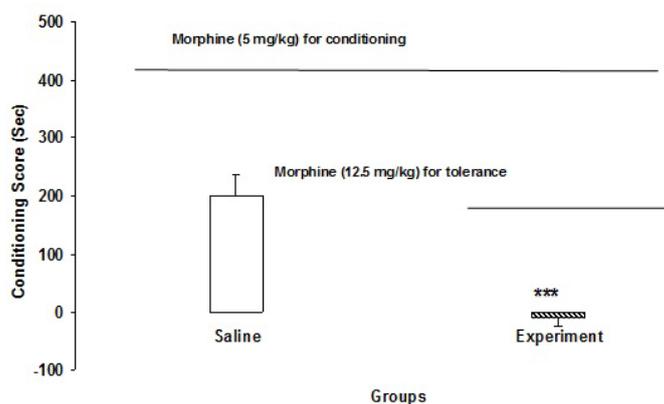


**Fig. 2:** Effect of morphine in inducing conditioned place preference (N8 data, data as mean  $\pm$  S.E.M and  $*p < 0.05$ ,  $**p < 0.01$ ,  $***p < 0.001$ ).

In this stage, after performing the surgery, the animals rested for 3 days in order for effect of the anesthetic drug to disappear. Then, the first day was getting familiar with the environment and, on the second day, beginning conditioning with morphine was done (subcutaneous injection of morphine at 9 am; after closing guillotine valve of the rats, they were placed in one part of the device for 40 min. Six h later, saline was injected into the animals and they were placed in the other part). On the third day, the animals were tested in the same way as the second day, with the only difference that they received saline in the morning and morphine in the evening. The fourth day was the same as the second day and the test was run on the fifth day.

#### Morphine CPP paradigm in morphine- Toleranced animals:

To determine these effects after making the animals tolerant, it was observed that, compared to the control group which received 5 mg/kg, 12.5 mg/kg dose could show very good tolerance in the animals and thus inhibit CPP (Figure 3) (\*\* $p < 0.001$  and two way ANOVA)

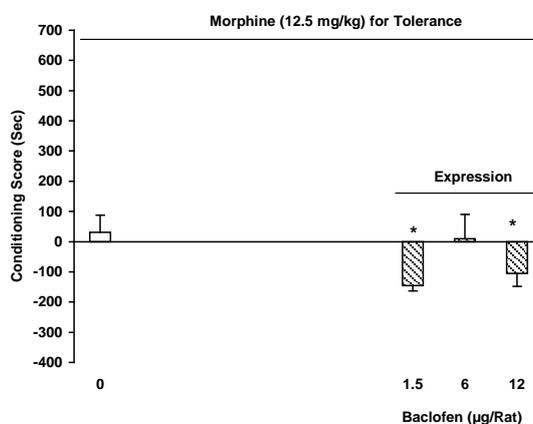


**Fig. 3:** Effect of morphine (subcutaneous 12.5 mg/kg) in inducing conditioned place preference in the group with induced tolerance.

In the control group, saline was injected subcutaneously during the first three days and CPP was done uninterruptedly with subcutaneous 5 mg/kg effective dose. The experimental group subcutaneously received 12.5 mg/kg morphine on first three days and rest of the stages were done similar to the control group.

#### Effects of Baclofen Injection (GABA-B receptor agonist) into Nucleus Accumbens Shell on Conditioned Place Preference Expression in Morphine-tolerant Rats:

To study CPP expression, tolerance was induced in the animals by injecting morphine (subcutaneous 12.5 mg/kg) for three consecutive days and subcutaneous 5 mg/kg effective dose was injected during conditioning stages. They received three separate doses of i-NAc (1.5, 6 and 12  $\mu\text{g}/\text{rat}$ ) baclofen on the testing day. Injecting 1.5 and 12  $\mu\text{g}/\text{rat}$  baclofen reinforced expression of conditioned place preference in tolerant rats (Figure 4) (two way ANOVA) and (\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ).

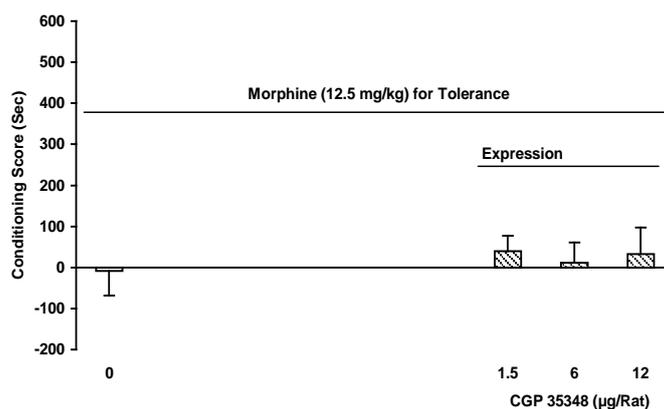


**Fig. 4:** Effect of baclofen (GABA-B agonist) in shell area of nucleus accumbens on expression of conditioned place preference in tolerant rats.

Rates of the control group subcutaneously received 12.5 mg/kg morphine for three consecutive days. Immediately afterward, CPP with effective dose of 5 mg/kg was subcutaneously injected. In the experimental group, in expression stage, the groups received 12.5 mg/kg subcutaneous morphine injection for three consecutive days. Then, uninterruptedly, CPP with effective dose of 5 mg/kg was subcutaneously injected and three separate doses of 1.5, 6 and 12 µg/rat with i-NAc baclofen were injected on the testing day.

*Effects of injecting CGP35348 (GABA-B receptor antagonist) into Nucleus Accumbens Shell on Conditioned Place Preference Expression in Morphine-tolerant Rats:*

In order to study CPP expression, tolerance was induced in the animals by injecting morphine (12.5 mg/kg, subcutaneously) for three consecutive days. 5 mg/kg effective dose was also injected subcutaneously during conditioning stages. On the testing day, three separate doses of 1.5, 6 and 12 µg/rat CGP35348 were injected in i-NAc method. Injecting CGP35348 did not induce any specific effect on acquisition and expression of conditioned place preference in morphine-tolerant rats (Figure 5).



**Fig. 5:** Effect of CGP35348 (GABA-B agonist) in shell area of nucleus accumbens on expression of conditioned place preference in tolerant rats.

In the control group, the rats received 12.5 mg/kg morphine subcutaneously for three consecutive days. Immediately afterward, CPP with effective dose of 5 mg/kg was subcutaneously injected. In the experimental group and in acquisition stage, on first three days and 5 min before injecting morphine, 12.5 mg/kg CGP35348 was subcutaneously injected in i-NAc way. After that, CPP was done with effective dose of 5mg/kg. In the expression stage, the groups received 12.5 mg/kg morphine subcutaneously for three consecutive days. Then, uninterruptedly, CPP with effective dose of 5 mg/kg was subcutaneously injected and three separate doses of 1.5, 6 and 12 µg/rat baclofen were injected on the testing day using i-NAc method.

*Discussion:*

In the current research, studies showed that subcutaneous injection of morphine induced significant conditioned place preference in the animals, which was consistent with previous results of other groups and researchers and showed that euphoric effects of morphine could be paired with environmental stimuli such as color, smelling, field material and also decorations of environmental walls and award them secondary reward property. It should be explained that numerous studies have been conducted on the reason for development of conditioned place preference and probable cellular molecular mechanisms and different researches have demonstrated that mesocorticolimbic reward pathway which is a dopamine pathway [1] is involved in the occurrence of this phenomenon. Also, other neurotransmitter systems like GABAergic system, glutamic system, nitric oxide system and serotonin system are also considered modulatory systems along the above mentioned dopamine system [13]. In the experiments, first, tolerance was induced in animals by injecting morphine for three consecutive days. In other words, when effective dose of morphine (as defined in the initial experiment) was injected into these animals, it could not induce conditioned place, which means morphine-tolerance. In the present experiment, using 25 and 50 mg/kg doses caused more than 50% mortality in the animals and, for this reason, 12.5 mg/kg was selected for inducing morphine tolerance. This dose could induce good tolerance in the animals. Emergence of morphine-tolerance has been observed among many effects of morphine, the most important of which include tolerance to painless effects, tolerance to euphoric effects, tolerance to occurrence of movement activity and tolerance to contraction in vas deferens of rats while no tolerance is generated to some effects of morphine like pupil miosis, respiratory depression and constipation.

Moreover, some reports have been in contrast to the present study stating that no tolerance is created against euphoric effects of morphine [14]. On the other hand, different studies have shown that, when tolerance to morphine occurs, the number of NMDA receptors in brain generally decreases and also the number of NMDA

receptors in nucleus accumbens decreases. In cellular molecular terms, it has been determined that, when tolerance occurs, strong changes are reported in the amount of types I, II and VIII adenylyltransferase, working as G protein-mediated inhibitory effect [1]. It should be noted that these three enzyme types are inhibited in case of acute morphine effect and therefore CAMP level decreases in cell. But, by chronic injection of morphine, these enzymes escape inhibitory effects of morphine and return cellular CAMP to its normal level. For this reason, in the case of tolerance, morphine gradually loses its effect [3,15].

Other mechanisms have been also presented for tolerance to morphine in cellular molecular terms, the most important of which include increased phosphorylation of opioid receptors [16], increased internalization of these receptors and therefore decreased number of receptors [16], decreased return of opioid receptors to membrane level and decreased activity of protein kinase A (PKA) [17,18]. Accordingly, what happens in cellular molecular terms during tolerance is somewhat evident. However, role of the neurotransmitters existing in reward pathway is not clear in development of this tolerance [19]. Different investigations have shown that one of the most important effective points for development of tolerance to narcotics is nucleus accumbens and, since main part of the neurons existing in nucleus accumbens are GABAergic neurons and there is an increased number of GABA-B receptors in this area [7], it seems that stimulation or inhibition of these receptors have some side effects on the tolerance [1,20]. In this study, depending on dose, injection of baclofen caused change in expression of conditioned place preference among morphine-tolerant rats. In low and very high doses, baclofen changed expression of conditioned place preference in morphine-tolerant rats. It seems that, according to the mechanisms mentioned in target neurons of morphine, the mechanisms change depending on GABA-G receptors in order to induce different responses when using baclofen. As stated earlier, injecting morphine decreased activity of adenylyltransferase enzyme and consequently increased activity of this enzyme in the case of recurrent morphine injection. When using baclofen, two types of responses were observed in the animals based on baclofen dosage. It seems that, in some values, baclofen had an effect consistent with morphine and thus it reinforced tolerance to morphine so that the animals treated with baclofen showed no tendency to morphine reception location in the conditioned place preference device. In other doses, the opposite effect was observed; meaning that morphine affected different subgroups of GABA-G by baclofen and thus different responses were observed. On the other hand, another implication could be considered for these reports which is that baclofen affects two presynaptic and postsynaptic types of receptors and the obtained responses are the result of activities of these presynaptic and postsynaptic receptors. However, presynaptic GABA-B receptors have not been identified in accumbens area thus far [13]. Injecting CGP35348 did not induce any specific effect for acquisition and expression of conditioned place preference in morphine-tolerant rats, which could mean that inhibition of GABA-G receptors does not probably play an important role in morphine-tolerant animals and therefore other antagonist drugs should be used in this field.

In a general summary, it appears that stimulation and inhibition of GABA-G receptors in nucleus accumbens play an important role in expression of conditioned place preference resulting from morphine in morphine-tolerant rats. This issue could have another effect on previous works related to baclofen and the obtained findings; also, it could confirm previous findings and broaden the current knowledge on operation of GABA-G receptors in morphine-tolerant animals.

## REFERENCES

- [1] Bowery, N.G., B. Bettler, W. Froestl, J.P. Gallagher, F. Marshall, M. Raiteri, T.I. Bonner, S.J. Enna, 2002. International Union of Pharmacology. XXXIII. Mammalian gamma-aminobutyric acid (B) receptors: structure and function. *Pharmacol. Rev.*, 54(2): 247-264.
- [2] Maccioni, P., P. Bienkowski, M. Carai, G. Gessa, G. Colombo, 2008. Baclofen attenuates cue-induced reinstatement of alcohol-seeking behavior in sardinian alcohol-preferring (sp) rats. *Drug Alcohol Depend.*, 95: 284-287.
- [3] Haney, M., C.L. Hart, R.W. Foltin, 2006. Effects of baclofen on cocaine self-administration: opioid- and nonopioid-dependent volunteers. *Neuropsychopharmacology*, 31(8): 1814-1821.
- [4] Fattore, L., M.S. Spano, G. Cossu, M. Scherma, W. Fratta, P. Fadda, 2009. Baclofen prevents drug-induced reinstatement of extinguished nicotine-seeking behaviour and nicotine place preference in rodents. *Eur. Neuro. psychopharmacol.*, 19(7): 487-498.
- [5] Bartoletti, M., A. Colantoni, V. De Luca, M. Gaiardi, 2010. Single and repeated baclofen treatment attenuates the discriminative stimulus effects of morphine in rats. *Pharmacol. Biochem. Behav.*, 97(2): 279-283.
- [6] Tyacke, R.J., A. Lingford-Hughes, L.J. Reed, D.J. Nutt, 2010. GABAB receptors in addiction and its treatment. *Adv. Pharmacol.*, 58: 373-396.
- [7] Fu, Z., H. Yang, Y. Xiao, G. Zhao, H. Huang, 2012. The gamma-aminobutyric acid type B (GABAB) receptor agonist baclofen inhibits morphine sensitization by decreasing the dopamine level in rat nucleus accumbens. *Behav Brain Funct.*, 8: 20.

- [8] Dayan, P., 2009. Dopamine, reinforcement learning, and addiction. *Pharmacopsychiatry*, 42(1): S56-65.
- [9] Cadoni, C., G. Di Chiara, 1999. Reciprocal changes in dopamine responsiveness in the nucleus accumbens shell and core and in the dorsal caudate-putamen in rats sensitized to morphine. *Neuroscience*, 90(2): 447-455.
- [10] Cicero, T.J., T. Ennis, J. Ogden, E.R. Meyer, 2000. Gender differences in the reinforcing properties of morphine. *Pharmacol. Biochem. Behav.*, 65(1): 91-96.
- [11] Paxinos, G., C.R. Watson, P.C. Emson, 1980. AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. *J. Neurosci. Methods*, 3(2): 129-149.
- [12] Karami, M., M.R. Zarrindast, H. Sepehri, H. Sahraei, 2002. Role of nitric oxide in the rat hippocampal CA1 area on morphine-induced conditioned place preference. *Eur. J. Pharmacol.*, 449(1-2): 113-119.
- [13] Bettler, B., K. Kaupmann, J. Mosbacher, M. Gassmann, 2004. Molecular structure and physiological functions of GABA(B) receptors. *Physiol. Rev.*, 84(3): 835-867.
- [14] Robinson, T.E., K.C. Berridge, 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Rev.*, 18(3): 247-291.
- [15] Pentney, R.J., A. Gratton, 1991. Effects of local delta and mu opioid receptor activation on basal and stimulated dopamine release in striatum and nucleus accumbens of rat: an in vivo electrochemical study. *Neuroscience*, 45(1): 95-102.
- [16] Ueda, H., M. Inoue, K. Mizuno, 2003. New approaches to study the development of morphine tolerance and dependence. *Life Sci.*, 74(2-3): 313-320.
- [17] Sklair-Tavron, L., W.X. Shi, S.B. Lane, H.W. Harris, B.S. Bunney, E.J. Nestler 1996. Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. *Proc. Natl. Acad. Sci. USA*, 93(20): 11202-11207.
- [18] Narita, M., H. Kato, K. Miyoshi, T. Aoki, Y. Yajima, T. Suzuki, 2005. Treatment for psychological dependence on morphine: usefulness of inhibiting NMDA receptor and its associated protein kinase in the nucleus accumbens. *Life Sci.*, 77(18): 2207-2220.
- [19] Schmidt, B.L., C.H. Tambeli, J. Barletta, L. Luo, P. Green, J.D. Levine, R.W. Gear, 2002. Altered nucleus accumbens circuitry mediates pain-induced antinociception in morphine-tolerant rats. *J. Neurosci.*, 22(15): 6773-6780.
- [20] Piepponen, T.P., K. Kiianmaa, L. Ahtee, 2002. Effects of ethanol on the accumbal output of dopamine, GABA and glutamate in alcohol-tolerant and alcohol-nontolerant rats. *Pharmacol. Biochem. Behav.*, 74(1): 21-30.