High-frequency electrical stimulation in the nucleus accumbens of morphine-treated rats suppresses neuronal firing in reward-related brain regions

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Summary

Background: Previous studies have reported that high-frequency stimulation (HFS) in the nucleus accumbens (NAc) is a potential treatment modality for drug craving and relapse. We aimed to explore the electrophysiological changes in reward-related brain regions during NAc stimulation and reveal the effects of stimulation frequency and target changes on NAc neuronal activities.

Material/Methods: Twenty-eight rats were randomized into saline (n=8) and morphine (n=20) groups. The morphine group was further divided into core (n=10, only the core of the NAc was stimulated) and shell (n=10, only the shell of the NAc was stimulated) subgroups. Conditioned place preference (CPP) behavior of the rats was evaluated to confirm morphine preference after morphine injection and CPP training for 10 days. We recorded NAc neuronal responses to NAc core stimulation at different frequencies, as well as changes in VP and VTA neuronal firing during NAc core stimulation, and changes in NAc neuronal firing during NAc shell stimulation.

Results: The results indicate that high frequency stimulation was more effective in suppressing NAc neuronal activities than low frequency stimulation and that core stimulation was more effective than shell stimulation. Most VP neurons were inhibited by NAc core stimulation, while VTA neurons were not.

Conclusions: The results suggest that electrical stimulation in the NAc can suppress neuronal firing in reward-related brain regions. The stimulation might be frequency-dependent in suppressing neuronal firing. The core and shell of the NAc play different roles in suppressing NAc neuronal firing as 2 stimulating targets.

key words: deep brain stimulation (DBS) • drug addiction • morphine • neuronal firing • nucleus accumbens (NAc) • rats

Abbreviations: NAc – nucleus accumbens; VP – ventral pallidum; VTA – ventral tegmental area; DA – dopamine; HFS – high-frequency stimulation; DBS – deep brain stimulation; PD – Parkinson’s disease; CPP – conditioned place preference; ISIH – interspike interval histogram; ANOVA – one-way analysis of variance; LFS – low-frequency stimulation; STN – subthalamic nucleus; GABA – gamma-aminobutyric acid

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**Background**

Drug addiction is a chronic and relapsing disorder which is characterized by drug-seeking and drug-taking behavior. Although drug withdrawal syndrome can be alleviated by medication, the effects of pharmacotherapy and psychotherapy on drug craving, which induces relapse, are usually minimal. The reward mesocorticolimbic dopamine (DA) circuit, consisting of the ventral tegmental area (VTA), the nucleus accumbens (NAc) and the ventral pallidum (VP), plays a critical role in drug reinforcement [1–3]. The NAc also appears to play a major role in drug-seeking. Some studies have suggested that glutamate release in the NAc core is necessary for heroin and cocaine craving [4,5]. These animal studies indicated the NAc was a potential target in the treatment of drug craving. A clinical study reported bilateral NAc lesions by stereotactic surgery alleviated opiate drug psychological dependence [6]. However, due to the invasiveness, irreversibility, intolerable adverse effects and ethical controversy, ablation surgery cannot be accepted as a routine treatment modality.

As an alternative technique, high-frequency stimulation (HFS) has also been proved to show similar blocking effects [7–9]. Compared with ablation surgery, deep brain stimulation (DBS) shows more advantages, such as minimal invasiveness, reversibility and adjustability. It has been widely applied in the treatment of multiple movement disorders and psychiatric diseases including Parkinson’s disease (PD) [10], dystonia [11], Tourette’s syndrome [12], obsessive-compulsive disorder [13] and depression [14]. Since the clinical presentations of addiction show some features of refractory psychiatric illness, DBS in the appropriate target might similarly serve to alleviate drug craving and relapse. A report suggested NAc stimulation significantly alleviated drug craving in 2 patients without any adverse effects or complications [15]. Some other clinical studies demonstrated that NAc stimulation attenuated alcohol and smoking dependency [16,17], and animal studies demonstrated HFS of the NAc was effective in reducing conditioned place preference (CPP) score induced by morphine [18] and alleviating cocaine craving in rats [19].

Wang et al. stimulated the NAc at 130Hz in rats with morphine preference, indicating that after stimulation for 7 days, the average CPP score (time spent in drug-paired side) of the stimulated group was lower than that of the sham stimulation group, while the CPP scores of the 2 groups had no significant differences before stimulation [20]. In their study, only behavioral changes were observed, while the changes in neuronal activities during stimulation were not revealed. Based on Wang’s study, we aimed to determine the effects of NAc stimulation on neuronal firing in reward-related brain regions of morphine-treated animals.

**Material and Methods**

**Animals**

Experiments were carried out on 28 adult male Sprague-Dawley rats weighing 250–270 g. Rats were housed in an environmentally controlled room (20–23°C, 12-hour light/12-hour dark cycle, lights on at 7:00 a.m.). Animals were provided with water and rat chow ad libitum. All animal experiments were performed in accordance with the Guidance for Animal Experimentation of the Capital Medical University and Beijing guidelines for the care and use of laboratory animals.

**Grouping**

Twenty-eight rats were randomized into saline (n=8) and morphine (n=20) groups, and the morphine group was further divided into core (n=10, only the core of the NAc was stimulated) and shell (n=10, only the shell of the NAc was stimulated) subgroups.

**CPP apparatus**

The CPP apparatus was a rectangular box (50×30×30 cm). A septum with a small sliding door divided the box into 2 chambers of equal size. One chamber was black with a rough wooden floor, and the other was white with a smooth PVC floor. Cameras were installed in the chambers to record the duration rats spent in each chamber. Light bulbs were used to keep the lighting levels in the 2 chambers at 20 lux.

**Drug administration**

All rats in the morphine group received intraperitoneal morphine hydrochloride (lot 070906, Shenyang Pharmaceutical Factory, China) injection at 10:00 a.m. and 7:00 p.m. for 10 days. The daily dose gradually increased from 8 mg/kg to 80 mg/kg. Three hours before morphine injection, saline of the same volume was also injected intraperitoneally. Rats in the saline group received the same administration, except that morphine hydrochloride was replaced by saline.

**CPP procedure**

The CPP test was designed to confirm that the dosage and duration of drug administration were sufficient to induce morphine preference in rats. The CPP procedure consisted of 3 phases including pretest, conditioning and test. On the pretest day (day 0), the rat was placed under the sliding door of the CPP apparatus. It was allowed to wander in the whole apparatus freely for 15 minutes, and the duration it spent in each chamber was recorded. The chamber in which the rat spent less time was designated as the drug-paired side. In the conditioning phase (day 1 to day 10), the sliding door was closed, the rat was placed in the drug-paired chamber for 1 hour after morphine injection, and in the other for the same duration after saline injection. After being trained for 10 days, the injection of both morphine and saline was discontinued. Since withdrawal syndrome usually disappeared 1 week after drug withdrawal, the test phase was designated on day 17. The test procedure was similar as the pretest procedure – the sliding door was removed. The rat was placed on the midline of
the apparatus and the duration the rat spent in each chamber was recorded for 15 minutes.

**Electrical stimulation of the NAc and extracellular single unit recording**

On day 17, immediately after the test phase, rats in the morphine group were anesthetized with 20% urethane solution (0.8 mL/100 g, intraperitoneally), and mounted in a stereotaxic apparatus (Northeast Optical Instrument Factory, China). An incision was made in the scalp. The skull was exposed and the part overlying the NAc, the VP and the VTA was drilled off. The stimulating electrode (FHC, outside diameter 0.50 mm, internal diameter 0.125 mm, USA) was implanted into the NAc core or shell obliquely at an angle of 10° in the parasagittal direction in order to keep enough distance from the recording electrode. The study consisted of 3 protocols: (1) to explore the electrophysiological changes in NAc neurons during NAc stimulation and the effects of frequency changes on NAc neuronal activities, we stimulated the NAc core at 20, 50, 80, 130 and 200 Hz and recorded the neuronal firing of the NAc; (2) to reveal how HFS affects neuronal activities in other reward-related brain regions, we stimulated the NAc core at 130 Hz (130 Hz is the most common HFS frequency in clinical application) and recorded neuronal firings of the VP and the VTA; and (3) to explore whether the core and shell of the NAc play different roles as stimulating targets, we stimulated the NAc shell at 130 Hz and recorded neuronal firings of the NAc. Regarding bregma, the coordinates of the NAc core and shell were chosen according to the stereotaxic atlas of Paxinos and Watson [21]. The NAc core: +2.2 mm anteroposterior (A/P), ±1.6 mm mediolateral (M/L), −6.7 mm dorsoventral (D/V); the NAc shell: +2.2 mm A/P, ±1.2 mm M/L, −7.5 mm D/V. Baseline firing was recorded for 2–3 minutes before electrical stimulation. Stimulation was delivered by A320R electrical stimulator (World Precision Instruments, USA). The stimulating parameters were: pulse width, 0.4 ms; intensity, 0.4 mA; train duration, 5 seconds [22]. After each train, the following one would not be delivered until the neurons recovered to the baseline status. The extracellular single unit discharge of the firing neurons was recorded with single barrel glass microelectrodes. The microelectrodes filled with 1% Chicago sky blue dye (Sigma, USA) in 3 mol/L NaCl were implanted with a WK-2 microelectrode propulsion device (Northwest Optical Instrument Factory, China). The coordinates of the NAc (for recording microelectrodes), the VP and the VTA were: the NAc: +1.6~+2.2 mm A/P, ±1.2~1.7 mm M/L, −3.9 mm ~ −8.1 mm D/V; the VP: −0.3~+0.3 mm A/P, ±1.5~2.4 mm M/L, −7.5 mm ~ −8.8 mm D/V; the VTA: −6.0 mm A/P, ±0.4~0.6 mm M/L, −7.4 mm ~ −8.4 mm D/V. Firing signals were amplified, filtered with a DAM80 preamplifier (World Precision Instruments, USA), displayed on an oscilloscope and recorded in a computer. Spike 2 biological signal acquisition system (CED, British) was used to analyze the data. Only stable discharges with signal-to-noise ratio >3/1 were recorded.

**Histology**

At the end of the experiment, Chicago sky blue was electroforeosed from the tip of the recording microelectrodes with −20 µA current for 20 minutes to localize the tip of the recording electrode. All rats were perfused transcardially with 0.1 mol/L phosphate-buffered saline and 4% paraformaldehyde under deep anesthesia. Brains were removed and fixed with 4% paraformaldehyde. With a freezing microtome, coronal sections at a thickness of 20 µm through the target were prepared and processed by Nissl staining to verify the location of the stimulating and recording electrode on coronal sections. Referring to the stereotaxic atlas of Paxinos and Watson [21], only rats with accurate implantation were included in data analysis.

**Data analysis**

The duration spent in the drug-paired chamber in the CPP test was converted to CPP score. Neuronal firing patterns were defined by interspike interval histogram (ISIH). According to ISIH analysis, regular firing pattern shows symmetrical density distribution, irregular firing pattern shows Poisson distribution, and burst firing pattern shows gradual decay of skewness distribution [23]. Since the neuronal spike duration (1.0~1.5 ms) is much longer than the period during which the amplifier was saturated (0.4 ms), the spikes could be accurately detected. The black bars indicated the stimulation artifacts.

The suppression rate was determined with the following formula: suppression rate = (firing rate during pre-stimulation period – firing rate during stimulation period) / firing rate during pre-stimulation period ×100%.

Results were presented as mean ±SEM, Paired-Samples t test, One-way analysis of variance (ANOVA) or Independent-Sample t test were used to analyze the data. Significance was defined as P<0.05. The SPSS statistical package 17.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.
The withdrawal syndrome had disappeared on day 17, and all rats with morphine administration showed preference to the drug-paired side (ie, spent more than 540 seconds in the drug-paired chamber), confirming the successful establishment of morphine preference (Independent-Sample t test: $t_{(26)}=-21.59, P<0.0001$) (Figure 3).

The neuronal activities of the NAc, the VP and the VTA

The implantations of stimulating electrodes in the core or shell of the NAc in the 20 rats were accurate. Spontaneous neuronal activities of the NAc, the VP and the VTA were recorded under the baseline condition and details were shown in Table 1.

Responses of the NAc neurons to core stimulation at different frequencies

Although only $40.7\%$ of neurons were inhibited by core stimulation at 20 Hz (Figure 4A), the general effect was inhibitory (Paired-Samples t test: $t_{(26)}=3.44, P=0.002$) (Figure 4B). The firing rates of most neurons tested in the NAc ($66.7\%$-$81.5\%$) showed a significant decrease in response to core stimulation at frequencies from 50 Hz to 200 Hz (Paired-Samples t test: $t_{(26)}=5.32, P<0.0001$; 80 Hz, $t_{(26)}=5.27, P<0.0001$; 130 Hz, $t_{(26)}=5.79, P<0.0001$; 200 Hz, $t_{(26)}=6.31, P<0.0001$). The suppression rate increased (from $25.80\pm7.04\%$ to $65.95\pm6.83\%$) as the frequency increased. Compared with stimulation at 130 Hz, only stimulation at 20 Hz showed a significantly lower suppression rate (ANOVA, Fisher’s LSD: $P=0.20 <0.05$) (Figure 4C). The neuronal firing rate recovered to baseline levels immediately after the electrical stimulation discontinued (Figure 4B).

To explore whether distance between recording and stimulating site might influence the inhibitory effect of HFS, we divided the recorded NAc neurons into an inhibited group and a no response group. The result showed that neurons in the inhibited group were much closer to the stimulating site than those in the no response group (Independent-Sample t test: $t_{(25)}=2.38, P=0.25$) (Figure 4D).

Response of the VP and the VTA neurons to core HFS

Similar to NAc neurons, VP neurons were significantly inhibited by core HFS. The mean firing rate decreased during stimulation (Paired-Samples t test: $t_{(22)}=5.86, P<0.0001$). HFS appeared to have no effect on VTA neurons (Paired-Samples t test: $t_{(12)}=-0.13, P=0.90$) (Figure 5).

Differences between the core and shell of the NAc as 2 stimulating targets

We stimulated the NAc shell at 130Hz and recorded the changes in neuronal activities in the NAc. Shell HFS also

| Brain region | n | Regular (%) | Irregular (%) | Bursting (%) | Minimal rate (spikes/s) | Maximal rate (spikes/s) | Mean rate (spikes/s) mean ± SEM |
|--------------|---|-------------|--------------|--------------|------------------------|------------------------|------------------------------|
| NAc          | 61*| 9 (14.8%)   | 40 (65.6%)   | 12 (19.7%)   | 2.11                   | 63.44                  | 16.65±1.57                  |
| VP           | 23 | 1 (4.3%)    | 11 (47.8%)   | 11 (47.8%)   | 3.66                   | 35.92                  | 15.08±1.46                  |
| VTA          | 13 | 1 (7.7%)    | 11 (84.6%)   | 1 (7.7%)     | 3.09                   | 27.27                  | 9.36±1.84                   |

* N of core stimulation group=27, n of shell stimulation group=34.

RESULTS

CPP test

The withdrawal syndrome had disappeared on day 17, and all rats with morphine administration showed preference to the drug-paired side (ie, spent more than 540 seconds in the drug-paired chamber), confirming the successful establishment of morphine preference (Independent-Sample t test: $t_{(26)}=-21.59, P<0.0001$) (Figure 3).

Figure 2. Removal process of stimulation artifacts by template subtraction method. (A). Raw data of recording traces. (B). Template subtraction method was used to classify waveforms, three types of waveform were identified and each one was marked. "01" or "00" indicated action potential, "02" indicated stimulation artifact. (C). Stimulation artifacts were removed by subtracting waves marked as waveform 02.

Figure 3. Comparison of the CPP scores between the saline group and morphine group in the pretest and test phase. Data was mean ±SEM, * stood for $P<0.05$, Independent-Sample t test

* N of core stimulation group=27, n of shell stimulation group=34.
showed an inhibitory effect on NAc neurons (Paired-Samples t test: \( t(33) = 3.89 \), \( P<0.0001 \)) (Figure 6A). Compared with core stimulation, the neurons recorded during shell stimulation were closer to the stimulating site \( (0.72 \pm 0.04 \text{ mm (shell)} \) vs. \( 0.96 \pm 0.30 \text{ mm (core)} \)). However, the former showed a higher suppression rate than the latter, supporting the idea that core stimulation had a more powerful inhibitory effect (Independent-Sample t test: \( t(59) = 2.59 \), \( P=0.01 \)) (Figure 6B).

**Discussion**

We found that electrical stimulation in the NAc could suppress neuronal firing in the NAc, and that HFS was more effective than low-frequency stimulation (LFS) in achieving this. HFS of the NAc core also showed an inhibitory effect on VP neurons while no effect was shown on VTA neurons. Although HFS of the shell also attenuated neuronal firing rate in the NAc, core HFS appeared to be more effective.

**During or after stimulation**

Numerous researchers have focused on revealing the changes in neuronal activities induced by electrical stimulation. However, due to the disturbance induced by large stimulation artifacts, it seems difficult, even impossible, to analyze single cell activity during stimulation. Some researchers then turned their attention to post-stimulation neuronal activities in early studies. Their conclusions were based on the presumption that neuronal activity seen immediately after stimulation would be similar to that during stimulation. However, what happens immediately after stimulation might not reflect what occurs during stimulation. Clinical evidence supports this viewpoint: when the stimulators implanted in PD patients are turned off, parkinsonian symptoms such as tremor and rigidity recur immediately. In the present study,
most NAc neurons were inhibited by NAc HFS, while the firing rates recovered to the baseline levels instantly after stimulation. Interestingly, in some neurons, post-stimulation firing rates were even higher than those of pre-stimulation, although to an insignificant extent. Nonetheless, when the artifacts during stimulation were removed, we found all neurons being excited instantly after stimulation were completely suppressed (no spontaneous spikes) by HFS during stimulation. Therefore, extrapolating from what happens following DBS to what occurs during DBS is unreasonable [26]. Although large stimulus artifacts might hamper investigations of physiological mechanisms underlying DBS effects using extracellular recording techniques, some researchers have found several methods to remove artifacts from raw data [25,27–29]. In the present study, template subtraction method was used to remove stimulus artifacts; the validity of this method has been confirmed in Hashimoto’s study [24].

**Inhibition mechanism of DBS and treatment of drug craving**

DBS is a highly effective treatment modality for numerous neurological and psychiatric disorders, but its therapeutic mechanisms are still controversial. Since the outcome of DBS was similar to that of ablation, the hypothesis of inhibition was put forward [7,8]. Some researchers demonstrated HFS of the NAc was effective in attenuating morphine reinforcement [18] and drug craving [15,19]. Based on the above results, we designed the experiment and postulated HFS might inhibit activities of neurons in the NAc. The results are in strong accordance with our previous assumption – HFS in the NAc elicited inhibitory effect on the target nucleus. Based on previous studies, activation of glutamatergic projection from the prefrontal cortex to the NAc core underlies cocaine or heroin-primed
reinstatement of drug-seeking behavior [4,5]. Excitation of NAc core neurons, which is elicited by glutamate release from the prefrontal cortex, plays a critical role in drug-seeking. This excitation could be blocked by the suppressive effects of HFS. The blockage further disturbs the functions of downstream nuclei by reducing efferent neurotransmitter released by NAc core neurons. Thus NAc ablation and DBS share the same mechanism of blockage. Since NAc ablation has been proved to be effective in attenuating morphine craving [30], and both ablation and HFS block the activities of the NAc, our electrophysiological findings can partly explain the results of Wang’s study [20] – high frequency stimulation of the NAc might reduce morphine preference by suppressing NAc neurons. Compared with ablation, DBS is a minimally invasive surgery and the stimulating contacts’ combination, current and frequency are adjustable.

The impact of frequency change on the suppressive effect

The efficacy of DBS in treating movement disorder is strongly dependent on the stimulation frequency [10,31]. Evidence showed that HFS of subthalamic nucleus (STN) led to motor function improvement, while 10 Hz LFS of STN led to deterioration of motor functions, and other LFS (5 Hz, 20 Hz and 45 Hz) had no effect on the symptoms [32]. According to our present knowledge, only HFS has been applied in drug addiction studies in NAc stimulation [18–20]. The effects of LFS on drug addiction are still unclear. In this study, the frequency of electrical stimulation ranged from 20 Hz to 200 Hz in order to reveal the impact of frequency change on neuronal discharge of the NAc. The results demonstrate that although stimulation at all frequencies attenuated neuronal firing rate in the target nucleus, the stimulation at 20 Hz showed less suppressive effects than that at 130 Hz. Although stimulation at frequencies of 50 Hz and 80 Hz induced lower suppression rates than stimulation at 130 Hz, the differences did not reach statistical significance. Thus we assume that stimulation at frequencies higher than 50 Hz might be effective in blocking the NAc to alleviate or terminate drug craving.

The effect of HFS on the VP and the VTA

Another finding in our study was that NAc core HFS not only changed the neuronal activities of the NAc itself, but also showed an effect on VP neurons. A previous study has demonstrated that NAc core neurons have a dense gamma-aminobutyric acid (GABA)ergic projection to the VP [33]. Therefore, HFS mediated suppression should reduce GABA release from NAc neurons to the VP, and then the disinhibition should lead to excitation of VP neurons. Contrary to this assumption, the result demonstrated that inhibition, instead of excitation, was observed in the majority of VP neurons during NAc core HFS. We postulate that NAc core HFS not only induced reduction of GABA release but also showed a suppressive effect on VP neurons directly. Since the VP is located adjacent to the NAc core, the direct effect mediated by core stimulation might cover VP neurons. The VTA also receive (GABA)ergic efferent fibers from the NAc [34]; while it did not respond to NAc HFS, we speculate some other mechanisms may be involved in this procedure and more studies should be done to explain it.

Differences between the core and shell of the NAc

The NAc can be divided into core and shell based on differences in anatomy and physiology [33,35,36]; anatomical studies demonstrate that the core and shell receive different prefrontal cortical afferents and project to different downstream nuclei. Projections to the core originate predominantly from the dorsal prefrontal cortex and the core projects to conventional basal ganglia circuitry. In contrast, the shell receives predominantly from the infralimbic and ventromedial prefrontal cortex and projects to subcortical limbic structures [37,38]. These anatomical differences suggest that the 2 subregions of the NAc may have independent functions – they play different roles in the reinstatement of cocaine seeking depending on the type of trigger used to elicit this behavior. Micro-infusion of GABA agonist or glutamate antagonist into core inhibited all forms of reinstatement [39,40]. Conversely, shell inactivation failed to suppress cocaine-primed reinstatement [40]; however, micro-infusion of dopamine or glutamate receptor antagonists into shell induced inhibition of this reinstatement [41]. Previous NAc ablation studies showed NAc core lesion reduced the acquisition of heroin self-administration, whereas shell lesion did not [42]. On the contrary, another study demonstrated NAc shell lesion was effective in attenuating morphine-primed CPP score, while core lesion was not effective [30]. Our results demonstrate that although both core and shell HFS suppressed NAc neuronal firing, and core HFS was more effective than shell HFS. A study of NAc HFS in the treatment of obsessive-compulsive disorder also demonstrated the difference between core and shell as 2 stimulating targets [43]. Based on this study and our results, we speculate that core and shell might act as different stimulating targets in the treatment of drug addiction.

Conclusions

In summary, the present study demonstrated electrical stimulation of the NAc could suppress the neuronal firing of the NAc and VP in morphine-treated rats. Three major conclusions could be drawn from our findings: (1) HFS of the NAc can block the activities of NAc neurons, which might underlie the mechanisms of NAc stimulation in alleviating drug addiction; (2) HFS is more effective in suppressing NAc neuronal firing than LFS; and (3) the core and shell of the NAc play different roles in suppressing NAc neuronal firing as 2 stimulating targets.

References:

1. Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. Trends Pharmacol Sci, 1992;13: 177–84
2. McBride WJ, Murphy JM, Ikemoto S: Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res, 1999;101: 129–52
3. Stefano GB, Kream RM, Esch T: Revisiting tolerance from the endogenous morphine perspective. Med Sci Monit, 2009; 15(9): RA189-98
4. McFarland K, Lapish CC, Kalivas PW: Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. Behav Brain Res, 1999;101: 129–52
5. LaLumiere RT, Kalivas PW: Glutamate release in the nucleus accumbens core is necessary for heroin seeking. J Neurosci, 2003; 23: 3531–37
6. Gao G, Wang X, He S et al: Clinical study for alleviating opiate drug psychological dependence by a method of ablating the nucleus accumbens with stereotactic surgery. Stereostat Funct Neurosurg, 2003; 81: 96–104
7. Haddad D, Swanson LW, divisions of the nucleus accumbens
8. Mirenowicz J, Nestler EJ: Genetic and pharmacological analysis of the nucleus accumbens
9. Watanabe K, Kitagawa M, Oono T, et al: Role of the nucleus accumbens in drug self-administration: a comparison with prefrontal cortex. Neurosci Lett, 2001; 307: 133–137
10. Matsumoto M, Niki T, Iwamura T, et al: Neuroanatomical and functional organization of the nucleus accumbens: a study in humans with functional magnetic resonance imaging. Brain Res, 2000; 866: 43–52
11. Swanson LW, Cowan WM, Young AB: The organization of the rat brain. In: Swanson LW, Cowan WM, Young AB (eds) The organization of the rat brain. Academic Press, New York, 1978: 1–60
24. Hashimoto T, Elder CM, Vitek JL: A template subtraction method for stimulus artifact removal in high-frequency deep brain stimulation. J Neurosci Methods, 2002; 115: 181-86

25. Montgomery EB Jr, Gale JT, Huang H: Methods for isolating extracellular action potentials and removing stimulus artifacts from microelectrode recordings of neurons requiring minimal operator intervention. J Neurosci Methods, 2005; 144: 107-25

26. Montgomery EB Jr, Gale JT: Mechanisms of action of deep brain stimulation (DBS). Neurosci Biobehav Rev, 2008; 32: 388-407

27. Helmer LF, Fallon JB: A novel stimulus artifact removal technique for high-rate electrical stimulation. J Neurosci Methods, 2008; 170: 277-84

28. Hashimoto T, Elder CM, Vitek JL: A template subtraction method for stimulus artifact removal in high-frequency deep brain stimulation. J Neurosci Methods, 2002; 115: 181-86

29. Montgomery EB Jr, Gale JT, Huang H: Methods for isolating extracellular action potentials and removing stimulus artifacts from microelectrode recordings of neurons requiring minimal operator intervention. J Neurosci Methods, 2005; 144: 107-25

30. Montgomery EB Jr, Gale JT: Mechanisms of action of deep brain stimulation (DBS). Neurosci Biobehav Rev, 2008; 32: 388-407

31. Helmer LF, Fallon JB: A novel stimulus artifact removal technique for high-rate electrical stimulation. J Neurosci Methods, 2008; 170: 277-84

32. Hashimoto T, Elder CM, Vitek JL: A template subtraction method for stimulus artifact removal in high-frequency deep brain stimulation. J Neurosci Methods, 2002; 115: 181-86

33. Montgomery EB Jr, Gale JT, Huang H: Methods for isolating extracellular action potentials and removing stimulus artifacts from microelectrode recordings of neurons requiring minimal operator intervention. J Neurosci Methods, 2005; 144: 107-25

34. Montgomery EB Jr, Gale JT: Mechanisms of action of deep brain stimulation (DBS). Neurosci Biobehav Rev, 2008; 32: 388-407