Behavioral/Cognitive

Field Potential Oscillations in the Bed Nucleus of the Stria Terminalis Correlate with Compulsion in a Rat Model of Obsessive-Compulsive Disorder

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The bed nucleus of the stria terminalis (BNST) is implicated in anxiety and reward processing, both of which are associated with obsessive-compulsive disorder (OCD). Specific neuronal groups in the BNST related to anxiety and reward have been identified, but quantitative data about the information carried by local field potential (LFP) signals in this area during obsession/compulsion are lacking. Here we investigate the BNST LFP in the schedule-induced polydipsia, an animal model of OCD. We implanted electrodes bilaterally in the BNST and random control brain regions in 32 male Wistar rats, and recorded corresponding LFP during compulsive and noncompulsive behavior. We first applied high-frequency (100 Hz) electrical stimulation through the implanted electrodes and analyzed its effects on compulsive behavior. We then performed time-frequency analysis of LFPs and statistically compared the normalized power of δ (1–4 Hz), θ (4–8 Hz), α (8–12 Hz), β (12–30 Hz), and lower γ (30–45 Hz) bands between different groups. Our data showed that the normalized δ, β, and γ powers in the right BNST were specifically correlated with compulsive behaviors. δ and γ oscillations increased and decreased during the initiation phase of compulsion, respectively, whereas β increased after compulsion stopped. Moreover, the effect of BNST electrical stimulation, in terms of suppression of compulsion, was significantly correlated with the percentage change of these bands during compulsion. Our research reveals potential biomarkers and underlying neurophysiological mechanisms of compulsion and warrants further assessment of the use of LFP for closed-loop neuromodulation in OCD.

Key words: bed nucleus of the stria terminalis; deep brain stimulation; local field potential; obsessive-compulsive disorder; schedule-induced polydipsia; time-frequency analysis

Significance Statement

Although specific neuronal groups in the bed nucleus of the stria terminalis (BNST) related to anxiety and reward circuitries have been identified, psychopathological information carried by local field potentials in the BNST has not yet been described. We discovered that normalized powers of the right BNST δ, β, and γ oscillations were highly correlated with compulsion. Specifically, δ and γ oscillations increased and decreased during the initiation phase of compulsion, respectively, whereas β increased after compulsion stopped. Such correlations were not found in other parts of the brain during compulsion, or in the BNST during noncompulsive behavior. Current findings reveal real-time neurophysiological biomarkers of compulsion and warrant further assessment of the use of local field potentials for closed-loop neuromodulation for OCD.

Introduction

The mechanisms of obsessions and compulsions are elusive, but elevated anxiety level and dysfunctional reward circuitry are important contributors (Bartz and Hollander, 2006; Figee et al., 2011). The bed nucleus of the stria terminalis (BNST), as part of the extended amygdala, is involved in anxiety and reward circuit-

Received May 14, 2015; revised Aug. 5, 2016; accepted Aug. 6, 2016.

Author contributions: H.W., K.v.K., J.-M.A., S.V.H., and B.N. designed research; H.W. performed research; H.W., T.T., I.N., K.v.K., J.-M.A., S.V.H., and B.N. contributed unpublished reagents/analytic tools; H.W., T.T., I.N., and M.D. analyzed data; H.W. wrote the paper.

This work was supported by Agency for Innovation by Science and Technology Baekeland Mandate 100683 and SBO BrainSTAR, Interdisciplinary Research Programmes of KU Leuven IDO/12/024, Research Foundation-Flanders 12L4812N, G.0729.09, and G.0A55.13, and Medtronic chair Neurosurgery for psychiatric disorders. We thank Frederik Ceyssens for the contribution in video recording setup; Marleen Welkenhuysen and Laura Luyten for sharing knowledge about the animal model and the extended amygdale; Steffen Fieuws for statistical advice; and Pei-Yen Hsiao for the artistic contribution in Figures 1 and 3b.

B.N. holds a chair Neurosurgery for psychiatric disorders, a donation from Medtronic and a chair Neuromodulation, an endowment from Medtronic. He has received grants for travel, research, and education from Medtronic, and

ries (Walker et al., 2003; Stuber et al., 2011; Lammel et al., 2012). Activation and suppression of specific cell groups in the BNST are related to anxiety-like behavior in rodents (Jennings et al., 2013). Pharmacological intervention targeting the BNST may induce or disrupt anxious behavior (Lee and Davis, 1997). Electrolytic and excitotoxic lesions of the BNST abolished anxious behaviors, and high-frequency electrical stimulation, which mimics the effect of ablation, suppresses compulsive behavior in rodents (van Kuyck et al., 2008; Davis et al., 2010; Luyten et al., 2011). Finally, deep brain stimulation in the BNST region proved effective in otherwise-refractory obsessive-compulsive disorder (OCD) patients, further suggesting the involvement of the BNST in obsession and compulsion (Nuttin et al., 1999, 2013).

Although the BNST is implicated in anxiety, reward processing, and OCD, quantitative data about the information carried by local field potential (LFP) signals in this area during obsession/compulsion is lacking. Here we performed time-frequency analysis of LFPs recorded in the BNST and random control brain regions, before, during, and after compulsive and noncompulsive drinking behaviors in the rat schedule-induced polydipsia (SIP) model, an animal model of OCD (Moreno and Flores, 2012).

Under the SIP conditioning, a rat receiving food pellets under fixed-time interval (usually between 30 and 120 s) will gradually develop polydipsic behavior over time. Similar to the clinical manifestation of OCD, there is a cue (i.e., the delivery of food pellet) triggering the compulsion in this model. A typical SIP-conditioned rat drinks repeatedly after receiving food pellets, and without the presence of such a cue, compulsive drinking is disrupted. Pharmaceutical intervention (e.g., serotonin reuptake inhibitor) and deep brain stimulation, which are used to treat OCD patients, could also effectively suppress compulsive drinking behavior in this model, further supporting the limbic origin of this behavior (Woods et al., 1993; van Kuyck et al., 2008).

Although clinical studies already showed that obsession and compulsion could be alleviated by surgical intervention in the BNST, the exact treatment mechanism and the role of the BNST in OCD remain unclear. The SIP model gave us a unique window to observe the dynamic neurophysiological changes in the BNST during the course of compulsion. We hypothesized that neural oscillations in the BNST were correlated with compulsive drinking behavior in SIP-conditioned rats. To test our hypothesis, we recorded LFPs (in the BNST and random control brain regions) and behavioral data in SIP- and control-conditioned rats, and performed time-frequency analysis of LFPs in the lower frequency range (1–45 Hz).

Materials and Methods

A total of 32 male adult Wistar rats were randomly allocated (service from http://www.random.org/) into three different groups: SIP BNST, SIP Random, and Control BNST (number of rats in each group confirmed after histological examination). Electrodes (twisted bipolar platinum, single-strand diameter: 0.203 mm, part number E363/8-2TW, Plastics One) were implanted at bilateral BNST (coordinates: at bregma, 3.4 mm lateral from midline, 20° insertion angle, 6.3 mm depth) in rats of SIP BNST and Control BNST groups, and at random brain regions in rats of SIP Random group (random targets were generated using the random integer generator from http://www.random.org/ with the following limitations: nonrepeating anatomical structures: within 2.5 mm range from bregma, 3.4 mm from midline, and 0–80 mm depth, with a 20° insertion angle, which does not trespass the BNST) (Paxinos and Watson, 2007). After electrode implantation, each rat underwent recovery period (3 d), acclimation period (3 d), conditioning period (19 d), and intervention period (6 d), followed by histological examination.

This research project was approved by the university ethics committee for laboratory experimentation (Project P165/2008 and P093/2012) and was in accordance with the Belgian and European laws, guidelines and policies for animal experimentation, housing, and care (Belgian Royal Decree, May 29, 2013 and European Directive 2010/63/EU on the protection of animals use for scientific purposes of 20 October 2010).

Surgical procedures. Similar surgical procedure has been described previously (van Kuyck et al., 2008; Luyten et al., 2011). In brief, after successful anesthesia induced by peritoneal injection of Anesketin (0.06 ml/100 g body weight) and Domitor (0.04 ml/100 g body weight), the rat was placed on a heating pad and fixed properly in the stereotactic frame. Midline incision and two burr holes were made based on implantation trajectory. Four additional burr holes surrounded the implantation sites (one for reference screw [E363/20, Plastics One] and three for anchoring screws). After implantation of electrodes and screws, dental cement was applied and a plastic pedestal (MS363, Plastics One) was fixed on top of the rat's head, with contacts of implanted electrodes placed inside. Antisedan was administered after operation was completed, and each rat was given 3 d of recovery and 3 d of acclimation periods before conditioning commenced.

Conditioning setup. Food at home cage was restricted until each rat reached 85% of its preoperative body weight, and a limited amount of food (~5 g each day) was given to each rat to maintain its body weight between 80% and 85% throughout the conditioning and intervention periods. Water supply was *ad libitum* in both home cage and conditioning cage. All rats first underwent 3 d of acclimation period. During the acclimation period, the rat was placed in the conditioning cage individually for 1 h each day, where it would receive 60 food pellets (45 mg, F0021-J, Bio-Serv) at once when it entered the cage (Control conditioning), and put back to its own home cage afterward. After acclimation period, rats in the SIP BNST and SIP Random groups underwent SIP conditioning, where pellets were delivered under fixed-time schedule in the conditioning cage (one pellet every minute; dispenser from Coulbourn Instruments) for 1 h daily (SIP conditioning). Rats in the Control BNST group, on the other hand, continued their Control conditioning. These conditionings last for 19 consecutive d (days 1–19). On days 20–25 (intervention period), each rat continued its original conditioning and received 3 d (in random order) of electrical stimulation in the implanted brain region (0.5 mA amplitude of 50 us pulse width at 100 Hz frequency, biphasic, bipolar stimulation, amplitude lowered if noticeable side effect due to stimulation, e.g., epilepsy, observed).

The pedestal cemented on top of the rat's head was connected to a swivel (swivel: SL6C, Plastics One; wire: 363–363 [CS], Plastics One) when the rat entered the conditioning cage. Water intake inside the conditioning cage was monitored, and a webcam (HD Webcam Pro C910, Logitech) was fixed on top of each conditioning cage to record behavior. LFPs in every rat during each conditioning session were recorded daily (NI USB-6341 with LabView SignalExpress 2010, National Instruments).

The control groups for the SIP model, for the electrode location, and for electrical stimulation were chosen as adequate as possible. With regard to controlling for the SIP model, differences in drinking behavior (e.g., frequency or total number of drinking bouts) may appear between Control BNST and SIP BNST conditions. But rats in the Control BNST condition are supposed to have normal drinking behavior, whereas SIP BNST condition provokes abnormal drinking behavior, modeling compulsion.

Electrode location is controlled by implanting electrodes in random brain targets, different from BNST. It can never be excluded that 1 randomly chosen brain target still is part of a relevant pathway for OCD. To ensure that most rats are stimulated in pathways not relevant for OCD, each rat received an electrode in a different randomly selected brain region in the SIP Random condition.

owns a patent on deep brain stimulation for obsessive-compulsive disorder. The remaining authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.1872-15.2016

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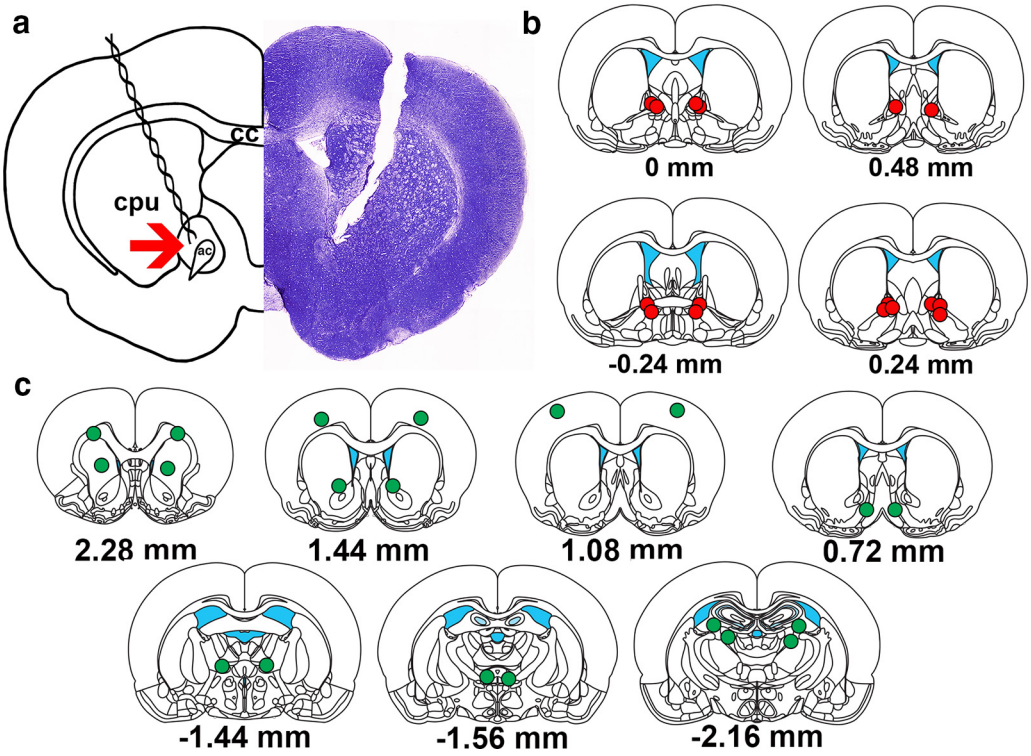


Figure 1. Histology examination. ***a***, Example coronal brain slice of cresyl violet staining (right) and graphic illustration (left) of anatomical structures, showing twisted bipolar electrode tip located in the BNST (indicated by the red arrow). ac, Anterior commissure; cc, corpus callosum; CPu, caudate-putamen. ***b***, ***c***, Histological reconstruction of (***b***) electrode placements in the BNST and (***c***) control brain regions. Numbers represent distance (± rostral/caudal) from bregma. Control brain regions include the following: corpus callous (2.28 mm), alveus of the hippocampus (−2.16 mm), nucleus of the vertical limb of the diagonal band (0.72 mm), reticular thalamic nucleus (−1.44 mm), laterodorsal thalamic nucleus (dorsomedial part) (−2.16 mm), nucleus accumbens core (1.44 mm), paraxiphoid nucleus of thalamus (−1.56 mm), primary motor cortex (1.44 mm), primary sensory cortex (hindlimb) (1.08 mm), and caudate-putamen (striatum) (2.28 mm). Figures adapted with permission from Paxinos and Watson (2007).

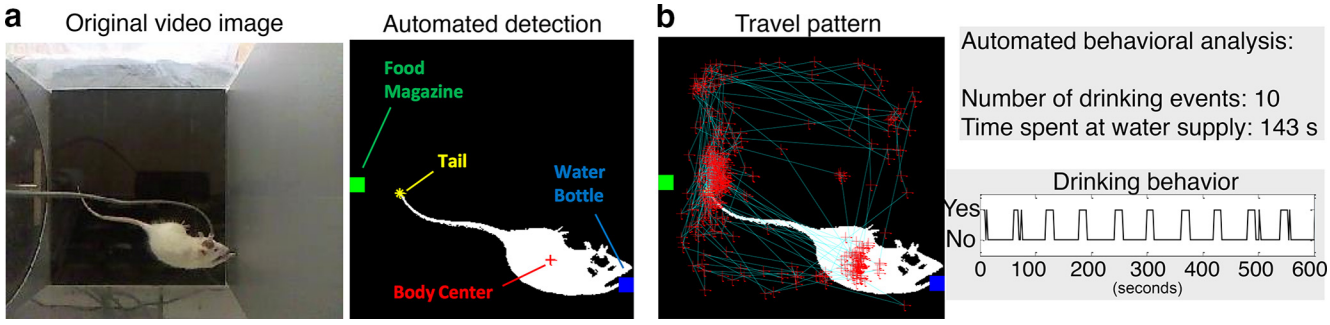


Figure 2. Automated rat detection and behavioral analysis. ***a***, Left, Original video image of a rat inside conditioning cage. Right, Automated recognition of rat's tail and body center, and locations of food and water supplies. ***b***, Behavioral data of 600 s of video showed repeated and patterned drinking behavior occurring in a rat subjected to schedule-induced polydipsia. Red crosses represent body centers of rat. Connecting blue lines indicate travel trajectories. Green and blue squares represent locations of food and water supplies, respectively.

Histology. Histology examination (cresyl violet staining) revealed the actual locations of implanted electrodes. Figure 1*a*, *b* shows an example of coronal brain slice with electrode implanted in the BNST, and the histological reconstruction of the electrode placements in the BNST, respectively. Random control brain regions included the following: corpus callosum, alveus of the hippocampus, nucleus of the vertical limb of the diagonal band, reticular thalamic nucleus, laterodorsal thalamic nucleus (dorsomedial part), accumbens nucleus core, paraxiphoid nucleus of thalamus, primary motor cortex, primary sensory cortex (hindlimb), and caudate-putamen (striatum).

The final numbers of rats in each group were as followed: SIP BNST, $N = 13$; SIP Random, $N = 10$; Control BNST, $N = 7$; 2 dropouts due to electrode breakage and misplacement of electrode in the Control group.

Video processing and behavioral analysis. The processing of video images consisted of two main steps:

Cage and animal detection: Based on the color properties of the cage floor (dark brown), the border of the cage and the exact locations of drinking and feeding tubes were extracted. The rat could be recognized using a specific threshold for near white pixel values, and for each video sample, the threshold was automatically recalculated depending on the contrast of the video. After fixing the threshold to distinguish between rat and background, a binary image was obtained (Fig. 2*a*).

Quantification of behavioral parameters: Based on automated rat detection, two main behavioral parameters were determined per second: rat location in cage in function of time (body center in x and y direction in centimeters), and drinking behavior in function of time (0 or 1). Every frame in which the rat touched the drinking tube with its head was defined as a drinking behavior (Fig. 2*b*).

With information regarding drinking behavior at hand, we were able to put time stamps on each drinking event. A drinking event could be

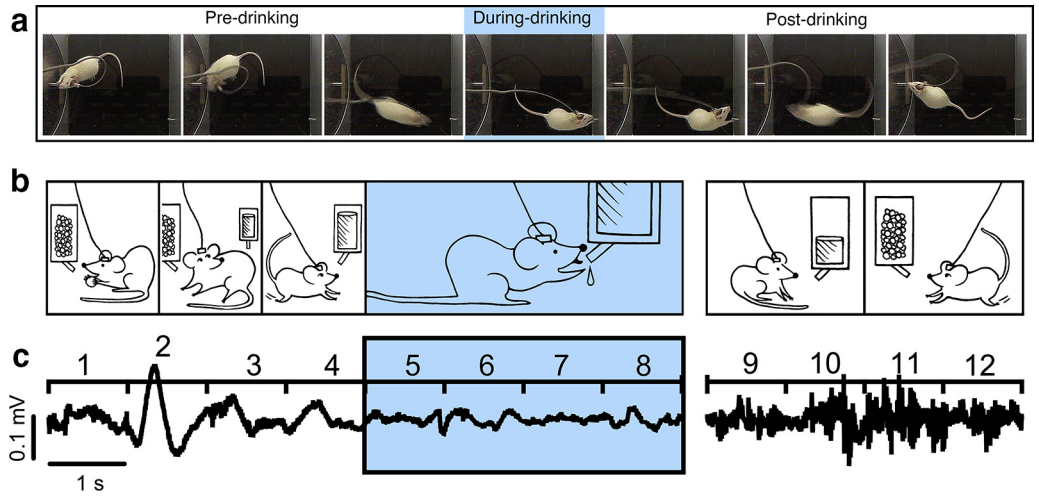
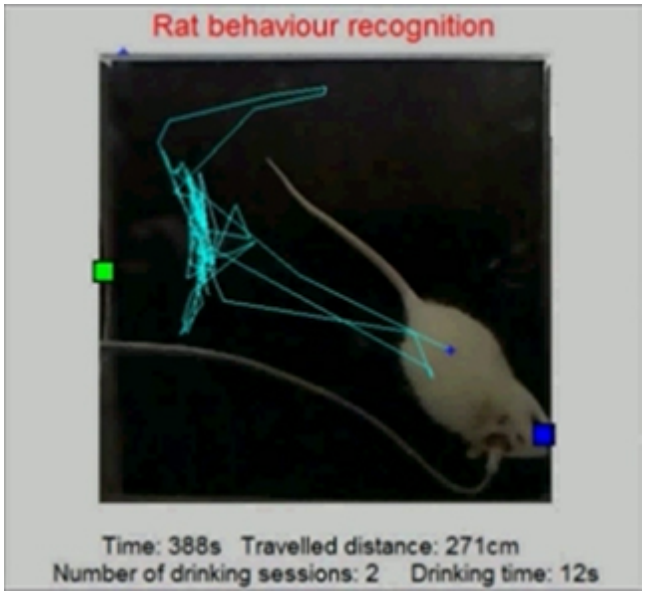


Figure 3. Examples of extracted drinking event and corresponding LFP. During drinking moments are indicated in blue background. ***a***, Example of a drinking event in video images. ***b***, Illustration of a typical SIP-conditioned drinking event, consisting of predrinking, during drinking, and postdrinking periods. ***c***, Example of extracted LFPs of one rat (from the SIP BNST group) corresponding to a drinking event (12 time windows, each window 1-s-long; windows 1–4: four consecutive seconds before drinking began; windows 5–8: first four consecutive seconds during drinking; windows 9–12: four consecutive seconds after drinking stopped).



Movie 1. Automated behavioral analysis of a SIP-conditioned rat. The rat waited in front of the food tube for the food pellet (green square represents location of food tube), and after receiving the food pellet, immediately traveled to the water tube (blue square) and drank water. +, body center of the rat; connecting −, travel trajectory. This movie was processed at 1 frame per second. The first 25 s of this movie is played at normal speed, and the rest is played at an increased speed.

either normal or compulsive, depending on the group and day, and consisted of the following 12 s: 4 consecutive seconds before drinking behavior began, first 4 consecutive seconds during drinking behavior, and 4 consecutive seconds after drinking behavior stopped (Fig. 3*a*), with the following constraint: the interval during which the rat's head touched the water tube must exceed 6 s. Video processing was performed using the MATLAB software package (The MathWorks) and custom scripts. Movie 1 shows an example of automated behavioral analysis of a SIP-conditioned rat.

LFP analysis. One hour daily recordings of brain activity at a frequency of 10 kHz were available for a set of 30 rats, from a pair of bipolar electrodes placed in each hemisphere.

To extract information in the LFP range, the signals were low-pass filtered with cutoff frequency of 200 Hz (fourth-order Butterworth filter). Power line interference was observed, and notch filters were implemented to reject noise at 50 Hz and the respective harmonics (up to 250 Hz). Given the frequency range of interest (1–45 Hz), we downsampled the signal to 500 Hz. An additional high-pass filter (cutoff frequency of 1 Hz) was implemented to reject possible movement-related artifacts (sixth-order Butterworth).

Based on the time stamps of drinking events, identification of relevant segments for the LFP analysis was possible. LFPs corresponding to drinking events from all rats during Baseline (first 3 d during conditioning period) and Conditioned periods (last 3 d during conditioning period) were extracted. Figure 3*b* shows the concept of synchronized 12 s behavioral and LFP data.

The short-time Fourier transform was used to approximate the power spectrum in time-frequency domain. We applied a window of 1 s, sliding it every 200 ms, on each 4 s interval independently (predrinking, during drinking, and postdrinking). The resolution in frequency was 1 Hz. For each time bin, we summed up the power content of the respective bands of interest: δ (1–4 Hz), θ (4–8 Hz), α (8–12 Hz), β (12–30 Hz), and lower γ (30–45 Hz) and normalized it with respect to the total content of power in the 1–45 Hz range. The result was an approximate time distribution of percentage power contributions per frequency band, averaged for each second, during all drinking events. For each rat, group means of power distributions per frequency band were generated for both hemispheres during Baseline and Conditioned periods. LFP processing was performed using the MATLAB R2015a software package and custom scripts.

Statistical analysis. We performed paired *t* tests on the mean water intake of Baseline and Conditioned to examine the effect of SIP and Control conditionings on drinking behavior, and the effect of high-frequency electrical stimulation on water intake (stimulation on vs off during intervention period).

For LFP analyses, we first compared the overall normalized powers during normal and compulsive drinking in the left and right BNST and control brain regions (ANOVA). To further investigate the change of frequency oscillation powers over the time course of compulsive and normal drinking, repeated-measures ANOVA was used to test the main effect of time (second, from precompulsion, during compulsion, to postcompulsion) on the normalized power of each frequency band in left and right BNST during compulsive drinking. Frequency bands in the BNST that showed significant changes during compulsive drinking were put to further repeated-measures ANOVA to test the main effect of groups (SIP BNST, SIP RND, and CON BNST) on normalized band powers, to de-

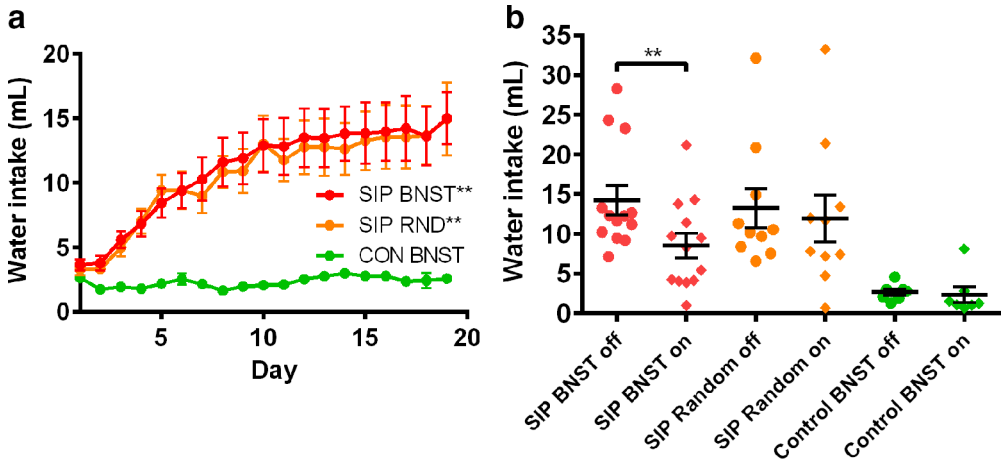


Figure 4. Effects of SIP and Control conditionings, and electrical stimulation on water intake. ***a***, Mean water intake in the SIP BNST group and the SIP Random group increased significantly after 19 consecutive days of conditioning. ***b***, Electrical stimulation in the BNST (SIP BNST on) significantly reduced compulsive drinking behavior but had no effect on normal drinking behavior (Control BNST on) or on compulsive drinking when applied in random control brain regions (SIP Random on). Data are mean ± SEM. $^{**}p < 0.01$.

termine whether these changes were specific to compulsive drinking and not normal drinking, and were in the BNST and not in other brain regions. Further repeated-measures ANOVA (main factor: Baseline vs Conditioned) was performed to investigate whether these changes in normalized power during compulsive drinking were present in baseline recordings in the same group of rats before they developed compulsive drinking. Finally, we performed one-way repeated-measures ANOVA *post hoc* pairwise comparison (with Bonferroni correction) in the SIP BNST group in frequency bands showing unique significant changes in previous analyses, to characterize the trend of normalized power before, during, and after compulsive drinking. To determine whether these changes in normalized band power can be used as an indicator for intervention efficacy, we calculated Pearson correlation coefficients (r) between percentage change (peak-to-peak value) in normalized power during compulsive drinking in the BNST and the effect of electrical stimulation (in terms of suppression of compulsive drinking). We used Statistica (StatSoft) to perform the statistical analysis (level of significance set at 0.05 for all tests).

Results

Effect of BNST high-frequency electrical stimulation on compulsion

We evaluated the effect of high-frequency electrical stimulation on drinking behavior as follows: Water intakes during Baseline (days 1–3) and Conditioned (days 17–19) periods: SIP BNST: Baseline (mean ± SEM): 4.34 ± 0.52 ml; Conditioned: 14.29 ± 2.21 ml; paired t test: $p = 0.0001$ ($t = 5.55$); SIP Random: Baseline: 3.88 ± 0.40 ml; Conditioned: 13.59 ± 2.48 ml; paired t test: $p = 0.001$ ($t = 4.85$); CON BNST: Baseline: 2.11 ± 0.18 ml; Conditioned: 2.47 ± 0.40 ml, paired t test: $p = 0.63$ ($t = 0.50$) (Fig. 4*a*).

Water intakes of SIP BNST, SIP Random, and Control BNST groups during electrical intervention period were as follows: SIP BNST: stimulation off: 14.25 ± 1.83 ml; stimulation on: 8.53 ± 1.56 ml; paired t test: $p = 0.008$ ($t = 3.19$); SIP Random: stimulation off: 13.21 ± 2.48 ml; stimulation on: 11.95 ± 2.95 ml; paired t test: $p = 0.24$ ($t = 1.26$); Control BNST: stimulation off: 2.66 ± 0.40 ml; stimulation on: 2.34 ± 0.99 ml; paired t test: $p = 0.67$ ($t = 0.45$) (Fig. 4*b*).

Based on individual responses to electrical stimulation, nonresponders (<25% decrease in water intake, $n = 5$) in the SIP BNST group were removed from further LFP analyses.

Frequency domain analyses of LFPs during compulsive and normal drinking

We first investigated the normalized power of each frequency band during the entire compulsive and normal drinking event in the left and right BNST and control brain regions (Fig. 5*a*,*b*). One-way ANOVA indicated that δ ($p = 0.001$, $F = 10.435$), θ ($p = 0.01$, $F = 5.775$), β ($p < 0.001$, $F = 14.711$), γ ($p = 0.009$, $F = 5.940$) oscillations in the right hemisphere, and θ ($p = 0.045$, $F = 3.575$) and β ($p = 0.014$, $F = 5.227$) in the left were significantly different between groups.

After identification of compulsive and normal drinking events, and extraction and frequency analysis of corresponding LFPs in the left and right BNST and control brain regions from all rats, we first wanted to identify frequency bands that were changing significantly during compulsive drinking in the BNST. One-way repeated-measures ANOVA showed that the normalized powers of δ ($p = 0.002$, $F = 14.243$), α ($p = 0.028$, $F = 4.231$), β ($p = 0.002$, $F = 11.717$), and γ ($p = 0.002$, $F = 14.164$) bands in the left BNST, and δ ($p = 0.007$, $F = 10.365$), β ($p = 0.004$, $F = 11.832$), and γ ($p = 0.002$, $F = 12.398$) bands in the right BNST fluctuated significantly during compulsive drinking (Mauchly's test of sphericity violated, and Greenhouse-Geisser corrected in all tests). We then performed two-way repeated-measures ANOVA on normalized powers of these frequency bands in the BNST and control brain regions, during compulsive and normal drinking, to test whether these changes were specific to compulsive drinking in the BNST. Statistical results indicated significant between-group effect in left β ($p = 0.014$, $F = 5.227$), and right δ ($p = 0.001$, $F = 10.435$), β ($p < 0.001$, $F = 14.711$), and γ ($p = 0.009$, $F = 5.940$) bands (Mauchly's test of sphericity violated, and Greenhouse-Geisser corrected in all tests; Fig. 5*c*–*i*). We further compared the changes in normalized powers in these bands in the BNST during compulsive drinking with baseline recordings (same rats, normal drinking before they developed compulsive drinking), and two-way repeated-measures ANOVA revealed that normalized powers of right δ ($p = 0.002$, $F = 21.713$), β ($p = 0.006$, $F = 15.563$), and γ ($p = 0.016$, $F = 10.012$) in the BNST during compulsive drinking were significantly different from baseline (Mauchly's test of sphericity violated, and Greenhouse-Geisser corrected in all tests; Fig. 6*a*–*c*).

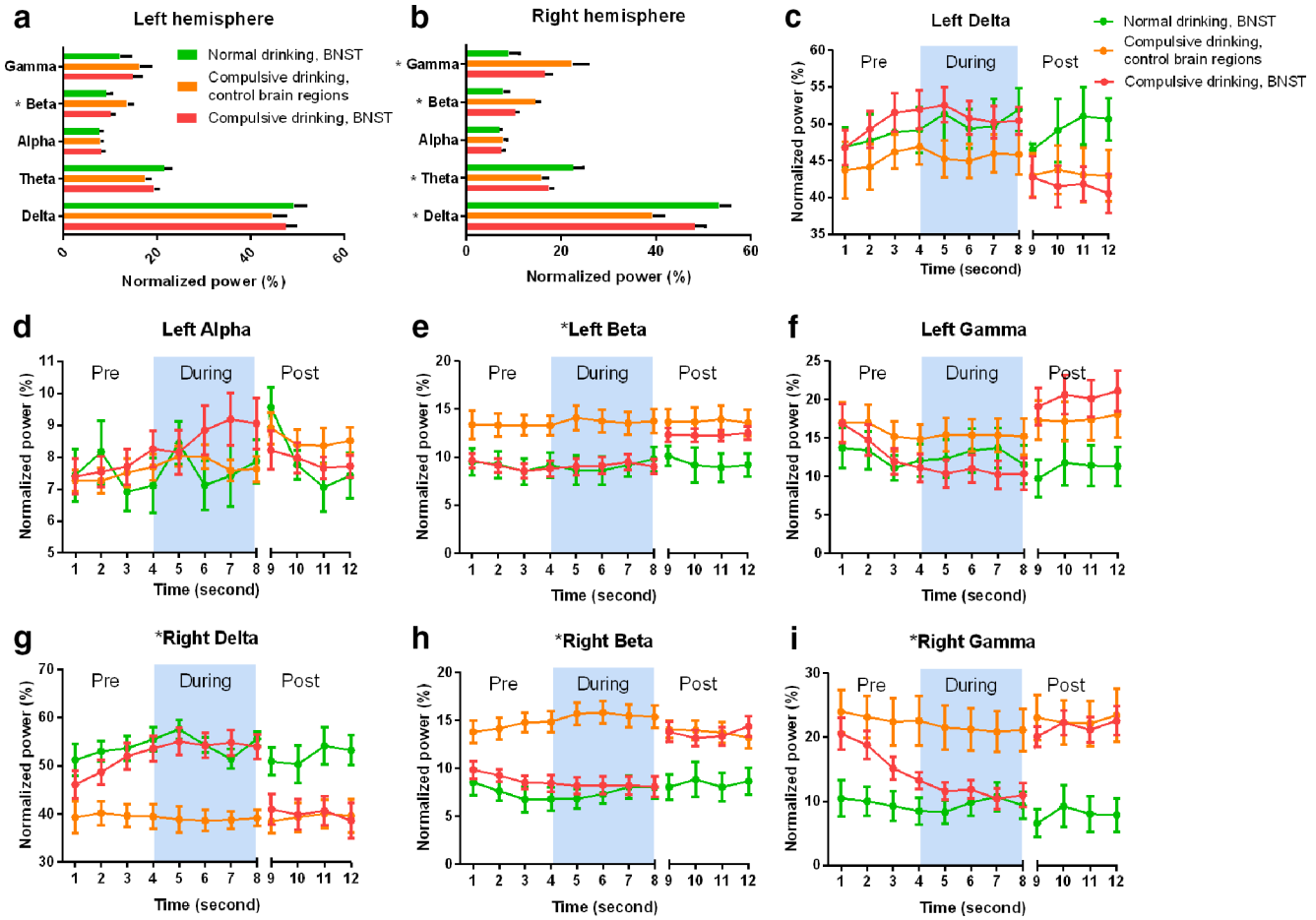


Figure 5. ***a***, ***b***, Gross comparisons of normalized power of δ, θ, α, β, and lower γ bands recorded in the BNST and control brain regions during compulsive and normal drinking. Generally speaking, normalized powers in the right hemisphere seemed to show significant differences between groups in more frequency bands than the left hemisphere. ***c–i***, Comparisons of change in normalized powers in the BNST and control brain regions before, during, and after compulsive and normal drinking showed that left β, right δ, right β, and right γ bands were significantly different between groups. Data are mean ± SEM. *$p < 0.05$.

Post hoc pairwise between-group comparisons showed that normalized powers of δ, β, and γ bands in the right BNST during compulsive drinking were significantly different from those of compulsive drinking in control brain regions (δ: $p = 0.021$, β: $p = 0.006$, γ: $p = 0.0407$), and normal drinking in the right BNST (δ: $p = 0.0488$, β: $p = 0.0379$, γ: $p = 0.0287$). Further *post hoc* pairwise between-group comparisons during the time course of drinking events suggested that normalized δ power in the right BNST was significantly higher than in control brain regions before and during compulsive drinking (Fig. 6*d*) and was significantly lower than normalized δ power in the right BNST after normal drinking (Fig. 6*e*). Normalized β and γ power in the right BNST, on the other hand, was showing the opposite trend: significantly lower than in control brain regions before and during compulsive drinking (Fig. 6*f*,*g*) and significantly higher than normalized powers in the right BNST after normal drinking (Fig. 6*h*,*i*). *Post hoc* pairwise within-group comparison revealed that normalized δ power in the right BNST was significantly higher during compulsive drinking compared with the postdrinking period, normalized β power in the right BNST was significantly lower predrinking and during compulsive drinking compared with postdrinking, and normalized γ power in the right BNST was significantly higher predrinking and postdrinking compared with during compulsive drinking (Fig. 7*a–c*, raw data included).

Last, the peak-to-peak change in the right BNST normalized δ, β, and γ powers during compulsive drinking correlated with the effect of electrical stimulation in the BNST on compulsive drinking (δ: $r = 0.71$, $p = 0.049$; β: $r = 0.84$, $p = 0.0091$; γ: $r = 0.89$, $p = 0.0029$; Fig. 7*d–f*; the locations of electrodes in relation to bregma in the SIP BNST group are also shown in these figures).

Discussion

Effects of different conditionings and electrical stimulation in different brain regions on drinking behaviors

After 19 consecutive conditioning days, SIP conditioning significantly increased water intake, whereas Control conditioning showed no significant effect. Results from electrical stimulation showed suppression of compulsive drinking but not normal drinking when stimulation was applied in the BNST. These results were in alignment with previous reports (van Kuyck et al., 2008; Moreno and Flores, 2012). Moreover, we have shown that electrical stimulation had no effect on compulsive drinking in SIP-conditioned rats when applied in random brain locations.

Interpreting LFPs in the BNST during compulsive drinking

Drinking events in Control-conditioned rats represented normal drinking behaviors, whereas in SIP-conditioned rats, they represented the course of compulsion during the last 3 d of conditioning period. Based on our hypothesis, the normalized power of

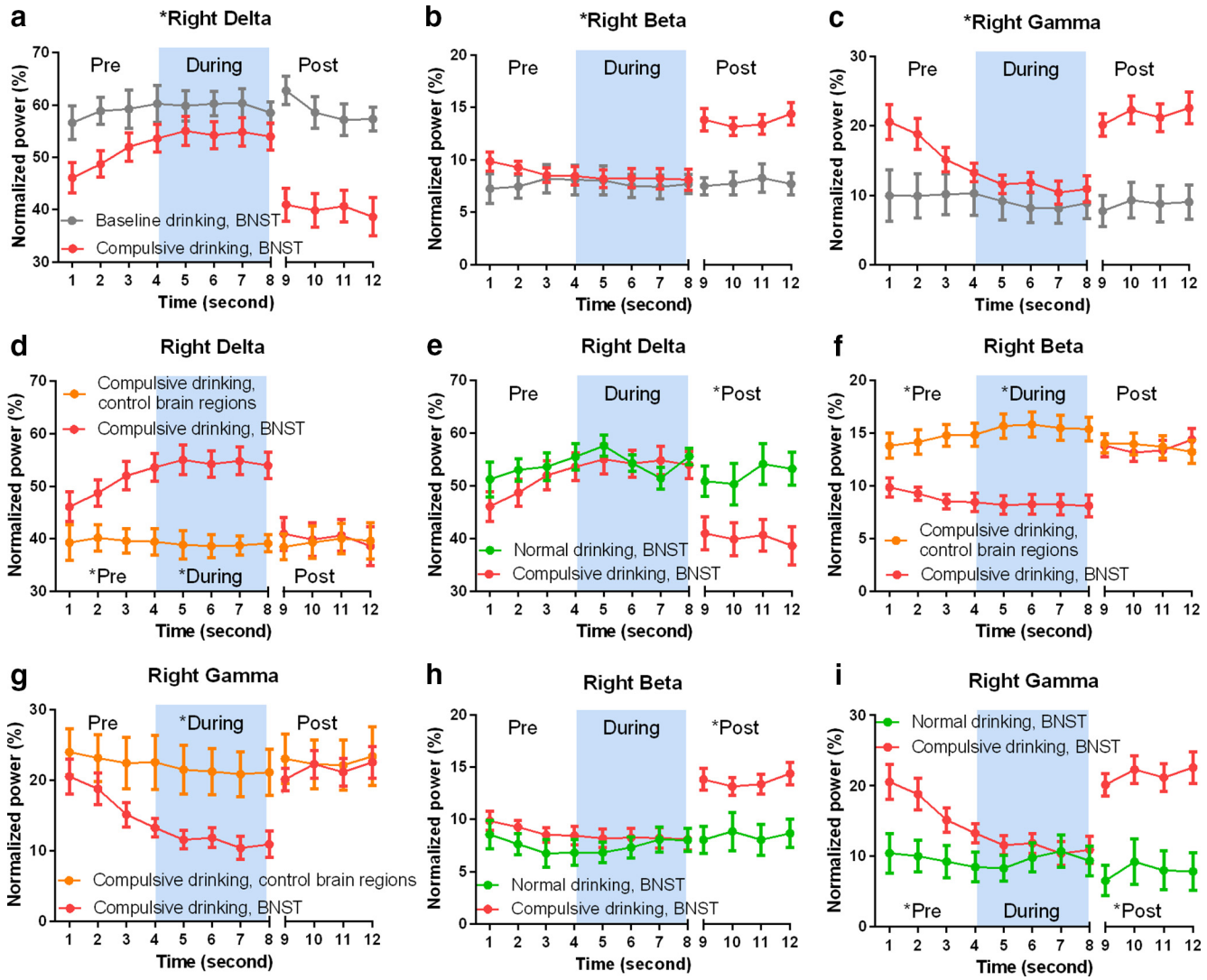


Figure 6. ***a–c***, By comparing with normal drinking during Baseline recordings, we found that normalized powers in δ, β, and γ bands in the right BNST were significantly different during compulsive drinking in the same cohort of rats. ***d***, ***e***, Normalized δ power in the right BNST was significantly higher than in control brain regions before and during compulsive drinking, and was significantly lower than normalized δ power in the right BNST after normal drinking. ***f–i***, Normalized β and γ powers in the right BNST, showing the opposite trend to δ: significantly lower than in control brain regions before and during compulsive drinking, and significantly higher than normalized powers in the right BNST after normal drinking. Data are mean ± SEM. *$p < 0.05$.

LFP frequency band(s) in the SIP BNST group should be exclusively associated with compulsive drinking.

Comparisons of normalized powers during the entire drinking event between groups indicated several frequency bands of potential interest (Fig. 5*a–c*). Initial statistical analysis indicated that δ, β, and γ power in the right BNST and δ, α, β, and γ in the left BNST showed unique changes during the course of compulsive drinking. Further analyses showed that only the normalized δ, β, and γ powers in the right BNST were specific to compulsive drinking (Figs. 5*d–i*, 6). Normalized δ power increased just seconds before compulsion (during which the cue, i.e., the food pellet, arrived), peaked during compulsion, and decreased after compulsion (Fig. 7*a*). Normalized β power remained at lower lever before and during compulsion but increased after compulsion (Fig. 7*b*). Normalized γ power decreased from before to during compulsion, and increased after compulsion (Fig. 7*c*). Welter et al. (2011) also reported similar results (higher and lower relative proportions of neurons displaying δ and β oscillations, respectively) recorded in the subthalamic nucleus in OCD patients. The identification of three seemingly inverse frequency band responses in the BNST during compulsion may complement recent studies (Jennings et al., 2013; Kim et al., 2013), showing multiple distinct neuronal groups, some activated while others inhibited, in the BNST during anxiety-like behavior. The correlations between the percentage change in the BNST δ, β, and γ powers during the course of compulsion and the effect of BNST electrical stimulation on compulsion further suggested a link between the BNST field potentials and compulsion (the more closely the electrodes were placed near the neural region related to compulsion, the more effective electrical stimulation was, and the more change in certain oscillations we observed; Fig. 7*d–f*). We also investigated the correlation between locations of implanted electrodes relative to bregma and stimulation efficacy in the SIP BNST group, and the results showed that electrical stimulation applied in electrodes implanted in the BNST caudal to bregma seemed more effective in suppressing compulsion than rostral to bregma.

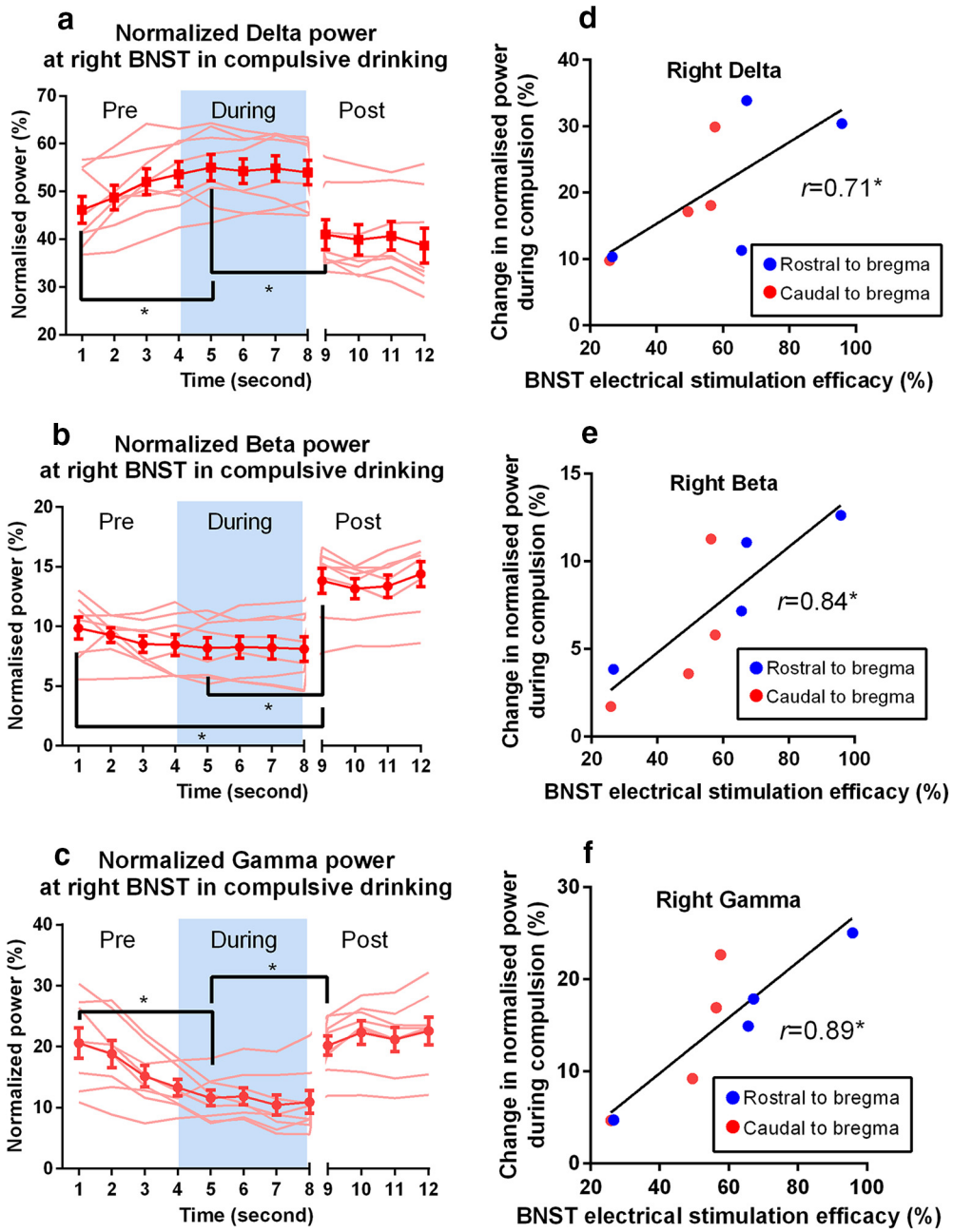


Figure 7. ***a–c***, Comparisons of normalized powers of δ, β, and γ bands in the right BNST before, during, and after compulsive drinking (raw data in pink). ***a***, Normalized δ power in the right BNST was significantly higher during than before and after compulsive drinking. ***b***, Normalized β power in the right BNST was significantly higher after than before and during compulsive drinking. ***c***, Normalized γ power in the right BNST was significantly lower during than before and after compulsive drinking. ***d–f***, Peak-to-peak change in the right BNST normalized δ, β, and γ powers during compulsive drinking showed correlation with the effect of electrical stimulation in the BNST on compulsive drinking, further suggesting a link between the BNST field potentials and compulsion. Colors of each data point represent locations of implanted electrodes in relation to bregma: blue represents caudal to bregma; red represents rostral to bregma. Data are mean ± SEM. $*p < 0.05$. *r*, Pearson correlation coefficient.

Figure 8 shows examples of raw LFPs recorded in the right BNST in compulsive and normal drinking, and in a control brain region in compulsive drinking. We could see the changes described above: δ oscillations occurring in the initiation phase of compulsion, reached its normalized peak during compulsion, and meanwhile, signals became "clean," which meant decreases in higher-frequency oscillations (i.e., γ band).

General remarks, conclusions, and future directions

The disease mechanism of OCD and the clinical treatment mechanism of high-frequency electrical stimulation in the BNST are not clearly understood at present. It is generally believed that anxiety level increases when the urge of obsession/compulsion appears, and decreases after obsession/compulsion is performed. By interfering pathological neural activity, high-frequency electrical stimulation in the limbic system may reduce the level of pathological anxiety in patients, which may explain its efficacy in OCD and anxiety disorders (Ressler and Mayberg, 2007).

The BNST is not only mediating the processing of anxiety (Davis et al., 2010) but is also involved in reward and motivation circuitry via direct and indirect connections to the basolateral

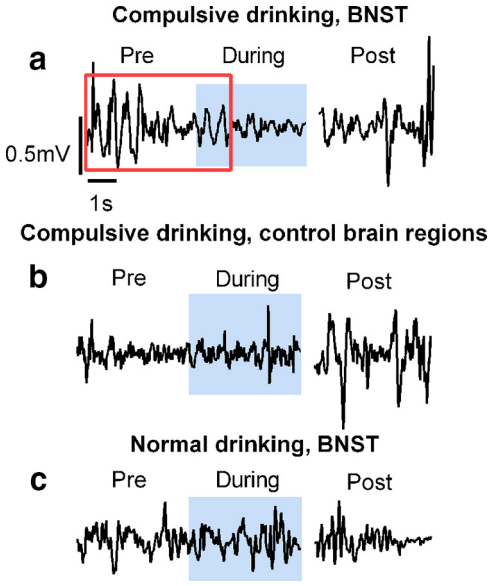


Figure 8. Examples of raw LFPs recorded from the right BNST during compulsive (***a***) and normal drinking (***c***), and from control brain regions during compulsive drinking (***b***). Strong δ oscillation was observed (***a***, red rectangle) in the right BNST during the initiation phase of compulsive drinking.

amygdala, the ventral tegmental area (VTA), and the nucleus accumbens (Walker et al., 2003; Poulos et al., 2010; Stuber et al., 2011; Lammel et al., 2012). With inputs from the infralimbic cortex and the ventral subiculum, projections from the BNST to the VTA dopaminergic neurons regulate reward and motivation circuitry (Georges and Aston-Jones, 2001; Jalabert et al., 2009; Kudo et al., 2012). Increased δ activity in the VTA in rats under aversive environment has been reported (Friedman et al., 2012). BNST glutamatergic projection neurons displayed a net enhancement of activity to aversive stimuli, whereas the firing rate of identified GABAergic projection neurons was suppressed (Jennings et al., 2013). Injection of orexin-A, a strong excitatory neuropeptide, in the BNST also induced anxiety-like measures (Lungwitz et al., 2012).

Our results indicated that the right BNST was more relevant to compulsive behavior, coinciding our previous research results reporting higher bust index in the right BNST in SIP-conditioned rats (Welkenhuysen et al., 2013). It has also been suggested clinically that right-hemisphere capsulotomy is more effective than left-hemisphere for refractory anxiety disorders (Rück et al., 2005).

Several clinical studies were conducted to record direct neural activity invasively in patients with OCD. Single-unit recordings in the subthalamic nucleus in OCD patients found the burst and oscillatory activities mainly in the associative-limbic part, with lower discharge frequency compared with recordings from patients with Parkinson's disease (Piallat et al., 2011; Welter et al., 2011). LFP recordings in the subthalamic nucleus in OCD patients showed significant changes during acute obsessive-compulsive symptoms in different frequency bands (Bastin et al., 2014). Our group previously participated in a clinical study recording LFP in the BNST in OCD and major depressive disorder, and found a distinct pattern of oscillatory activity significantly higher in the α band in patients with major depressive disorder but not in OCD (Neumann et al., 2014). However, the LFP was recorded under resting state without symptom provocation.

Many questions arise from our experimental results. If the δ, β, and γ powers were the biomarkers of OCD, could they be influenced via means of pharmaceutical or neurosurgical intervention? Which subdivision of the BNST is most related to OCD? Which groups of the BNST neurons are responsible for these power changes in OCD? Do these biomarkers exist in other animal models of OCD? Is it a distinguished characteristic of OCD, or does it also exist in other psychiatric disorder (e.g., anxiety disorders)? Is it specifically pathological, or does it exist in normal psychophysiological conditions, too?

In conclusion, we have shown that the LFPs in the BNST contain relevant information associated with compulsion. Often there is a symptom trigger in OCD (a stairway triggering obsessive counting; a door triggering compulsive checking). This event-dependent characteristic makes OCD an ideal indication for intervention with real-time feedback, such as closed-loop deep brain stimulation. Stimulation in the BNST region has already been applied to treat otherwise-refractory OCD patients (Greenberg et al., 2010), but currently only with constant stimulation settings. Superiority of closed-loop over fixed deep brain stimulation has been shown in Parkinson's disorder (Rosin et al., 2011). Our current findings reveal neurophysiological biomarkers and underlying mechanism of compulsion, and warrant further research of the use of LFP for closed-loop neuromodulation in OCD.

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