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Amygdala Deep Brain Stimulation Is Superior to Paroxetine Treatment in a Rat Model of Posttraumatic Stress Disorder

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ABSTRACT

Background: Posttraumatic stress disorder (PTSD) is a very debilitating disease refractory to current treatment with selective serotonin reuptake inhibitors (SSRIs) in up to 30 percent of patients, illustrating the need for new treatments of PTSD. Neuroimaging studies have shown increased activity of the amygdala of patients with PTSD.
Objective/hypothesis: To investigate amygdala deep brain stimulation (DBS) as a possible novel treatment for PTSD and compare it to current treatment with a commonly used SSRI, paroxetine, in a rat PTSD model.
Methods: A PTSD model was created by subjecting rats to inescapable foot shocks in the presence of a conspicuous ball. Response to treatment was measured as a decreased burying behavior when presented with the same ball 1 and 2 weeks after the shocks. Rats were treated with either daily intraperitoneal paroxetine injections or amygdala DBS via an electrode implanted 1 week prior to shocks. Generalized anxiety was assessed using an elevated plus maze.
Results: Animals treated with amygdala DBS showed less ball burying at 2 weeks relative to the animals treated with paroxetine. The animals treated with paroxetine, however, had a lower general anxiety level compared to the DBS-treated group.
Conclusions: In this PTSD model, paroxetine was found to decrease the measured general anxiety level of rats that underwent the PTSD protocol, but did not counteract shock-induced hyper-vigilance toward the trauma-associated object (ball). Amygdala DBS, however, did decrease shock-induced hyper-vigilance as measured by a lower burying time, but had no effect on general anxiety assessed in the elevated plus maze. By attenuating amygdala function, DBS may act to treat the cause of PTSD, hyperactive amygdala function, and may be a promising novel alternative in cases of PTSD refractory to current pharmacological treatments.

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Introduction

Posttraumatic stress disorder (PTSD) is a severe anxiety disorder triggered after exposure to a traumatic event that produces intense fear, helplessness, or horror. The lifetime prevalence of PTSD in the US population is estimated to be 6.8% [1], and despite current treatments, approximately 30% of PTSD patients will still exhibit symptoms 10 years after diagnosis [2]. An estimated 5–15% of the 1.64 million military veterans returning from Iraq and Afghanistan have PTSD [3]. The total cost to society for these veterans is estimated at up to $2.3 billion over a two-year period [4].

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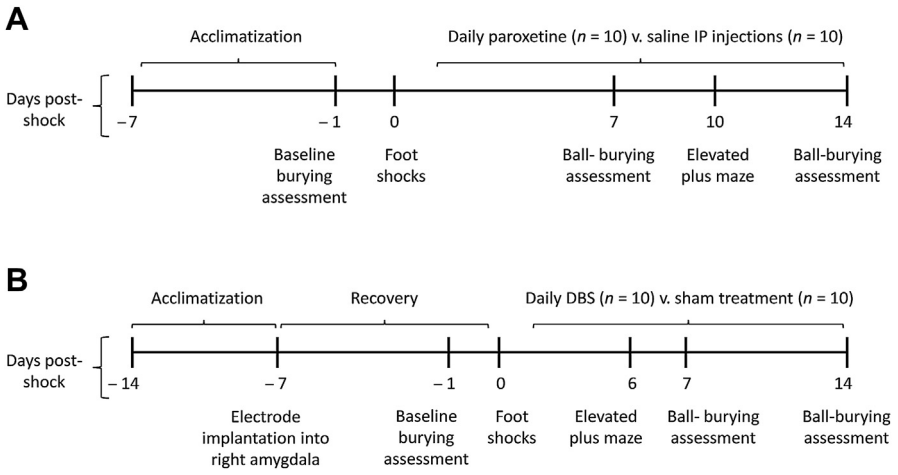

Figure 1. Protocol timeline. A: Paroxetine group. The animals were allowed to acclimatize for approximately 1 week. A baseline burying assessment was performed one day prior to subjecting the animals to inescapable foot shocks in the presence of a small blue toy car. The assessment was repeated on post-shock days 7 and 14 using the same miniature tennis ball that was present during the shocks. An anxiety assessment was performed on day 10 post-shock. B: DBS group. Again, the animals were allowed to acclimatize for 1 week. DBS electrodes were then implanted and the animals were allowed to recover from surgery for 1 week. Behavioral assessments were performed at one day prior to inescapable foot shocks and on post-shock days 7 and 14.

Functional imaging of war veterans has implicated the amygdala as a key brain structure involved in PTSD. Functional MRI studies [5,6] and PET-CT scans [7,8] demonstrate increased activity in the amygdala of PTSD patients relative to controls. Moreover, the intensity of the amygdala activity closely correlates with the severity of PTSD symptoms [8,9]. Remarkably, the incidence of PTSD of combat veterans who sustained brain injury that included the amygdala was zero [10]. Collectively, these studies show a clear correlation between amygdala activity and PTSD symptoms, suggesting that the symptoms of PTSD may be the result of abnormal amygdala function [11]. We hypothesize that PTSD may be treated by attenuating amygdala activity.

Deep brain stimulation (DBS) is a surgical treatment used in a growing list of psychiatric and neurodegenerative movement disorders involving the stereotactic placement of an electrode into a predefined brain region. Attenuation of neuronal activity at the targeted region with high frequency electrical current delivered through the electrode has been shown to be effective in psychiatric conditions such as depression, obsessive-compulsive disorder, aggression, and Tourette syndrome [12–14]. Relief of treated symptoms is optimized and side effects are minimized by adjusting the stimulation variables including pulse amplitude, width, and frequency. While DBS has been shown to successfully treat a variety of neurological pathologies, its exact mechanism of action is still unclear, but may be akin to a transient functional inactivation [15]

Amygdala DBS represents a potential novel, reversible treatment for PTSD patients who are refractory to current treatment with SSRIs. We compare amygdala DBS to paroxetine using a well-established rat model of PTSD in which animals experience inescapable foot shocks in the presence of a unique, innocuous object [16]. These animals then exhibit avoidance and hypervigilant behaviors when later presented with the same innocuous object in the absence of foot shocks. This avoidance and hypervigilant behavior manifests itself as a robust burying behavior which can be easily quantified. The differences in the time spent burying can be used to quantify the effect of either amygdala DBS therapy or paroxetine treatments in the PTSD model [16,17]. The outcome of this study is to demonstrate that amygdala DBS therapy may be superior to treatments with paroxetine.

Materials and methods

Experimental design

Animals were divided into a paroxetine IP injection group and a DBS group (Fig. 1, panels A and B respectively). After 7 days of acclimatization, the baseline burying behavior of 20 rats assigned to the IP injection group was assessed by scoring video recordings of the animal behaviors in the presence of a novel object, a small blue plastic car. On the following day, the 20 animals were subjected to inescapable foot shocks in the presence of a different unfamiliar innocuous object, a miniature tennis ball. The animals were then randomly divided into 2 subgroups of 10 animals each. One subgroup was treated with daily 5 mg/kg IP paroxetine injections, a dose that has been demonstrated to reduce avoidance and hypervigilant behavior in a similar PTSD rat model [18]. The second subgroup of 10 animals served as a control and received daily IP injections of the vehicle solution without paroxetine. Burying behavior of both subgroups was then assessed on post-shock days 7 and 14. The elevated plus maze (EPM), a well-established apparatus to measure anxiety-like behavior in rodent models [19–21], was used as a measure of generalized anxiety on post-shock day 10.

The DBS treatment group was designed as a crossover study (Table 1). After 7 days of acclimatization, 20 rats were implanted with DBS electrodes and allowed to recover for 6 days. Baseline behavior assessments of the 20 implanted animals were performed as with the IP injection group and on the following day the animals were subjected to inescapable foot shocks in the presence of a novel miniature tennis ball. The 20 animals were then divided into 2 subgroups, a sham-DBS subgroup and a DBS-sham subgroup. Over the next 7 days, the DBS-sham subgroup received daily DBS therapy

Table 1
DBS treatment schedule (crossover design).

	Post-shock week 1	Post-shock week 2
Sham-DBS subgroup ($n = 10$)	Do not receive DBS therapy	Receive DBS therapy
DBS-sham subgroup ($n = 10$)	Receive DBS therapy	Do not receive DBS therapy

DBS therapy: 2.5 V, 160 Hz, 120 μs pulse width, 4 h per day.

while the sham-DBS subgroup served as a control. Behavior assessments were then performed on post-shock day 7. During the next 7 days, the sham-DBS subgroup received daily DBS therapy while the DBS-sham subgroup served as a control. Again, behavioral assessments were performed on post-shock day 14. Generalized anxiety of both subgroups was assessed on post-shock day 6 with an EPM test.

Animals

A total of forty animals were used for this work. The animals were 3 month old male Sprague–Dawley rats weighing approximately 350–400 g. The animals were housed in individual cages in a reversed 12-h light/dark cycle with lights off at 9:00 AM. The animals were acclimatized for 7 days and had free access to food and water throughout the duration of the experiments. The experimental protocol was reviewed and approved by the University of Arizona Institutional Animal Care and Use Committee.

Inescapable foot shocks

Our rat model of posttraumatic stress disorder was created using a similar method first described by Mikics et al. [16] A miniature, 2-inch diameter tennis ball (Kong, Golden, CO) was first placed into a 30 × 30 × 25 cm shock box (Coulbourn Instruments, Whitehall, PA) fitted with a metal grid floor. The miniature tennis ball served as a neutral object which was handled only with a gloved hand and each animal was assigned its own miniature ball. The animals were then placed into the shock box with the miniature ball present and allowed to explore the cage and ball for 60 s. After this brief period, the animals were exposed to a 1-s shock of 2.0 mA intensity every 30 s over a 5-min period for a total of 10 shocks. The animals were then immediately returned to their home cage without the miniature ball present. The ball was placed in a zip-locked bag, labeled with the identification number of the animal.

Paroxetine administration

Paroxetine was obtained as a gift through the NIMH Chemical Synthesis and Drug Supply Program. A 3.2 mg of paroxetine/ml stock solution was prepared by dissolving 160 mg of paroxetine in 5 ml dimethyl sulfoxide (DMSO) and adding sterile saline to a total volume of 50 ml. Animals within the paroxetine treatment group received daily 5 mg/kg IP paroxetine injections in the lower left peritoneal quadrant. The injected amount was usually 0.6 ml of paroxetine stock. Animals in the control group were administered 0.6 ml of a vehicle containing a 10% DMSO solution by volume in normal saline without paroxetine.

Surgical DBS electrode implantation

General anesthesia was induced and maintained with isoflurane. The top of the head was clipped free of hair then secured to a stereotactic frame. The skin was prepped with iodine and a sagittal incision was made over the scalp off the midline to the right exposing the underlying cranium. A small craniotomy was made using a high speed drill over the point overlying the right basolateral nucleus (BLn) of the amygdala at −2.4 mm posterior to bregma and 4.8 mm lateral to the sagittal suture. A 10-mm insulated, monopolar electrode (Plastics One, Roanoke, VA) attached to a plastic pedestal was lowered 7.2 mm below the surface of the brain and a ground wire attached to the same pedestal was subcutaneously tunneled along the dorsum of the rat. The craniotomy was sealed with silicone and 4 stainless steel jeweler screws were fixed to the exposed skull. Dental cement was used to anchor the electrode construct to the screws and skull. The scalp was closed over the dried dental cement with suture.

Deep brain stimulation

For DBS treatments, the animals were kept in their individual home cages and an external pulse generator was connected to the intracranial electrode with enough wire length to allow the rats to move freely in their cage. DBS was administered daily for 4 consecutive hours for 7 days. The stimulation settings were monopolar, 120 μs pulse width, 160 Hz frequency, and 2.5 V using a Model 2100 programmable pulse generator (A-M Systems, Sequim, WA) or an AFG310 programmable pulse generator (Tektronix, Beaverton, OR). Signals were verified using a TDS 2014 oscilloscope (Tektronix, Beaverton, OR).

Behavioral assessment

Burying behavior was measured before and after exposure to inescapable foot shocks (Fig. 2). The animals were kept in their individual home cages and placed on a bench top in a fully lit, quiet area of the laboratory. To measure baseline burying activity prior to administration of inescapable foot shocks, a small blue plastic car used as a novel conspicuous object was placed into the housing cage and the animal's behavior was video-recorded for 10 min one day before shock. The recordings were scored off-line to measure the time spent burying the object defined as any attempt to move the cage bedding toward the object. This definition included moving bedding with paws or using the head to shovel bedding toward the object. For comparison, exploratory behavior was also assessed and was defined as behaviors such as sniffing, biting, or moving the novel object. The rats spent time exploring the cage or sleeping when not engaging the novel object. The toy car was wiped clean with 70% dilute ethyl alcohol and thoroughly dried after each assessment.

Post-shock burying assessments were conducted on days 7 and 14 after the animal was subjected to inescapable foot shocks. The assessment was performed using the same procedure as the pre-shock assessment with the exception that instead of using the blue plastic car, the miniature tennis ball that was present during the inescapable shocks was presented to the animals in their home cages. Each rat was presented with the same miniature tennis ball that was originally placed in the shock box during the inescapable shocks so as to conserve possible olfactory cues. The behavior was again recorded on video and later scored for time spent burying and exploring the ball (Fig. 2). Each behavioral assessment was conducted after scheduled DBS or paroxetine treatments. When first presented with the miniature tennis ball, the animals initially were still for the first 30–60 s and when not observed burying the tennis ball, the majority of rats spent time in the opposite corner of the cage grooming and sleeping.

Elevated plus maze

The EPM used to assess the level of anxiety in the animals was a wooden apparatus with 4 straight arms forming pathways projecting away from a common center at 90° from adjacent arms forming a cross. The dimensions of each arm were 10 × 50 cm. Two arms opposite each other were open to the surroundings and the other two arms were enclosed with 40 cm tall walls except where they opened to the center. The maze was elevated 60 cm above the floor.

Anxiety was measured as the fraction of time the animal spent in an open arm over a 5-min period. The EPM was completely surrounded with black curtains. The animals were initially placed in

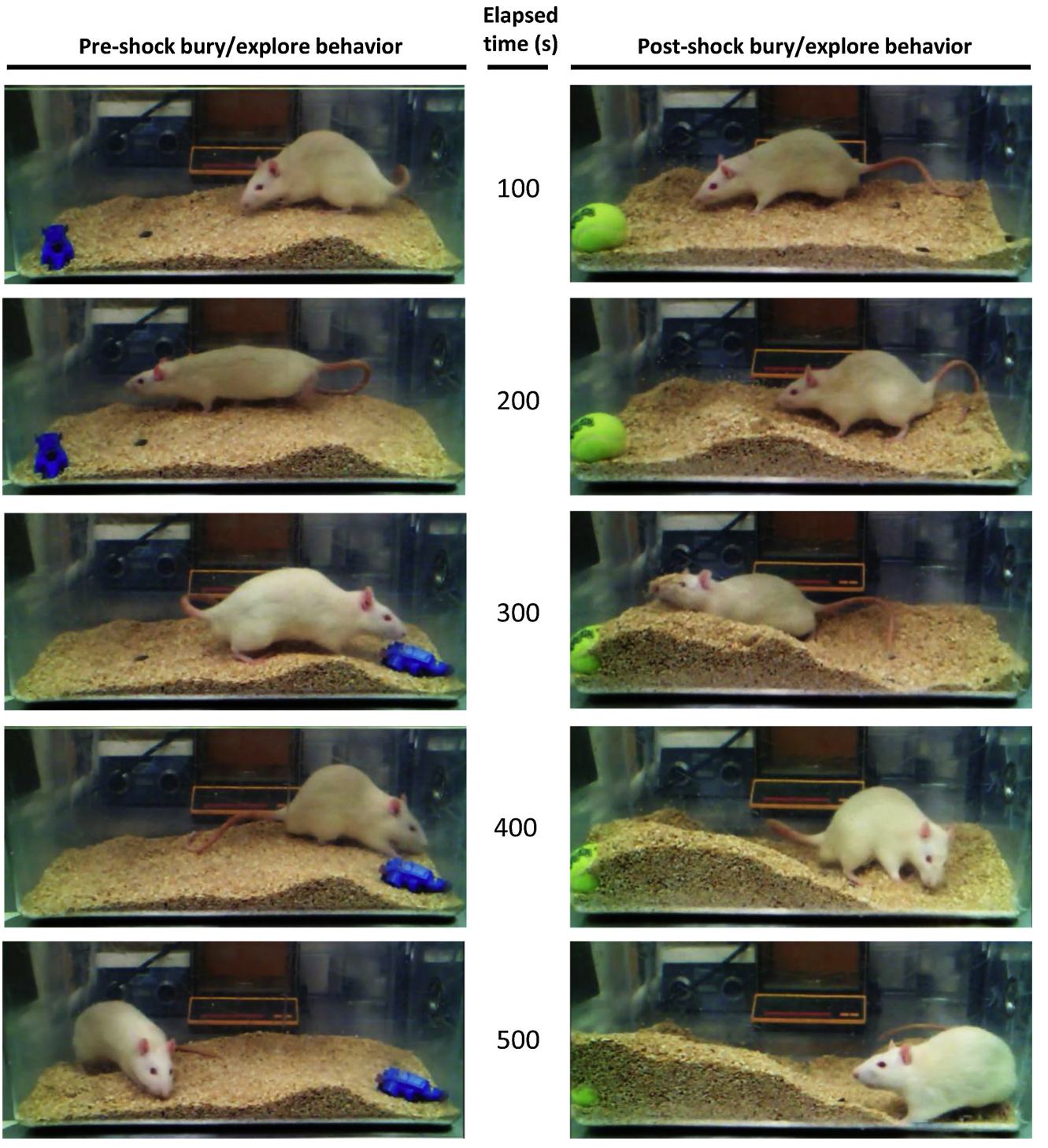

Figure 2. Cue-specific behavioral assessment. Behavior toward a novel object was assessed by placing either a blue plastic car (pre-shock assessment, left column) or a miniature tennis ball (post-shock assessment, right column) into the rat's home cage and recording its behavior on video for 10 min. Post-shock behavior was assessed on post-shock days 7 and 14 using the same miniature tennis ball that was present during the inescapable foot shocks. The animals exhibited normal exploratory behavior prior to exposure to foot shocks (left column), and burying behavior after the foot shocks (right column). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the center of the maze with the head facing an open arm. A video recording was made of the movement of the animal from an overhead camera. The video recording was analyzed with the ANY-maze video tracking software version 4.3 (Stoelting Co., Wood Dale, IL). With the software package, the areas within the closed arms and open arms were defined and the time spent within each arm, number of entries into the arms, distance traveled within the arms, and movement time within the arms were tabulated. Further details can be found elsewhere [22].

Histology

At the conclusion of the experiment, the animals were deeply anesthetized, then perfused transcardially with saline followed by

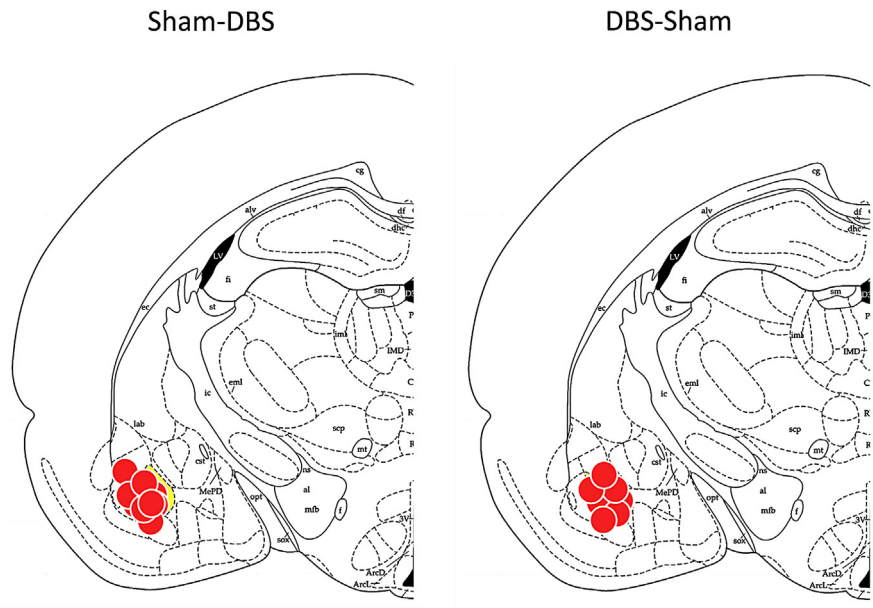

Figure 3. Schematic section of rat brain illustrating the termination points of DBS electrodes in the right basal lateral amygdala (BLA) for the sham-DBS and DBS-sham groups. The red dots indicate the termination points of individual electrodes as assessed by histology. The BLA is highlighted yellow on the schematic diagram. (Adapted from the rat brain atlas of Paxinos and Watson [23]) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

0.4% paraformaldehyde and the brains were extracted. The brains were then frozen and thin slices (40 μm) were obtained to assess the electrode insertion points. The slices were mounted and stained with cresyl violet (NISSL stain). Confirmation of proper electrode placement was then made under a light microscope (Fig. 3). Histology showed that the stimulation parameters used caused no significant damage to the amygdala.

Statistical analyses

Quantitative data were reported as means ± standard error of mean (SEM). All statistical analyses were conducted using Sigma-Stat software (SYSTAT, San Jose, California). The Mann–Whitney test or Student's *t*-test were used to test for significance of non-parametric and parametric data sets respectively. Means were considered statistically significant at a value of $P < 0.05$.

Results

Effective administration of paroxetine was measured as a small change in weight over the course of the protocol. SSRIs have been used to treat eating disorders in humans [24–26] and have been shown to cause weight loss in rats [27]. Weights were recorded before any test or handling on the day of exposure to inescapable foot shocks and on post-shock days 3, 7, 10, and 14. Figure 4 shows the mean weight change for animals either receiving IP paroxetine or IP vehicle injections. The data were normalized by calculating the difference of each individual data point relative to the baseline weight recorded on the day when the inescapable shocks were administered. A least square linear fit was added to the paroxetine and control data sets. The control subgroup gained an average of 5.0 g over the 14 day period and the paroxetine subgroup lost an average of 4.4 g in the same timespan ($P < 0.05$ on days 7, 10, and 14). This result shows that the paroxetine dosage we used yielded well documented and reasonable side effects, and hence was physiologically active.

Generalized anxiety-like behavior using an EPM was assessed for the Paroxetine treatment groups on post-shock day 10 and in the DBS treatment groups on post-shock day 6. There was no significant difference in the time spent in the closed arms in either group (Fig. 5A). There was also no significant difference spent in the open arms between the sham-DBS and DBS-sham subgroups (Fig. 5B). There was a statically significant difference, however, between the mean times that the control and paroxetine animals spent in the open arms ($P < 0.05$). This implies that the paroxetine-treated animals had a lower level of general anxiety than the control, sham-DBS, and DBS-sham subgroups. There was no significant difference between groups in the open arm entry ratio (not shown).

All subgroups showed a large increase in mean burying time between the pre-shock and post-shock assessments (Fig. 6). There was no significant difference between the mean burying times of the control and paroxetine subgroups at the 1-week and 2-week post-shock assessments time points. There was also no significant difference between the sham-DBS subgroup and the paroxetine treatment group at the 1-week post-shock assessment, but there was approximately a 50% decrease in mean burying time observed

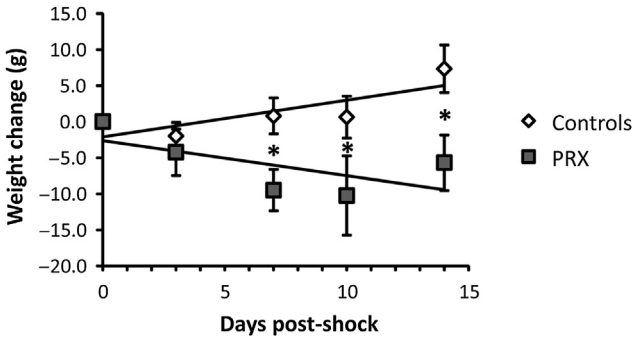

Figure 4. Physiological effects of paroxetine. Weight change of the animals in the paroxetine treatment group is shown over the course of IP injections. The data were normalized to the baseline weight measured on the day of the foot shocks for both the control and paroxetine groups ($n = 10$ for each subgroup, $^*P < 0.05$). A least squares linear fit was added and standard errors are indicated by the bars.

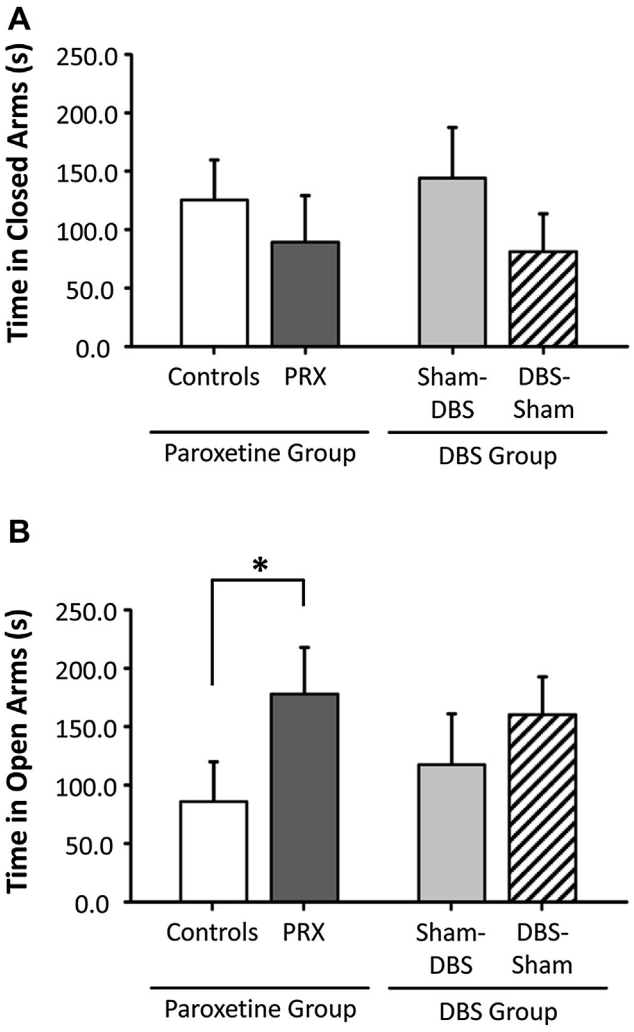

Figure 5. Changes in general anxiety as measured by the elevated plus maze. Mean times spent in the closed arms (A) and open arms (B) of the elevated plus maze are shown for both the paroxetine and DBS arms of the protocol ($n = 10$ for each subgroup, $^*P < 0.05$).

for the DBS-sham subgroup at the 1-week post-shock assessment relative to the other subgroups ($P < 0.05$). By the 2-week post-shock assessment day, the mean burying times of both the sham-DBS and DBS-sham subgroups were significantly lower than that of the control and paroxetine subgroups ($P < 0.05$). These results indicate that amygdala DBS reduces PTSD-like avoidance as measured by burying behavior in this animal model, whereas paroxetine treatment had no effect. The results also show that the benefit of amygdala DBS was sustained beyond the stimulation period. The reduction in burying behavior expected by the DBS-treated animals during the first week after the foot shocks was maintained in the second week even after daily DBS treatments were stopped. Also, the animals that received DBS during the second week and not the first week showed decreased PTSD-like avoidance, suggesting that amygdala DBS was still beneficial even if the start of the treatment was delayed by 1 week.

All animals exhibited a significant decrease in exploration time from the baseline assessment to the 1-week assessment as depicted in Fig. 7 ($P < 0.05$). This decreased in exploration remained significant for the control, paroxetine, and sham-DBS subgroups at the 2-week post-shock assessment ($P < 0.05$). However, the amount of time that the DBS-sham subgroup spent exploring the miniature tennis ball at the 2-week post-shock assessment was not statistically

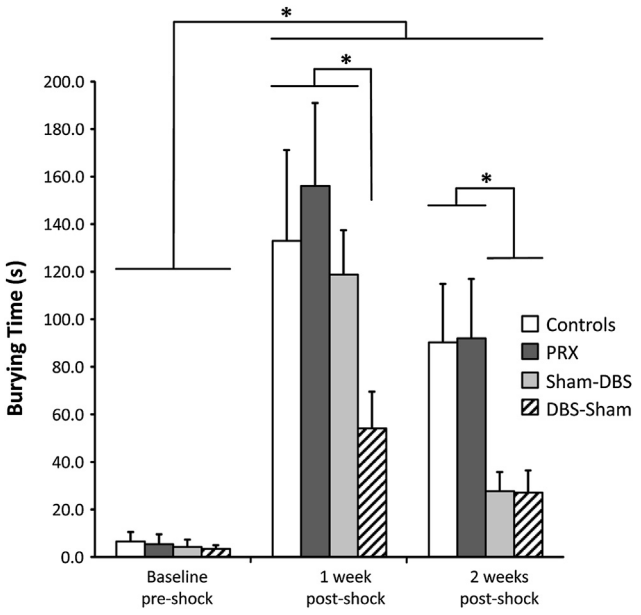

Figure 6. Changes in burying times. One day prior to the inescapable shocks, the animals were assessed for baseline burying behavior by observing their reaction to a novel object, a small plastic blue car, placed in their home cage over a period of 10 min ("Baseline pre-shock"). Burying behaviors were also assessed on post-shock days 7 and 14 using the same miniature tennis ball present during the shock administration ($n = 10$ for each subgroup, $^*P < 0.05$).

different from that of the animals at the baseline pre-shock assessment.

Altogether, these results indicate that, in contrast to the reduction of general trauma-independent anxiety assessed by the EPM [22], paroxetine had little effect on burying behavior of the trauma-related object. DBS treatment, however, had the opposite effect, and appeared to be effective in reducing anxiety related to cues that were present during the traumatic event.

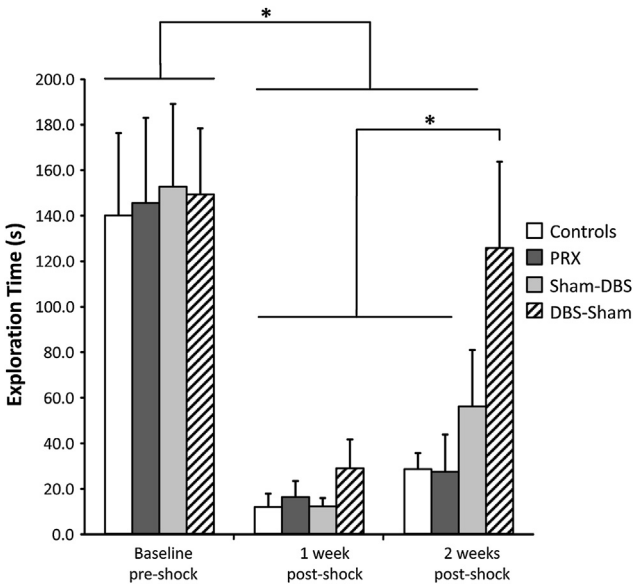

Figure 7. Changes in exploration times. One day prior to the inescapable shocks, the animals were assessed for baseline exploratory behavior by observing their interactions with a novel object, a small plastic blue car over a period of 10 min ("Baseline pre-shock"). Exploratory behaviors were assessed on post-shock days 7 and 14 using the same miniature tennis ball present during the shock administration ($n = 10$ for each subgroup, $^*P < 0.05$).

Discussion

Our data strengthen previous findings that DBS to the right amygdala BLn reduces burying behavior in a PTSD animal model [17]. We found that animals treated with right amygdala DBS (the DBS-sham subgroup) had a significant 2-fold decrease of burying time 1 week after inescapable foot shocks relative to sham animals (the sham-DBS subgroup) with implanted electrodes that did not receive DBS treatment during the first week after foot shocks (Fig. 6). The exploration time for the animals also correlated with previous findings [17]

The crossover design of the DBS treatment group demonstrated that amygdala DBS was effective at reducing burying behavior even when initiated in a delayed fashion after the establishment of abnormal behavior. The sham-DBS subgroup did not receive DBS treatments during the week following exposure to inescapable foots shocks and spent a significantly longer time burying at the 1-week behavioral assessment time point compared to the DBS-sham subgroup that did receive daily DBS treatments during the week following the foot shocks (Fig. 6). This burying time difference was negligible, however, at the end of the second week when the sham-DBS subgroup in turn received DBS treatments and the DBS-sham subgroup did not. This further suggests that the effect of DBS treatment lasts at least 1 week after the cessation of treatment. The DBS-sham subgroup did not receive DBS therapy during the second week post-shock, yet still had a small average burying time at the 2-week behavioral assessment. This finding indicates that DBS may have facilitated the extinction of fear toward the object. Once extinction was achieved, it lasted even after cessation of the therapy. This is important in the context of PTSD which is often understood as a failure of fear extinction due to the lack of inhibitory control of the amygdala by the medial prefrontal cortex [28].

Symptoms of PTSD are currently treated by a combination of psychopharmacology, psychotherapy, education, and supportive measures [29]. Paroxetine is a selective serotonin reuptake inhibitors (SSRIs) associated with improved outcomes in a randomized controlled trial of 551 patients with PTSD [30], and has been shown to decrease PTSD symptoms of intrusion, avoidance, and hyper-arousal in a human placebo-controlled trial [31]. Paroxetine has also been successfully used to decrease avoidance and hyper-vigilance activities in a PTSD rat model [18].

Amygdala DBS is superior to IP paroxetine at reducing burying behavior in the PTSD animal model. The behavioral assessment 1 week after inescapable foots shocks showed no significant difference between the mean burying times of animals receiving daily IP vehicle injections, daily IP paroxetine injections, or animals with an implanted intracranial electrode that did not receive DBS treatment (Fig. 6). The DBS-sham subgroup did receive daily DBS treatments over the first week and had a significantly lower mean burying time than all 3 previous subgroups suggesting that DBS treatment provided a significant benefit for PTSD. At the 2-week behavioral assessment time point, both the sham-DBS and the DBS-sham subgroups had significantly lower burying times than either the animals treated with IP vehicle or IP paroxetine, further supporting the finding that amygdala treatment is superior to paroxetine in this PTSD animal model. This conclusion is also supported by exploration times recorded for the animals (Fig. 7). Paroxetine therapy was not shown to alleviate the PTSD avoidance symptom measured by this PTSD rat model, in contrast to the sustained benefit noted by high frequency amygdala DBS therapy.

The action of paroxetine on PTSD is likely the result of reduced general anxiety. Paroxetine was shown to cause a lower level of general anxiety as assessed by the EPM (Fig. 5) when compared to DBS and the control groups. Paroxetine-treated rats spent significantly more time in the open arms of the apparatus than the animals treated with IP vehicle. There was no statistically significant difference found for the EPM times recorded for the DBS group of animals. Though DBS treatment was shown to dramatically reduce avoidance of a stimulus associated with a previous trauma in the PTSD rat model, DBS therapy appeared to have no effect on the overall anxiety level of the animals. In contrast, paroxetine did lower the general anxiety level, but appeared to have no effect on the measured PTSD avoidance stimulus (burying). This is an important finding implying that paroxetine can only alleviate PTSD by reducing generalized anxiety. In this animal model, SSRIs appear to lack specificity when treating the symptoms of PTSD and thus may lead to a lack of efficacy in more severe cases. This may explain the failure of SSRIs in recent trials involving combat PTSD [32]. Conversely, DBS of the BLn reduced the more specific PTSD behavior (ball burying) without interfering with generalized anxiety when measured with the EPM. BLn DBS may carry this effect by facilitating fear extinction.

The basolateral nucleus of the amygdala was chosen as in ideal target for DBS. The human amygdala is a complex group of nuclei that form an almond-shaped structure in each mesial temporal lobes, rostral to the hippocampus. The amygdala has been shown to have a primary role in regulating the strength of memories in relation to their emotional significance [33]. In the rat and monkey, the amygdala is composed of more than 10 individual nuclei. The rat amygdala can be divided into the basolateral, cortical, and centromedial groups. The basolateral nucleus processes afferent information from the other amygdala nuclei and thus is the main relay nucleus within the amygdala, an ideal target for intervention [17]. Moreover, functional imaging studies have demonstrated that the increased activity of the amygdala in PTSD patients was primarily located in the basal portion of the amygdala [34].

Caution must be taken when translating findings obtained in a rodent model to possible treatments. Our work, for example, does not explain the individual differences in paroxetine responses seen in humans. The significant time differences seen for the EPM open arm exploration between the Control and PRX subgroups for example (Fig. 5B) may be due in part to the better controlled genetic makeups of the animals, or to a more fundamental difference in the effect of paroxetine. Also, the possible side effects of amygdala DBS on other types of behaviors such as classical fear conditioning need to be assessed in further work. Nonetheless, our results provide further evidence that BLn DBS may be a novel and more specific treatment for severe PTSD.

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