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An Avoidance-Based Rodent Model of Exposure With Response Prevention Therapy for Obsessive-Compulsive Disorder

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ABSTRACT

BACKGROUND: Obsessive-compulsive disorder is treated with exposure with response prevention (ERP) therapy, in which patients are repeatedly exposed to compulsive triggers but prevented from expressing their compulsions. Many compulsions are an attempt to avoid perceived dangers, and the intent of ERP is to extinguish compulsions. Patients failing ERP therapy are candidates for deep brain stimulation (DBS) of the ventral capsule/ventral striatum, which facilitates patients' response to ERP therapy. An animal model of ERP would be useful for understanding the neural mechanisms of extinction in obsessive-compulsive disorder.

METHODS: Using a platform-mediated signaled avoidance task, we developed a rodent model of ERP called extinction with response prevention (Ext-RP), in which avoidance-conditioned rats are given extinction trials while blocking access to the avoidance platform. Following 3 days of Ext-RP, rats were tested with the platform unblocked to evaluate persistent avoidance. We then assessed if pharmacologic inactivation of lateral orbitofrontal cortex (lOFC) or DBS of the ventral striatum reduced persistent avoidance.

RESULTS: Following Ext-RP training, most rats showed reduced avoidance at test (Ext-RP success), but a subset persisted in their avoidance (Ext-RP failure). Pharmacologic inactivation of lOFC eliminated persistent avoidance, as did DBS applied to the ventral striatum during Ext-RP.

CONCLUSIONS: DBS of ventral striatum has been previously shown to inhibit lOFC activity. Thus, activity in lOFC, which is known to be hyperactive in obsessive-compulsive disorder, may be responsible for impairing patients' response to ERP therapy.

Keywords: Deep brain stimulation, Obsessive-compulsive disorder, Orbitofrontal cortex, Platform-mediated avoidance, Rat, Ventral striatum

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Obsessive-compulsive disorder (OCD) is a devastating illness affecting an estimated three million individuals in the United States alone (1). Many of the compulsive behaviors in OCD (e.g., hand washing, lock checking) are viewed as protective against perceived threats (e.g., infection, intruders) (2). The standard behavioral therapy for OCD is exposure with response prevention (ERP), in which patients are repeatedly exposed to triggers for their compulsions but are prevented from expressing the compulsion (3). The goal of repeated sessions of ERP is to extinguish compulsive behaviors (2). ERP is effective in the majority of OCD patients; however, approximately 40% either drop out or fail ERP (4,5). Little is known about the mechanisms of ERP therapy failure or the interactions between extinction and compulsive behaviors, thereby necessitating an animal model.

Patients failing to respond to ERP, as well as to pharmacotherapies, are candidates for deep brain stimulation (DBS) of the ventral capsule/ventral striatum (VC/VS). DBS of VC/VS reduces OCD and anxiety symptoms (6) and facilitates patients' response to ERP therapy (7,8). OCD is associated with excessive activity in the orbitofrontal cortex (OFC) [for reviews see (9,10–12)], and DBS has been shown to reduce blood oxygen level–dependent signaling in OFC together with compulsions (13–15). In rodents, DBS of dorsal VS (a rodent homologue of VC/VS) has been shown to reduce the firing rate of neurons within the lateral OFC (lOFC) (16). However, the role of lOFC in ERP has not been studied. Therefore, to further explore the roles of lOFC and DBS in ERP, we developed a rodent model of ERP therapy using a platform-mediated avoidance task (17). This allowed us to characterize persistent avoidance and its response to manipulations of OFC, both directly and indirectly via DBS of the ventral striatum.

METHODS AND MATERIALS

Subjects

One hundred ten male Sprague Dawley rats (~325 g; Harlan Laboratories, Indianapolis, IN) were housed and handled as previously described (18). Rats were fed standard rat chow in

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a restricted manner (18 g/day) to facilitate pressing a bar for food on a variable-interval schedule of reinforcement (variable interval 30 seconds). 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.

Behavior

Rats were initially trained to press a bar to receive food pellets on a variable-interval reinforcement schedule (variable interval 30 seconds) inside standard operant chambers (Coulbourn Instruments, Whitehall, PA) located in sound-attenuating cubicles (MED Associates, St. Albans, VT). Bar pressing was used to maintain a constant level of activity against which avoidance and freezing could reliably be measured. Food was available throughout all phases of the experiment.

For platform-mediated avoidance, rats were trained as previously described (17). Briefly, rats were conditioned with a pure tone (30 seconds, 4 kHz, 75 dB) coterminating with a shock delivered through the floor grids (2 seconds, 0.4 mA). The intertrial interval was variable, averaging 3 minutes. The platform was fixed to the floor and was present during all stages of training (including bar–press training). Rats were conditioned for 10 days, with nine tone–shock pairings per day with the reinforcement schedule changed to a continuous schedule of reinforcement during the tone. The availability of food on the side opposite to the platform motivated rats to leave the platform during the intertrial interval, facilitating trial–by–trial assessment of avoidance.

Once platform-mediated avoidance was learned, rats underwent extinction (tones with no shock) in the presence of a transparent Plexiglas barrier that prevented access to the platform. During extinction with response prevention (Ext-RP), rats underwent sessions consisting of 15 tone-alone presentations across 3 consecutive days with a variable interval 30 seconds food schedule. After 3 days of Ext-RP, the barrier was removed and rats were again exposed to the tone.

Surgery and Histology

Rats were initially anesthetized with isoflurane inhalant gas (5%) in an induction chamber and positioned in a stereotaxic frame. Isoflurane (2% to 3%) was delivered through a facemask for anesthesia maintenance. For our inactivation experiment, rats were bilaterally implanted with 26-gauge guide cannulas (Plastics One, Roanoke, VA) in the lOFC using the following coordinates: +3.20 mm anterior-posterior; ±3.30 mm medial-lateral; and +4.40 mm dorsal-ventral to bregma (19). Cannulas were fixed to the skull with anchoring screws and acrylic cement. After surgery, a topical triple antibiotic was applied around the surgery incision, and an analgesic (ketoprofen, 5 mg/kg) was injected intramuscularly. Stainless steel obturators (33 gauge) were inserted into the guide cannulas to avoid obstructions until infusions were made. Rats were allowed 5 to 7 days to recover from surgery before behavioral testing.

For our DBS experiment, a similar surgical procedure as above was used, except that rats were implanted with concentric bipolar stimulating electrodes (NEX-100; Rhodes Medical Instruments, Santa Barbara, CA) as previously described (20). Electrodes were aimed at a dorsal VS site (+1.2 mm anterior-posterior, ±2.0 mm medial-lateral, and −6.5 mm dorsal-ventral to bregma). Rats were allowed 5 days to recover from surgery before behavioral testing.

After behavioral experiments, rats were deeply anesthetized with sodium pentobarbital (450 mg/kg intraperitoneal) and transcardially perfused with 0.9% saline followed by a 10% formalin solution. Brains were removed from the skull and stored in 30% sucrose for cryoprotection for at least 72 hours before sectioning and Nissl staining. Histology was analyzed and correct placement of cannulas and stimulating electrodes was assessed.

Pharmacologic Inactivation

Fluorescent muscimol (MUS) (0.2 μL; BODIPY TMR-X Conjugate; Sigma-Aldrich, St. Louis, MO) was infused to enhance gamma-aminobutyric acid type A receptor activity, thereby temporally inactivating lOFC. On the day of infusion, 0.2 μL of MUS or saline (SAL) (vehicle) was infused at a rate of 0.2 μL/min. Injector tips extended 1.0 mm beyond the guide cannula. After infusion, injectors were left in place for 1 minute to allow the drug to diffuse. Eight rats were eliminated from our MUS experiment because the injections were located outside of our area of interest (lOFC).

Deep Brain Stimulation

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 have been used in previous rat models studying DBS-like stimulation (16,20–22). Stimulation was generated with an S88X stimulator (Grass Instruments, Warwick, RI) and a constant-current unit (SIC-C Isolation Unit; Grass Instruments). Three rats were eliminated from DBS experiments because placements were not located in dorsal VS, as previously described (20).

Data Collection and Analysis

Behavior was recorded with digital video cameras (Micro Video Products, Bobcaygeon, Ontario, Canada). Freezing was quantified from digitized video images using commercially available software (Freezescan; Clever Systems, Reston, VA). Platform avoidance was quantified by observers blinded to experimental group, where avoidance was defined as the rat having at least two paws on the platform. The time spent avoiding during the tone (percent time on platform) was used as our avoidance measure. We calculated percent suppression of bar pressing for each tone as previously described (17): (pretone rate − tone rate) / (pretone rate + tone rate) × (100). A value of 0 indicates no suppression, whereas a value of 100% indicates complete suppression. To calculate pretone rates, we used the 60 seconds before tone onset. Avoidance, freezing, and suppression of bar pressing to the tone were expressed as a percentage of the 30-second tone presentation. Statistical significance was determined with Student two-tailed *t* tests, Wilcoxon matched pairs test, one-way analysis of variance (ANOVA), or repeated-measures ANOVA, followed by Tukey post hoc analysis, when appropriate (STATISTICA; StatSoft, Inc., Tulsa, OK).