Deep brain stimulation of the nucleus accumbens reduces alcohol intake in alcohol-preferring rats

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Object. The authors tested the hypothesis that deep brain stimulation (DBS) in the nucleus accumbens (NAcc) decreases alcohol intake in alcohol-preferring (P) rats after each animal has established a stable, large alcohol intake and after P rats with an established intake have been deprived of alcohol for 4–6 weeks.

Methods. Bipolar stimulating electrodes were bilaterally placed in the NAcc using stereotactic coordinates. In the first study, P rats (9 animals) were allowed to establish a stable pattern of alcohol intake (about 5–7 g/day) over approximately 2 weeks, and the acute effects of DBS in the NAcc (140–150 Hz, 60-μsec pulse width, and 200-μA current intensity) on alcohol intake and alcohol preference were studied. Each animal acted as its own control and received 1 hour of DBS followed by 1 hour of sham-DBS or vice versa on each of 2 sequential days. The order of testing (sham-DBS vs DBS) was randomized. In the second study, each animal was allowed to establish a stable alcohol intake and then the animal was deprived of alcohol for 4–6 weeks. Animals received DBS (6 rats) or sham-DBS (5 rats) in the NAcc for 24 hours starting when alcohol was reintroduced to each animal.

Results. Deep brain stimulation in the NAcc, as compared with a period of sham-DBS treatment in the same animals, acutely decreased alcohol preference. Furthermore, alcohol consumption and preference were significantly reduced in the DBS group compared with the sham treatment group during the first 24 hours that alcohol was made available after a period of forced abstinence.

Conclusions. The NAcc plays a key role in the rewarding and subsequent addictive properties of drugs of abuse in general and of alcohol in particular. Deep brain stimulation in the NAcc reduced alcohol consumption in P rats both acutely and after a period of alcohol deprivation. Therefore, DBS in the NAcc coupled with other neurophysiological measurements may be a useful tool in determining the role of the NAcc in the mesocorticolimbic reward circuit. Deep brain stimulation in the NAcc may also be an effective treatment for reducing alcohol consumption in patients who abuse alcohol and have not responded to other forms of therapy. (DOI: 10.3171/2010.4.FOCUS10105)

Key Words • deep brain stimulation • nucleus accumbens • reward • alcohol abuse

Deep brain stimulation is a surgical procedure in which an electrode is implanted in 1 or more specific areas of the brain and high-frequency electrical stimulation (130–180 Hz) is delivered to target sites. This procedure ameliorates symptoms associated with some movement disorders and has been a moderately effective treatment for intractable pain. The use of DBS is being extended to include a variety of psychiatric disorders such as obsessive-compulsive disorder and depression. The NAcc has a central role in the pathogenesis of drug dependence and is an important element in the mesocorticolimbic reward circuit. It is involved in establishing the salience and reward of drugs of abuse. Many investigators believe that dysregulation of the neurophysiological processes involved in establishing the quality or intensity of rewarding experiences contributes to addiction.

For these reasons, the NAcc is an attractive target for DBS, and early studies are promising. Deep brain stimulation in the NAcc has selectively blocked the reinstatement of psychostimulant use and attenuated morphine-induced place preference. In a patient who received DBS for the primary purpose of alleviating severe anxiety and depression, stimulation in the NAcc had the unintended consequence of improving the patient’s comorbid alcohol dependence. Data from a subsequent animal study showed that brief periods of DBS in either the core or the shell of the NAcc reduced alcohol consumption in rats trained to drink alcohol.
There are many rat models of alcohol-seeking behavior. Rats that would not "naturally" imbibe alcohol are often trained through variations in the Samson sucrose-fading procedure to consume high quantities of alcohol. Alcohol-prefering rats, on the other hand, are selectively bred. These animals spontaneously consume alcohol in large quantities and do not require reinforcement schedules with sucrose to entice them to drink alcohol. The P rats fulfil all the criteria of a valid model of alcohol-seeking behavior proposed by Cicero: P rats drink sufficient amounts of ethanol to develop metabolic tolerance; they consume large quantities of ethanol and achieve pharmacologically relevant blood alcohol levels; and they increase ethanol consumption after a period of abstinence, which is called the "alcohol deprivation effect" (ADE). The ADE is robust and observed in myriad models of alcohol abuse including rats, mice, and monkeys, and humans. Its underlying basis is unknown, but ADE may approximate relapse drinking in otherwise abstinent alcoholics.

To our knowledge, DBS has not been used in P rats to suppress or prevent alcohol intake. The NAcc plays a crucial role in alcohol dependence, and lesioning the NAcc shell reduces alcohol preference in P rats already drinking alcohol. Since DBS in PD is thought to mimic the effects of lesioning, we sought to determine whether DBS in the NAcc in P rats would have an effect similar to lesioning and suppress alcohol intake. We studied the effect of DBS on alcohol intake in P rats in 2 experiments. In the first study, we tested the hypothesis that DBS would decrease alcohol intake in P rats after each animal had established a stable and large alcohol intake. In the second study, we allowed P rats to establish stable ethanol intake. We then deprived each animal of alcohol for 4–6 weeks and tested the hypothesis that DBS would prevent or reduce the burst of excess alcohol consumption usually seen in P rats when alcohol is made available after a period of abstinence. Thus, the purpose of our study was to show that DBS is effective in reducing both alcohol preference and ADE in rats known to prefer alcohol with no sucrose fading or any other behavioral modification to induce alcohol consumption.

Methods

The experiments were approved by the Institutional Animal Care and Use Committee of Dartmouth College in accordance with National Institutes of Health guidelines for the use of animals in research. We conducted 2 studies. In the first, 9 male P rats weighing between 172 and 365 g (mean ± SEM, 310 ± 10 g) were used; 11 male P rats weighing between 440 and 580 g (528 ± 12 g) were included in the second study. All rats were housed in a temperature-controlled room (21°C) under a 12/12 light/dark cycle (light on at 6:00 a.m.). The rats had ad libitum access to food pellets, water, and 10% ethanol except for 24 hours before and 24 hours after surgery to prevent interactions with the anesthetics used.

Acquisition of Alcohol Preference

Rats were given free access to alcohol for 3–7 weeks to establish a baseline level of alcohol consumption (approximately 5–7 g/kg/day). Access was discontinued in the perioperative period, when stimulating electrodes were placed in the NAcc as described below. Animals were subsequently allowed free access to alcohol for 4–7 days to reestablish baseline levels of alcohol consumption before beginning the DBS treatment.

Surgical Procedures

Each animal was anesthetized with inhaled isoflurane (2–5%, Webster Veterinary). We maintained body temperature at 37°C by using a rectal thermometer and a servo-controlled heating pad placed under the animal. Each rat’s head was fixed in a stereotactic frame (Model 1430, David Kopf Instruments), and a midline incision was made starting just caudal to the eyes and ending just rostral to the ears. We placed concentric bipolar stainless steel electrodes (outer diameter 0.005 in, Plastics One, Inc.) bilaterally in the outer shell of the NAcc. The locations of the stimulating electrodes were calculated from the bregma and the brain surface using stereotactic coordinates: anteroposterior +1 mm, mediolateral ±3 mm, and dorsoventral –8 mm. Electrodes were positioned in a custom-made holder before implantation to guarantee a consistent separation between the electrode tips. We secured electrodes to the skull using dental cement (Dentsply International Inc.). After surgery animals were given buprenorphine for 48 hours to alleviate pain.

Study I: Assessment of the Effect of DBS on Established Alcohol Intake

Prior to DBS treatment, animals were moved to operant chambers (Med-Associates, Inc.) and were kept on the same light/dark cycle and access regimen for alcohol and water as previously described. Animals were tethered for 1 hour on the day before DBS-treatment days to acclimate them to the tether before DBS treatment. Animals were treated with DBS for 2 days using a 2-hour treatment cycle (1 hour on/1 hour off DBS). Whether an animal received stimulation in the 1st hour or the 2nd hour on Day 1 of treatment was randomly determined, and the treatment order was reversed on the next day. Thus, each animal acted as its own control every day, and each animal experienced both DBS treatment orders. Prior to the stimulation/no stimulation treatment, animals were tethered to a cable used to deliver DBS to the electrodes implanted in each animal. Animals were given time to acclimate to the tether for at least 1 hour before treatment. Rats underwent a conditioning period of DBS 1 hour before the beginning of the dark cycle consistently at 6:00 p.m., that is, the time of day that the rats were seen to be the most active. For this DBS conditioning period, alcohol—not water and food—was withheld from the rats. At the start of the dark cycle, 10% alcohol was reintroduced to the rats, and DBS was either continued during the stimulation treatment or the pulse was discontinued during the no stimulation treatment. Both drinking bottles containing either water or 10% ethanol were weighed prior to and just after each 1-hour stimulation/no stimulation period.

Stimulation was delivered using a pulse code generator (Master-8-vp, A.M.P.I., Ltd.) and an Isoflex stimulus

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isolation device (A.M.P.I., Ltd.). The stimulation current during the DBS conditioning period and treatment period consisted of monophasic square wave pulses. Stimulation was delivered at a frequency of 140–150 Hz with a pulse width of 60 μsec and a current intensity of 200 μA.

**Study II: DBS Treatment of ADE**

The second study was designed to show the effect of DBS on alcohol consumption in P rats after a period of abstinence from alcohol. Rats were given free access to alcohol for 4–6 weeks to establish a baseline level of consumption. Access to alcohol was then discontinued for 4–6 weeks. During the abstinence period, bipolar stimulating electrodes were bilaterally placed in the NAcc, as described above. After surgery, each animal was given a week to recover and acclimate to the testing chamber and tethering. Animals were randomly assigned to a control group (sham stimulation) or a DBS treatment group. Animals in the control group did not receive DBS during the conditioning period or when they had free access to 10% ethanol, water, and food for 24 hours. Animals in the DBS treatment group received DBS at a frequency of 140–150 Hz, a pulse width of 60 μsec, and a current intensity of 200 μA for 1 hour before they were allowed free access to 10% ethanol, water, and food and received continuous DBS stimulation for the ensuing 24 hours after the reintroduction of alcohol. In both treated and control animals, the ethanol and water volumes were recorded at the beginning and the end of the 24-hour treatment period.

**Brain Histology and Electrode Placement Confirmation**

At the conclusion of each experiment, each animal was given an overdose of phenobarbital and was perfused through the apex of the heart with 250 ml of saline followed by 250–300 ml of 4% paraformaldehyde in phosphate-buffered saline to fix the brain in situ. After perfusion, the brain was removed, fixed overnight in 4% paraformaldehyde, cryoprotected in 30% sucrose for 36–72 hours, and frozen at −70°C in Tissue-Tek (Sakura Finetek U.S.A., Inc.). Subsequently, 50-μm coronal sections were cut through the basal ganglia using a cryostat (Leica CM3050, Leica Microsystems, Inc.). Tissue sections were mounted on glass slides and counterstained with cresyl violet. Locations of the electrodes were identified under light microscopy based on the insertion track and tissue damage caused by the tips of the electrodes.

**Statistical Analysis**

In the first study, we determined the alcohol preference for each rat by calculating the grams of alcohol consumed during the stimulation-on or stimulation-off phases of the experiment. The mean and standard error of the mean quantity of alcohol and water consumed (g/hour) and the alcohol preference (the ratio of alcohol consumed/total fluid consumed) were calculated from each treatment condition over the 2-day study period. Values for the stimulation-on period were compared with values representing the stimulation-off periods by using a paired t-test. The values for the study of alcohol deprivation reflect the mean quantity of alcohol and water consumed in a 24-hour testing period (g/kg/24 hrs) and the alcohol preference while each animal received DBS or no DBS. Because treated and control rats were 2 separate groups, the results of the alcohol deprivation experiment were analyzed using an unpaired t-test. A p < 0.05 was considered statistically significant.

**Results**

**Effect of DBS on Established Alcohol Consumption**

At the beginning of the studies, rats were given free access to alcohol for 3–7 weeks to establish a sufficient baseline level of alcohol consumption. The mean quantity of alcohol consumed at the end of the alcohol acquisition period in animals in the first study was 5.5 ± 1.6 g. The values were similar to those observed for male P rats in previous studies. The rats behaved normally and continued to eat and gain weight before and after surgery.

The sites of the stimulating electrode tips were easily identified from the insertion tracks. Electrodes were successfully placed in the region of or adjacent to the NAcc and ranged from 0.6 to 2.7 mm anterior to the bregma, from 1.9 to 3.7 mm dorsal to the interaural line, and from 0.9 to 2.5 mm laterally on either side of the midline.

Average individual values of alcohol preference with or without DBS are shown in Fig. 1 for each of the 9 P rats studied. Over the 2 periods of DBS treatment on 2 separate days, 7 of 9 animals exhibited decreased alcohol consumption, 1 showed no effects from DBS, and 1 increased its alcohol consumption during DBS. No unusual behaviors were noted during the DBS period; that is, animals explored the cage and moved about with no outward manifestation indicating that DBS was or was not being given.

The average alcohol and water consumption levels and the alcohol preference of the rats are summarized in Fig. 2. Water consumption increased by approximately 50% during periods of DBS compared with that during sham treatments, but this change was not statistically significant (p = 0.090). Alcohol consumption decreased approximately 30% during periods of DBS in the NAcc as compared with the unstimulated study periods in the

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**Fig. 1.** Bar graph depicting the effects of DBS in the NAcc on alcohol preference in 9 individual rats. EtOH = ethanol.
same animals, although the decrease was not statistically significant \((p = 0.071)\). On the other hand, the increase in water intake and the decrease in alcohol intake during DBS treatment periods generated alcohol preference values (alcohol consumed/total fluid consumed) that decreased significantly during DBS compared with the sham-DBS period \((p = 0.016)\). The combined amounts of alcohol and water consumed during both the DBS stimulation period and the sham-DBS period on each day were similar; there was no evidence that the order of testing affected the response to either the DBS stimulation or the sham-DBS testing.

**Effect of DBS on Alcohol Consumption After Alcohol Deprivation**

The average amount of alcohol and water consumed and the alcohol preference of 2 groups of animals receiving DBS or sham-DBS for 24 hours after alcohol had been reintroduced following a 4- to 6-week period of abstinence are shown in Fig. 3. Water consumption was greater in the DBS treatment group (6 rats) than in the sham-DBS group (5 rats), but this difference was not statistically significant \((p = 0.088)\). Alcohol consumption over 24 hours was significantly reduced in the DBS treatment group compared with the sham treatment group \((p = 0.016)\). Alcohol preference was also significantly reduced during DBS compared with during the sham treatment \((p = 0.021)\).

**Discussion**

Deep Brain Stimulation Attenuates Alcohol Preference in \(P\) Rats

The purpose of these experiments was to show that DBS in the NAcc in \(P\) rats was capable of acutely attenuating each animal’s preference for alcohol as well as the increase in alcohol consumption following a period of abstinence. Deep brain stimulation in the NAcc acutely decreased alcohol preference compared with a period of sham-DBS treatment in the same animals, and alcohol consumption and alcohol preference were significantly reduced in a DBS treatment group compared with a sham treatment group during the first 24 hours that alcohol was made available after a period of forced abstinence.

**Technical Limitations of the Study**

The stimulating electrodes were large relative to the size of the NAcc in rats. We selected stereotactic coordinates aimed at the shell of the NAcc, but it is unlikely that stimulation was restricted to the shell. Not all the electrodes were in or directly adjacent to the NAcc shell, and the current spread was relatively large during DBS so that stimulation of even those electrodes in the shell of the NAcc probably affected closely adjacent tissue outside the shell. Therefore, the most conservative conclusion from these data is that bilateral HFS in the region of the NAcc blunts alcohol consumption acutely, even after a period of alcohol deprivation, in \(P\) rats.

A related limitation is that our neuroanatomical localization of the electrodes was imperfect. There was a surprising amount of tissue damage at the tip of the stimulating electrodes, which tended to leave a hole or tear during tissue slicing. Thus, we know the general location within particular nuclei based on our neuroanatomical analysis, but the tissue damage precludes a more detailed description of exactly where within particular nuclei the major focus of stimulation was located. One might be concerned that electrode placement actually lesioned the NAcc, but the relative lack of effect of sham-DBS in the control animals argues in favor of the conclusion that the suppression of alcohol consumption was a response to the HFS of the nucleus and not simply to electrode placement.

Finally, the alcohol preference values are much lower in the first study than in the second. The duration of these...
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However, that the ratios were consistent from day to day in this study, and the effect of DBS on alcohol preference was still apparent despite the relatively low alcohol preference ratios.

Suppression of Alcohol Intake by DBS in P Rats

Ikemoto et al. demonstrated that 6-OHDA lesions in the shell of the NAcc in P rats attenuated alcohol-seeking behavior; therefore, one might hypothesize that DBS in the NAcc is mimicking the effect of these lesions. Deep brain stimulation was originally thought to work like a lesion in movement disorders. Previous work by others in which DBS delivered to the NAcc (core and shell) in rats trained to imbibe alcohol had the effect of significantly decreasing alcohol consumption, and the results of our study are consistent with the hypothesis that DBS mimics the effect of a 6-OHDA lesion in the NAcc. However, the mechanism of action of DBS is hotly debated, and it is not clear that DBS achieves its lesionlike effects by actually creating functional lesions in the target sites of stimulation.

Most of the work elucidating the mechanism of action of DBS has been done in the basal ganglia in an effort to understand the beneficial effects of HFS in the STN of patients with PD. Just as is true for attenuated alcohol consumption during DBS in P rats, the therapeutic effects of DBS in PD resemble those of lesions, and DBS may silence neurons in the STN as well as other nuclei within the basal ganglia. The neuronal soma may be inhibited by HFS, but axons within the region of stimulation may be activated even at high frequencies. Perhaps reflecting this stimulatory effect, the activity of dopaminergic neurons increases in the substantia nigra compacta during HFS of the STN. Moreover, neurotransmitter levels rise within the basal ganglia during HFS of the STN: dopamine levels rise in the striatum, γ-aminobutyric acid increases in the globus pallidus interna, and glutamate levels increase within the STN and globus pallidus interna. The similarities between the effects of surgical lesions, which ought to eliminate neuronal activity, and the effects of DBS on the symptoms of PD are hard to reconcile with evidence of persistent neuronal activity and increased neurotransmitter levels in the basal ganglia during DBS. Thus, neurotransmitter levels are modified throughout the basal ganglia as a result of DBS in the STN, but the relationship of any one of these individual changes to the therapeutic benefit of DBS has not been established unequivocally. This same mechanistic dilemma—DBS elicits neurotransmitter release, but also seems to silence some neurons while activating others—confronts us as we try to understand how HFS in the NAcc reduces alcohol consumption in P rats. Notwithstanding the current difficulties understanding the mechanism of action of DBS, HFS of the NAcc and other nuclei in the mesolimbic reward circuit may be used as a powerful probe to understand the basic biology of the reward circuit and to elucidate the mechanism of action of DBS, especially when DBS is combined with measurements of electrical activity and neurotransmitter release in other nuclei in the reward circuit.

We believe that alcohol-seeking behavior originates
from a deficiency in the dopamine-mediated mesocorticolimbic reward circuit. Thus, DBS in the NAcc may be supplanting ethanol and its effects on reward circuitry or may be affecting the “wiring” of the mesocorticolimbic circuitry so that ethanol no longer has a heightened salience or an abnormal reward value. Such a process might be mediated by an increase in dopamine release during HFS of the NAcc—just the opposite of the predicted effect of 6-OHDA lesions in the NAcc. The DBS-mediated increase in dopamine in the NAcc may eliminate the need to consume ethanol, which also causes increased dopamine release in the NAcc. Alternatively, abnormalities in the reward circuit may be functionally silenced or “rebalanced” by DBS such that ethanol is not capable of producing as robust a reward signal in the presence of DBS.

Regardless of the mechanism of action, P rats are thought to approximate many features of alcoholism found in patients with a genetic predisposition toward alcoholism. For this reason, DBS, which is already effective and approved for use in humans in other settings, may be a beneficial therapy in patients with severe alcoholism resistant to other forms of therapy. If DBS proves to be effective at reducing the salient effect of alcohol in abstinent drinkers, it may also decrease the risk of relapse. Thus, DBS may serve as a solitary or an adjunctive therapy in patients resistant to current treatments for alcoholism.

Conclusions

The main finding in the current study was that DBS reduced alcohol intake in 2 models of alcohol consumption in P rats; alcohol preference was acutely reduced for a short duration of DBS in animals with established patterns of alcohol consumption, and alcohol consumption and alcohol preference were reduced over 24 hours of DBS when alcohol was made available after a period of abstinence. The P rats exhibit many characteristics of a valid model of alcohol-seeking behavior as proposed by Cicero, and they fulfill a seventh criterion: relapse-related ADE. As far as we know, this study is the first in which DBS was used to attenuate the ADE in P rats and is the only experimental intervention to have such a robust effect. The results indicated that ADE may be mechanistically related to the NAcc. And DBS in the NAcc, however it works, seems to blunt alcohol consumption both acutely and in previously abstinent animals. We concluded, as have many others, that the NAcc plays a key role in the rewarding and subsequent addictive properties of drugs of abuse in general and of alcohol in particular. Moreover, DBS coupled with other neurophysiological measurements may be a useful tool in determining the role of the NAcc in the mesocorticolimbic reward circuit.

Disclosure

This work was supported by a COSAT grant from Johnson & Johnson (J.C.L.). Dr. Roberts holds a patent with Advanced Neuromodulation Systems, Inc., and is a consultant for Medtronic. Dr. Leiter holds a patent with Advanced Neuromodulation Systems, Inc. Dr. Green owns stock in Johnson & Johnson, Pfizer, and Mylan; has received support from Janssen and Eli Lilly; and is a member of Data Safety Monitoring Board at Eli Lilly.

Author contributions to the study and manuscript preparation include the following. Conception and design: Leiter, Henderson, Chau, Roberts, Green. Acquisition of data: Henderson, Bradford. Analysis and interpretation of data: Leiter, Henderson, Bradford, Roberts, Green. Drafting the article: Leiter, Henderson, Green. Critically revising the article: Leiter, Green. Reviewed final version of the manuscript and approved it for submission: Leiter, Henderson, Green. Statistical analysis: Leiter, Henderson. Administrative/technical/material support: Leiter, Green. Study supervision: Leiter, Roberts, Green.

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