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Dopamine Responsiveness in the *Nucl. Accumbens* Shell and Parameters of the Heroin-Influenced Conditioned Place Preference in Rats

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Previous evidence demonstrated that drug-induced extracellular dopamine (DA) concentrations in the *nucl. accumbens* shell (AcbSh) might underlie different vulnerabilities to heroin addiction in inbred mice strains. We investigated a potential role of the responsiveness of the DA system in the AcbSh with respect to the vulnerability to heroin-influenced conditioned place preference (CPP) in rats. Animals were randomly assigned to the heroin and saline (control) groups. Heroin-group rats were then re-classified into two groups according to the degree of heroin-induced CPP, high preference (HP) and low-preference (LP) ones. The levels of extracellular DA and dihydroxyphenyl acetic acid (DOPAC) were estimated dynamically by *in vivo* microdialysis. Compared with the saline group, extracellular DA and DOPAC concentrations in the heroin-treated groups were significantly higher 30 min after the last injection, but the DA level decreased sharply in these groups on days 1 and 3 and became lower than that of the saline group. Compared with LP-group rats, HP-rats displayed a higher heroin-induced increase in the DA concentration 30 min after the last heroin injection and higher DOPAC and DOPAC/DA ratios 14 days after such injection. These results suggest that differences in the DA system responsiveness in the AcbSh may determine individual differences in vulnerability to heroin addiction.

Keywords: *nucl. accumbens* shell, heroin addiction, vulnerability, dopamine, conditioned place preference (CPP).

INTRODUCTION

Individual vulnerability to the reinforcing effects of drugs appears to be a crucial factor in the development of addictions in humans. The mesolimbic dopamine (DA) system has been implicated as an important substrate for reinforcing effects of most drugs of abuse [1] (like opioids, including heroin). The rewarding effects of addictive drugs are thought to be mediated by increased DA-ergic transmission in the projections originating from the ventral tegmental area (VTA) that innervate the *nucl. accumbens* (Acb) and prefrontal cortex [2, 3].

Two subregions of the Acb, the dorsolateral core and the ventromedial shell, are thought to subserve different functions related to the reinforcing properties of drug rewards. Some studies suggest that the Acb shell (AcbSh) plays an important role in the reward function of DA [4]. Rats can learn self-administration by perfusing DA uptake inhibitors (nomifensine [5] and cocaine [6]) and also mixtures of D1 and D2 receptor agonists [7] into the shell but not the core of the above nucleus. In addition, systemic D-amphetamine-influenced conditioned place preference (CPP), a measure of reward, can be attenuated by selective lesions of DA-ergic terminals in the AcbSh but not in the core of this structure [8].

The link between DA-ergic functioning and behavioral processes has been extensively studied in the field of drug abuse [9]. The results allowed researchers to suggest that individual differences in the DA-ergic function can result in varying degrees of susceptibility to drug abuse [10].

In animal studies, it has been argued that the intrinsic properties of drugs of abuse do not account *per se* for individual variability in the occurrence of drug addiction, and different extracellular DA concentrations in the AcbSh may underlie the above specificities [11]. Thus, individual differences in the responsiveness of the DA system to novelty and

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stress have been shown to predict the susceptibility of individual animals to drug addiction [12]. According to this hypothesis, individuals with a hyperresponsive DA system would be more prone to drug addiction. Furthermore, this difference in individual vulnerability to addiction may be based on neural substrates and genetic background [11].

Previous studies of addiction susceptibility differences focused on different rat strains. Among such different strains, dissimilar susceptibility to addictive drugs, in particular amphetamine [13], cocaine [14], alcohol [15], and opioids [16], has been found, and the vulnerability difference was interrelated with drug-induced changes in the DA concentrations. Some studies seem to suggest that highly-vulnerable animals have a higher basal DOPAC/DA ratio in the Acb and higher extracellular concentrations of DA in this structure in response to the action of addictive drugs [17, 18].

Conditioned place preference (CPP) is an evaluation measure of the rewarding effect [19]. Rats of different strains showed different intensities of drug-induced CPP, and it was suggested that genetic differences may underlie dissimilar sensitivities to the place-conditioning procedure [20]. However, different CPP dynamics can be seen in the same rat strains, which is related to the response to novel environment. Animals with high responses to novel environment tend to have higher scores of the CPP development [21]. Nevertheless, it is not clear whether differences in the CPP development and responses to novel environment are also related to the DA responsiveness in the AcbSh of rats of the same strain. Furthermore, there is a lack of studies on the dynamic variety of extracellular DA concentrations and DA update rate in the AcbSh the same-strain rats, especially with respect to heroin addiction.

In our study, we used brain microdialysis in freely moving rats to examine the effect of heroin on changes in the extracellular DA and DOPAC concentrations in the AcbSh between same-strain rats demonstrating different vulnerability within the addiction and withdrawal phases of the respective experiment.

METHODS

Animals. Male Sprague–Dawley rats (n = 48, body mass 250 to 300 g) were obtained from the Animal Center of the Xiangya Medical College, Central South University. They were housed in standard breeding cages (27×21×13.5 cm) maintained at 20-25°C, relative humidity 55%, with an automatic 12-h light/dark cycle. (8 a.m. to 8 p.m.). All rats were allowed to acclimatize for one week before the experiment.

Behavioral Tests. In the CPP experiments, there were four identical two-chamber Plexiglas boxes with two equal-size compartments (30×30×30 cm). One compartment was painted white and had a mesh floor, while the other compartment was painted black and had a smooth floor. Two replaceable clapboards with white-black sides were used to separate the compartments. One replaced clapboard contained a 10 × 10 cm opening that allowed free access to the two compartments.

The rats were randomly assigned into heroin-treated and control (saline) groups (n = 40 and n = = 8, respectively). The apparatus was located in a room separate from the colony room, which was supplied with white noise (ambient background of 70 dB) in order to mask extraneous sounds. A video camera and a remote computer monitor allowed us to measure time intervals spent by rats in the compartments of the CPP apparatus.

The pre-conditioning phase was 3 day long. Every day, all rats were placed into the CPP apparatus for 30 min with the replaced clapboard. On the 2nd and 3rd days, the time the rats spent in each compartment was recorded for 15 min, with each entry and exit being defined as both front paws in the respective compartment. The average of the two times was considered the baseline CPP (Pre). Then heroin was paired with the nonpreferred compartment, and another side was paired with saline injection. The conditioning phase was 7 days, and the heroin-group rats were subjected to a randomly balanced order of conditioning in which either heroin or saline was first or repeated. Everyday conditioning training was conducted with twice heroin and twice equal-volume saline injections, respectively. Every time the rats were injected with saline or heroin, they were then placed immediately for 30 min into the paired compartment. The time interval between two trains was not shorter than 4 h. Heroin was administered according to an escalating dose schedule. Doses within 7 days increased from 0.5 mg/kg on day 1 to 3.5 mg/kg bid i.h. on day 7. Saline-group rats were injected only with an equal volume of saline and subjected to the same CPP procedure.

On the next after the last conditioning day, each

rat was subjected to a preference test in a drug-free state. The rat was placed within the middle opening of the replaced clapboard to allow free access of the animal to the entire apparatus for 15 min. Total times spent in the white and black compartments were measured. The difference (sec) between the time spent in the drug-paired compartment within the testing phase and the preconditioning phase was considered a measure of the degree of heroin-induced conditioning. The drug-influenced preference was taken into account if the difference was positive.

When the CPP testing phase was terminated, animals of the studied groups were reclassified into two groups according to the degree of heroin-induced conditioning. These were the high-preference group (HP, n = 12) and the low preference group (LP, n = = 12); each group included 30% of the total number of animals examined. The HP- and LP-group rats were injected with heroin (3.5 mg/kg bid i.h) until microdialysis sample collection was started.

Microdialysis Procedures. Brain microdialysis experiments were performed as was previously described. Rats were stereotaxically implanted under 4% pentobarbital sodium (30-35 mg/kg) anesthesia with a CMA/11 guide cannula. This cannula was fixed in position with three stainless steel screws and dental acrylic plastic with its tip close to the AcbSh (FP 1.7 mm, ML 0.7 mm; and DV 7.0 mm from the bregma). A dummy probe was then inserted into the guide cannula to prevent obstruction.

After recovery from surgery (48 h), the rats were connected to a microperfusion pump (CMA/110; CMA/Microdialysis AB, Sweden) and placed into a cylindrical Plexiglas transparent microdialysis container (30 cm in diameter; 35 cm in height), where they were allowed to move freely. The dummy probe was replaced with a concentric microdialysis probe (CMA/11, CMA/Microdialysis AB, Sweden; membrane, 1.0 mm; cut-off, 6000 Dalton; shaft length, 14 mm). They were inserted through the guide cannula, extending beyond the cannula tip to maximize the contact of the dialysis membrane-exposed surface area with the AcbSh.

Ringer solution (140 mM NaCl, 1.2 mM $CaCl_2$, 3.0 mM KCl, and 1.0 mM $MgCl_2$) was perfused through the syringe pump connected to the probe via a fluorinated ethylene-propylene tubing (FEP, 0.005″ ID) at a flow rate of 2.0 µl/min for at least 90 min prior to the start of sample collections. Samples were collected 30 min and on days 1, 3, 7, and 14 after the last heroin injection into refrigerated (4°C) microcentrifuge tubes containing 2.0 µl of hydrochloric acid to prevent enzymatic breakdown.

Analyses of the dialysate samples were performed by high-performance liquid chromatography with a coulometric electrode array system (HPLC-EC CoulArrar5600A; ESA, USA). A standard curve was plotted according to 100, 50, 20, 10, 1, 0.5, and 0.05 µg/l dopamine standards (Sigma, USA). Correlation coefficients (r^2) for the peak area of concentrations of the standard curve were calculated using linear regression. The results showed that the standard linear curve had satisfactory r^2 values (DA: y = = 16.157 x, r^2 = 0.9998, and DOPAC: y = 16.619 x, r^2 = 0.9992). An output from the detector was analyzed with a computer program, and the levels were determined by comparison with a standard curve. The lower sensitivity limit for DA was approximately 0.1 µg/l, and for DOPAC it was 0.2 µg/l.

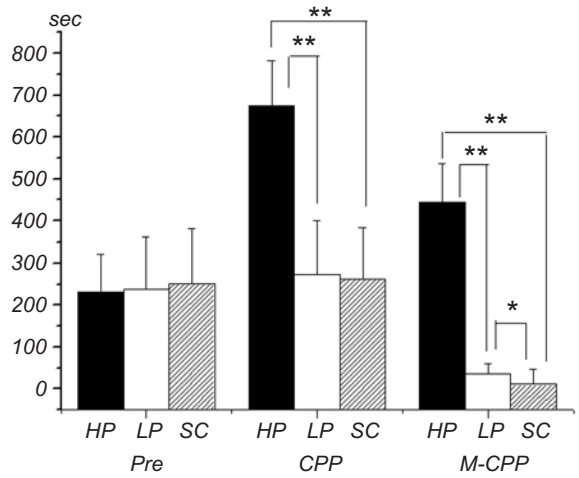
Histology. After completion of the experimental, the rats were anesthetized with sodium pentobarbital and perfused transaortally with 0.9% NaCl for 5 min followed by 4% paraformaldehyde for 10 min. The brains were removed, placed in 4% paraformaldehyde for at least 6 h, and immersed in 30% v/v sucrose until they sank completely. Coronal 40-µm-thick sections were cut with a cryostat, and the placement of the cannula tip was confirmed by microscopic examination. Only animals with correctly placed probes were included in the statistical analyses.

Statistical Analysis. Microdialysis and CPP numerical data among the experiment (HP and LP) and control groups were statistically analyzed using one-way ANOVA followed by *post-hoc* analysis by means of the Fisher's protected least significant difference (PLSD) test. Differences with $P < 0.05$ were considered significant.

RESULTS

Changes in the Chronic Heroin- or Saline-Influenced CPP. The baseline CPP values (Pre) in the three groups were comparable (P = 0.94). A 7-day-long heroin treatment significantly increased the time spent in the heroin-paired side (M-CPP), compared with that in the control group (P < < 0.01, P < 0.05). The M-CPP of the HP group was significantly greater than that in the LP group (P < < 0.01) (Fig. 1).

Effects of Heroin on DA and DOPAC in the AcbSh. One-way ANOVA was conducted with

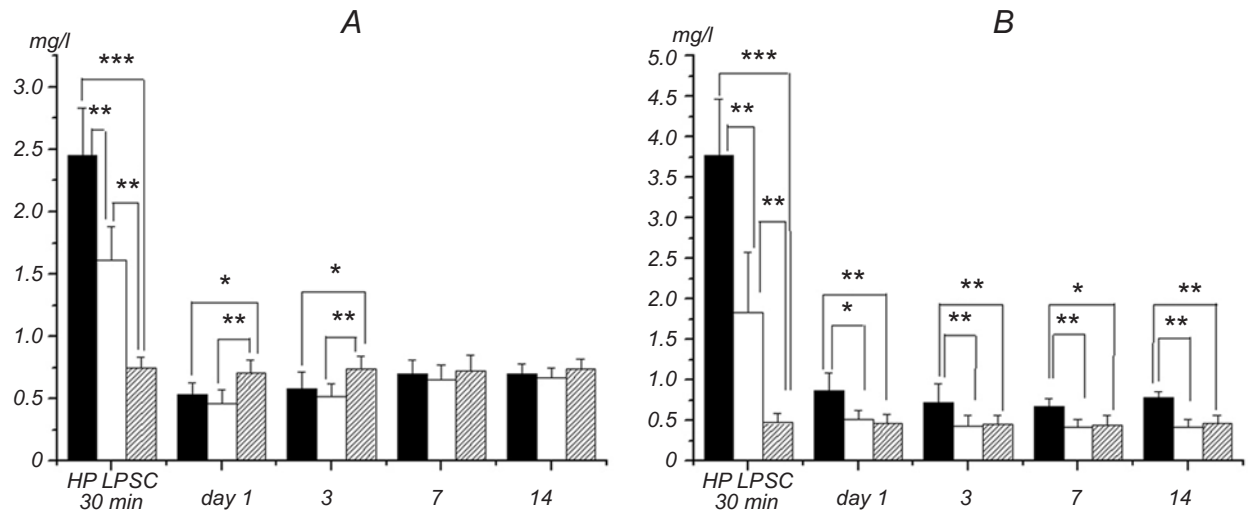


F i g. 1. Differences in the conditioned place preference (CPP) influenced by chronic heroin or saline treatment. Vertical scale) Actual total times (sec) spent at the non-preferred side(s). Pre are values before the treatments, CCP are those after heroin treatment in the respective group, and M-CCP are differences between the baseline and test CCP values. HP, LP, and SC are the high-preference group (n = 12), low-preference group (n = 12), and saline control group (n = 8), respectively. *P < 0.05,**P < 0.01 in the comparisons shown. Data in this and subsequent Figures are presented as means ± s.e.m.

respect to changes in the microdialysis data in different time points among the HP, LP, and saline control (SC) groups. Compared with the SC group, the concentrations of extracellular DA (Fig. 2A) and DOPAC (B) in the AcbSh were significantly higher at 30 min after the last heroin injection (P < 0.001). However, the DA concentrations in both HP and LP groups decreased sharply on days 1 and 3 after the last injection, while these indices were significantly lower than in the control group (P < 0.05, P < 0.01). The DA concentrations in heroin-treated rats recovered gradually in a week after the last injection. On days 7 and 14, those were still lower, but there was no significant difference compared to that in the SC group. Compared with the LP group, the DA concentration in the AcbSh of the HP group was significantly higher after the last injection (P < 0.01), and the two groups demonstrated no significant differences with respect to withdrawal (A).

Although the DOPAC level decreased significantly after withdrawal, this index in the AcbSh of HP rats was significantly higher than that in LP- and SC-group rats at all five time points (P < 0.05, P < 0.01). At the same time, there were no significant differences in the DOPAC concentration between the LP- and SC-groups at many time points within 14 days of withdrawal (Fig. 2B).

Comparison of the DOPAC/DA Ratios in the AcbSh. The DOPAC/DA value is believed to be an important indicator of the DA update rate. One-way ANOVA was conducted among the experimental (HP and LP) groups and SC group with respect to this ratio at different time points. The DOPAC/DA ratios in the AcbSh in both HP and LP groups were significantly higher 30 min after the last heroin injection compared with that in the SC group and then gradually decreased after withdrawal.



F i g. 2. Dynamics of changes in the concentrations of extracellular DA (A) and DOPAC (B) in the *nucl. accumbens* shell (AcbSh) measured in different experimental groups by *in vivo* microdialysis at different time points (30 min, days 1, 3, 7, and 14) after the last heroin injection. In HP, LP, and SC groups, n = 8, n = 7, and n = 6, respectively. ***P < 0.001 (comparison by one-way ANOVA). Other designations are similar to those in Fig. 1.

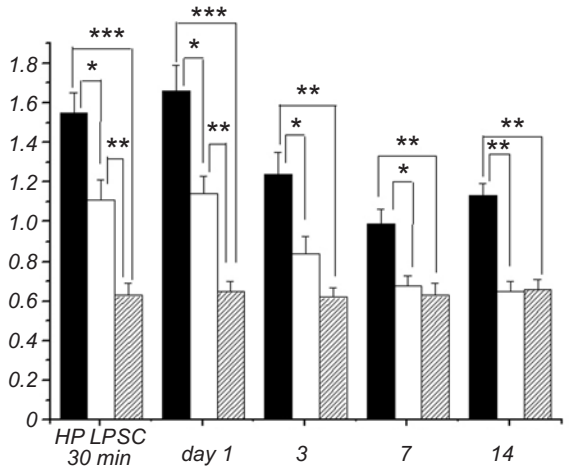


Fig. 3. Dynamics of the DOPAC/DA ratios in the AcbSh in different experimental groups. Designations are similar to those in Figs. 1 and 2.

However, the LP group demonstrated no significant difference from the SC group 3 days after the last injection. At all five monitored time points, the HP rats had, however, significantly higher DOPAC/DA ratios than those in LP- and SC-group rats ($P < 0.05$, $P < 0.01$, $P < 0.001$) (Fig. 3).

DISCUSSION

In this study, all rats of the same strain and kept in the same environment were treated by chronic heroin conditioning training. Some rats stayed much longer at the drug side, while this index for other rats did not increase considerably (and even decrease). The dissimilar tendency of the CPP development in these animals may be considered related to different vulnerabilities to drug addiction.

Activation of the limbic DA system is an important mechanism of opioid psychological dependence. Previous studies also showed that the rewarding effects of opioids were parallel with the DA concentration increase in the limbic system. The results showed that, after chronic heroin treatment in both HP and LP groups, the concentrations of extracellular DA and DOPAC in the AcbSh were significantly higher than those in the control group, indicating that heroin can induce DA release and its increased metabolism. This is consistent with previous studies related to opioids [22].

We also found that the examined DA concentration significantly decreased after withdrawal, and on days 1 and 3 of withdrawal it was even significantly lower than that in the control group. Then this index gradually recovered after a week, which is consistent with the majority of observations [23]. This suggests that the DA concentration gradually decreased after withdrawal because of the lack of sustained heroin stimulation. At the same time, the sustained high-concentration state of DA within the addiction phase led to down-regulation of the postsynaptic membrane DA receptor function by a negative feedback mechanism. Then, the low functional state of the DA central system could be the neurobiological basis of the withdrawal symptoms [1].

The DOPAC concentration and DOPAC/DA ratio are believed to be effective indicators of activity of the DA system or DA update rate. We found that, within the addiction period, the DOPAC/DA ratios in the HP and LP groups were significantly higher than that in the control (SC) group, indicating that the DA update rate in the AcbSh is significantly higher. After withdrawal, the DOPAC level and DOPAC/DA ratio in rats with different addiction vulnerabilities demonstrated clear differentiation. The DOPAC/DA index in the HP group was significantly higher than that in the control and LP groups, and so did the DOPAC level, while the latter after withdrawal and the DOPAC/DA ratio after 3 days of withdrawal in the LP group showed no significant difference from the control (SC) group, which suggested that rats with high addiction vulnerability had a higher DA reactivity to drugs compared with low-vulnerability rats. However, a higher DA update rate also leads to stronger craving for heroin. Results of other studies on the addiction susceptibility in rats to alcohol [15], cocaine [14, 18], amphetamine [13], and morphine [16] also agree with this finding.

The findings in our study indicate that the difference in susceptibility to heroin addiction in rats depends on the responsiveness of the DA system to drug exposure. Heroin HP rats have a higher DA system responsiveness in the AcbSh, which is possibly one of the neurobiochemical factors responsible for the heroin vulnerability and is an individual marker of the respective differences.

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The study was carried out in accordance with the statements of the Council Directive regarding the protection of animals used for experimental and other scientific purposes (86/609/EEC, 1986, Strasbourg) and respective regulations of the Ethics Committee at the Xinxiang Medical University.

The authors of this study, Ch. Wang, H. Chen, X. Wang, R. Zhang, and W. Hao, confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

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