Pharmacology, Biochemistry and Behavior 102 (2012) 249–256

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Transient inactivation of the nucleus accumbens reduces both the expression and acquisition of morphine-induced conditioned place preference in rats

Mohammad-Hossein Esmaeili [a], Hedayat Sahraei [b,*], Hengameh Ali-Beig [c], Mojgan Ardehari-Ghaleh [c], Zahra Mohammadian [c], Homeira Zardooz [d], Seyed Hassan Salimi [e], Jamal Shams [f], Ali Noroozzadeh [g]

[a] Department of Physiology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran
[b] Neurosciences Research Center, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran
[c] Department of Biology, School of Science, Tehran North Branch, AZAD Islamic University, Tehran, Iran
[d] Department of Physiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[e] Department of Psychology, School of Medicine, Baqyiatallah (a.s.) University of Medical Sciences, Tehran, Iran
[f] Department of Psychiatry, School of Medicine, and Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[g] Behavioral Sciences Research Center, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 10 August 2011
Received in revised form 24 April 2012
Accepted 29 April 2012
Available online 3 May 2012

Keywords:
Morphine
Conditioned place preference
Shell and core parts of nucleus accumbens
Rat

ABSTRACT

In the present study, the effects of transient inhibition of the shell and core parts of the nucleus accumbens by lidocaine on the expression and acquisition of morphine-induced conditioned place preference in male Wistar rats were investigated. In addition, the number of bouts of sniffing, rearing, and compartment crossing was scored. Lidocaine hydrochloride was injected into different parts of the nucleus accumbens 5 min before each morphine session for the transient inhibition of particular anatomical regions. Subcutaneous (s.c.) injection of morphine (2.5 and 5 mg/kg) induced place preference. Transient inhibition of the left and/or right side of the shell part of nucleus accumbens reduced morphine place conditioning. However, when both sides of the nucleus were inhibited, inhibition was weaker when compared to the results when only one side was inhibited. Also, the number of compartment crossings in these animals reduced significantly. Nevertheless, the number of rearing occurrences was reduced only when both sides of the shell part of the nucleus accumbens were inhibited. In contrast, the number of sniffing bouts increased in all three groups. The results for the core part of the nucleus accumbens also indicated that place preference was inhibited after transient inhibition of the left, right, and both sides. However, although the number of total compartment crossings was reduced in all experimental groups, the reduction was not statistically significant. The data obtained was similar to the number of rearings, yet the number of sniffing bouts increased in the experimental groups compared to the control. In conclusion, these results confirmed the involvement of the left and right sides and core and shell parts of the nucleus accumbens in morphine place conditioning.

1. Introduction

It has been shown that the mesolimbic dopaminergic pathway originates from the ventral tegmental area, projects to the nucleus accumbens (NAc) and prefrontal cortex, and plays a key role in opioid and other abused drug reward, dependence, and sensitization (Manzanedo et al., 2005; Di Chiara, 2002; Spanagel and Welss, 1999; Vanderschuren et al., 1999). The NAc in particular is thought to play a critical role in both natural and drug-induced reward (Di Chiara and Imperato, 1988; Di Chiara et al., 1999; Imperato and Di Chiara, 1986). Studies have revealed that the principal neurons of the NAc are γ-aminobutyric acid (GABA) medium-spiny neurons that comprise over 90% of the total neuronal population (Brog et al., 1993; Smith and Bolam, 1990). The axons of these neurons form projections of the NAc as well as the dense network within the NAc, thereby overlapping their own dendrites (McFarland and Kalivas, 2001; Kawaguchi et al., 1995; Pennartz and Gronenwegen, 1994; O'Donnell and Grace, 1994; Chang and Kitai, 1985). These local axon collaterals account for most of the GABA synapses in the NAc (Smith and Bolam, 1990), suggesting that medium-spiny neurons mediate the main information processing functions of the NAc as well as convey the information to projection areas. The NAc also receives excitatory inputs from cortical afferents and modulatory dopaminergic input from the ventral tegmental area (Smith and Bolam, 1990; Sesack and Pickel, 1990; Di Chiara et al., 1994).

Based on anatomical and cytochemical differences, the NAc can be divided into core and shell subregions (Jongen-Relo et al., 1993, 1994; Meredith, 1999; Meredith et al., 1993), each having different roles in drug dependence (Pontieri et al., 1995, 1996; Smith et al., 2009). It

* Corresponding author at: Department of Physiology and Biophysics, School of Medicine, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Iran, P.O. Box 19395-6558. Tel./fax: +98 21 2612725.
E-mail address: h.sahraei@bmsu.ac.ir (H. Sahraei).

0091-3057/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2012.04.015

has been reported that while administering abused drugs, such as morphine, an increase in dopamine release in the shell subregion of the NAc is observed. The core subregion has been shown to play a role in conditioning models, such as the Pavlovian conditioning model (Di Ciano et al., 2001). The data from past decades have revealed that both dopaminergic and non dopaminergic substrates may be involved in the mediation of nucleus accumbens function (Joyce and Koob, 1981; Robbins and Koob, 1980; Koob et al., 1978; Robbins et al., 1983; Kalivas et al., 1982, 1983; Kalivas, 1984; Kalivas and Bronson, 1985; Kalivas and Miller, 1985; Kalivas et al., 1989). For example, Swerdlow and Koob showed that dopamine terminal destruction in the NAc by 6-hydroxydopamine can block D-amphetamine—but not caffeine-induced locomotor activity (Swerdlow and Koob, 1985). In this regard, Kalivas et al. (1983) showed that enkephalin microinjection in the nucleus accumbens could increase locomotor activity which is not antagonized by neuroleptic pretreatment or dopamine terminal destruction by 6-hydroxydopamine .

Previous studies also are indicative of an asymmetry between the right and left sides of the NAc during locomotive activity. Belcheva et al. (1990) showed that a microinjection of two dopamine receptor agonists – apomorphine and SKF38393 – into the right NAc induced more hyperactivity than the injection into the left NAc. From these findings, they postulated that the activity of the NAc is mediated by a varied distribution of dopamine D_1 and D_2 receptors in the left and right NAc (Belcheva et al., 1990). Asymmetry in the NAc also appears to affect other neurotransmitter/neuropeptide systems. In this regard, Belcheva et al. (1994) found that a microinjection of cholecystokinin-8 (CCK-8) into the right NAc increased dopamine activity more than an identical injection into the left NAc. Collectively, these findings may indicate an anatomical and functional asymmetry between the left and right NAc.

However, research on the NAc has not focused on this asymmetry. Considering that the left and right NAc have different connections with other regions of the central nervous system (Heimer et al., 1991), the role of the shell and core subregions of the NAc in drug dependence and reward may vary between the two sides; therefore, the role of the right vs. left side and shell vs. core part of the NAc in drug dependence and reward may be different. Previous studies have used toxic agents, such as 6-hydroxydopamine and kainic acid, for the removal of dopaminergic or glutamatergic neurons or electrical lesions in order to investigate the role of the NAc in drug abuse (Tzschentke, 2007). However, these methods can affect other activities of the NAc, such as its role in feeding in rats. This may interfere with the obtained results; moreover, since the chemical agents failed to remove all neurons in the nucleus, these methods may seem to be ineffective for the investigation mentioned above. The aim of this study was to investigate the potential effects of blocking the left or right or both sides of the shell and core subregions of the NAc on both morphine-induced conditioned place preference and conditioned dopamine-related behaviors, including rearing, sniffing, and compartment crossing. Our study attempted to address the issues mentioned above by using 2% lidocaine as the local anesthetic that could transiently block neuronal activity for up to 30 min (Moaddab et al., 2009; Mrose and Ritchie, 1978; Ragsdale et al., 1994; Ritchie, 1979; Sommer and Tehovnik, 1997; 1999; Tehovnik and Sommer, 1997).

2. Material and methods

2.1. Animals

Male Wistar rats (250±20 g, Pasture Institute, Tehran, Iran) were used throughout the study (6–8 rats for each experiment). The animals were housed in groups of four per cage under a 12 h/12 h light/cycle (lights on at 7:00 AM), with ad libitum food and water available. The animals were randomly allocated to different experimental groups. All of the experiments were conducted in accordance with standard ethical guidelines and approved by the local ethics committee (The Baqiyatallah [a.s.] University of Medical Committee on the Use and Care of Animals, 81/021, July 10, 2002).

2.2. Drugs

The following drugs were used throughout the experiments: morphine sulfate (TEMAD, Iran), lidocaine hydrochloride (Sigma, St. Louis, MO, USA), ketamine hydrochloride, and xylazine (Alfasan Worden, Holland). All of the drugs were dissolved in sterile saline. Morphine was injected subcutaneously in a volume of 1 ml/kg, and 2% lidocaine (Moaddab et al., 2009; Ragsdale et al., 1994; Sommer and Tehovnik, 1997; 1999) was prepared and then administered intra-NAc in a volume of 1 μl/rat 5 min before the morphine injection. The duration of action for lidocaine is around 30 min, and the duration of action for morphine is 10–25 min; therefore, the 5 min lag between the lidocaine and morphine injections provided adequate drug coverage (Moaddab et al., 2009). The control groups received saline.

2.3. Surgical procedures

The rats were anesthetized with ketamine hydrochloride (70 mg/kg, i.p.) + xylazine (10 mg/kg, i.p.), and one or two 23-gauge stainless steel cannulas were placed stereotaxically (Stolting Instruments, USA) into the shell or core part of the NAc. Stainless steel, 23-gauge guide cannulas were implanted bilaterally 0.5 mm above the intended site of injection according to the atlas of Paxinos and Watson (1987). The stereotaxic coordinates for the shell part of the NAc were the following: incisor bar (−3.3 mm), 1.2 mm anterior to bregma, ±0.8 mm lateral to the sagittal suture, and 6.8 mm down from top of skull. The coordinates for the core part of the NAc were the following: incisor bar (−3.3 mm), 0.8 mm anterior to bregma, ±1 mm lateral to the sagittal suture, and 7 mm down from top of skull. The cannulas were secured with jewelers' screws and dental acrylic. After surgery, a dummy inner cannula was inserted into the guide cannula and left in place until the injections were made. The length of the dummy cannula was identical to the guide cannula. The animals were allowed 7 days to recover from the surgery and anesthesia.

For drug infusions, 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 μl/rat (0.5 μl in each side) over a period of 60 s. The injection needles were left in place for an additional 60 s to facilitate the diffusion of the drugs. The effect of lidocaine as a reversible Na^+ channel blocker is the greatest around 8 min after the infusion and may last for up to 30 min (Moaddab et al., 2009; Ragsdale et al., 1994; Sommer and Tehovnik, 1997; 1999).

2.4. Apparatus

A two-compartment place preference apparatus (30×60×30 cm) was used in our experiments (Sahraei et al., 2009). The apparatus was made of wood with two identical compartments (the apparatus was divided into two compartments of equal size by means of a removable white guillotine door) and similar shading (both were white), but distinguishable by texture and olfactory and visual cues. To provide a tactile difference between compartments, one of the compartments had a smooth floor while the other had a white nylon mesh floor. Olfactory differences between the compartments were achieved by placing a drop of menthol at the right center of the textured compartment (nylon mesh). For visual differences, the compartments were differentially striped black on their sides. Using this apparatus, the animals showed no consistent preference for either compartment, supporting our un-biased place conditioning paradigm.

2.5. Behavioral testing

2.5.1. Measurement of conditioned place preference

Conditioned place preference consisted of three phases: pre-conditioning, conditioning, and post conditioning. Place conditioning was conducted using an unbiased procedure, with minor changes to the design as previously described (Sahraei et al., 2009).

2.5.1.1. Pre-conditioning. On day 1 (pre-conditioning), each rat was separately placed into the apparatus for 10 min, with free access to both compartments, and later, the time spent by rats in each compartment was measured. Our data showed that the animals did not show any consistent preference for either compartment (data not shown).

2.5.1.2. Conditioning. This phase consisted of a 3-day schedule of conditioning sessions. In this phase, the animals received three trials in which they experienced the effects of morphine while confined to one compartment for 45 min and three trials in which they experienced the effects of saline while confined to the other compartment for 45 min. On the first day of conditioning sessions, the animals received morphine at 9:00 AM and saline at 4:00 PM. On day 2, the time order of receiving morphine and saline was reversed. On day 3, the animals received morphine and saline as on day 1. Immediately after each morphine or saline injection, the animals were placed in their relative compartment for 45 min. Access to the other compartment was blocked on these days. In addition, the morphine and saline compartments were randomly assigned to each animal in a counterbalanced way. Thirty minutes before each morphine administration, 5% lidocaine was injected into the core, shell, and/or both parts according to the experiment procedure.

2.5.1.3. Post-conditioning phase. On the 5th day (the preference test day), the partition was removed, and each rat was placed in the middle part of the apparatus where it could access both compartments. The behavior of each animal was digitally videotaped for 10 min. The offline video files were later analyzed by a person who was not familiar with the experiments. The time spent in each compartment was recorded. The difference between the post-conditioning and pre-conditioning scores was denoted as the "change in preference" score. Sniffing and rearing were two stereotyped behaviors, described by Molloy and Waddington (1995); non-stereotyped behavior, compartment crossing, was also recorded. Compartment crossing is defined as the total number of crossings between compartments, which is considered a good indicator of locomotor activity (Sahraei et al., 2009; Fedele et al., 1998). Previous researchers suggested that locomotor activity may influence place preference responses and recommended evaluating locomotor activity in future experiments (Moaddab et al., 2009; for review see Tzschentke, 2007). We decided to record sniffing and rearing occurrences to investigate if our treatment resulted in an overall disruption of striatal circuitry.

2.6. Histology

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

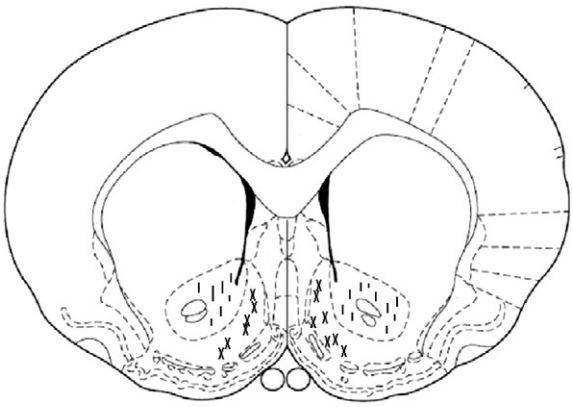

Fig. 1. Location of the cannula tips in the nucleus accumbens of animals used in the dose–response studies and experiments involving transient inhibition. Symbols (x and |) indicate where the cannula tips were placed in the shell and core part of the nucleus accumbens, respectively.

2.7. Data analysis

All data are expressed as mean ± SEM. The morphine dose–response was analyzed using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. A three-way ANOVA was applied to evaluate the differences between the lidocaine-treated groups using side, pre-treatment, and treatment as factors. When the three-way ANOVA showed a significant difference, the Tukey HSD test was applied to demonstrate the difference. Differences with $P<0.05$ were considered significant.

3. Results

3.1. Morphine dose–response in place conditioning paradigm

The effects of morphine on place preference are shown in Fig. 2. Different doses of morphine sulfate (1, 2.5, 5 and 7.5 mg/kg, s.c.) were injected into rats and caused a significant place preference to the drug-paired compartment at doses of 2.5 and 5 mg/kg [$F(5,30)=5.34$, $P<0.01$]. Based on these data, the dose of 5 mg/kg of morphine was selected as an effective dose for the rest of the experiments. In addition, morphine administration increased the number of sniffings [$F(5,30)=11.2$, $P<0.001$], rearings [$F(5,30)=10.42$, $P<0.0001$], and

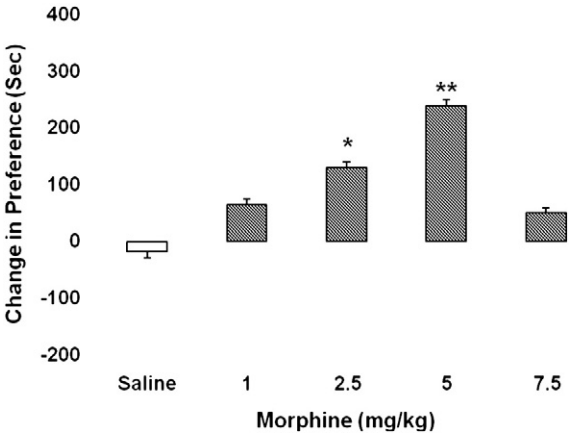

Fig. 2. Conditioned place preference induced by morphine. Animals received different doses of morphine (1, 2.5, 5, and 7.5 mg/kg, s.c.). Each point shows the mean ± SEM conditioning score for 6–8 rats. *$P<0.05$, **$P<0.01$ different from the saline control group.

compartment crossings [$F(5,30)=12.58$, $P<0.0001$] in a dose-dependent manner as compared with the saline control (Table 1).

3.2. Morphine place conditioning paradigm in rats when right or left and/or complete shell part of nucleus accumbens was transiently inactivated

In this part of our experiments, the place conditioning paradigm was conducted with morphine (5 mg/kg, s.c.), but 5 min before each morphine administration, 2% lidocaine was injected into the right, left, or both sides of the shell part of the NAc. The control group received intra-accumbal sterile saline. Our results showed that the place preference for the drug-paired compartment was significantly reduced when the right or left side of shell part of the nucleus accumbens was inhibited [three-way ANOVA within-group comparison: Side effect: $F(5,35)=5.12$, $P<0.001$; Pretreatment effect: $F(1,35)=3.432$, $P<0.001$; Treatment effect: $F(5,35)=4.34$, $P<0.001$; Side × Pretreatment × Treatment interaction: $F(8,73)=9.34$, $P<0.0001$]. Interestingly, when both sides of the nucleus accumbens were inhibited, the effect of morphine did not change significantly (Fig. 3A). In addition, the total compartment crossings were reduced among all groups compared with the control, but the inhibition was greater when the left side or left + right side was inhibited [three-way ANOVA within-group comparison: Side effect: $F(5,35)=3.41$, $P<0.01$; Pretreatment effect: $F(1,35)=2.11$, $P<0.05$; Treatment effect: $F(5,35)=3.25$, $P<0.01$; Side × Pretreatment × Treatment interaction: $F(8,73)=4.34$, $P<0.001$; Fig. 3B]. In relation to rearing, the behavior was slightly increased when the left or right side of the nucleus accumbens was inhibited alone, but when the left side was inhibited alone, the number of rearings was reduced significantly [three-way ANOVA within-group comparison: Side effect: $F(5,35)=1.12$, $P>0.05$; Pretreatment effect: $F(1,35)=1.09$, $P>0.05$; Treatment effect: $F(5,35)=1.21$, $P<0.05$; Side × Pretreatment × Treatment interaction: $F(8,73)=2.11$, $P<0.05$; Fig. 3C]. Furthermore, the number of sniffings in all groups increased compared with the control [three-way ANOVA within-group comparison: Side effect: $F(5,35)=6.11$, $P<0.001$; Pretreatment effect: $F(1,35)=4.32$, $P<0.001$; Treatment effect: $F(5,35)=3.54$, $P<0.01$; Side × Pretreatment × Treatment interaction: $F(8,73)=3.23$, $P<0.01$; Fig. 3D].

3.3. Effects of transient inhibition of right or left and/or complete core part of NAc on morphine place preference

Our data indicated that the place preference for the drug-paired compartment was significantly reduced when the right, left, or both sides of the core part of the nucleus accumbens were transiently inhibited [three-way ANOVA within-group comparison: Side effect: $F(5,30)=4.32$, $P<0.001$; Pretreatment effect: $F(1,30)=3.67$, $P<0.001$; Treatment effect: $F(5,30)=6.43$, $P<0.001$; Side × Pretreatment × Treatment interaction: $F(8,65)=8.04$, $P<0.0001$]. The inhibition was less significant when the right side of the nucleus accumbens was inhibited (Fig. 4A). Moreover, the number of total compartment crossings was reduced in all groups but it was only significant for the group with both-side inhibition [three-way ANOVA within-group comparison: Side effect: $F(5,30)=1.26$, $P<0.1$; Pretreatment effect: $F(1,30)=1.3$, $P<0.1$; Treatment effect: $F(5,30)=0.98$, $P<0.1$; Side × Pretreatment × Treatment interaction: $F(8,65)=2.43$, $P<0.05$; Fig. 4B]. Regarding rearings, the behavior was slightly increased when either the right or both sides of the nucleus accumbens were inhibited, but when the left side was simultaneously inhibited, the number of rearings was reduced significantly [three-way ANOVA within-group comparison: Side effect: $F(5,30)=1.07$, $P<0.1$; Pretreatment effect: $F(1,30)=1.2$, $P<0.1$; Treatment effect: $F(5,30)=1.21$, $P<0.1$; Side × Pretreatment × Treatment interaction: $F(8,65)=2.19$, $P<0.05$; Fig. 4C]. Similar to the shell part, the number of sniffings also increased in all experimental groups [three-way ANOVA within-group comparison: Side effect: $F(5,30)=3.12$, $P<0.01$; Pretreatment effect: $F(1,30)=2.54$, $P<0.05$; Treatment effect: $F(5,30)=3.43$, $P<0.01$; Side × Pretreatment × Treatment interaction: $F(8,65)=4.75$, $P<0.01$; Fig. 4D].

Table 1
Effects of morphine conditioning on dopamine-related behaviors in rats. Morphine treatment significantly increased locomotor activity (as shown by compartment crossings) and numbers of rearings and sniffings. Each point shows the mean ± SEM conditioning score for 6–8 rats. $^{c}P<0.001$, $^{b}P<0.01$, $^{a}P<0.05$, different from the saline control group.

Morphine (mg/kg)	Sniffings	Rearings	Compartment crossings
0	13 ± 3.2	10 ± 3.43	7 ± 3.17
1	24 ± 6.1^{b}	14 ± 8.3	9 ± 3.42
2.5	27 ± 8^{b}	24 ± 5.11^{b}	17 ± 4.3^{b}
5	26 ± 5.1^{b}	30 ± 2.34^{c}	25 ± 3.12^{c}
7.5	17 ± 4.21	21 ± 5.64^{a}	17 ± 2.1^{b}

4. Discussion

Our findings of the present study may offer new insights into morphine reward (e.g., conditioned place preference) and the role of the nucleus accumbens (NAc). The transient inhibition of different parts of the NAc or the entire complex may help discover the role of this part of the central nervous system in relation to drug abuse. Our data indicated that both the core and shell subregions of the NAc were involved in morphine place preference and that the right and left sides may have different roles in the place preference paradigm, and even in conditioned dopamine-related behaviors such as sniffing, locomotion, and rearing.

Our data also showed that morphine can induce place preference in a dose-dependent (bell-shaped dose response) manner. Our results were in agreement with those of previous studies in which the subcutaneous and intraperitoneal administration of opioids was found to induce a place preference in both rats and mice (For review see Tzschentke, 2007). It is clear that morphine can induce place conditioning by reducing the tonic inhibition of dopaminergic neurons through actions at μ-opioid receptors on GABAergic interneurons (Johnson and North, 1992). Data also confirmed that morphine elevates the extracellular concentration of dopamine in the nucleus accumbens (Imperato and Di Chiara, 1986). However, when the dose of opioid increases, the affinity of morphine shifts from μ-opioid receptors toward κ-opioid receptors, and the aversive, sedative, and/or cognition-impairing effects of a high dose of morphine may overcome the rewarding effects of the drug (McClung and Nestler, 2008; Tzschentke, 2007). In addition, our data indicated that morphine at a dose of 7.5 mg/kg did not induce any place preference as reported by other investigators (Moaddab et al., 2009). The possible explanation seems to be associated with difference in the apparatus used in the two studies (Moaddab et al., 2009). We used a two-compartment apparatus, whereas Moaddab et al. used a three-compartment apparatus. In addition, the effect of the apparatus on the results obtained in the place-conditioning paradigm has been mentioned in previous studies (for review see Tzschentke, 2007). Our data also demonstrated that morphine-treated animals showed an increase in their conditioned dopamine-related behaviors as well as compartment crossings, which can be considered an indicator of locomotion (Sahraei et al., 2009). However, the possible relationship between morphine place conditioning and conditioned dopamine-related behaviors has not been investigated. We have previously shown that compartment crossings may be distinguished as an indicator of locomotor activity in animals and may be useful in research on animal activity. In the present study, we measured other conditioned dopamine-related behaviors (i.e., sniffing and rearing), which are regarded as proper indicators of mesolimbic system activity (Fedele et al., 1998). It is necessary to be noted that as mentioned in the Introduction section above, the behaviors that were recorded in our study may have a non-dopaminergic source, as revealed by Koob et al. in the late 1980s

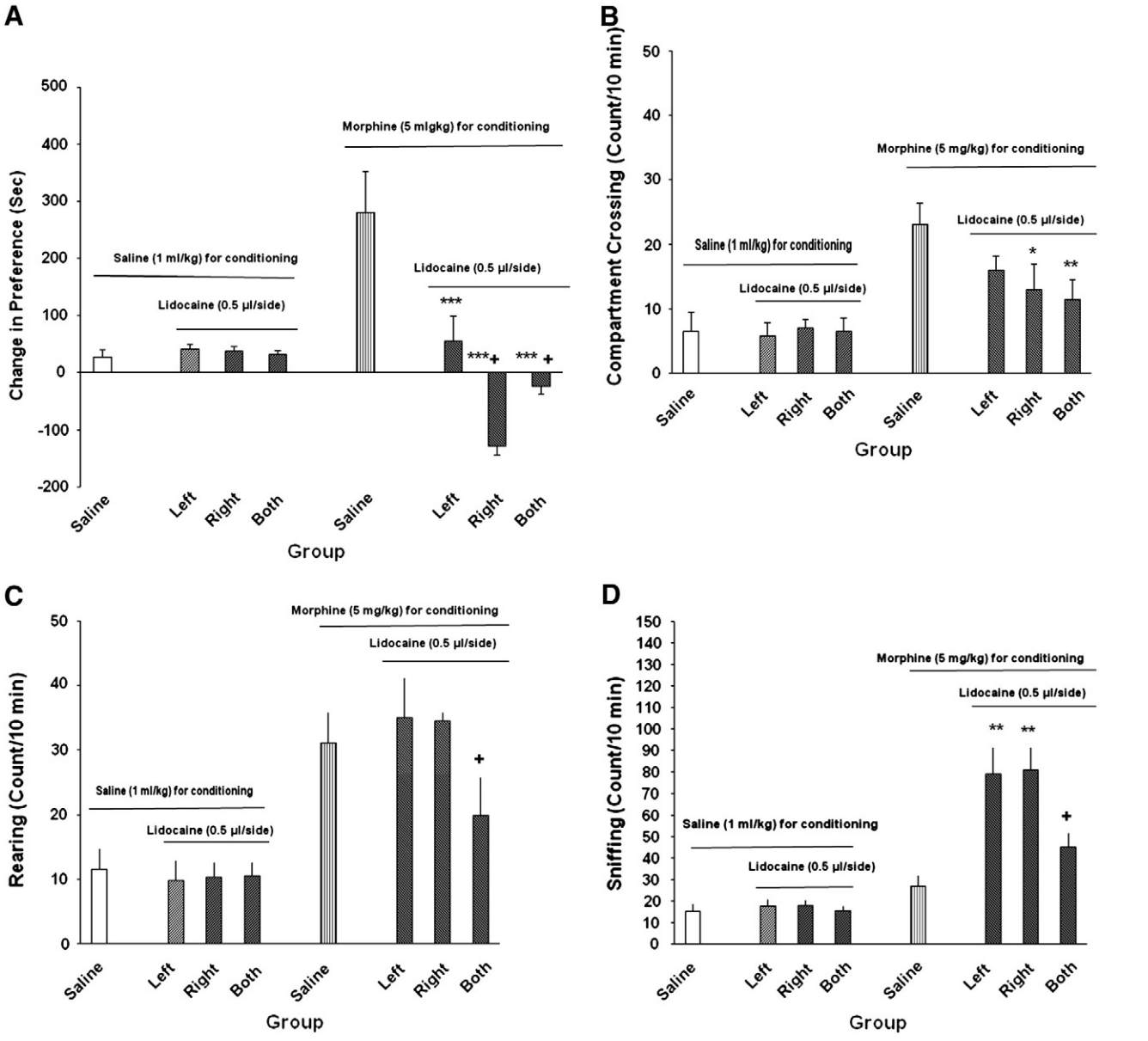

Fig. 3. (A) Effects of left, right, and/or both side transient inhibition of the shell part of the nucleus accumbens on morphine place preference. Each point shows the mean ± SEM conditioning score for 8 rats. ***P<0.001, **P<0.01, different from the control group; +P<0.05, different from the right side. (B–D) Effects of transient inhibition of left, right, or both sides of the shell part of the nucleus accumbens on dopamine-related behaviors in rats during place conditioning test. Dopamine-related behaviors were monitored in the post-conditioning phase during the test. Compartment crossings reduced when the right part or both sides of the shell subregion were transiently inhibited (B). In addition, the number of rearings also decreased when both sides of the shell subregion were inhibited (C). Interestingly, the number of sniffings in the animals increased (D). Each point shows the mean ± SEM conditioning score for 6–8 rats. **P<0.01, *P<0.05, different from the saline control group; +P<0.05, different from the right side.

(Joyce and Koob, 1981; Robbins and Koob, 1980; Koob et al., 1978; Robbins et al., 1983).

In these experiments, every single behavior studied increased following the administration of different doses of morphine, except the dose of 7.5 mg/kg that caused a decline in the behaviors. However, since these behaviors were not measured in previous studies of morphine place conditioning, no direct comparisons could be made (Tzschentke, 2007). On the other hand, a number of studies have been conducted on the effects of morphine on dopamine-dependent behaviors, such as sniffing, rearing, and locomotion (Vanderschuren et al., 1996; Vezina and Stewart, 1987; Harris et al., 2004). Previous studies have shown that administering morphine may yield different behaviors; however, all such studies have assessed the animals immediately after the administration, whereas in our study an interval of 24 h was used between the last administration and evaluation. Based on our results, one can conclude that these behavioral changes occur in response to the paradigm used for morphine place conditioning; nevertheless, since some of the behaviors decreased while the others increased, it could be assumed that these behaviors may not always change in parallel with the animal activity and preference, indicating that different mechanisms might be involved in the expression of these behaviors.

As mentioned above, the NAc is a heterogeneous compartment that plays a role in the relationship between emotion and movement and also in the expression of emotions. However, despite the intense studies focusing on the role of different parts of the NAc in drug dependence and addiction as well as the role of different neurotransmitters within the nucleus, the exact role of the nucleus accumbnes associated with drug dependence remains unknown. This was the primary objective of our study, and attempts were made to gain a better understanding of the role of the NAc.

Our data showed that the transient inactivation of the shell subregion of the NAc on either the right side or both sides was sufficient to

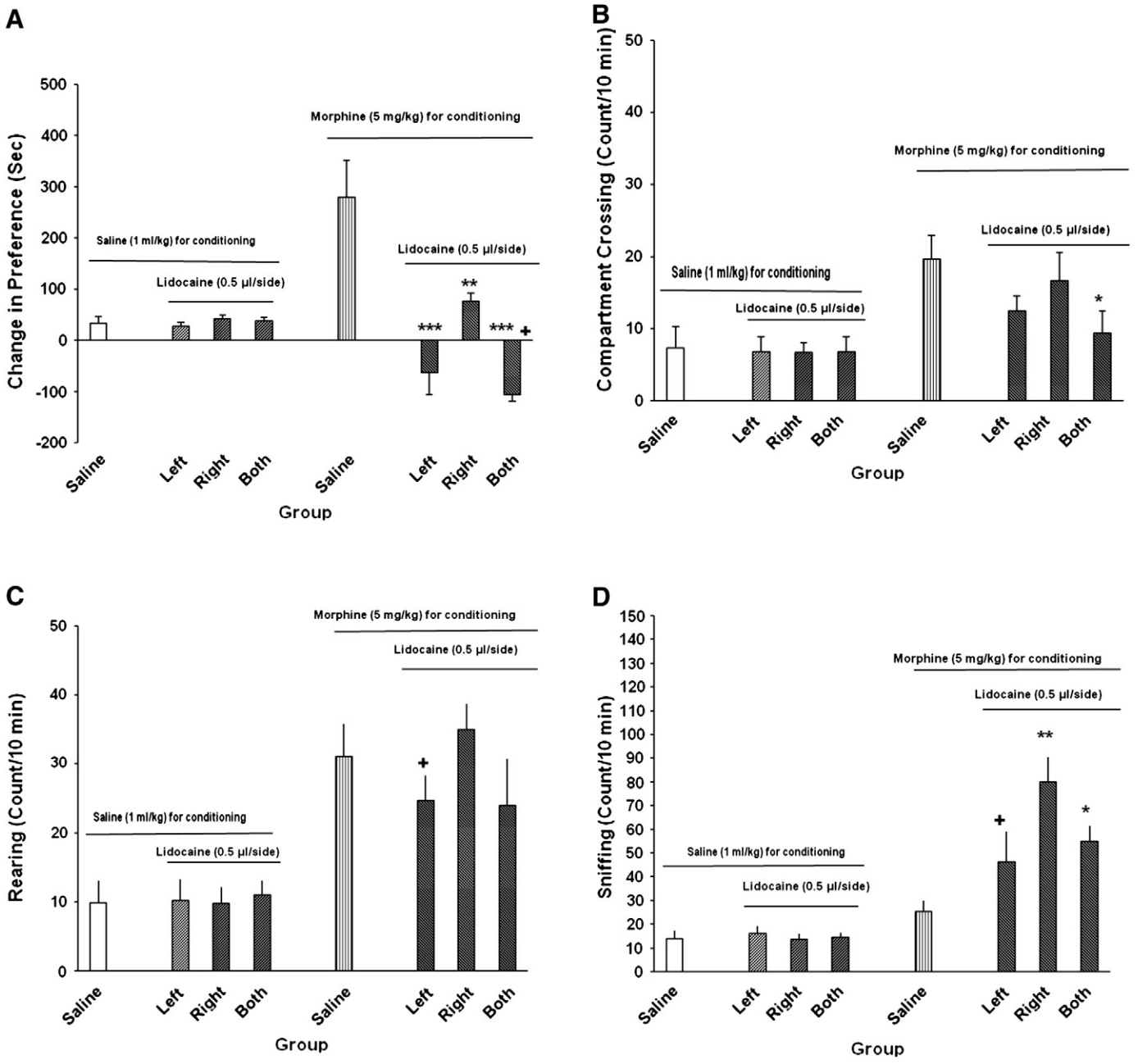

Fig. 4. (A) Effects of left, right, and/or both side transient inhibition of the core part of the nucleus accumbens on morphine place preference. Each point shows the mean ± SEM conditioning score for 8 rats. ***P<0.001, **P<0.01, different from the saline control group; +P<0.05, different from the right side. (B–D) Effects of transient inhibition of left, right, or both sides of the core part of the nucleus accumbens on dopamine-related behaviors in rats during place conditioning test. Compartment crossings were reduced only when both sides of the core subregion were inhibited (B). In addition, the number of rearings decreased only when the left side was inhibited (C). Interestingly, the number of sniffings in the animals increased (D). Each point shows the mean ± SEM conditioning score for 6–8 rats. **P<0.01, *P<0.05, different from the saline control group; +P<0.05, different from the right side.

reduce morphine place conditioning and even shift it to place aversion. However, inhibition of the right subregion hampered morphine's effect more profoundly, indicating the importance of the right subregion in this regard. Since in our experiments morphine tended to induce place aversion while the right part of the shell subregion was transiently inactivated, one can conclude that lidocaine within this subregion may interact with morphine's effect in such a way that cellular activity is blocked. In this regard, several experiments have shown that lidocaine can inhibit neuronal activity by blocking Na^+ channels (Ragsdale et al., 1994; Sommer and Tehovnik, 1997, 1999). It must be noted that Na^+ channels are necessary for action potential separation and subsequent neurotransmitter release, and inhibition of these channels can block these activities. Hence, the shell subregion of the NAc should be a target of future studies using different testing methods, including electrophysiology, pharmacology, microdialysis, and toxic agents for the further evaluation of the role of this part of the nucleus accumbens in drug dependence.

Regarding locomotion activity, it was only reduced significantly when the right and the total shell were inactivated, suggesting that the right part of the shell subregion might be more important than the left part for the expression of locomotor activity. On the other hand, one may postulate that the differences reported for the left or right side originated from the greater variability in the left side condition, resulting in reduced statistical power, as opposed to any true difference between the right and left sides of the shell of the nucleus

accumbens associated with their contribution to locomotor activity. However, more experiments seem to be needed to further evaluate this matter. In addition, the number of sniffings also increased significantly when the right or left subregion was inhibited. However, rearings did not change after inhibiting the right or left subregion, indicating that this behavior may not be directly linked to the morphine effect. Alternatively, since stereotyped behaviors are considered striatal conditioned dopamine-related behaviors (Di Chiara et al., 2004; Diaz Heijtz and Castellanos, 2006; Eklund et al., 2009; Kepecs et al., 2006; Luther, 2005), it could be concluded from our results that rearings are unlikely linked to morphine place preference.

It is clear from the previous experiments that dopamine levels increased in the shell subregion of the NAc following morphine administration into rats and decreased during drug-seeking behavior (Pontieri et al., 1995), which is related to the behaviors induced by morphine, including self-administration and place conditioning (for review see Tzschentke, 2007). Compartment crossings also decreased when the shell subregion of the NAc was transiently inhibited on the left or right or both sides. In addition, it seems that a functional segregation between the ventral and medial shell subregions of the NAc exists, with increased effective activity in the ventral shell (Sellings and Clarke, 2003). Since our experiments focused on the ventral portion of the shell subregion, the role of the medial portion was not studied.

An interesting finding regarding the inhibition of the shell subregion of the NAc was the trend toward a reduction in all behavioral signs, except sniffing, which were initially measured for the left side and followed for the right side and finally for both sides. This trend, observed after analyzing the behavioral signs, may indicate that the shell subregion on the left side of the NAc is less important than the right side regarding morphine place preference and reward.

Our results pertaining to the core subregion of the NAc indicated that the transient inhibition of this part also inhibited the mean time spent on the morphine-paired side, which, in fact, reflects the importance of the core subregion in morphine place preference. In contrast to the observation for the shell subregion, the core subregion on the left side was found to be more important than the right as the transient inhibition of the left part induced a pronounced inhibition of morphine place conditioning, shifting it to place aversion. To date, no investigation concerning the side-specific effect of the NAc core in the place-conditioning paradigm has been reported. Previous studies have shown that the chemical destruction of different parts of the NAc produces diverse effects in the responses to morphine, amphetamine, and cocaine place preference and locomotion (Bardo et al., 1990, 1995; Bardo and Neisewander, 1987; Bardo et al., 1988, 1997; Bossert et al., 2007; Ikemoto et al., 2005; Sellings and Clarke, 2003). Our results also indicated that the inhibition of the core subregion of the NAc on the left or both sides reduced the total compartment crossings; however, inhibition on the right side did not affect this conditioned dopamine-related behavior. Interestingly, this trend was also observed in other conditioned dopamine-related behaviors, including rearing and sniffing. The importance of our finding was associated with an increase in sniffing when only the right subregion was inhibited. However, one could argue that the shell is a critical site for conditioned place preference and that the core injections diffuse to this area. The evidence against this explanation is that the right core injections are less effective than the left, demonstrating an opposite pattern compared to that observed in the shell.

In conclusion, it should be emphasized that conditioned dopamine-related behaviors are the most important indicators of striatal dopamine function that are differentially integrated in the shell and core subregions of the NAc (Di Chiara, 2002). Our results indicated that the animals with a history of morphine administration showed different conditioned dopamine-related behaviors, and the transient inactivation of the core and shell subregions of the NAc differentially influenced these behaviors. These findings also revealed the importance of the different parts of the NAc. Because of their importance in activity monitoring by the NAc, it is suggested that these behaviors with respect to drug abuse should be investigated in further experiments. However, since none of the behaviors we measured in these experiments are completely dopamine-dependent, it must be mentioned that the role of other neurotransmitter systems, including peptidergic, cholinergic, and glutamatergic, should not be excluded.

It should be noted that our study was restricted to morphine, and since the effects of other abused drugs are not identical to those of morphine, the effects of other drugs must be investigated separately. Based on these findings, we suggest that research on the shell or core subregion of the NAc should involve the analysis of both sides to accomplish a complete and better understanding.

Acknowledgments

This work was supported by a grant from the Behavioral Sciences Research Center (BSRC), Baqiyatallah (a.s.) University of Medical Sciences.

References

Bardo MT, Neisewander JL. Chronic naltrexone supersensitizes the reinforcing and locomotor-activating effects of morphine. Pharmacol Biochem Behav 1987;28:267–73.
Bardo MT, Neisewander JL, Ennis RB. Chronic treatment with naltrexone enhances morphine-stimulated dopamine neurotransmission: neurochemical and behavioral evidence. Neuropharmacology 1988;27:1103–9.
Bardo MT, Bowling SL, Pierce RC. Changes in locomotion and dopamine neurotransmission following amphetamine, haloperidol, and exposure to novel environmental stimuli. Psychopharmacology (Berl) 1990;101:338–43.
Bardo MT, Bowling SL, Rowlett JK, Manderscheid P, Buxton ST, Dwoskin LP. Environmental enrichment attenuates locomotor sensitization, but not in vitro dopamine release, induced by amphetamine. Pharmacol Biochem Behav 1995;51:397–405.
Bardo MT, Robinet PM, Hammer Jr RF. Effect of differential rearing environments on morphine-induced behaviors, opioid receptors and dopamine synthesis. Neuropharmacology 1997;36:251–9.
Belcheva I, Bryer JB, Starkstein SE, Honig M, Moran TH, Robinson RG. Hemispheric asymmetry in behavioral response to D1 and D2 receptor agonists in the nucleus accumbens. Brain Res 1990;533:286–91.
Belcheva I, Belcheva S, Petkov VV, Petkov VD. Asymmetry in behavioral responses to cholecystokinin microinjected into rat nucleus accumbens and amygdala. Neuropharmacology 1994;33:995-1002.
Bossert J, Poles GC, Wihbey KA, Koya E, Shaham Y. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. J Neurosci 2007;27:12655–63.
Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluro-gold. J Comp Neurol 1993;338:255–78.
Chang HT, Kitai ST. Projection neurons of the nucleus accumbens: an intracellular labeling study. Brain Res 1985;347:112–6.
Di Chiara G. Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 2002;137:75-114.
Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 1988;85:5274–8.
Di Chiara G, Morelli M, Consolo S. Modulatory functions of neurotransmitters in the striatum: Ach/dopamine/NMDA interaction. Trends Neurosci 1994;17:228–33.
Di Chiara G, Loddo P, Tanda G. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. Biol Psychiatry 1999;46:1624–33.
Di Chiara G, Bassareo V, Fenu S, De Luca MA, Spina L, Cadoni C, et al. Dopamine and drug addiction: the nucleus accumbens shell connection. Neuropharmacology 2004;47(Suppl. 1):227–41.
Di Ciano P, Cardinal RN, Cowell RA, Little SJ, Everitt BL. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of Pavlovian approach behavior. J Neurosci 2001;21:9471–7.
Diaz Heijtz R, Castellanos FX. Differential effects of a selective dopamine D1-like receptor agonist on motor activity and c-fos expression in the frontal–striatal circuitry of SHR and Wistar–Kyoto rats. Behav Brain Funct 2006;2:18.
Eklund MB, Johansson LM, Uvnas-Moberg K, Arborelius L. Differential effects of repeated long and brief maternal separation on behaviour and neuroendocrine parameters in Wistar dams. Behav Brain Res 2009;203:69–75.
Fedele E, Varnier G, Ansaldo MA, Raiteri M. Nicotine administration stimulates the in vivo N-methyl-D-aspartate receptor/nitric oxide/cyclic GMP pathway in rat hippocampus through glutamate release. Br J Pharmacol 1998;125:1042–8.

Harris GC, Wimmer M, Byrne R, Aston-Jones G. Glutamate associated plasticity in the ventral tegmental area is necessary for conditioning environmental stimuli with morphine. Neuroscience 2004;129:841–7.
Heimer L, Zahm DS, Churchill K, Kalivas PW, Wohltmann C. Specificity in the projection patterns of accumbal core and shell in the rat. Neuroscience 1991;41:89-125.
Ikemoto S, Qin M, Liu Z. The functional divide for primary reinforcement of D-amphetamine lies between the medial and lateral ventral striatum: is the division of the nucleus accumbens core, shell, and olfactory tubercle valid? J Neurosci 2005;25:5061–5.
Imperato A, Di Chiara G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 1986;239:219–28.
Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 1992;12:483–8.
Jongen-Relo AL, Groenewegen HJ, Voorn P. Evidence for a multi-compartmental histochemical organization of the nucleus accumbens in the rat. J Comp Neurol 1993;337:267–76.
Jongen-Relo AL, Voorn P, Groenewegen HJ. Immunohistochemical characterization of the shell and core territories of the nucleus accumbens in the rat. Eur J Neurosci 1994;6:1255–64.
Joyce EM, Koob GF. Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Psychopharmacology 1981;73:311–3.
Kalivas PW. Evidence for interactions of endogenous peptides with the mesolimbic dopamine system. Psychopharmacol Bull 1984;20:354–7.
Kalivas PW, Bronson M. Mesolimbic dopamine lesions produce an augmented behavioral response to enkephalin. Neuropharmacology 1985;24:931–6.
Kalivas PW, Miller JS. Dopamine microinjection into the nucleus accumbens: correlation between metabolism and behavior. Biochem Pharmacol 1985;34:284–6.
Kalivas PW, Nemeroff CB, Prange Jr AJ. Neuroanatomical site specific modulation of spontaneous motor activity by neurotensin. Eur J Pharmacol 1982;78:471–4.
Kalivas PW, Widerlöv E, Stanley D, Breese G, Prange Jr AJ. Enkephalin action on the mesolimbic system: a dopamine-dependent and a dopamine-independent increase in locomotor activity. J Pharmacol Exp Ther 1983;227:229–37.
Kalivas PW, Duffy P, Barrow J. Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. J Pharmacol Exp Ther 1989;251:378–87.
Kawaguchi Y, Wilson CJ, Augood SJ, Emson PC. Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci 1995;18:527–35.
Kepecs A, Uchida N, Mainen ZF. The sniff as a unit of olfactory processing. Chem Senses 2006;31:167–79.
Koob GF, Riley SJ, Smith SC, Robbins TW. Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. J Comp Physiol Psychol 1978;92:917–27.
Luther F. The research component in orthodontic education: sniffing out rats (SnOR). J Orthod 2005;32:73–4.
Manzanedo C, Aguilar MA, Rodriguez-Arias M, Minarro J. Sensitization to the rewarding effects of morphine depends on dopamine. Neuroreport 2005;16:201–5.
McClung CA, Nestler EJ. Neuroplasticity mediated by altered gene expression. Neuropsychopharmacology 2008;33:3-17.
McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. J Neurosci 2001;21:8655–63.
Meredith GE. The synaptic framework for chemical signaling in nucleus accumbens. Ann N Y Acad Sci 1999;877:140–56.
Meredith GE, Pennartz CM, Groenewegen HJ. The cellular framework for chemical signaling in the nucleus accumbens. Prog Brain Res 1993;99:3-24.
Moaddab M, Haghparast A, Hassanpour-Ezatti M. Effects of reversible inactivation of the ventral tegmental area on the acquisition and expression of morphine-induced conditioned place preference in the rat. Behav Brain Res 2009;198:466–71.
Molloy AG, Waddington JL. Sniffing, rearing and locomotor responses to the D-1 dopamine agonist R-SK & F 38393 and to apomorphine: differential interactions with the selective D-1 and D-2 antagonists SCH 23390 and metoclopramide. Eur J Pharmacol 1995;108:305–8.
Mrose HE, Ritchie JM. Local anesthetics: do benzocaine and lidocaine act at the same single site? J Gen Physiol 1978;71:223–5.
O'Donnell P, Grace AA. Tonic D2-mediated attenuation of cortical excitation in nucleus accumbens neurons recorded in vitro. Brain Res 1994;634:105–12.
Paxinos G, Watson D. The rat brain in stereotaxic coordinates. 2nd edition. New York: Academic Press; 1987.
Pennartz CM, Gronenwegen HJ, Lopes de Silva FH. The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioral, electrophysiological and anatomical data. Prog Neurobiol 1994;42:719–61.
Pontieri FE, Tanda G, Di Chiara G. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. Proc Natl Acad Sci U S A 1995;92:12304–8.
Pontieri FE, Tanda G, Orzi F, Di Chiara G. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. Nature 1996;382:255–7.
Ragsdale DS, McPhee JC, Scheuer T, Catterall WA. Molecular determinants of state-dependent block of Na + channels by local anesthetics. Science 1994;265:1724–8.
Ritchie JM. A pharmacological approach to the structure of sodium channels in myelinated axons. Annu Rev Neurosci 1979;2:341–62.
Robbins TW, Koob GF. Selective disruption of displacement behaviour by lesions of the mesolimbic dopamine system. Nature 1980;285:409–12.
Robbins TW, Roberts DC, Koob GF. Effects of d-amphetamine and apomorphine upon operant behavior and schedule-induced licking in rats with 6-hydroxydopamine-induced lesions of the nucleus accumbens. J Pharmacol Exp Ther 1983;224:662–73.
Sahraei H, Etemadi L, Rostami P, Pourmotabbed A, Zarrindast MR, Shams J, et al. GABA(B) receptors within the ventral tegmental area are involved in the expression and acquisition of morphine-induced place preference in morphine-sensitized rats. Pharmacol Biochem Behav 2009;91:409–16.
Sellings LH, Clarke BP. Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. J Neurosci 2003;23:6295–303.
Sesack SR, Pickel VM. In the rat medial nucleus accumbens, hippocampal and catecholaminergic nerve terminals converge on spiny neurons and are in apposition to each other. Brain Res 1990;527:266–79.
Smith AD, Bolam JP. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. Trends Neurosci 1990;13:259–65.
Smith KS, Tindell AJ, Aldridge JW, Berridge KC. Ventral pallidum roles in reward and motivation. Behav Brain Res 2009;196:155–67.
Sommer MA, Tehovnik EJ. Reversible inactivation of macaque frontal eye field. Exp Brain Res 1997;116:229–49.
Sommer MA, Tehovnik EJ. Reversible inactivation of macaque dorsomedial frontal cortex: effects on saccades and fixations. Exp Brain Res 1999;124:429–46.
Spanagel R, Welss F. The dopamine hypothesis of reward: past and current status. Trends Neurosci 1999;22:521–7.
Swerdlow NR, Koob GF. Separate neural substrates of the locomotor-activating properties of amphetamine, heroin, caffeine and corticotropin releasing factor (CRF) in the rat. Pharmacol Biochem Behav 1985;23:303–7.
Tehovnik EJ, Sommer MA. Effective spread and time course of neural inactivation caused by lidocaine injection in monkey cerebral cortex. J Neurosci Methods 1997;74:17–26.
Tzschentke MT. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 2007;12:227–462.
Vanderschuren LJ, Spruijt BM, Hol T, Niesink RJM, Van Ree JM. Sequential analysis of social play behavior in juvenile rats: effects of morphine. Behav Brain Res 1996;72:89–95.
Vanderschuren LJ, Schoffelmeer AN, Mulder AH, De Vries TJ. Dopaminergic mechanisms mediating the long-term expression of locomotor sensitization following pre-exposure to morphine or amphetamine. Psychopharmacology 1999;143:244–53.
Vezina P, Stewart J. Conditioned locomotion and place preference elicited by tactile cues paired exclusively with morphine in an open field. Psychopharmacology 1987;91:375–80.