

## Original article

# Inhibition of the reinstatement of morphine-induced place preference in rats by high-frequency stimulation of the bilateral nucleus accumbens

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**Keywords:** stimulation; nucleus accumbens; morphine; drug dependency

**Background** Opiate addiction remains intractable in a large percentage of patients, and relapse is the biggest hurdle to recovery. Many studies have identified a central role of the nucleus accumbens (NAc) in addiction. Deep brain stimulation (DBS) has the advantages of being reversible, adjustable, and minimally invasive, and it has become a potential neurobiological intervention for addiction. The purpose of our study was to investigate whether high-frequency DBS in the NAc effectively attenuates the reinstatement of morphine seeking in morphine-primed rats.

**Methods** A morphine-dependent group of rats was given increasing doses of morphine during conditioned place preference training. A control group of rats was given equal volumes of saline. After the establishment of this model, withdrawal syndromes were precipitated in these two groups by administering naloxone, and the differences in withdrawal symptoms between the groups were analyzed. Electrodes for DBS were implanted in the bilateral shell of the NAc in the experimental group. The rats were stimulated daily in the NAc for 5 hours per day over 30 days. Changes in the conditioned place preference test and withdrawal symptoms in the rats were investigated and place navigation studies were performed using the Morris water maze. The data were assessed statistically with one-way analysis of variance (ANOVA) followed by Tukey's tests for multiple post hoc comparisons.

**Results** High-frequency stimulation of the bilateral NAc prevented the morphine-induced reinstatement of morphine seeking in the conditioned place preference test. The time spent in the white compartment by rats following 30 days of DBS ( $268.25 \pm 25.07$  seconds) was not significantly different compared with the time spent in the white compartment after relapse was induced by morphine administration ( $303.29 \pm 34.22$  seconds). High-frequency stimulation of the bilateral NAc accelerated the innate decay of drug craving in morphine-dependent rats without significantly influencing learning and memory.

**Conclusion** Bilateral high-frequency stimulation of the shell of the NAc may be useful as a novel therapeutic modality for the treatment of severe morphine addiction.

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Opiate drugs such as morphine are commonly used for relieving pain. However, this class of psychoactive agents can also elicit intense euphoric effects followed by feelings of well-being when taken in high doses, which can lead to drug abuse and may ultimately result in addiction.<sup>1</sup> The mesocorticolimbic dopamine reward circuit, which consists of the ventral tegmental area (VTA), nucleus accumbens (NAc) and ventral pallidum (VP),<sup>2</sup> plays a critical role in drug reinforcement. The NAc has a central role in the pathogenesis of drug dependence and is an important element in the mesocorticolimbic reward circuit,<sup>2-4</sup> which has been well described in animal studies.<sup>5</sup> The NAc is involved in establishing the reward and salience of drugs of abuse.<sup>6</sup> Therefore, the NAc is an attractive target for treatment. Wang et al<sup>7</sup> reported that a bilateral NAc lesion had excellent effects in opiate drug-dependent patients, and the relapse rate decreased significantly after 13 months of follow-up. However, the patients appeared to have impaired memory, dyscalculia, misbehavior, and other complications. Accordingly, ablation of the NAc cannot be used as a routine treatment because of its irreversibility, intolerable adverse effects, and ethical implications. Deep brain stimulation (DBS) is a surgical procedure in which an electrode is implanted in one or more specific areas

of the brain and high frequency electrical stimulation (130–180 Hz) is administered at target sites.<sup>8</sup> Over the past decade, there has been increasing interest in neurosurgical procedures using DBS to treat Parkinson disease, dystonia, depression, and obsessive compulsive disorder.<sup>9-10</sup> In addition, a case report of NAc DBS in a patient with severe depression, anxiety, and co-morbid alcoholism found significant improvements in alcohol dependency.<sup>11</sup> Xu et al<sup>12</sup> reported bilateral NAc DBS in an opiate drug-dependent patient had excellent effects on sustained abstinence

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without significant complications. DBS has the advantages of being reversible, adjustable, and minimally invasive. Therefore, it is a potential neurobiological intervention for addiction. Based on this potential, our purpose in this study was to investigate whether high-frequency DBS in the NAc effectively attenuates reinstatement of morphine seeking in morphine-primed rats while not affecting learning and memory.

## METHODS

### Experimental animals

Male Sprague-Dawley (SD) rats weighing 280–320 g were provided by the Experimental Animal Center of Military Medical Sciences, China. The animals were individually housed in cages (constant temperature, 22–25°C; humidity, 60–70%; illumination, 12/12-hour cycles) with free access to food and water. All experiments were performed during the light phase of the cycle. The animals that preferred the black compartment in a conditioned place preference (CPP) apparatus were selected and randomized into a control group ( $n=5$ ), sham DBS group ( $n=10$ ), and DBS group ( $n=10$ ). The care and handling of animals was conducted in compliance with the Chinese Animal Welfare Act, and this study was approved by the responsible governmental agency at Capital Medical University affiliated Beijing Neurosurgical Institute.

### Drug administration

All rats in the DBS and sham DBS groups received intraperitoneal morphine hydrochloride (lot 080403, Shengyang Pharmaceutical Factory, China) injections at increasing doses (5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90 mg/kg) once daily for 12 days. Rats in the control group received equal volumes of saline without undergoing surgery or electrical stimulation.

### CPP apparatus

The CPP apparatus was a rectangular box (50 cm×30 cm×30 cm). A partition with a small sliding door divided the box into two chambers of equal size. One chamber was black with a rough wooden floor, and the other chamber was white with a smooth polyvinyl chloride floor. Cameras were installed in the chambers to record the amount of time rats spent in each chamber. Light bulbs kept the lighting levels in the two chambers at 20 lux. The box was placed in a soundproof room with ventilation and dim illumination. The CPP procedure consisted of pretest, conditioning, and test phases. On the three consecutive pretest days (day 1 to day 3) (Figure 1), the rats were placed under the sliding door of the CPP apparatus and given access to both compartments for 15 minutes. The time the rats spent in each compartment was recorded. Because these rats preferred the black chamber, the white chamber was designated as the drug-paired side. In the conditioning phase (day 4 to day 16), the sliding door was closed, and the rats were placed in the drug-paired chamber for 30 minutes after morphine or saline injection

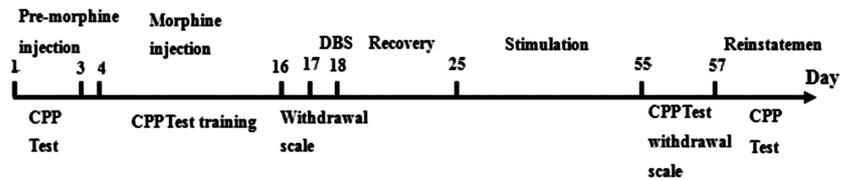


Figure 1. Diagram of the experimental timeline.

twice daily. This phase continued until rats in the morphine group spent more time in the white compartment than in the black compartment according to a schedule of double conditioning sessions. On day 17, rats were given naloxone hydrochloride (2 mg/kg, Beijing Sihuan Pharmaceutical Factory, China) intraperitoneally. Each rat was observed for withdrawal syndrome for 20 minutes, and evaluated using a withdrawal scale.<sup>13</sup> In addition, weight loss was evaluated by weighing the animals just before and after the behavioral observations.

### Implantation of stimulation electrodes

On day 18, the rats were anesthetized by intraperitoneal injections of urethane (1 g/kg) and mounted in a stereotaxic apparatus (Kopf instruments, USA). Bipolar stainless steel electrodes (outer diameter 200  $\mu$ m, inner diameter 50  $\mu$ m, CBBPF50, FHC, USA) were implanted bilaterally in the shell of the NAc according to the following coordinates relative to bregma: +2.0–2.2 mm anteroposterior (A/P),  $\pm$ 1.2–1.5 mm mediolateral (M/P), –6.5–7.5 mm dorsoventral (D/V).<sup>14</sup> Electrodes were cemented in place by affixing dental acrylic to two stainless steel screws fastened to the skull. After a seven-day (day 19 to day 25) recovery period from surgery, the stimulation was delivered with an A320R electrical stimulator (World Precision Instruments, USA). The stimulating parameters were an intensity of 2 V, pulse width of 60  $\mu$ s, and frequency of 130 Hz. Each train of pulses continued for 60 minutes at intervals of 15 minutes, five times a day for 30 days (day 25 to day 55). After each stimulation, the rats were placed in the white chamber for 30 minutes. The rats in the sham DBS group underwent the same procedures except they were not exposed to any electrical stimulation. On day 56, the postconditioning tests were carried out. On the test day, the sliding door was opened to allow free access to both chambers for 15 minutes. The amount of time the rats spent in each compartment was recorded to assess individual preferences. Then, the rats were given naloxone hydrochloride (2 mg/kg, Beijing Sihuan Factory, China) intraperitoneally. Each rat was observed for withdrawal syndrome for 20 minutes, and evaluated using a withdrawal scale.<sup>13</sup> After stimulation was completed (day 57), the DBS and sham DBS groups were again given morphine hydrochloride (2.5 mg/kg) intraperitoneally. After 15 minutes, the rats were placed in the CPP chamber and the sliding door was opened to allow free access to both sides of the chamber for 15 minutes. The amount of time the rats spent in each compartment was recorded.

### Morris water maze

Learning and memory were assessed in a Morris water

maze that consisted of a black circular pool 180 cm in diameter and 60 cm in height filled with water at 25°C. A circular transparent Plexiglas platform, 10 cm in diameter, was permanently placed in the middle of the northeast quadrant, 40 cm into the pool, and 1.5 cm below the water surface. One week after the stimulations were completed, testing of the rats in the DBS group, DBS sham group and control group for place navigation with the Morris water maze began, and this testing continued for 8 consecutive days. Each session included four trials of searching for the platform, with different starting positions for each trial. The order of the starting positions was randomly varied from day to day, but it was the same for all rats each day. The rats were placed in the pool facing the sidewall, and had a maximum of 2 minutes to search for the platform. The performance of the rats was monitored with an overhead video camera connected to an image analyzer (HVS Image, UK) and analyzed using the water maze software HVS WATER 2020. Experiments were performed at 8:00 a.m.

#### Verification of electrode placement

After completion of all experiments, the rats were anesthetized with urethane (1 g/kg) and perfused intracardially with 0.1 mol/L phosphate-buffered saline followed by 4% paraformaldehyde. The brains were removed and coronal sections (30  $\mu$ m) through the target were prepared and processed by violet stain derived from coal tar to confirm the location of the stimulating electrode. Only rats with electrode placements in the area of interest were included in subsequent data analysis.

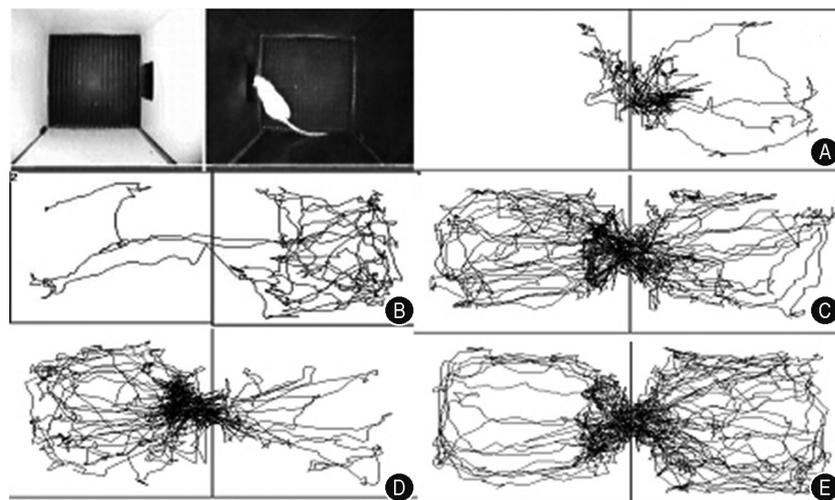
#### Statistical analysis

All results are expressed as the mean  $\pm$  standard error (SE). The data were assessed statistically with one-way analysis of variance (ANOVA) followed by Tukey's test for multiple post hoc comparisons using SPSS 13.0 for Windows (SPSS Inc., USA). A *P* value of less than 0.05 was accepted as statistically significant.

## RESULTS

#### CPP test (Figure 2)

All rats in the DBS and sham DBS groups that received morphine demonstrated a preference for the morphine-paired white chamber and exhibited morphine withdrawal syndrome on day 17, which confirmed the successful establishment of a morphine preference (*P* < 0.001). After 30 days of DBS, the amount of time the DBS-treated rats spent in the white compartment ((268.25 $\pm$ 25.07) seconds) of the CPP apparatus was not significantly different compared with the pretest phase ((252.46 $\pm$ 50.14) seconds) (*P* > 0.05). For the sham DBS group ((276.83 $\pm$ 40.04) seconds), there was also no significant difference compared



**Figure 2.** CPP test. **A:** The trajectory of innate preferences in rat under the conditions used in this study. **B:** Preference trajectory after reinstatement in control rats. **C:** Preference trajectory after morphine administration. **D:** Preference trajectory after reinstatement in rats treated with morphine and sham stimulation. **E:** Preference trajectory after reinstatement in rats treated with morphine and active deep brain stimulation.

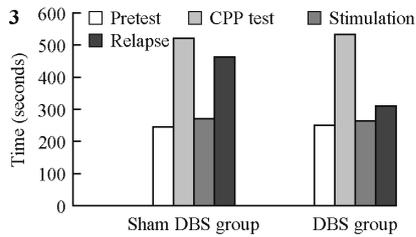
with the pretest phase (*P* > 0.05). After the final stimulation, the rats in the DBS and sham DBS groups were given morphine hydrochloride (2.5 mg/kg) intraperitoneally. The time spent in the white compartment by rats following 30 days of DBS ((268.25 $\pm$ 25.07) seconds) was not significantly different compared with the time spent in the white compartment after relapse was induced by morphine administration ((303.29 $\pm$ 34.22) seconds). In contrast, the time the sham DBS rats spent in the white compartment after 30 days of sham DBS ((276.83 $\pm$ 40.04) seconds) was significantly different compared with the time spent in the white compartment after relapse was induced by morphine ((466.26 $\pm$ 40.23) seconds) (*P* < 0.05) (Figure 3).

#### Withdrawal syndrome

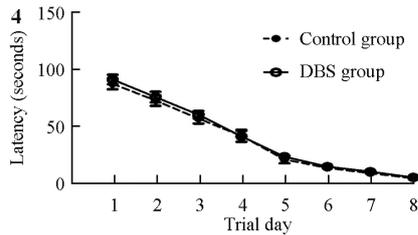
Before the final stimulation, the control group showed no significant withdrawal syndrome. In contrast, the sham DBS and DBS groups showed significant withdrawal syndromes, including abnormal posture, irritation, diarrhea, weight loss, ptosis, and other symptoms. The withdrawal syndrome scores in the sham DBS and DBS groups were 44.05 $\pm$ 4.16 and 44.72 $\pm$ 5.35, respectively. The withdrawal syndrome scores after the final stimulation in the DBS group 8.04 $\pm$ 1.85 were significantly lower than those before stimulation 44.05 $\pm$ 4.16 when given naloxone hydrochloride intraperitoneally. These scores were also significantly different compared with those in the sham DBS group 12.85 $\pm$ 3.59 (*P* < 0.01), but not those in the control group 6.25 $\pm$ 0.79 (*P* > 0.05). These results demonstrate that withdrawal symptoms in morphine-dependent rats fade with time, but high-frequency stimulation of the bilateral NAc can facilitate the rate of loss of withdrawal symptoms in morphine-dependent.

#### Morris water maze (Figure 4)

The latency to find the hidden platform in the Morris water maze in the DBS and control groups decreased with time,



**Figure 3.** Comparison of performances in the conditioned place preference test between the sham and active deep brain stimulation groups during the pretest, test, stimulation, and relapse phases.



**Figure 4.** Latency to find the hidden platform in the Morris water maze, presented as the mean±SE. The two groups of animals started swimming 8 minutes after completing deep brain stimulation. The maximum time allowed for each swim was 120 seconds. Learning in the deep brain stimulation group was not significantly different from that in the control group ( $P > 0.05$ ).

and there was no significant difference between the DBS and control groups on this measure ( $P > 0.05$ ). However, the speed of learning in the DBS group was slightly slower than in the control group. As such, that high-frequency stimulation of the bilateral NAc had no significant influence on learning and memory under the conditions used in this study.

## DISCUSSION

The results of this study indicate that high-frequency stimulation of the bilateral NAc of rats can prevent morphine-induced reinstatement in the morphine CPP test and accelerate the rate of decay of drug craving in morphine-dependent individuals without significantly influencing learning and memory.

The most important DBS parameters are the amplitude, pulse width, and frequency. Frequency plays an important role in treatment. Wen et al<sup>15</sup> reported that low frequency stimulation (80 Hz) can strengthen drug seeking in morphine-primed rats. Many authors have attempted to elucidate the mechanism underlying DBS. This has been done in the basal ganglia in an effort to understand the beneficial effects of high-frequency stimulation on patients with Parkinson's disease. The clinical effects of high-frequency DBS may be partly related to suppression of neuronal activity via activation of inhibitory interneurons or depolarization-based inactivation.<sup>16,17</sup> High-frequency DBS can excite the axon terminals of striatal and/or external pallidal neurons, resulting in release of gamma-aminobutyric acid (GABA) and inhibition of globus pallidus

internus (Gpi) neurons.<sup>18</sup> Consistent with the view that DBS produces its effects by reversible neuronal inactivation, administration of an  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor antagonist into the NAc blocks excitatory neurotransmission and attenuates cocaine-induced reinstatement of drug craving.<sup>19</sup> However, although there is also evidence that the neuronal soma is inhibited by high-frequency stimulation, axons within the region of stimulation may be activated, even at high frequencies.<sup>20</sup> Consistent with this notion, DBS of the NAc core suppressed neuronal activity in the orbitofrontal cortex, apparently via antidromic excitation of inhibitory cortical interneurons.<sup>21</sup> Together, these results suggest that high-frequency stimulation of the NAc attenuates morphine craving by inhibiting neuronal activity in accumbal output neurons and/or inactivation of accumbal afferents.

We believe that morphine-induced craving originates from deficits in the dopamine mesocorticolimbic reward circuit. Drugs of abuse produce excessive dopamine neurotransmission in the ventral striatum, where the NAc is located.<sup>22</sup> Microdialysis studies in animals have shown that addictive drugs preferentially increase extracellular dopamine in the NAc. High-frequency DBS of the NAc may supplant morphine and its effects on reward circuitry or may affect the "wiring" of the mesocorticolimbic circuitry so that morphine no longer has a heightened salience or an abnormal reward value. Such a process may be mediated by increased dopamine release during high-frequency stimulation of the NAc. DBS-mediated increases in dopamine in the NAc may eliminate the drive to consume morphine, which also causes increased dopamine release in the NAc. Alternatively, abnormalities in the reward circuit may be functionally silenced or "rebalanced" by high-frequency stimulation such that morphine-related reward signals are attenuated in the presence of DBS.

NAc shell dopamine acting through D1 receptors is also involved in Pavlovian learning through pre-trial and post-trial consolidation mechanisms and in the use of spatial short-term memory for goal-directed behavior.<sup>1</sup> It has been reported that a bilateral NAc lesion can weaken memories.<sup>7</sup> In this study, we investigated the effects of high-frequency DBS of the bilateral NAc on cognition in rats using the Morris water maze task. Our results demonstrate that the latencies to find the hidden platform in the DBS and control groups decreased with time, and there was no significant difference between DBS group and control group. Therefore, high-frequency stimulation of the bilateral NAc had no significant influence on learning and memory in rats.

In conclusion, the present results indicate that high-frequency DBS of the bilateral NAc blunts reinstatement of drug craving produced by re-exposure to morphine. In addition, high-frequency stimulation of the bilateral NAc has no significant influence on learning and memory in rats. Together, these results suggest that high-frequency DBS of the NAc may be useful as a novel therapeutic modality for

the treatment of severe morphine addiction.

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