

Enhancement of Fear Extinction with Deep Brain Stimulation: Evidence for Medial Orbitofrontal Involvement

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Deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS) reduces anxiety, fear, and compulsive symptoms in patients suffering from refractory obsessive-compulsive disorder. In a rodent model, DBS-like high-frequency stimulation of VS can either enhance or impair extinction of conditioned fear, depending on the location of electrodes within VS (dorsal vs ventral). As striatal DBS activates fibers descending from the cortex, we reasoned that the differing effects on extinction may reflect differences in cortical sources of fibers passing through dorsal-VS and ventral-VS. In agreement with prior anatomical studies, we found that infralimbic (IL) and anterior insular (AI) cortices project densely through ventral-VS, the site where DBS impaired extinction. Contrary to IL and AI, we found that medial orbitofrontal cortex (mOFC) projects densely through dorsal-VS, the site where DBS enhanced extinction. Furthermore, pharmacological inactivation of mOFC reduced conditioned fear and DBS of dorsal-VS-induced plasticity (pERK) in mOFC neurons. Our results support the idea that VS DBS modulates fear extinction by stimulating specific fibers descending from mOFC and prefrontal cortices.

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INTRODUCTION

Deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS) reduces fear and anxiety symptoms in patients suffering from refractory obsessive compulsive disorder (OCD; Denys *et al*, 2010; Greenberg *et al*, 2010a), but little is known about the mechanisms. Previous studies have proposed that the primary effects of DBS are on cortical fiber bundles passing through the striatum (see Greenberg *et al*, 2010b for review). Furthermore, OCD patients show deficient extinction of conditioned fear, which depends on specific areas of the prefrontal cortex (Milad *et al*, 2013). Using a rat model, we previously found that high-frequency DBS-like electrical stimulation of the dorsal-VS (just above the anterior commissure) enhanced fear extinction, whereas stimulation of the ventral-VS impaired fear extinction (Rodriguez-Romaguera *et al*, 2012). Interestingly, these striatal sub-regions did not follow core/shell compartmentalization. Furthermore, DBS effects were not observed with pharmacological inactivation of dorsal-VS or ventral-VS (Rodriguez-Romaguera *et al*,

2012), suggesting that DBS modulates different sets of cortical fibers passing through these VS areas. We therefore sought to determine the cortical sources of fibers passing through the dorsal-VS and ventral-VS sub-regions.

The infralimbic(IL) cortex and prelimbic (PL) cortex project through VS (Berendse *et al*, 1992; Mailly *et al*, 2013; McGeorge and Faull, 1989; Sesack *et al*, 1989; St Onge *et al*, 2012; Vertes, 2004) and could potentially mediate DBS effects on extinction. It is well established that inhibition of IL impairs fear extinction (Burgos-Robles *et al*, 2007; Laurent and Westbrook, 2009; Quirk *et al*, 2000; Sierra-Mercado *et al*, 2011), whereas stimulation of IL enhances extinction (Kim *et al*, 2010; Maroun *et al*, 2012; Milad and Quirk, 2002; Milad *et al*, 2004). In contrast, inhibition of PL reduces fear expression (Corcoran and Quirk, 2007; Laurent and Westbrook, 2009; Sierra-Mercado *et al*, 2011), whereas PL stimulation increases fear expression and impairs extinction (Vidal-Gonzalez *et al*, 2006).

Other areas projecting through the VS include the orbitofrontal cortex (OFC) and the anterior insular (AI) cortex (Gabbott *et al*, 2005; Hoover and Vertes, 2007, 2011; Mailly *et al*, 2013; McGeorge and Faull, 1989; Schilman *et al*, 2008), but their role in fear extinction has not been studied. Furthermore, the projection patterns of these areas through dorsal-VS and ventral-VS are not clearly defined. We therefore injected the retrograde tracer wheat germ agglutinin (WGA) into the dorsal-VS or ventral-VS, to assess retrograde labeling in the cortex. Cortical areas

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projecting predominantly through the dorsal-VS site (in which DBS enhanced fear extinction) were further studied using pharmacological inactivation and immunocytochemistry.

MATERIALS AND METHODS

Subjects

Fifty five male Sprague-Dawley rats (~325 g; Harlan Laboratories) were housed and handled as previously described (Quirk *et al*, 2000). Rats used in the fear conditioning experiment were fed standard rat chow in a restricted manner (18 g/day), to facilitate pressing a bar for food on a variable interval schedule of reinforcement (VI-60) and to maintain a constant behavioral baseline against which freezing could be reliably measured. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Puerto Rico—School of Medicine in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

Surgery and Drug Infusion

Rats were initially anesthetized with isoflurane inhalant gas (5%) in an induction chamber and positioned in a stereotaxic frame. Isoflurane (2%–3%) was delivered through a face mask for anesthesia maintenance. For our neuroanatomical study, unilateral infusions of WGA were aimed at the dorsal-VS (–6.5 mm DV, ±2.0 mm ML, and +1.2 mm AP) or ventral-VS (–8.0 mm DV, ±2.0 mm ML, and +1.2 mm AP; Paxinos and Watson, 1998). A small volume of 20 nl was used to restrict the tracer from spreading into adjacent sites. Using a 0.5- μ l Hamilton syringe, we infused WGA at a rate of 1 nl/min during a 20-min period. After infusion, the syringe was left in place for an additional 20 min to allow for diffusion and avoid backflow through the syringe track. The incision was then sutured using wax-coated braided silk. After surgery, a topical triple antibiotic was applied around the surgery wound and an analgesic (Ketoprofen, 5 mg/kg) was injected intramuscularly. Animals were killed 7 days after infusions, to allow for adequate tracer transport.

For our inactivation experiment, fluorescent muscimol (MUS, 0.2 μ l, 0.11 nmol; BODIPY TMR-X Conjugate; Sigma-Aldrich, St Louis, MO) was infused to enhance GABA-A receptor activity, thereby temporally inactivating the region. A similar surgical procedure as above was used, except that rats were chronically implanted with 26-gauge bilateral guide cannulas (Plastics One, Roanoke, VA) between medial OFC (MO) and the rostral part of the ventrolateral OFC (rVLO): +4.68 mm AP, ±0.60 mm ML, +4.00 mm DV; Paxinos and Watson, 1998), in order to target the area that resembles the projection to dorsal-VS (Figure 2). Acrylic cement was used to affix cannulas to the skull. Stainless steel obturators (33 gauge) were inserted into the guide cannulas to avoid obstructions until infusions were made. Injector tips extended 1.0 mm beyond the guide cannula. Rats were allowed 7 days to recover from surgery before behavioral testing. On the day before the behavioral experiment, obturators were removed, injectors were

inserted into the cannulas without infusion, and rats were acclimated for handling. On the day of infusion, 0.2 μ l of MUS or saline (SAL, vehicle) was infused at a rate of 0.2 μ l/min. After infusion, injectors were left in place for 2 min, to allow the drug to diffuse. After behavioral experiments, rats were perfused and placements were verified for correct placement as previously described (Rodríguez-Romaguera *et al*, 2012). Nine rats with placements and MUS spread outside of mOFC were eliminated.

Immunocytochemistry

For our neuroanatomical study, sections (50 μ m thickness, 200 μ m apart) were initially treated with 10% methanol and 3% H₂O₂ in 0.1 M PB, to inhibit endogenous peroxidase, and rinsed extensively in PBS with Triton X-100 (PB-TX; Sigma, St Louis, MO). Preincubation occurred in PB-TX with 10% normal goat serum (NGS) for 30 min and incubation in primary anti-WGA (1:50 000; Sigma-Aldrich) in PBS-TX with 10% NGS for four nights at 4 °C. The tissue was then treated with biotinylated secondary antibody (1:200; Vector Laboratories, Burlingame, CA) and incubated with avidin–biotin complex solution (Vectastain ABC kit; Vector Laboratories). Standard DAB procedures were used to react the tissue and visualization was optimized by using cobalt chloride and nickel ammonium sulfate. Sections were mounted onto gel-coated slides, dehydrated, defatted in xylene, and coverslipped with mounting medium (Permount, Fisher Scientific, Fair Lawn, NJ). To visualize the cytoarchitectonic subregions of cortical area, adjacent sections were reacted with neuronal marker NeuN. Sections were incubated with primary anti-NeuN antibody (1:1000; Chemicon, Rosemont, IL) overnight at room temperature and treated with the same procedures described above.

For our pERK immunocytochemistry experiment, alternate sections were initially blocked in a solution of 2% NGS (Vector Laboratories, USA) plus 0.3% triton (Triton X-100, Sigma-Aldrich) in 0.12 M potassium buffer SAL for 1 h, as previously described (Rodríguez-Romaguera *et al*, 2012). The sections were incubated overnight at room temperature with anti-pERK serum raised in rabbit (1:100; p-44/42MAPK, 4370L; Invitrogen, Carlsbad, CA) and treated with the same procedures described above.

For our neuroanatomical study, the tissue from a total of 24 WGA-injected animals was examined. Striatal injections with either contamination or weak labeling were eliminated from the analysis. Contamination refers to injections in which the tracer was not limited to the dorsal-VS or ventral-VS regions, but had leaked into the adjacent striatal regions. Weak labeling refers to poor uptake of the tracer as indicated by poor labeling in the midbrain. A total of four animals (two in dorsal-VS and two in ventral-VS) had injection sites that were confined to the dorsal or ventral region with adequate uptake. The cortex was divided into specific regions based on the rat atlas (Paxinos and Watson, 1998) and labeled cells were plotted with stereological analysis (see below). Cortical areas were defined according to cytoarchitectonic boundaries in adjacent sections, as indicated by NeuN labeling (Paxinos and Watson, 1998). We analyzed the number of labeled cells in the medial (mPFC), OFC, and AI prefrontal cortices. The mPFC was subdivided into cingulate (Cg1), PL, and IL cortices, the

OFC into MO and VLO OFCs, and the AI into ventral (AIv) and dorsal (AId) AI cortices.

The number of cells labeled with WGA was quantified by using an unbiased stereology method (Stereoinvestigator Software, Micro Bright Field, Williston, VT), with a modified Nikon microscope (Nikon E800) under dark field illumination. Contours were drawn for each cortical area leaving a small transitional space ($\sim 200 \mu\text{m}$) between areas. A fractionator probe was used to estimate the number of labeled cells with systematic random sampling. Quantification of cells was performed at $\times 20$ magnification in $100 \mu\text{m}^2$ counting frames, which constituted 64% of the reference space. We used the PL cortex to confirm that stereology estimates were accurate by comparing with manual counts of the entire structure. For each cortical region, the fraction of cells (% of total cells counted) was used to analyze the distribution of cortical cell labeling. The fraction of cells between each injection site was compared for dorsal-VS and ventral-VS regions.

For our pERK immunocytochemistry experiment, images were generated for mOFC and the number of pERK immunoreactive neurons in each brain hemisphere was quantified as previously described (Rodriguez-Romaguera *et al*, 2012). The density of pERK-positive neurons was calculated by dividing the number of positive neurons by the total area counted.

Behavior

Rats underwent bar-press training, auditory fear conditioning, and extinction in standard operant chambers (Coulbourn Instruments, Whitehall, PA) inside sound-attenuating boxes (Med Associates, St Albans, VT). Further details regarding the apparatus have been previously described (Quirk *et al*, 2000). On day 1, rats were presented with five non-reinforced tones (4 kHz, 75 dB, 30 s; habituation) followed by seven tones that co-terminated with footshocks (0.5 s, 0.56 mA; conditioning). On day 2, rats were infused with either SAL or MUS 30 min before being presented with 15 tones in the absence of footshocks (extinction). On day 3, rats were presented with eight tones in the absence of footshocks (extinction test). The first trial of habituation, the last trial of conditioning, and the last trial of extinction were eliminated from analysis to make two trial blocks (according to Rodriguez-Romaguera *et al*, 2012). During all phases of the experiment, the intertone interval was variable (~ 3 min) and food was available on a variable interval schedule (VI-60).

Deep Brain Stimulation

DBS was delivered through concentric bipolar electrodes (NEX-100; Rhodes Medical Instruments, Tujunga, CA) with contacts measuring 0.5 mm in length and separated by 0.5 mm as previously described (Rodriguez-Romaguera *et al*, 2012). Stimulation was monophasic, with the deeper contact as negative. We used DBS parameters similar to those used in humans (100 μA , 0.1-ms pulse duration, 130 Hz), which were also used in previous rat models studying DBS-like stimulation (Do-Monte *et al*, 2013; McCracken and Grace, 2007; Rodriguez-Romaguera *et al*, 2012). DBS was generated with an S88X stimulator (Grass

Instruments, Warwick, RI) and a constant-current unit (SIC-C Isolation Unit; Grass Instruments).

Data Collection and Analysis

Behavior was recorded with digital video cameras (Micro Video Products; Bobcaygeon, Ontario, Canada) and freezing was quantified from digitized video images using commercially available software (Freezescan; Clever Systems, Reston, VA). The amount of time rats spent freezing to the tone was expressed as a percentage of the tone presentation. Statistical significance was determined with Student's two-tailed *t* tests, one-way ANOVA, or repeated-measures ANOVA, followed by Tukey *post-hoc* analysis, when appropriate (STATISTICA; Statsoft, Tulsa, OK).

RESULTS

Figure 1a shows findings from our previous study (Rodriguez-Romaguera *et al*, 2012), in which DBS of ventral-VS impaired fear extinction, and DBS of dorsal-VS enhanced fear extinction. To identify the cortical areas projecting through these different VS subregions, we injected the retrograde tracer WGA into either dorsal-VS or ventral-VS, and used stereological techniques to count the number of retrogradely labeled cells in different cortical areas (Figure 1b and c).

Ventral-VS Receives Projections from PL, IL, and AI

Figure 2a and b show drawings of retrogradely labeled neurons resulting from WGA injections into dorsal-VS or ventral-VS. Group data revealed that there were no significant differences between the two striatal sites in the number of labeled cells in PL (dorsal-VS: 46%, ventral-VS: 45%, $t_{(3)} = 0.59$, $p = 0.62$; Figure 2c), Cg1 (dorsal-VS: 4%, ventral-VS: 3%, $t_{(3)} = 0.96$, $p = 0.44$), or caudal VLO (cVLO; dorsal-VS: 15%, ventral-VS: 16%, $t_{(3)} = -0.02$, $p = 0.98$). Regarding PL, the ventral-VS projections originated largely from middle and deep layers, but not superficial layers. In contrast to PL, Cg1, and cVLO, there were significant differences between striatal sites in the number of labeled cells in the IL and AI (AId + AIv) cortices. Specifically, ventral-VS injections yielded significantly more IL and AI labeling than dorsal-VS injections (IL = dorsal-VS: 3%, ventral-VS: 24%, $t_{(3)} = -4.90$, $p = 0.04$; AI = dorsal-VS: 4%, ventral-VS: 17%, $t_{(3)} = -5.66$, $p = 0.03$; Figure 2b and c). This suggests that modulation of IL and AI may contribute to the impairment of extinction by DBS of ventral-VS, perhaps through DBS-induced inhibition of IL, as previously proposed for lateral OFC (McCracken and Grace, 2007).

Dorsal-VS Receives Projections from PL and mOFC

In contrast to ventral-VS, projections from PL to dorsal-VS originated largely from middle and superficial layers, but not deep layers. Furthermore, dorsal-VS tracer yielded significantly more retrograde labeling in the mOFC (MO + rVLO), compared with ventral-VS (mOFC; dorsal-VS: 43%, ventral-VS: 11%, $t_{(3)} = 10.99$, $p = 0.008$; Figure 2a and c). Thus, DBS enhancement of extinction observed with the

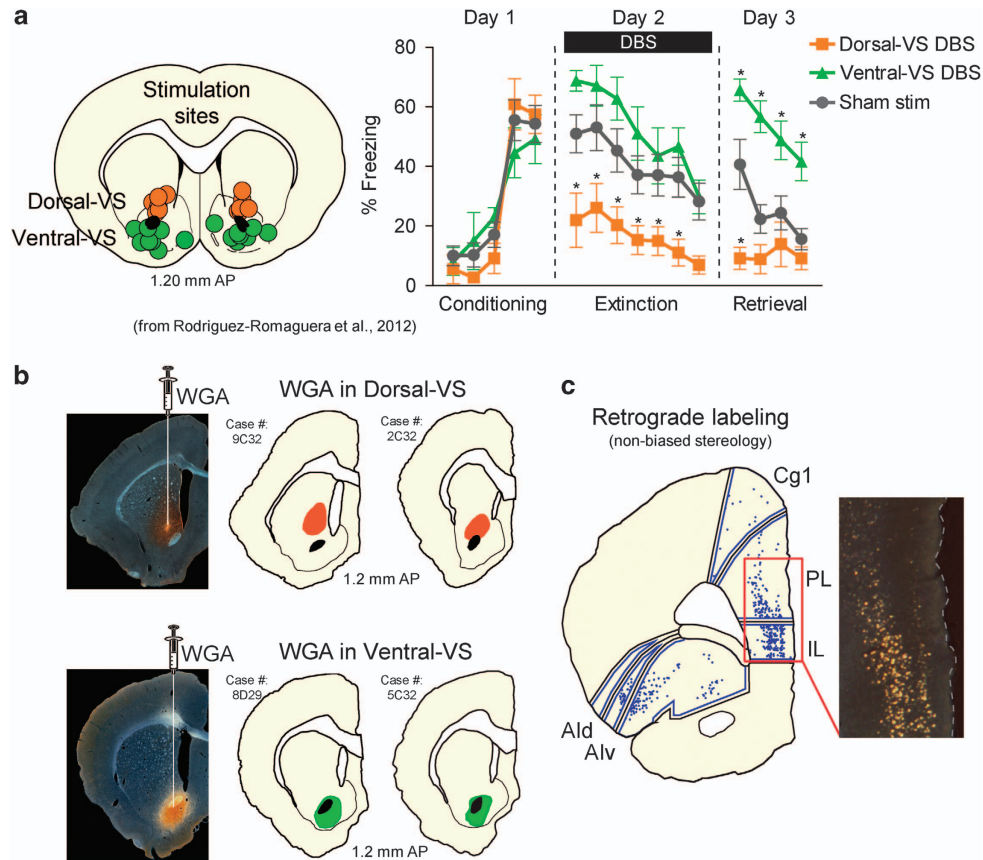


Figure 1 Stereological analysis of cortical neurons projecting through dorsal- and ventral-ventral striatum (VS). (a) Left: deep brain stimulation (DBS) electrode placements in VS. Circle diameter indicates the estimated spread of current from electrode tip. DBS of dorsal-VS was applied dorsal to anterior commissure (orange circles) and DBS of ventral-VS was applied ventral to anterior commissure (green circles). Right: freezing plots for Sham ($n = 16$), dorsal-VS DBS ($n = 6$), and ventral-VS DBS ($n = 8$) groups. Dorsal-VS DBS reduced freezing on day 2 and facilitated extinction recall on day 3. Ventral-VS DBS had the opposite effects. Data are shown in blocks of two trials as mean \pm SEM. * $p < 0.05$. Adapted from Figure 1b and c of Rodríguez-Romaguera *et al*, 2012. (b) Left: representative micrographs showing the spread of wheat germ agglutinin (WGA) into dorsal-VS (top) or ventral-VS (bottom). Right: Injection sites and spread of all cases used in Figure 2c. (c) Illustration of the cortical maps generated using non-biased stereology techniques. Sample obtained from a rat with a WGA injection into ventral-VS.

dorsal-VS site could be due to DBS-induced inhibition of mOFC (McCracken and Grace, 2007), if mOFC normally opposes extinction. Prior studies, however, have not examined the role of mOFC in fear expression or fear extinction.

Pharmacological Inactivation of mOFC Reduces Fear Expression

To determine the role of mOFC in fear expression and extinction, we infused the fluorescently labeled GABA-A agonist MUS into mOFC just before extinction training (on Day 2). We included only rats showing spread of MUS to MO and rVLO subregions of mOFC (Figure 3a). Inactivation of mOFC significantly reduced freezing from the start of the extinction session (Figure 3b). Repeated-measures ANOVA revealed a main effect of group ($F_{(1, 12)} = 12.46$, $p = 0.004$). *Post-hoc* comparisons confirmed that MUS-infused rats showed significantly less freezing than controls in trial blocks 1, 2, 3, 5 and 7 (all p 's < 0.05), suggesting that mOFC activity acts to augment fear expression. The following day, both SAL and MUS rats showed significantly less freezing than the previous day ($t_{(7)} = 2.47$, $p = 0.04$), demonstrating

extinction retrieval (Figure 3b). There was no difference, however, between MUS and SAL groups in extinction retrieval ($F_{(1, 12)} = 0.04$, $p = 0.84$), indicating that mOFC is not a site of extinction memory.

DBS of Dorsal-VS Induces Plasticity in mOFC

Our mOFC MUS finding is consistent with the idea that DBS-induced inhibition of mOFC could account for reduced freezing observed when DBS is on (see Figure 1a), but what accounts for reduced freezing the following day when DBS is off? One possibility is that DBS (but not extinction itself) induces plasticity in inhibitory circuits within mOFC (Gradinaru *et al*, 2009; McCracken and Grace, 2007), which could account for the long-lasting reduction in freezing. To investigate whether DBS alone induces plasticity in mOFC, we measured the expression of the neuronal plasticity marker pERK (Sweatt, 2004). Naive rats were given 3 h of DBS or sham stimulation in either dorsal-VS or ventral-VS (same as Figure 1a), and were killed immediately after. We observed that DBS of dorsal-VS (but not ventral-VS) triggered pERK labeling in mOFC neurons. One-way ANOVA revealed a significant effect of treatment in the

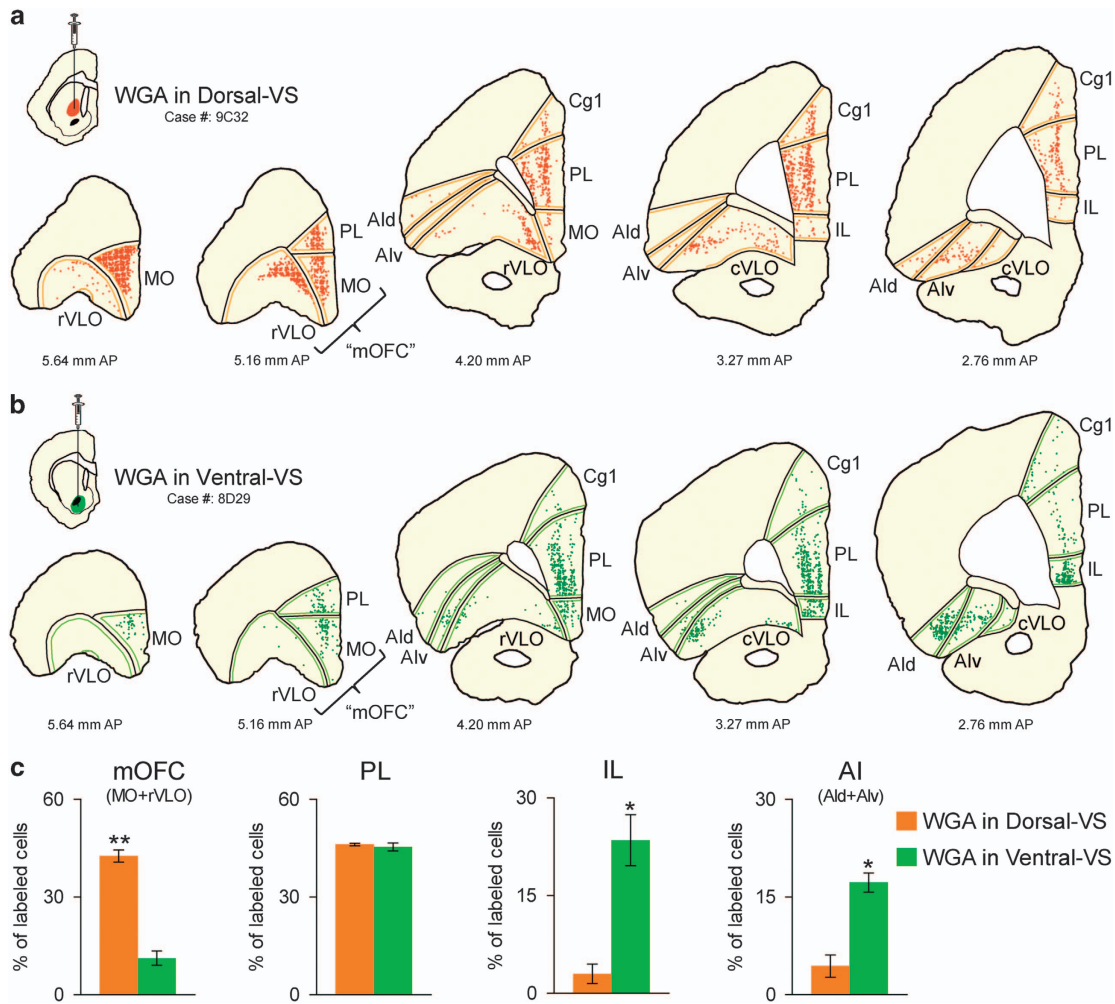


Figure 2 Medial orbitofrontal cortex (mOFC) projects through dorsal-ventral striatum (VS), whereas infralimbic (IL) and anterior insular (AI) cortices project through ventral-VS. (a) Stereological maps of cortical neurons that project through dorsal-VS (orange dots) at various anterior-posterior (AP) levels from bregma. Top left inset: micrographs showing the spread of wheat germ agglutinin (WGA) into dorsal-VS that generated the stereological maps. The boundary of the cortical area counted is defined using orange lines. (b) Stereological maps of cortical neurons that project through ventral-VS (green dots) at various AP levels from bregma. Top left inset: Micrographs showing the spread of WGA into ventral-VS that generated the stereological maps. The boundary of the cortical area counted is defined using green lines. (c) Comparison of fraction of cells (% of total cells counted) retrogradely labeled by WGA infusions into either dorsal-VS or ventral-VS ($n=4$). Data presented as mean \pm SEM. * $p < 0.05$, ** $p < 0.01$. Ald, dorsal portion of anterior insular; Alv, ventral portion of anterior insular; Cg1, cingulate area 1; cVLO, caudal portion of ventrolateral orbitofrontal; MO, medial orbitofrontal; PL, prelimbic; rVLO, rostral portion of ventrolateral orbitofrontal.

number of pERK-labeled neurons within mOFC ($F_{(2,31)} = 15.90$, $p < 0.001$; Figure 4). *Post-hoc* tests confirmed that DBS of dorsal-VS significantly increased the number of pERK-labeled neurons compared with sham-operated controls ($p < 0.001$). Further studies are needed to discriminate between plasticity in excitatory vs inhibitory networks within mOFC.

DISCUSSION

We investigated cortical projections through the dorsal and ventral subregions of ventral striatum, to better understand the effects of DBS on extinction of conditioned fear (Rodríguez-Romaguera *et al*, 2012). We found that dorsal and ventral subregions of VS receive different sets of inputs from mPFC and OFC. The dorsal-VS receives strong projections from mOFC, whereas the ventral-VS receives

strong projections from IL and AI. Thus, enhancement of fear extinction with DBS of dorsal-VS could be due to modulation of mOFC fibers, whereas impairment of fear extinction with DBS of ventral-VS could be due to modulation of IL/AI fibers.

Pharmacological inactivation of mOFC reduced freezing, demonstrating that mOFC is part of fear expression circuitry. mOFC could drive fear through its projections to the basolateral nucleus of the amygdala or PL (Hoover and Vertes, 2011). Therefore, one could speculate that DBS of dorsal-VS inhibits the mOFC. McCracken and Grace (2007) have shown that DBS-like stimulation of dorsal-VS reduces OFC firing rate, and suggest that this occurs via activation of recurrent collaterals projecting onto inhibitory interneurons in OFC. Thus, reduced freezing in the presence of DBS could be due to inhibition of mOFC. Projections from deep layers of PL through dorsal-VS may also be

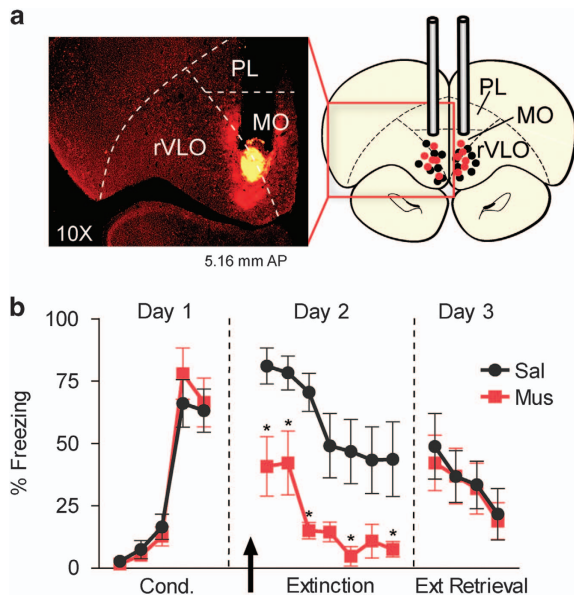


Figure 3 Inhibition of medial OFC (mOFC) neurons reduces fear expression. (a) Left: representative micrograph showing the spread of muscimol (MUS) in mOFC. Right: placement of cannula tips within the mOFC. (b) Freezing plots for saline (SAL, $n=8$) and MUS ($n=6$) groups. MUS reduced freezing on day 2 and did not alter extinction retrieval on day 3. Data are shown in blocks of two trials as mean \pm SEM. $*p < 0.05$.

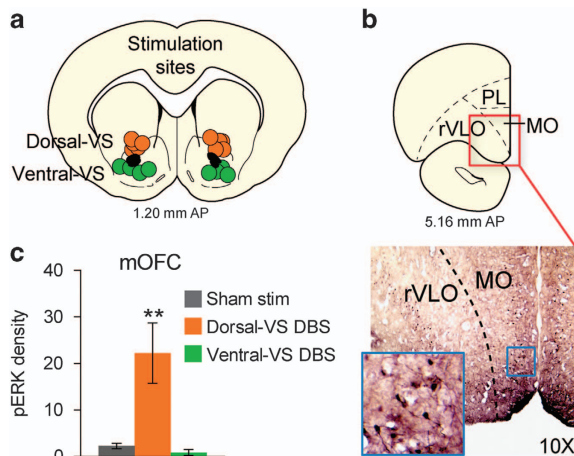


Figure 4 Deep brain stimulation (DBS) of dorsal-ventral striatum (VS) increases pERK expression in medial OFC (mOFC) neurons. (a) DBS electrode placements in ventral striatum. Circle diameter indicates the estimated spread of current from electrode tip. DBS of dorsal-VS was applied dorsal to the anterior commissure (orange circles) and DBS of ventral-VS was applied ventral to the anterior commissure (green circles). (b) Representative micrograph showing pERK labeling in mOFC after DBS of dorsal-VS. (c) Comparison of pERK density (counts/0.1 mm²) after rats underwent 3 h of Sham DBS ($n=9$), dorsal-VS DBS ($n=4$) or ventral-VS DBS ($n=4$). Data presented as mean \pm SEM. $**p < 0.01$.

involved. Deep layers of PL project to the lateral hypothalamus (Gabbott *et al*, 2005), which mediate conditioning-induced increases in autonomic responses (LeDoux *et al*, 1988). Another target of PL deep layers is the dorsal midline thalamus (Gabbott *et al*, 2005), an area shown to be

important for fear expression (Padilla-Coreano *et al*, 2012; Do-Monte *et al*, 2015). Thus, the beneficial effects of DBS in dorsal-VS (rather than ventral-VS) may rely on mOFC and PL convergence at this site, which has been observed in rats, monkeys, and humans (Draganski *et al*, 2006; Haber *et al*, 2006; Maily *et al*, 2013).

In addition to reducing freezing, DBS of dorsal-VS has also been shown to strengthen extinction memory (Rodríguez-Romaguera *et al*, 2012). Potentiation of inhibitory circuitry within OFC or PL by DBS may account for strengthening of extinction. Consistent with this, we observed expression of pERK in both mOFC (this study) and PL (Rodríguez-Romaguera *et al*, 2012) following DBS. As pharmacological inactivation of mOFC (this study) or PL (Sierra-Mercado *et al*, 2011) does not alter extinction memory, DBS-induced inhibition of these areas likely does not replace extinction. In support of this, administering DBS alone does not induce extinction (Rodríguez-Romaguera *et al*, 2012). Alternatively, DBS may strengthen extinction memory via indirect effects on IL, as suggested by DBS-induced increases in Fos, pERK, and BDNF levels in IL (Do-Monte *et al*, 2013; Rodríguez-Romaguera *et al*, 2012).

There is considerable debate over the homology of orbitofrontal areas in rodents and primates. Cross-species comparisons of connectivity suggest that rodent mOFC is homologous to the medial region of the OFC in humans (Brodman area 14) (Price, 2007; Wallis, 2012; Wise, 2008). Hyperactivity within the human mOFC is thought to drive the excessive compulsive behavior that is the hallmark of OCD (for reviews, see Baxter *et al*, 1996; Menzies *et al*, 2008; Milad and Rauch, 2012). DBS of the VC/VS increases BOLD signaling in mOFC of OCD patients (Rauch *et al*, 2006); however, whether this represents activation of excitatory or inhibitory networks is not known. Furthermore, a recent study showed that optogenetic activation of mOFC neurons induces OCD-like behaviors in mice (Ahmari *et al*, 2013). Therefore, DBS-induced inhibition of mOFC may explain the reduction in symptoms observed in refractory OCD patients receiving DBS (Denys *et al*, 2010; Greenberg *et al*, 2010a; Haber and Behrens, 2014). In support of this, it was recently shown that DBS of VC/VS reduces functional connectivity in cortico-striatal circuits in OCD patients (Figeo *et al*, 2013). Thus, augmentation of exposure-based therapies with DBS (Denys *et al*, 2010) may be due to enhancement of fear extinction via mOFC inhibition.

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The authors declare no conflict of interest.

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