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Archival Report

Biological Psychiatry

Bidirectional Modulation of Extinction of Drug Seeking by Deep Brain Stimulation of the Ventral Striatum

Freddyson J. Martínez-Rivera, Jose Rodríguez-Romaguera, Mario E. Lloret-Torres, Fabricio H. Do Monte, Gregory J. Quirk, and Jennifer L. Barreto-Estrada

ABSTRACT

BACKGROUND: Recent research in humans and rodents has explored the use of deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VS) as a possible treatment for drug addiction. However, the optimum electrode placement and optimum DBS parameters have not been thoroughly studied. Here we varied stimulation sites and frequencies to determine whether DBS of the VS could facilitate the extinction of morphine conditioned place preference in rats.

METHODS: Rats were implanted with DBS electrodes in the dorsal or ventral subregions of the VS and trained to the morphine conditioned place preference. Subsequently, rats received extinction sessions over 9 days, combined with 60 min of either high- (130 Hz) or low- (20 Hz) frequency DBS. To study circuit-wide activations after DBS of the VS, c-fos immunohistochemistry was performed in regions involved in the extinction of drug-seeking behaviors.

RESULTS: High-frequency DBS of the dorsal-VS impaired both extinction training and extinction memory, whereas high-frequency DBS of the ventral-VS had no effect. In contrast, low-frequency DBS of the dorsal-VS strengthened extinction memory when tested 2 or 9 days after the cessation of stimulation. Both DBS frequencies increased c-fos expression in the infralimbic prefrontal cortex, but only low-frequency DBS increased c-fos expression in the basal amygdala and the medial portion of the central amygdala.

CONCLUSIONS: Our results suggest that low-frequency (rather than high-frequency) DBS of the dorsal-VS strengthens extinction memory and may be a potential adjunct for extinction-based therapies for treatment-refractory opioid addicts.

Keywords: Amygdala, CPP, DBS, Extinction, Morphine, Ventral striatum

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Deep brain stimulation (DBS) of the ventral capsule (VC)/ventral striatum (VS) has been shown to reduce symptoms in treatment-refractory patients with obsessive-compulsive disorder (1), as well as to facilitate their responses to extinction-based therapies (2,3). Recently, DBS of the VC/VS has been suggested as a promising target for treatment-refractory drug addiction (4,5). Drug addiction is characterized by the persistence of maladaptive behaviors such as compulsion to seek the drug (6), suggesting a deficit in circuits that regulate the extinction of addictive behaviors (7,8). Thus, given that VC/VS represents a central region of the reward circuit that receives projections from areas that regulate the extinction of drug seeking (7,9,10), DBS of this region could represent a clinical tool for understanding the mechanisms of drug addiction.

DBS is usually delivered at frequencies of 90 Hz or more (11). In humans, high-frequency DBS of the VC/VS has been shown to reduce symptoms of addiction to alcohol (12–14), nicotine (13,15), and heroin (16). Preclinical studies in rats have demonstrated that high-frequency DBS of the VS is effective in reducing drug-seeking behaviors for ethanol (17,18), cocaine (19,20), heroin (21), and morphine (22,23). However, in some patients, addictive symptoms remain unchanged or worsen after high-frequency DBS (12,24,25). As an alternative to high-frequency DBS, low-frequency DBS (≤20 Hz) has been recently suggested as a treatment for addiction (26–28). In rats, low-frequency DBS of the VS attenuated cocaine relapse (29) and abolished cocaine sensitization when combined with pharmacologic treatments (28).

Although previous studies of DBS have focused on the expression and relapse of addictive behaviors (4,5), none have examined the effects of DBS on the extinction of addiction, which is the basis of exposure-based therapies for addictive disorders (30,31). Extinction is a form of learning in which associations between cues and the drug are weakened by repeated exposure to the cues in the absence of the drug (30,32). Thus, strengthening the extinction of drug-associated memories in addicted patients may reduce the risk of relapse. We therefore assessed the effects of DBS of the VS on extinction of morphine conditioned place preference (CPP) in rats. We applied either high- or low-frequency DBS to distinct

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subregions of the VS previously shown to either enhance or impair extinction of conditioned fear (33). Using an immunohistochemical approach, we also assessed the effects of high- and low-frequency DBS on cellular activity within regions necessary for extinction of drug seeking.

METHODS AND MATERIALS

Subjects

Eighty-seven adult male Sprague Dawley rats (~350 g; Harlan Laboratories, Livermore, CA) were individually housed with food and water available ad libitum (12:12 hour light/dark cycle; 64°F, 30% humidity). The behavioral experiments were performed during the light phase of the cycle, and all procedures were in accordance with the Institutional Animal Care and Use Committee of the University of Puerto Rico, Medical Sciences Campus, and the Association for Assessment and Accreditation of Laboratory Animal Care.

Surgery

Rats were anesthetized with isoflurane inhalant gas (5% for induction) and positioned in a stereotaxic frame (2%–3% for maintenance). Rats were bilaterally implanted with concentric bipolar stimulating electrodes (NEX-100; Rhodes Medical Instruments, Summerland, CA) as previously described (33,34). Electrodes in the dorsal-VS site were aimed at –6.5 mm dorsoventral, ±3.5 mm mediolateral, and +1.2 mm anteroposterior, whereas the ventral-VS site coordinates were –8.0 mm dorsoventral, ±3.5 mm mediolateral, and +1.2 mm anteroposterior (35). Electrodes were fixed to the skull with anchoring screws, C&B-metabond (Parkell, Inc., Edgewood, NY), and acrylic cement. Finally, a topical antibiotic (neomycin sulfate, Certi-Sporyn), and an analgesic (5 mg/kg, intramuscular, Ketofen; Zoetis, Inc., Florham Park, NJ) were applied. Rats were allowed 7 to 10 days of recovery.

Drug

Morphine sulfate (5 mg/kg; Sigma-Aldrich, St. Louis, MO) was dissolved in saline (0.2 ml/100 g of body weight) and administered to both sham and stimulated groups. The dose, time course (4 days), and route of administration (subcutaneous) were selected based on studies showing efficacy in inducing CPP without affecting locomotion (32,36).

Conditioned Place Preference

CPP was performed as previously described (37). Briefly, the CPP apparatus consists of an acrylic chamber (42 cm long × 30 cm high × 42 cm high) separated into two compartments with an entry containing a removable guillotine door. Compartments consisted of grated-texture flooring with black-and-white-checkered walls, or smooth flooring with black-and-white-lined walls. All CPP protocols were performed at semidarkness conditions (~10 lux). Behavioral data was acquired using the Any-Maze tracking system (Stoelting Co., Wood Dale, IL).

On days 1 (habituation) and 2 (baseline), animals were allowed to move freely between compartments for 20 minutes, and the preferred and nonpreferred sides were noted. During the conditioning phase, rats were injected with morphine and restricted to the nonpreferred side (drug-paired side) for 45 minutes, or injected with saline and restricted to the preferred side (saline-paired side) for the same amount of time. A total of four injections of either morphine or saline were administered during the conditioning sessions (days 3–10, alternating the days). Three days after the last conditioning session (day 13, expression test), rats were given a drug-free test of 20 minutes in which they were allowed to move freely between both compartments of the CPP chamber. The amount of time spent (%) in the drug-paired side was calculated as an index of conditioning.

On the extinction phase, DBS was continuously delivered for 60 minutes in three blocks of 20 minutes: the first block in the home cage; the second in the CPP chamber combined with extinction sessions; and the last block in the home cage again. This duration of stimulation was selected based on previous studies showing that 60 minutes of DBS is sufficient to produce behavioral and neuroplasticity changes in rodents (4,38,39). We therefore divided the 60 minutes of stimulation in three blocks of 20 minutes between the home cage and the CPP chamber for the following reasons. 1) Our CPP-extinction sessions consisted of 20 minutes; 2) DBS effects of the VS usually start after a period of adjustment that may take several minutes (~30) (38,39); and 3) previous studies in rodents have applied DBS during a range of time that includes not only the behavioral test, but also the pretest and posttest periods (18,23,33). A total of 11 or 9 extinction sessions were performed for high- (days 14–24) or low- (days 14–21) frequency DBS, respectively. Similar to the expression test, rats were allowed to move freely between compartments during each extinction session. The percentage of time spent in the previous drug-paired side was measured as an index of extinction. In the last 2 days of extinction, the stimulator was turned off to evaluate long-lasting effects of DBS.

Deep Brain Stimulation

DBS was delivered as previously reported (33,34). Briefly, the VS was bilaterally aimed with a concentric bipolar electrode with each polar end measuring 0.5 mm and 0.5 mm apart (NEX-100; Rhodes Medical Instruments). The DBS parameters used for high- and low-frequency stimulation were similar to those used in humans and rodents (100–200 μA, 0.1-ms pulse duration, 130 Hz or 20 Hz) (29,33,40,41). The stimulator (S88X) and constant current unit (SIC-C Isolation Unit; Grass Instruments) were connected to a commutator (Plastics One, Roanoke, VA) through a cable that delivered the stimulation to the implanted electrodes.

Histology

Rats were deeply anesthetized with sodium pentobarbital (450 mg/kg intraperitoneally) and transcardially perfused (10 minutes) with saline (0.9%) followed by paraformaldehyde (10%,vol/vol). Brains were stored in a 30% sucrose-formalin solution for 48 hours before sectioning. Coronal sections (40 μm thick) were cut in a cryostat and mounted on gelatin-coated slides. Sections were stained for with cresyl violet, coverslipped with a distrene 80–plasticizer–xylene mounting media, and examined in a microscope. Placements outside the VS were excluded.

Immunohistochemistry

Similar to the behavioral experiments, naïve rats received daily 1 hour of high-frequency, low-frequency, or sham stimulation to the dorsal-VS for 6 consecutive days in their home cages. We used naïve rats to determine the effects of DBS on neural circuits independent of the effects of morphine exposure or extinction training. Although the effect of high-frequency DBS of the VS on the expression of c-fos has been previously evaluated (20,34), no previous study has investigated the effect of low-frequency DBS. We therefore decided to match the stimulation regimen (i.e., frequencies and duration) used in our behavioral experiments to address this question. On day 6, rats were deeply anesthetized with sodium pentobarbital (450 mg/kg intraperitoneally) 1 hour after receiving 1 hour of DBS or sham stimulation. They were perfused transcardially with 100 mL of 0.9% saline followed by 500 mL of 4% paraformaldehyde in 0.1 mol/L phosphate buffer at pH 7.4. Brains were transferred to a solution of 30% sucrose in 0.1 mol/L phosphate buffer at 4°C during 48 hours for cryoprotection. Brains were frozen and coronal sections (40 μm) were cut on a cryostat (CM 1850; Leica Biosystems, Inc., Buffalo Grove, IL) at the level of frontal cortex, VS, and amygdala areas. For c-fos immunohistochemistry, sections were blocked in 2% normal goat serum (Vector Laboratories, Inc., Burlingame, CA) plus 0.3% triton X-100 (Sigma-Aldrich) in 0.12 mol/L potassium buffer saline for 1 hour, as previously described (34). The sections were then incubated overnight at room temperature with rabbit anti-c-fos serum (1:10,000 Ab-5; Oncogene Science, Inc., Uniondale, NY). Sections were then incubated for 2 hours at room temperature in a solution of biotinylated goat antirabbit immunoglobulin G (Vector Laboratories) and placed in a mixed avidin-biotin horseradish peroxidase complex solution (ABC Elite Kit; Vector Laboratories) for 90 minutes. Black immunoreactive nuclei labeled for c-fos were visualized after 10 minutes of exposure to DAB/peroxidase substrate kit (Vector Laboratories). Sections were mounted in coated-gelatin slides, dehydrated, and coverslipped. Counter-sections were collected, stained, coverslipped, and used to determine electrode placement, as well as the anatomical boundaries of each structure analyzed.

Immunoreactivity Quantification

Counts of the number of c-fos immunoreactive neurons were carried out at 20× magnification with an Olympus microscope (Model BX51; Center Valley, PA). We restricted our analysis to several areas involved with the extinction of drug-seeking behaviors (10). Images were generated for prelimbic (PL) and infralimbic (IL) subregions of the prefrontal cortex, core and shell subregions of the nucleus accumbens (NAc)/VS, basal nucleus of the amygdala (BA), lateral portion of the central nucleus of the amygdala, and medial portion of the central nucleus of the amygdala (CeM). Positive c-fos-like immunoreactivity showed brown-black staining distinct from the background. The c-fos positive cells were automatically counted and averaged for each hemisphere at three or four different rostrocaudal levels of each structure (MetaMorph software 6.1; MetaMorph, Inc., Nashville, TN). The density of c-fos–positive neurons was calculated by dividing the number of c-fos–positive neurons by the total area of each region.

Statistical Analyses

Student *t* tests and analyses of variance (ANOVAs) were performed to determine statistical differences in behavioral and immunohistochemistry experiments. Tukey post hoc analyses were used for multiple comparisons. Data are presented as mean ± SEM and statistical significance was established as $p \leq .05$. All statistical analyses were performed using the Statistica software (version 6.0; Statsoft, Tulsa, OK).

RESULTS

High-Frequency DBS of the Dorsal-VS Impairs Extinction of Morphine Place Preference

Rats were first conditioned to exhibit place preference for morphine over 8 days. Morphine was injected and rats were placed in their nonpreferred side, similar to previous studies (22,23). Next, rats were given 9 days of extinction, during which high-frequency DBS was delivered to the VS for 60 minutes (Figure 1B). DBS was delivered either to the dorsal ($n = 11$) or ventral ($n = 9$) VS and compared with sham-stimulated control rats ($n = 23$). Because control rats that were implanted with electrodes into the dorsal ($n = 12$) or ventral ($n = 11$) regions of the VS showed no statistical differences across the 9 days of extinction ($F_{1,21} = 0.33$; $p = .56$), they were combined into a single group. Control rats extinguished to chance levels of preference (50%) by day 9 (49.8 ± 6.1) compared with day 1 (71.80 ± 3.36; $F_{2,40} = 5.31$; $p = .008$; Tukey post hoc; $p = .001$). Rats exposed to high-frequency DBS of the dorsal-VS maintained a preference to the morphine-paired side throughout the 9 days of extinction training, as revealed by a significant main effect of treatment (Figure 1C, orange line; repeated-measures ANOVA; $F_{2,40} = 5.31$; $p = .008$). The impairment of extinction in the dorsal-VS group persisted for 2 days following the cessation of DBS (main effect of treatment; $F_{2,40} = 5.24$; $p = .009$; Tukey post hoc, day 1: $p = .02$ for dorsal-VS vs. sham; day 2: $p = .0002$ for dorsal-VS vs. sham).

High-frequency DBS of the ventral-VS had no effect on the extinction of morphine-CPP, either during the 9 days of training (with DBS on) or in the 2 days afterward (with DBS off; Figure 1C, green line; all $ps > .05$). High-frequency DBS of either the dorsal or the ventral sites did not affect locomotion, as measured by the number of transitions between compartments (Figure 1D; one-way ANOVA; DBS on: $F_{2,40} = 1.59$; $p = .21$; DBS off: $F_{2,40} = 0.28$; $p = .75$).

One possible explanation for the apparent impairment in extinction is that DBS of dorsal-VS may itself be rewarding, causing rats to remain on the drug side in the presence of DBS. Arguing against this, however, a separate group of rats showed that high-frequency DBS of dorsal-VS was not sufficient to induce a CPP (% time on DBS side during test day – sham: 47.71 ± 5.78; DBS: 44.76 ± 4.36; $p = .68$). Considering that DBS was continuously applied between the home cage and the CPP chambers during extinction training, it is possible that DBS in the home cage alone would be sufficient to induce extinction impairment. Although we have not tested the effects of high-frequency DBS alone on the extinction of morphine-CPP, a previous study found that high-frequency DBS of the dorsal-VS in the home cage had no

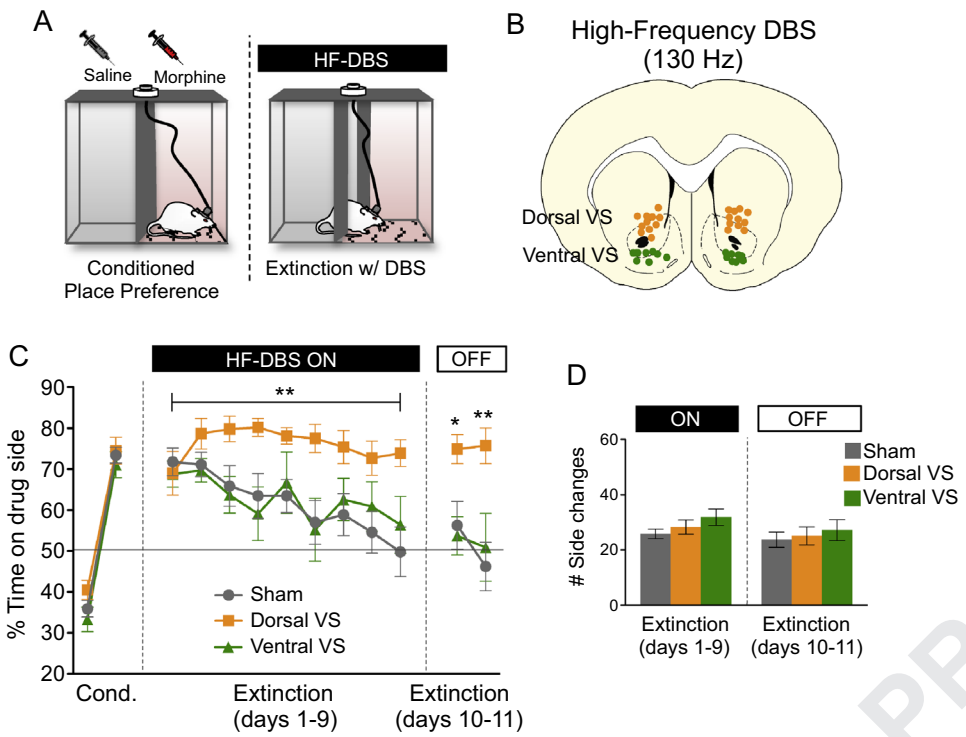

Figure 1. High-frequency deep brain stimulation (HF-DBS) of dorsal–ventral striatum (VS) impairs extinction of morphine conditioned place preference (CPP). **(A)** Diagram showing the CPP-DBS protocol. **(B)** Representative coronal section of DBS electrode placement in dorsal-VS and ventral-VS (dorsal and ventral to the anterior commissure). **(C)** HF-DBS of the dorsal-VS impaired the extinction of morphine-induced CPP, whereas HF-DBS of the ventral-VS had no effect. **(D)** There was no effect of DBS on exploratory behavior/locomotion (side changes/transitions) during the CPP-DBS protocol. Sham: $n = 23$; dorsal-VS: $n = 11$; ventral-VS: $n = 9$. Data shown as mean ± SEM. $^{*}p < .05$; $^{**}p < .01$.

effect on the expression or the extinction of conditioned fear (33). Nonetheless, further studies are needed to investigate the effects of DBS alone on the extinction of drug-seeking behavior.

Low-Frequency DBS of VS Strengthens Memory for Extinction of Morphine Place Preference

Previous studies have demonstrated that low-frequency DBS can induce effects opposite to those seen with high-frequency DBS in the motor system (11) and in addiction (28,42). Given that high-frequency DBS showed extinction impairment in morphine-CPP, we then hypothesized that low-frequency DBS of this same region would have an opposite effect (extinction facilitation). Because high-frequency DBS of the ventral portion of the VS had no effect on extinction, rather than investigating the effects of low-frequency DBS in this region, we decided to focus on the dorsal-VS (Figure 2A), the site where extinction was impaired by high-frequency DBS (see Figure 1). Following CPP training, rats were given partial extinction training of 6 days together with low-frequency DBS of the dorsal-VS ($n = 14$) or sham stimulation ($n = 15$) (Figure 2B). A partial extinction protocol was used to avoid floor effects caused by complete extinction. Repeated measures ANOVA showed that low-frequency DBS of the dorsal-VS had no observable effects across the 6 days of extinction training (Figure 1C; blue line; $F_{1,27} = 0.973$; $p = .33$). Two days later, however, in the absence of DBS, extinction memory was strengthened as indicated by low levels of side preference (repeated-measures ANOVA; $F_{1,27} = 5.013$; $p = .033$; Tukey post hoc; day 1: $p = .003$ dorsal-VS vs. sham and day 2: $p = .009$ dorsal-VS vs. sham). Enhanced extinction was still observed 1 week later (extinction day 15; unpaired t test; $t_{12} = 2.27$; $p = .041$; DBS: $n = 7$; sham: $n = 7$), suggesting that the DBS-induced enhancement in extinction was long lasting. Low-frequency DBS of the dorsal-VS did not affect transitions between CPP compartments (Figure 2D; unpaired t tests; DBS on: $t_{27} = 1.49$; $p = .145$; DBS off: $t_{27} = 0.12$; $p = .904$), ruling out locomotion effects.

DBS of VS Modulates Activity in Areas Mediating Extinction of Drug-Seeking Behavior

We observed frequency dependent effects of DBS capable of either impairing or enhancing extinction memory. We therefore assessed the effects of both high- and low-frequency DBS on the activity of reward circuits, as indicated by expression of the immediate early gene c-fos (43–45). To assess the effects of DBS independently from any effects of morphine or CPP training, untrained rats were given high-frequency ($n = 4$), low-frequency ($n = 5$), or sham stimulation ($n = 6$) in the dorsal-VS for 6 days, 60 minutes per day (Figure 3A). Rats were sacrificed 60 minutes following the last stimulation session.

Compared to the sham group, DBS altered c-fos expression in the IL cortex ($F_{2,9} = 6.42$; $p = .018$) (Figure 3B), BA ($F_{2,10} = 8.53$; $p = .006$) (Figure 3D), and CeM ($F_{2,10} = 9.73$; $p = .004$) (Figure 3D). Tukey post hoc analyses showed that high-frequency DBS increased c-fos expression only in IL ($p = .032$), whereas low-frequency DBS increased c-fos expression in IL ($p = .030$), BA ($p = .015$), and CeM ($p = .009$). Neither high- nor low-frequency stimulation altered activity in the NAc (core: $F_{2,10} = .31$; $p = .734$; shell: $F_{2,11} = .18$; $p = .836$) (Figure 3C). Similarly, no changes in c-fos expression were observed in the prelimbic cortex

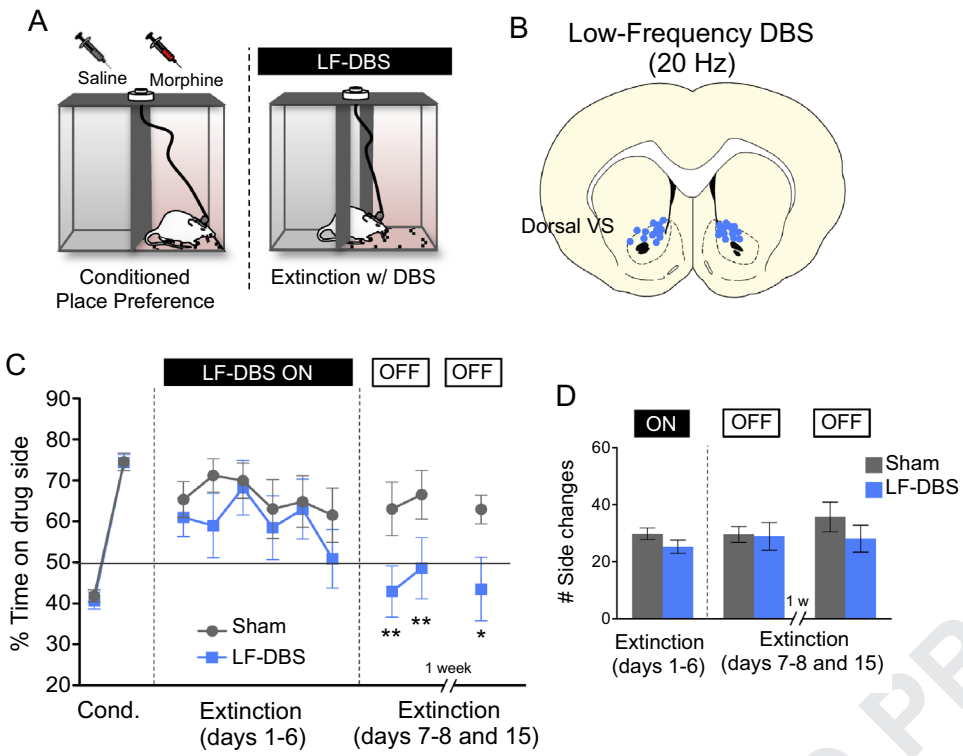

Figure 2. Low-frequency deep brain stimulation (LF-DBS) of the dorsal–ventral striatum (VS) strengthens memory for extinction of morphine-induced conditioned place preference (CPP). **(A)** Diagram showing the CPP-DBS protocol. **(B)** Representative coronal section of DBS electrode placement in the dorsal-VS (dorsal to the anterior commissure). **(C)** LF-DBS of the dorsal-VS reduced the time spent in the drug-paired side during the extinction test (DBS off phase). This effect persisted 9 days after stimulation. **(D)** There was no effect of DBS on exploratory behavior/locomotion (side changes/transitions) during the CPP-DBS protocol. Sham: $n = 15$; DBS: $n = 14$. For 1-week test: sham: $n = 7$; DBS: $n = 7$. Data shown as mean ± SEM. $^{*}p < .05$; $^{**}p < .01$.

($F_{2,9} = 1.45$; $p = .283$) (Figure 3B) or in central nucleus of the amygdala ($F_{2,10} = .34$; $p = .713$) (Figure 3D). Thus, while both high- and low-frequency DBS activated IL, only low-frequency DBS activated the amygdala (BA and CeM).

DISCUSSION

Using a rodent model of DBS treatment of addictive disorders, we demonstrated that facilitation of extinction of morphine-CPP is specific to both the targeted striatal subregion and the frequency of DBS. We observed an impairment in extinction with high-frequency DBS of the dorsal-VS, whereas no effect was observed in the ventral-VS. Conversely, low-frequency DBS of the dorsal-VS strengthened extinction memory. In the dorsal-VS, low-frequency, but not high-frequency, DBS increased c-fos expression in the amygdala (BA and CeM), an area commonly associated with the expression and extinction of drug-seeking behaviors (10).

Previous studies in rats have investigated the effects of DBS on drug-seeking behaviors (4,5). However, only two studies have tested the effects of DBS on extinction of drug seeking. Levy *et al.* (26) reported that either high- or low-frequency DBS of the prelimbic cortex, a region associated with drug seeking, facilitated the extinction of cocaine self-administration. Friedman *et al.* (42) demonstrated that a combined pattern of high-/low-frequency DBS stimulation of the lateral habenula facilitated the extinction of cocaine self-administration. In our study, we demonstrated that high-frequency and low-frequency DBS of the dorsal-VS have opposite effects on the extinction of drug-associated memories. Differences between our study and the previous studies may be due to differences in the region of stimulation (e.g., prefrontal cortex vs. VS), the drug studied (e.g., cocaine vs. morphine), and/or the behavioral paradigms used (e.g., self-administration vs. CPP). Nevertheless, our findings agree with previous work showing that DBS effects on extinction depend on both frequency and placement. Regarding opioids, high-frequency DBS of the VS during either morphine conditioning (23) or abstinence of morphine or heroin (21,22) reduced drug-seeking behaviors. Thus, potential differences between these findings and ours may be attributable to the recruitment of important brain regions for the extinction of morphine-seeking behaviors (9,10).

DBS can modulate local and/or distant sites. Locally, high-frequency DBS has been shown to induce depolarization blockade due to activation of local interneurons (46,47), as well as synaptic depression associated with neurotransmitter depletion and inhibition (47). Consistent with this, previous studies have reported that, similar to electrolytic lesions (48), high-frequency DBS of the NAc core reduces the acquisition of morphine place preference (23). High-frequency DBS has also been shown to induce antidromic spikes that activate inhibitory interneurons in cortical areas (38). Because IL projects densely to the dorsal-VS (49,50), impaired extinction with high-frequency DBS could be attributed to distal activation of inhibitory interneurons in IL (5,38), which is a critical region for the expression of extinction of morphine place preference (10). Consistent with this idea, high-frequency DBS of the NAc shell attenuated cocaine reinstatement, an effect that was also observed after pharmacologic inactivation of IL (20). Furthermore, pharmacologic inactivation of IL impairs extinction of cocaine seeking (51), and extinction of

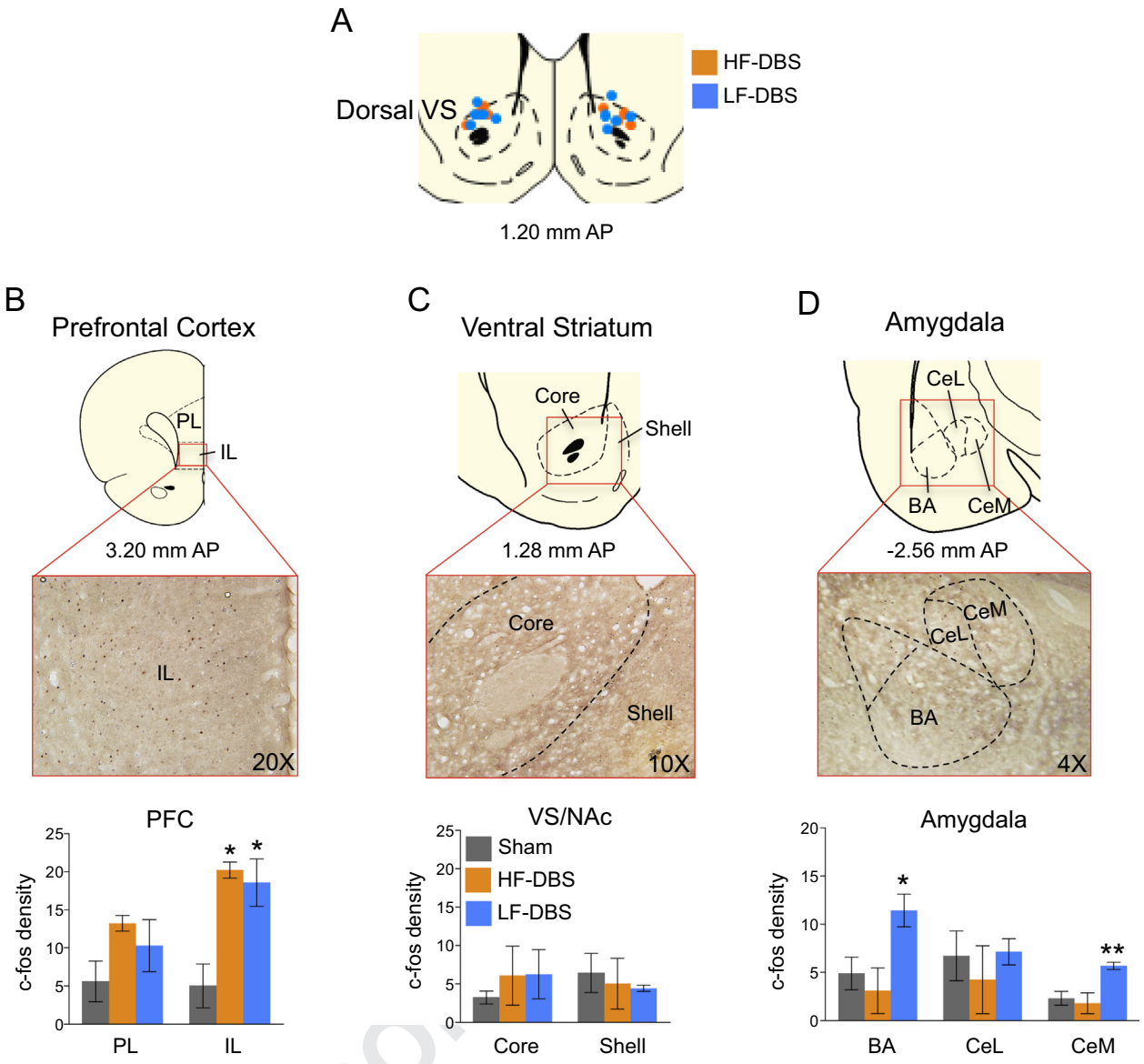

Figure 3. The effects of high-frequency (HF) and low-frequency (LF) deep brain stimulation (DBS) on c-fos expression in prefrontal, striatal, and amygdala regions. **(A)** Diagram showing a representative coronal section of DBS electrode placements in the dorsal–ventral striatum (VS), for both HF and LF groups. **(B, C)** Both HF- and LF-DBS increased c-fos immunoreactivity in infralimbic cortex (IL), whereas neither affected nucleus accumbens (NAc) subregions. Magnification = 20× **(B)** and 10× **(C).** (**D**) Only LF-DBS increased c-fos labeling in the amygdala (basal nucleus [BA] and medial portion of the central nucleus [CeM]). Magnification = 4×. Sham: $n = 6$; HF-DBS: $n = 4$; LF-DBS: $n = 5$. Data represented as mean ± SEM. $^{*}p < .05$; $^{**}p < .01$. AP, anteroposterior; CeL, lateral portion of the central nucleus of the amygdala; core, nucleus accumbens core; shell, nucleus accumbens shell; PFC, prefrontal cortex; PL, prelimbic cortex.

morphine-CPP is enhanced by intra-IL activation of protein kinase C zeta (52) and Narp (53), two proteins involved in long-term potentiation via upregulation of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors.

In contrast to high-frequency DBS, low-frequency DBS (20–60 Hz) has been associated with distal activation rather than distal inhibition (54). In the thalamus, low-frequency DBS activates inputs (terminals) from cortical pyramidal neurons (54). Thus, low-frequency DBS of the dorsal-VS might be activating IL pyramidal neurons, rather than activating IL inhibitory interneurons. Using a model of cocaine sensitization, it was recently shown that low-frequency DBS of the VS/NAc, combined with antagonists of D_1 dopamine receptors in the same brain region, produced long-lasting abolishment of behavioral sensitization (28). Although we did not observe changes in c-fos expression in the VS/NAc, our data showed that low-frequency DBS increased c-fos expression in the amygdala and IL, both regions that project to VS (49,55–57) and are critical for the acquisition and expression of drug-extinction memories (7,9,10,58). Although activity in IL is

necessary for the expression of extinction of drug-associated memories (7,51,52), plasticity in the basolateral amygdala via activation of p-Erk cascade (59) or enhancement of glutamatergic transmission (9,10) has been correlated with the acquisition of drug extinction.

We also observed that low-frequency DBS increased c-fos expression in CeM, suggesting that the central amygdala might play a role in the facilitation of extinction memory. Overexpression of GluA1 (Gria1) subunits of alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors in central amygdala facilitated the extinction of morphine place preference, whereas downregulation of GluA1 subunits in this same region had the opposite effect (60). In addition, drug-seeking behaviors can be attenuated by intra–central amygdala infusion of agonists of metabotropic glutamate receptors (61), D_2/D_3 dopaminergic receptors (62), or 5-HT-2C serotonergic receptors (63). Thus, the cooperative integration of activity in IL, BA, and CeM could underlie the strengthening of extinction memory observed after the cessation of low-frequency DBS. Nevertheless, because c-fos findings were obtained from naïve rats, it is possible that additional circuits are recruited in drug-exposed animals receiving extinction training.

In summary, our results demonstrate that stimulation of the dorsal-VS at the frequencies most often used for clinical DBS (high frequency) impaired morphine extinction, whereas low-frequency DBS of the same area strengthened extinction memory. Thus, whereas high-frequency DBS of the VS appears to disrupt the extinction circuitry perhaps by local interruption of extinction afferents and feed-forward inhibition of IL, low-frequency DBS might be recruiting brain regions (i.e., amygdala and IL) necessary for extinction behaviors through the stimulation of afferent terminals.

However, further studies are needed to determine the effects of low-frequency DBS in the ventral portion of the VS, as well as to determine whether low-frequency DBS could reverse the extinction impairment induced by high-frequency DBS.

Because addiction is a chronic relapsing brain disorder (6), DBS could be a promising alternative for treatment-resistant patients who do not respond to conventional therapies. Given that rodent models of drug extinction resemble exposure-based therapies in humans (30), low-frequency DBS of dorsal-VS may represent a potential adjunct for extinction-based therapies in opioid addiction. Accordingly, Kuhn *et al.* (64) reported that DBS intervention combined with pharmacologic treatment (levomethadone) reduced the consumption of both the drug of abuse (heroin) and the prescribed drug (levomethadone). Thus, the incorporation of DBS as a potential therapy for substance-related disorders might represent a valuable tool to enhance the response to standard procedures.

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ARTICLE INFORMATION

From the Department of Anatomy and Neurobiology (FJM-R, MEL-T, GJQ, JLB-E); Department of Psychiatry (JR-R, FHDM, GJQ), School of Medicine, Medical Sciences Campus, University of Puerto Rico, San Juan, Puerto Rico.

Address corresponding to Jennifer L. Barreto-Estrada, Ph.D., University of Puerto Rico, School of Medicine, Medical Science Campus, Department of Anatomy and Neurobiology, P.O. Box 365067, San Juan, Puerto Rico 00936-5067; E-mail: jennifer.barreto1@upr.edu.

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