

BEHAVIOURAL NEUROSCIENCE

Deep brain stimulation in the central nucleus of the amygdala decreases ‘wanting’ and ‘liking’ of food rewards

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Abstract

We investigated the potential of deep brain stimulation (DBS) in the central nucleus of the amygdala (CeA) in rats to modulate functional reward mechanisms. The CeA is the major output of the amygdala with direct connections to the hypothalamus and gustatory brainstem, and indirect connections with the nucleus accumbens. Further, the CeA has been shown to be involved in learning, emotional integration, reward processing, and regulation of feeding. We hypothesized that DBS, which is used to treat movement disorders and other brain dysfunctions, might block reward motivation. In rats performing a lever-pressing task to obtain sugar pellet rewards, we stimulated the CeA and control structures, and compared stimulation parameters. During CeA stimulation, animals stopped working for rewards and rejected freely available rewards. Taste reactivity testing during DBS exposed aversive reactions to normally liked sucrose tastes and even more aversive taste reactions to normally disliked quinine tastes. Interestingly, given the opportunity, animals implanted in the CeA would self-stimulate with 500 ms trains of stimulation at the same frequency and current parameters as continuous stimulation that would stop reward acquisition. Neural recordings during DBS showed that CeA neurons were still active and uncovered inhibitory-excitatory patterns after each stimulus pulse indicating possible entrainment of the neural firing with DBS. In summary, DBS modulation of CeA may effectively usurp normal neural activity patterns to create an ‘information lesion’ that not only decreased motivational ‘wanting’ of food rewards, but also blocked ‘liking’ of rewards.

Introduction

Could deep brain stimulation (DBS) treat addiction (Rouaud *et al.*, 2010; Heldmann *et al.*, 2012) or obesity (Sani *et al.*, 2007; Melega *et al.*, 2012)? Here, we assess the central nucleus of the amygdala (CeA) as a potential target for neuromodulation of reward dysfunction to mimic DBS treatments for Parkinson’s disease (Schwalb & Hamani, 2008; Gubellini *et al.*, 2009; Weaver *et al.*, 2009; Doshi, 2011) and other disorders (Chang, 2004; Gubellini *et al.*, 2009) including OCD (Haber & Brucker, 2009; Greenberg *et al.*, 2010), depression (Mayberg *et al.*, 2005; Lozano *et al.*, 2008), and epilepsy (Halpern *et al.*, 2008).

DBS mechanisms remain an enigma although it is thought to be due to cell inhibition and axon activation (Garcia *et al.*, 2005a; Kringelbach *et al.*, 2007; Okun, 2012). Modeling suggests that

high-frequency stimulation (> 100 Hz) may impose a regular pattern in neural circuits effectively ‘masking’ pathological activity patterns (Grill *et al.*, 2004; Johnson & McIntyre, 2008). Neural recording during stimulation could provide useful insight into DBS mechanisms.

Here, we targeted the central nucleus of the amygdala (CeA), which has connections to cortical and subcortical areas involved in processing taste, food consumption, and reward including the lateral hypothalamus, ventral tegmental area (Sah *et al.*, 2003), parabrachial nucleus and nucleus of the solitary tract (Ricardo & Koh, 1978) and indirect connections with the nucleus accumbens and ventral pallidum (Price & Amaral, 1981). The CeA is composed largely of medium-sized spiny neurons, like the ventral striatum (McDonald & Augustine, 1993) and its afferents and efferents are primarily GABA-ergic (Swanson & Petrovich, 1998). The CeA is involved in reward learning, reward processing, and regulation of feeding (Baxter & Murray, 2002), although its specific role is still debated (Cai *et al.*, 2014). Incentive motivational mechanisms may play a role as

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decreased food consumption occurs with CeA lesions and inactivation (Kemble *et al.*, 1979; Seeley *et al.*, 1993; Touzani *et al.*, 1997; Will *et al.*, 2004, 2009) along with increased food intake with opioid stimulation (Mahler & Berridge, 2009; DiFeliceantonio & Berridge, 2012). Furthermore, CeA activation may focus and intensify incentive motivation (Robinson *et al.*, 2014). The hedonic functions of CeA are less clear with reports of both increased aversive reactions to bitter tastes with CeA lesions (Touzani *et al.*, 1997), as well as reports of unchanged 'liking' or 'disliking' reactions (Kemble *et al.*, 1979; Galaverna *et al.*, 1993; Mahler & Berridge, 2011) although incentive control by DBS would still be fortuitous.

In this study, we stimulated CeA with DBS-like parameters hypothesizing and demonstrating that we could modulate (i) working for, (ii) approach and (iii) consumption of food rewards. Specifically, we showed that lever pressing for sucrose pellet rewards was reduced by DBS in CeA. The effect was not due to motor disruption, but could be due in part to observed changes in hedonic evaluation of tastes. CeA stimulation made taste stimuli aversive in general. Simultaneous neural recordings during DBS showed fewer neural responses to reward-related behavior.

Materials and methods

Male Sprague-Dawley rats, ~ 8–13 weeks old (250–400 g, Charles River, US) were used as subjects ($n = 20$ total, 11 in experiment 1, 9 in experiment 2). Rats were housed individually on a 9:30 a.m. to 7:30 p.m. reversed light/dark schedule with unrestricted access to standard rat chow and water (animals were paired-housed prior to electrode implantation). Testing was carried out during their dark (active) period. Two experiments were performed to investigate the effects of deep brain stimulation (DBS) in the central nucleus of the amygdala (CeA) on reward behavior. Experiment 1 assessed the effects of DBS on an operant responding task. Experiment 2 assessed the effects of DBS on 'liking' and 'disliking' reactions to tastes. All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee, in accordance with the National Institute of Health's guidelines for the care and use of laboratory animals.

Experiment 1 (operant task)

Apparatus

All training and testing were conducted in a 28 cm × 35 cm × 60 cm plastic chamber illuminated by red light from below with a metal grid (to ground the animal) over a glass floor. The rat's electrode was connected to the recording and stimulating system via a headstage cable and commutator. The commutator enabled the rat to freely move around and explore the chamber. For the operant responding task, two retractable levers (Coulbourn Instruments, Whitehall, PA, US) were attached to one wall of the chamber and located on either side of a food bowl. Touch detectors were connected to both levers in order to assess lever contacts. One of the levers was designated as the reward lever and delivered a sucrose pellet to the food bowl contingent upon lever contacts. The other lever served as a control for general lever-pressing behavior, where responses were recorded, but had no consequences. The location of the reward and control lever was counter-balanced across rats and remained consistent for each rat throughout the study.

In a separate self-stimulation study, levers were retracted and two 2.5-cm-diameter nose-poke holes with photo-beam detectors were exposed on the cage wall. Poking in a designated reward hole

(counter-balanced between animals), triggered intracranial stimulation. Poking the control hole did nothing (timing was recorded).

Delivery of all stimulus and reward presentations, as well as recording of responses were managed by MTASK (in-house program). Electrical stimulation was delivered using a constant-current digital stimulus isolator (A-M systems, Carlsborg, WA, US) with timing (frequency and pulse width) controlled by a LabVIEW (National Instruments, Austin, TX, US) program developed in the lab. Neural activity was recorded during the test sessions using an in-house program, DataTask (written in LabVIEW). Neural signals were amplified ($\times 1000$) and bandpass-filtered between 300 Hz and 6 kHz. This relatively low gain was used to prevent amplifier saturation and consequent lock-out by the large amplitude stimulation artifacts. A video camera underneath the glass floor recorded all behavior. Timestamp clocks (10 μ s accuracy) for the behavior control program, video recording, and neural recording were synchronized to enable subsequent analysis of neural activity related to stimulus presentations and behavioral responses.

Behavioral training

Habituation. Three to five days prior to the start of training, rats were handled for 10–15 min daily and given free access to banana-flavored sucrose pellets (used throughout experiment 1) in their home cages (10–15 pellets per day). One day prior to training, rats were placed in the chamber for 30 min with the levers retracted to acclimatize them to the testing chamber.

Food self-administration paradigm. Ten minutes after an animal was placed in the chamber, both levers extended into the chamber where they remained for the rest of the 40-min session. Training began with a fixed ratio schedule one (FR1) in which each contact of the reward lever delivered one sucrose pellet, followed by a 500-ms timeout. When the rats delivered more than 10 pellets and touched the reward lever three times more than the control lever, they were then trained on a limited progressive ratio paradigm (5 FR1 trials, 5 FR5 trials, and then FR10 trials for the remainder of the session). The successful delivery of five pellets triggered the shift from FR1 to FR5 and again from FR5 to FR10. There was a 500-ms timeout period between lever contacts (Fig. 1a.i). Once animals had obtained average lever responses across three consecutive days, they were implanted with electrodes.

Surgical procedures

Electrodes consisted of two moveable bundles of six wires (two 75- μ m stainless steel stimulating wires, four 50- μ m tungsten recording wires). Each bundle could be lowered independently. Under 1–2% isoflurane anesthesia with oxygen maintenance (1–1.5 L/min), electrodes were fixed to the skull stereotaxically (Tindell *et al.*, 2004) targeting the central nucleus of the amygdala (AP -2.0 mm, ML ± 3.4 mm to ± 3.5 mm, DV 7.0 mm to 8.5 mm) in seven rats. Four surgical/stimulation control rats had electrodes in the ventral pallidum, extended amygdala, medial anterior dorsal nucleus of the amygdala, amygdala striatum transitional area, and inferior to the CeA. Thermal support was provided and vital signs were monitored during the procedure. For pain management, animals were given Flunixin meglumine (2.5 mg/kg subcutaneously) prior to removal from anesthesia and also twice the next day (and subsequent days if needed). A thin layer of lidocaine jelly and bacitracin was placed around the wound edge after closure and the animal was given 1–2 mL of warm sterile saline (0.9%) intraperitoneally to help prevent dehydration. After anesthesia recovery, animals were

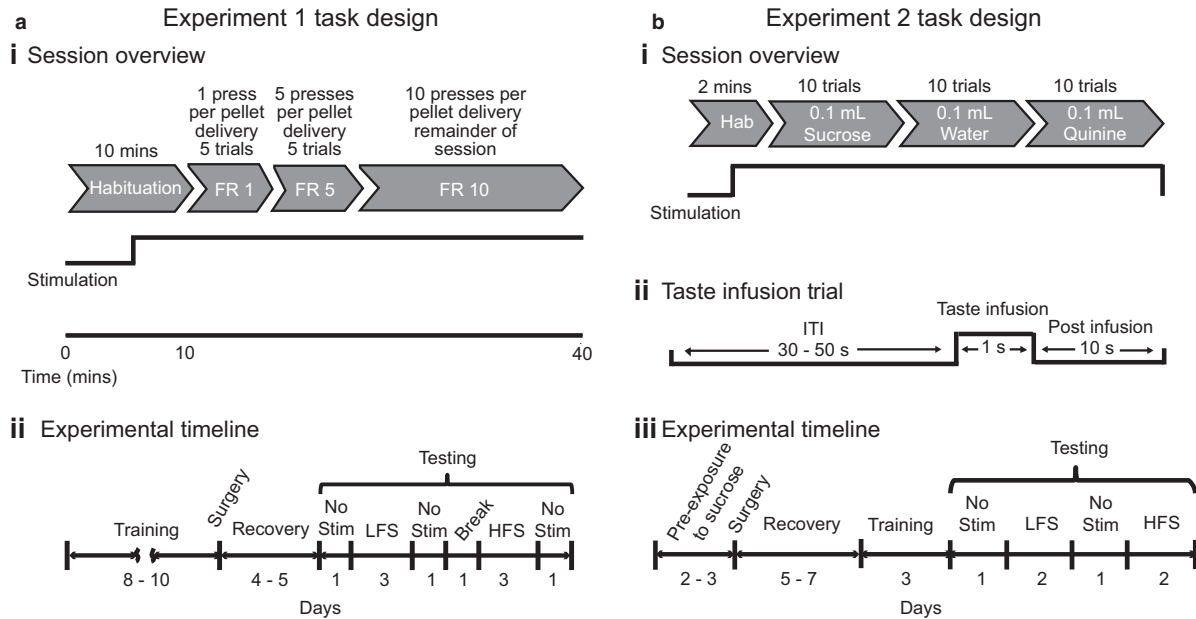


FIG. 1. (a) Experiment 1 task design. (i) Schematic of a limited progressive ratio (FR – fixed ratio) test session. Stimulation was turned on 5 min into the habituation period for DBS test days. (ii) Experimental timeline. (b) Experiment 2 task design. (i) Experimental paradigm for a given test session. For DBS test sessions, stimulation was turned on 1 min into the habituation period. (ii) Schematic of a taste-infusion trial showing timing of the different events (ITI – inter-trial interval). (iii) Experimental timeline. No Stim – test days where no stimulation was delivered, LFS – low frequency stimulation at 20 Hz, HFS – high-frequency stimulation at 130 Hz.

housed individually with free access to food and water. Penicillin G benzathine (15 000 units/daily subcutaneously) was given immediately after surgery and the following day. Rats were given at least 4 days to recover before screening tests.

Screening

Screening tests were used to search for the optimal electrode depth location and lowest effective current. Each screening session consisted of a 1-min habituation period and 10-min operant response paradigm (described in *Behavioral training*). Stimulation was delivered continuously at 130 Hz (see *Stimulation parameters*). If stimulation had no effect on pellet delivery or produced motor side effects (e.g. paw twitching, turning), both electrodes were lowered in increments of 0.16 mm. Electrode advancement stopped when stimulation caused a cessation of pellet delivery and there were no adverse motor effects. Searching was also stopped at 8.5 mm below dura if no site was found. Stimulation current started at 250 μ A but was increased up to 400 μ A, if no effect was observed, or decreased to a lower current. Four screening tests were done per day with stimulation absent for one of the sessions (to provide a daily baseline response). Once the effective site and stimulation amplitude were found, rats were given 2 days off and then moved on to the limited progressive ratio test described below.

Testing DBS-like stimulation

The effect of stimulation on a limited progressive ratio operant responding task (described above under *Food self-administration paradigm*) was tested in 40-min sessions, one per day (Fig. 1a.i). A testing sequence consisted of three consecutive days of high-frequency stimulation (130 Hz), three consecutive days of low frequency stimulation (20 Hz), and 3 days without stimulation (Fig. 1a.ii). A two-day break was given between high and low frequency days to minimize any potential lingering effects of DBS

(Gubellini *et al.*, 2009). Rats were randomly selected to begin testing with either the high or low frequency stimulation block and no stimulation days were interspersed throughout the 2-week testing period. On stimulation days, stimulation was turned on 5 minutes into the habituation period to allow animals to acclimatize to the stimulation and to provide a period of assessment with stimulation before the behavioral task began. In some cases, extra test days were used to record more neural data.

In order to determine the threshold frequency that resulted in decreased operant responses, additional tests were run on two of the rats implanted in the CeA. These two rats were given a break of several days after the main experiment, then they were tested on the same behavioral task as described above but with lower frequencies of stimulation: 2 Hz, 4 Hz, 10 Hz, and 16 Hz. Each frequency was tested (on a separate day) and repeated and the number of lever contacts and pellets consumed were measured. Rats were also retested with 20 Hz stimulation to verify that stimulation at this frequency still resulted in decreased operant responses and that the effects of stimulation had not changed over time. They were also tested again in the absence of stimulation.

Intracranial self-stimulation

A few days after the food self-administration test, rats were trained to poke their nose in a hole to receive a 500 ms, 130 Hz stimulation train (500 ms timeout period between pokes). Self-stimulation was acquired when the subject poked his nose into the reward hole three times more than the control hole for two consecutive days (note, three of the rats had been trained at 100 Hz and their self-stimulation training and testing days were done before FR testing). To determine the frequency of stimulation that best supports self-stimulation, subjects were then tested on frequency sweeps. Frequencies of 20 Hz, 60 Hz, and 130 Hz were tested each for 15 min and the numbers of nose-pokes for the reward and control holes were quantified for each frequency. Order of frequency testing was randomized.

Stimulation parameters

Stimulation values were controlled by an A-M systems stimulator and were set to continuous monopolar, biphasic stimulation with pulse width of 100 μ s per phase and amplitude 250–400 μ A. Stimulation was split between two identical bilateral electrodes each 75 μ m in diameter so current delivered to the target on each side of the brain was \sim half of the set amplitude, that is, around 125 μ A to 200 μ A. Each rat was tested at its minimum threshold current determined in the screening test. Pilot current studies (starting from 20 μ A) had also been performed which also gave a starting amplitude range (see Supporting information, Data S1 and Fig. S1). The majority of the rats had a minimum effective current of 250 μ A (or 125 μ A per electrode/location) resulting in a current density of \sim 28.3 mA/mm² at the electrode tip (see Supporting information for current density calculations). At a distance of \sim 0.2 mm and \sim 0.5 mm from the tip, this current density would approximate the threshold to activate cell bodies and axons of passage (Ranck, 1975; Nowak & Bullier, 1998) with a volume of activation of up to \sim 0.5 mm³ (see also Data S1).

Given the frequency dependencies in clinical DBS applications, we wanted to test this out as well in our study. Hence, we chose to assess two 'extreme' frequencies – a high and low frequency. We selected a standard high frequency (130 Hz) shown to be therapeutic in clinical DBS (Okun, 2012) and a low frequency (20 Hz) shown to have either no effect or an activating effect (Kuncel *et al.*, 2007).

Histology

Anatomical localization of electrode sites was done after completion of testing. Rats were anesthetized with isoflurane gas. A lesioning DC current (0.1 mA) was passed for 10 s to mark the electrode location in the brain. After euthanizing the rats with pentobarbital (dose: 200 mg/kg, intraperitoneal), the brains were removed, frozen in an isopentane and isopropyl alcohol solution, sliced into 40 μ m sagittal sections using a CM 1850 cryostat (Leica Microsystems, Buffalo Grove, IL, US), and stained with cresyl violet (Fisher Scientific, Pittsburgh, PA, US). Electrode placement was confirmed by examining the lesion sites in stained slices under a light microscope (Nikon, Melville, NY, US).

Behavioral assessment and data analysis

To determine the effects of stimulation on motivation, lever contacts, pellets consumed and approaches to the food bowl were counted. Lever contacts on the control and reward levers were compared over each 30-min testing session. Pellet consumption was calculated (delivered minus unconsumed pellets).

Motor and exploratory behavior and approaches to the food bowl were assessed by frame-by-frame video analysis with DataRat (in-house video scoring software) during the last 5 min of the habituation period and for 30-s intervals every 5 min during operant testing. Horizontal and vertical midline chamber crossings, rearing movements, and 'vacuum' taste reactions (Berridge & Valenstein, 1991) were counted.

Neural analysis

Single neural units were discriminated from each other and background noise (Tindell *et al.*, 2004) using Offline Sorter (Plexon, Inc., Dallas, TX, US). Digitized spikes in the database were analyzed with Epoch Builder (in-house neural and behavioral analysis program) and Neuroexplorer (Nex Technologies, Madison, AL, US). The effects of

stimulation on neural activity were assessed after stimulation artifacts were removed. Firing rate calculations during stimulation periods were corrected for loss of neural data due to the artifact dead time (Bar-Gad *et al.*, 2004). Stimulation artifacts were removed using a custom-built LabVIEW program that fitted each artifact independently with a 7th degree polynomial and subtracted the fit from the recording (using an approach based on Wagenaar & Potter (2002)).

The immediate effects of stimulation were determined by peristimulus time histograms (PSTHs, bin width = 0.5 ms) aligned to the onset of each stimulation pulse (for all stimulation pulses in the session). Increases or decreases in firing rate were deemed significant if two consecutive bins were greater than ± 2 standard deviations from the mean firing rate of the unit measured during the first 5 min of habituation (before stimulation). Control 'PSTH' plots were computed in an identical manner from the 5-min prestimulation period by aligning to 'sham stimulation' pulses simulating 20 Hz or 130 Hz stimulation.

Perievent time histograms (PETHs) and rasters were analyzed for reward-related events: pellet delivery, first lever contact (in each trial) on the reward lever, and on the control lever. The firing rate reference for all unit responses was the average rate during the 5 s just before the habituation period ended. To determine whether a unit was responsive to pellet delivery, firing rates during the 0.5-s epoch after the stimulus event was compared to the reference period. Neural activity before and following lever response was also evaluated by comparing the 0.5-s period just before and after the first lever contact to the 5-s reference period.

Experiment 2 (taste reactivity)

All training and testing were conducted with rats in a 25-cm diameter plastic cylinder over a clear floor in white light for video recording from below (Tindell *et al.*, 2006). Taste reactions (Grill & Norgren, 1978) were quantified from a frame-by-frame analysis of video recordings.

Habituation and exposure to sucrose solution

Prior to surgery, rats were handled for 10–15 min and then given daily access to 20-mL, 17% sucrose solution in their home cage for 4 days. Rats that did not consume at least 15 mL of sucrose solution per day by day 4 were excluded from the study. For 2 days before surgery, rats were placed in the chamber for 10 min to acclimatize to the experimental set-up. Rats then underwent oral cannula and electrode implantation surgery as described below.

Surgical procedures

Bilateral oral cannula implantation was performed as described in a previous study (Tindell *et al.*, 2006). In the same surgery, rats were then implanted with a recording and stimulating electrode targeting the central nucleus of the amygdala (AP -2 mm, ML ± 3.4 mm to ± 3.5 mm, DV 7.5–8.5 mm) on each side of the brain. Electrode construction, electrode implantation procedure and peri-operative care were the same as described in experiment 1 with the addition that penicillin was given pre-operatively as well as daily for up to 5 days after surgery.

Taste reactivity training

After surgical recovery (5–7 days), rats underwent 3 days of taste-infusion training in the testing chamber. Sessions consisted of a 2-

min habituation period, followed by trials of intraoral infusions of sucrose, water, and quinine with a variable interval of 40 s to 60 s between trials. For each session, rats were given blocks of 10 trials per taste, starting with 17% sucrose solution, followed by tap water, and then 0.01% quinine (Fig. 1b.i). Between blocks, an extra infusion of tap water was given in order to rinse the mouth between tastes. For each trial, 0.1 mL of the tastant was infused over a period of 1 s directly into the rat's mouth using a similar set-up as described by Tindell *et al.* (2006) (Fig. 1b.ii). After taste reactivity training but before testing, rats were also given screening tests to determine effective electrode depth and minimum effective current that produced mouth movements. Screening tests proceeded similar to those done in experiment 1 (see *Screening*), although instead of doing an operant response task, the animals' facial reactions were observed. After screening, rats received 2 days off before taste reactivity testing began.

Taste reactivity testing with DBS

Rats were tested over a period of 6 days. Video recordings of taste reactions were made with the camera zoomed in and focused on the mouth and forelimb region. Testing conditions were similar to training conditions, although rats received stimulation during testing on four of the 6 days and neural activity was recorded. For three of the nine rats used in experiment 2, stimulation was delivered using a Medtronic pulse generator (Minneapolis, MN, US) (3–5 V or ~72 to ~150 μ A, monophasic 100 μ s pulses). Stimulation frequencies tested were the same as in experiment 1. For all other rats, the same stimulation parameters as in experiment 1 were used. For DBS sessions, stimulation was turned on one minute into the habituation period and remained on until the end of the session. Rats were tested first with a no stimulation session, followed by 2 days of either high or low frequency, then another day of no stimulation, followed by 2 more days of the other frequency of stimulation (Fig. 1b.iii: experimental timeline). The order of stimulation was counter-balanced across rats. Anatomical localization of electrode sites was done after completion of testing as described in experiment 1.

Behavioral assessment and data analysis

Taste reactions (Grill & Norgren, 1978) were scored offline using frame-by-frame video analysis and an in-house DataRat scoring program. For each taste, five out of ten trials were analyzed. Hedonic, neutral, and aversive taste reactions were scored and summed (Berridge, 2000) during the 10-s period beginning at the onset of the infusion. To assess if stimulation alone produced any 'vacuum' facial and body reactions (specifically mouth, tongue, or forelimb movements) without any actual taste present (Berridge & Valenstein, 1991), two 10 s periods (immediately before taste infusion and 30 s before infusion) were also scored for reactions. These two periods were later combined when statistical testing revealed no significant difference between them.

Neural analysis

Discrimination and stimulation artifact removal with dead time compensation was the same as experiment 1. Baseline firing rates were computed in the habituation period before and after stimulation started. As in experiment 1, PSTHs and PETHs were computed. PETHs were analyzed around taste onset. To determine whether a unit was responsive to taste onset, firing rates during 1 s epochs after the stimulus event were compared to the 1 s period before the event.

Statistical analysis (experiment 1 and 2)

Numbers are reported as mean \pm standard error. Unless otherwise stated two-way mixed models ANOVAS were used to test the main effects of stimulus location (between-subjects) and frequency of stimulation (within-subjects, repeated measure) for the different behavioral measures in experiment 1. The differences between reward and control lever contacts for each test condition were assessed to determine overall effects of frequency and/or structure being targeted. Paired t-tests were also carried out to compare differences between reward and control lever contacts for a given frequency and between reward and control nose-pokes for self-stimulation results. For experiment 2, three-way mixed models ANOVAS were carried out comparing taste reactions (hedonic, neutral, and aversive), frequency of stimulation, and period (before and during taste infusion) for a given taste. For each taste reaction, the tastes, frequency of the stimulation, and period were compared. For both experiments, Mann–Whitney U tests were used to determine if a response was significant to a given event for individual units. Also, given the limited number of units to begin with, all units in a given test condition were pooled across rats. Two-way ANOVAS were carried out comparing different epochs and stimulation conditions (0 Hz, 20 Hz, and 130 Hz) for a given event of interest. Statistical tests were implemented with SPSS vs. 22 (IBM, Armonk, NY, US). Alpha was set to 0.05 and specific comparisons were made using Holm–Sidak *post hoc* tests when significant effects were found. The standardized effect size is also reported for major comparisons. For a mixed models ANOVA, the standardized effect size (ES) was computed from Eqn (1) in Mathematics and Equations (Spybrook *et al.*, 2011).

$$ES = \frac{\hat{\beta}}{\sqrt{\hat{\tau}^2 + \hat{\sigma}^2}}, \text{ where } \hat{\beta} = \text{fixed coefficient, } \hat{\tau}^2 = \text{variance of the intercept, and } \hat{\sigma}^2 = \text{covariance} \quad (1)$$

Results

Experiment 1 (operant responding task)

DBS in the central nucleus of the amygdala (CeA) decreased working for sucrose pellets

Stimulation of CeA ($n = 7$ rats, Fig. 2a) significantly decreased engagement with the reward lever (Fig. 3a and b) compared to no stimulation and to stimulation in control sites ($n = 4$ rats, Fig. 2a) [stimulation site \times frequency: $F_{2,102} = 22.47$, $P < 0.001$]. Screening for the effective sites (Fig. 2) indicated that the volume of tissue activated by stimulation was about 0.4 mm³ (Fig. S1). Both 20 Hz and 130 Hz decreased reward lever contacts, relative to control lever contacts, when compared to no stimulation ($P < 0.001$, ES = 2.95 and $P < 0.001$, ES = 2.10 respectively). There was no difference between high and low frequency ($P = 0.08$, ES = 0.885). For rats implanted in the control sites, there was no effect on lever contacts of either 20-Hz stimulation or 130-Hz stimulation ($P = 0.94$, ES = 0.22 and $P = 0.95$, ES = 0.03 respectively) and no difference between frequencies ($P = 0.94$, ES = 0.25). With stimulation off, the number of reward lever contacts (relative to control lever contacts) was similar for electrodes in CeA and non-CeA (control) sites ($P = 0.24$, ES = 0.15).

DBS in the CeA decreases consumption of sucrose pellets

Pellet delivery and consumption. Stimulation of the CeA also resulted in dramatically fewer lever presses and thus, decreased sugar pellet delivery and consumption (Fig. 3c) [stimulation site \times frequency: $F_{2,105} = 19.941$, $P < 0.001$]. DBS in CeA diminished

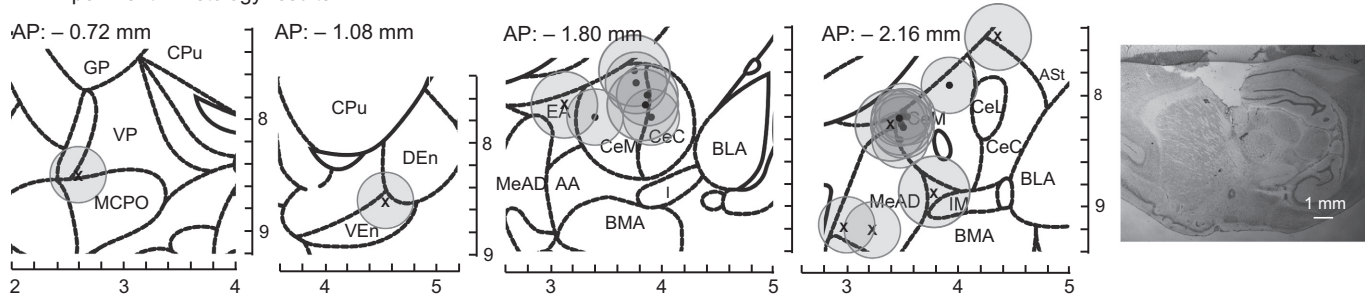
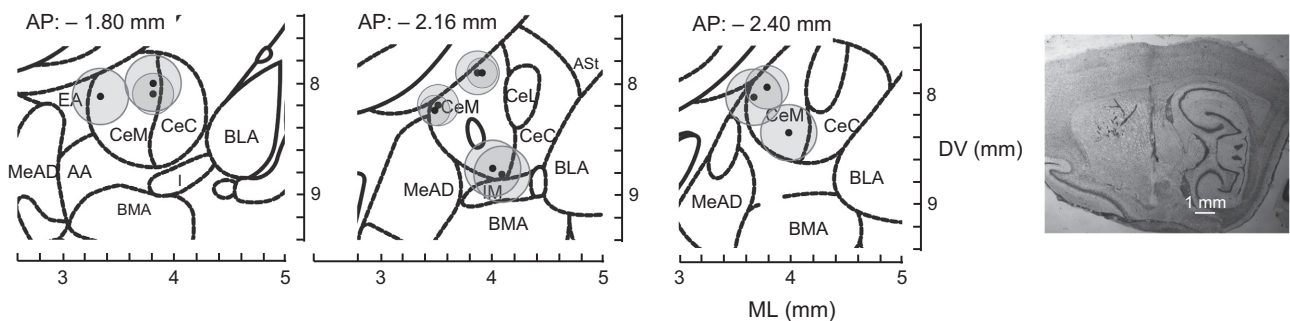
a Experiment 1 histology results**b** Experiment 2 histology results

FIG. 2. Coronal slices showing electrode placements of rats implanted in the CeA (black-filled circles, $n = 7$ rats) and in the controls sites (x's, $n = 4$ rats) for (a) experiment 1 and (b) experiment 2 ($n = 6$ rats). Gray circles represent estimated current density that would exceed the threshold to activate axons (for cell bodies, circles would be $\sim 1/2$ the size of these). See Data S1 for detailed information. Note, rats were implanted bilaterally, but for presentation purposes both left and right electrode locations are plotted on the same coronal image in the figure. Images are modified from the Paxinos and Watson rat atlas, 6th edition (Paxinos & Watson, 2007). See Abbreviations section, for names of the structures. Images on the far right are examples of brain slices showing electrode tracks and lesion marks from a rat implanted in the CeA in experiment 1 (top, far right) and another in experiment 2 (bottom, far right). Slices stained with cresyl violet.

pellet consumption to less than one-sixth of that was eaten when stimulation was absent (0 vs. 20 Hz: $P < 0.001$, ES = 3.87; 0 vs. 130 Hz: $P < 0.001$, ES = 3.43), but there was no difference between frequencies ($P = 0.29$, ES = 0.44). In contrast, stimulation in control sites had no effect on pellet consumption (0 vs. 20 Hz: $P = 0.16$, ES = 1.09; 0 vs. 130 Hz: $P = 0.92$, ES = 0.07) and no difference between frequencies ($P = 0.11$, ES = 1.16) (Fig. 3c). Baseline consumption was the same between rats implanted in the CeA and in control sites ($P = 0.20$, ES = 0.87).

During CeA stimulation, even the few pellets earned were rarely eaten, and the occasional pellets that rats did put in their mouths were often expelled (Movie S1) [stimulation site \times frequency: $F_{2,105} = 12.60$, $P < 0.001$]. On average, rats ate only $21.9 \pm 12.1\%$ of the pellets delivered during 20 Hz stimulation into the CeA and $57.5 \pm 19.3\%$ during 130 Hz stimulation compared to $99.8 \pm 0.1\%$ during no stimulation (0 vs. 20 Hz: $P < 0.001$, ES = 1.85; 0 vs. 130 Hz: $P = 0.02$, ES = 1.01). There was no difference between 20 Hz and 130 Hz stimulation ($P = 0.06$, ES = 0.84). Rats implanted in control sites ate almost 100% of pellets delivered whether receiving stimulation or not (0 Hz: $99.4 \pm 0.2\%$, 20 Hz: $99.8 \pm 0.2\%$, 130 Hz: $99.5 \pm 0.3\%$; $P = 1$, ES ≤ 0.01 for all comparisons).

Approach. The approach rate after pellet delivery was compared as an index of motivation. Rats typically approached the food bowl immediately after lever pressing to retrieve the pellets. However, with CeA stimulation at both 20 Hz and 130 Hz, rats approached the food bowl less frequently than when stimulation was absent ($P < 0.001$, ES = 4.57 and $P < 0.001$, ES = 3.70 respectively, Fig. 3d). There was a significant interaction effect between stimulation site and frequency [$F_{2,103} = 24.48$, $P < 0.001$]. Stimulation at control sites had no effect on approach behavior ($P = 0.059$, ES = 1.09 and

$P = 0.745$, ES = 0.09 for 20 Hz and 130 Hz respectively). With stimulation absent, there was no difference in approaches to the bowl between CeA and non-CeA rats ($P = 0.29$, ES = 0.77).

'Vacuum' taste reactions. Stimulation in the CeA appeared to induce a state of taste aversion (see experiment 2) as shown by mouth gaping movements. Twenty-Hertz stimulation in the CeA produced on average 22.9 ± 12.5 gapes per 30-min test session. With 130-Hz stimulation, this number was 31.2 ± 8.0 gapes in 30 min. No gapes were observed during sessions with no stimulation or for control sites.

General exploratory behavior

Chamber crossing and rearing. Stimulation had little or no effect on general motor behavior (assessed during the last 5 min of the habituation period). Chamber crossing did not change significantly with CeA stimulation [main effect: stimulation site $F_{1,105} = 0.58$, $P = 0.45$, ES = 0.17; main effect: frequency $F_{1,105} = 4.68$, $P = 0.03$, ES = 0.26; stimulation site \times frequency $F_{1,105} = 2.69$, $P = 0.10$]. Average rate of chamber crossing for CeA rats was 6.45 ± 0.62 crosses/min during no stimulation and 3.20 ± 0.82 crosses/min with stimulation. Rats implanted in control sites did 4.16 ± 0.12 crosses/min and 3.86 ± 0.30 crosses/min during no stimulation and stimulation respectively. Measures of rearing behavior also confirmed the absence of stimulation effects on general movement [main effect: stimulation site $F_{1,105} = 1.02$, $P = 0.32$, ES = 0.23; main effect: frequency $F_{1,105} = 2.89$, $P = 0.09$, ES = 0.30; stimulation site \times frequency $F_{1,105} = 1.42$, $P = 0.24$]. Average rears per minute were 3.44 ± 0.60 (no stimulation) and 1.78 ± 0.37 (during stimulation) for rats implanted in the CeA. Similarly, the average rears

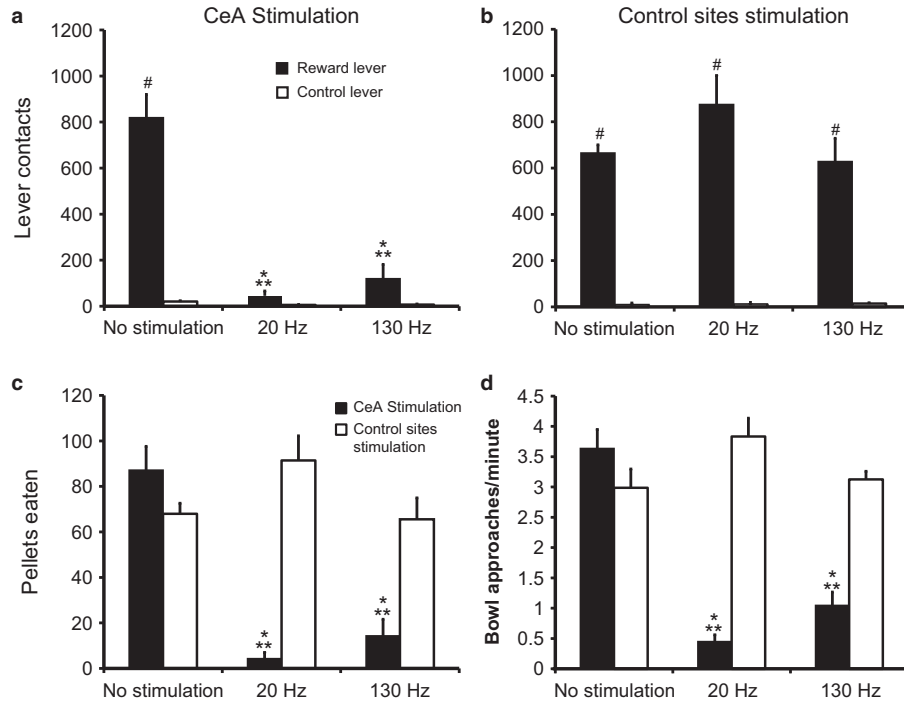


FIG. 3. DBS in the CeA decreases motivation to work for and consume sucrose pellets. (a and b) Average number of reward (black bars) and control lever (unfilled bars) contacts for no stimulation, 20 Hz stimulation, and 130 Hz stimulation. (a) Rats implanted in the CeA ($n = 7$). Reward lever contacts were significantly reduced during stimulation compared to no stimulation, $*P < 0.001$ and compared to rats receiving stimulation in control (non-CeA) sites, $**P < 0.001$. Reward lever contacts were significantly greater than control lever contacts, $^{\#}T(1) = 77.000$, $P < 0.001$ during no stimulation, but not for 20 Hz [$T(1) = 68.000$, $P = 0.053$] or 130 Hz [$T(1) = 73.000$, $P = 0.007$] stimulation. (b) Rats implanted in areas outside of the CeA ($n = 4$). There was a significant decrease in control lever contacts compared to reward lever contacts for each test condition, $^{\#}T(1) = 78.000$, $P < 0.001$, but no effect of stimulation. (c) Average number of sucrose pellets consumed in 30 min for rats implanted in the CeA (black bars) and control sites (unfilled bars). Stimulation decreased pellet consumption for rats implanted in the CeA ($*P < 0.001$) compared to no stimulation and to stimulation control sites ($**P < 0.001$). (d) Average rate of bowl approaches. Stimulation decreased rate of bowl approaches for rats implanted in the CeA ($*P < 0.001$, black bars) compared to no stimulation and to stimulation in control sites ($**P < 0.001$, unfilled bars). Error bars represent standard error.

per minute were 2.11 ± 0.44 (no stimulation) and 1.94 ± 0.32 (during stimulation) for rats implanted in control sites.

Stimulation frequencies lower than 16 Hz had no effect

Both 'high' frequency (130 Hz) and 'low' frequency (20 Hz) decreased operant responses and pellet consumption, that is, decreased motivational 'wanting', although we had expected 20 Hz to not have a 'blocking effect'. Hence, further testing was carried out in two of the CeA rats to determine if there was a threshold frequency, below which this 'blocking' effect is not observed. These two rats had previously been subjected to the same 30-min operant responding 130-Hz and 20-Hz tests described above. Stimulation at 16 Hz and 20 Hz decreased reward lever contacts [lever type \times frequency: $F_{5,26} = 5.32$; $P = 0.002$] compared to no stimulation and stimulation at 2 Hz, 4 Hz, and 10 Hz ($P < 0.001$, ES = 3.03, $P < 0.001$, ES = 3.27; $P = 0.008$, ES = 2.95, $P = 0.004$, ES = 3.19; $P = 0.001$, ES = 3.29, $P < 0.001$, ES = 3.53; $P = 0.003$, ES = 3.32, $P = 0.002$, ES = 3.55) (Fig. 4a). Stimulation at 16 Hz and 20 Hz reduced pellet delivery and consumption [$F_{5,13} = 8.27$; $P = 0.001$, 0 vs. 16 Hz: ES = 2.12; 0 vs. 20 Hz: ES = 2.41] (Fig. 4b). Stimulation at 10 Hz did not differ from no stimulation ($P > 0.9$, ES < 0.2) (Fig. 4b).

CeA supports self-stimulation

Despite the reward blocking effects of continuous stimulation, when given the opportunity, all rats would nose-poke to turn on

stimulation delivered in brief (500 ms) bursts [reward nose-pokes vs. control nose-pokes: $t_{10} = 7.895$, $P < 0.01$]. Self-stimulation at 130 Hz frequency was supported at CeA and control locations (Fig. 4c, results of self-stimulation training). Animals were then further tested at different frequencies (20 Hz, 60 Hz, and 130 Hz). Twenty-Hertz frequency (Fig. 4d) supported self-stimulation, but higher frequencies generally evoked more robust responses. Stimulation at 130 Hz resulted in a greater number of nose-pokes compared to 60 Hz and 20 Hz ($P < 0.001$, ES = 1.03 and $P < 0.001$, ES = 1.42). Sixty-Hertz stimulation also elicited more responses than 20 Hz ($P < 0.001$, ES = 0.39) (Fig. 4d) [nose-poke \times frequency: $F_{2,102} = 19.23$, $P < 0.001$]. Rats nose-poked in the rewarded hole significantly more than the unrewarded nose-poke hole (20 Hz: $P < 0.001$, ES = 0.15; 60 Hz: $P < 0.001$, ES = 0.56; and 130 Hz: $P < 0.001$, ES = 1.6) indicating that self-stimulation behavior was not a simple arousal effect.

Experiment 2 (taste reactivity)

DBS attenuates hedonic value of sucrose and increase aversive reactions to taste

Taste reactivity was tested in six rats with CeA stimulation sites (Fig. 2b). Histology revealed three of the nine rats were not implanted in the CeA and were excluded from analysis. Typically, sucrose taste infusions evoked hedonic reactions. With deep brain stimulation, however, sucrose taste reactions switched to aversion (0 vs. 20 Hz: $P = 0.004$, ES = 0.86; 0 vs. 130 Hz: $P < 0.001$, ES = 1.30) (Fig. 5a). The numbers of hedonic reactions to sucrose

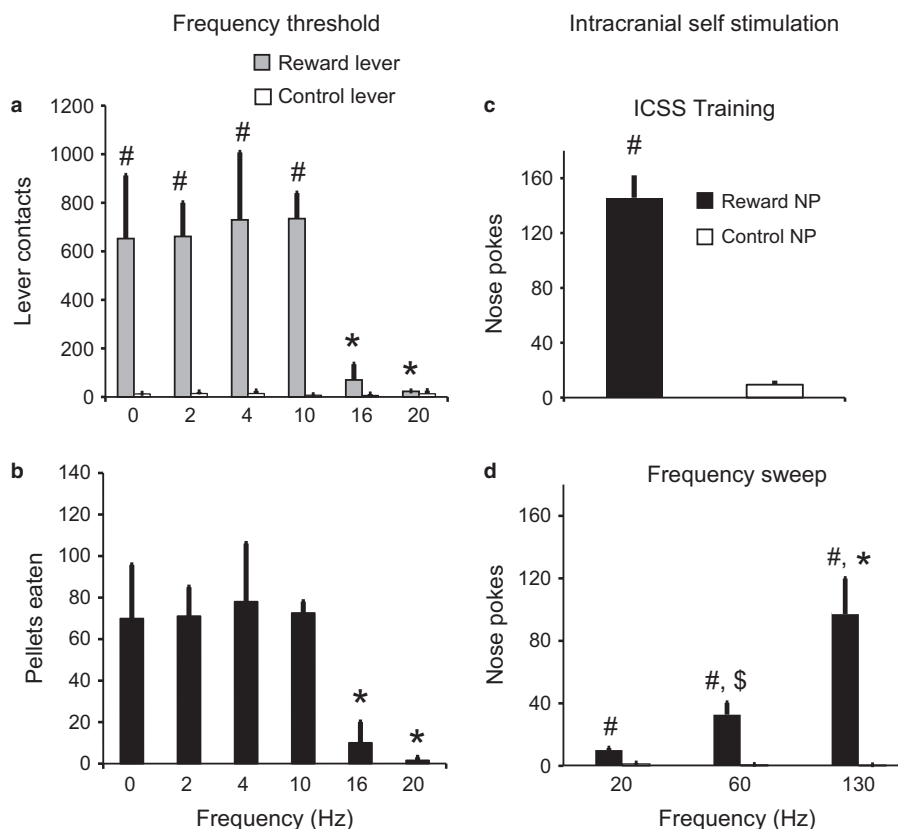


FIG. 4. (a and b) DBS at frequencies below 16 Hz had no effect on operant responses or pellet consumption. (a) Average number of reward (gray bars) and control lever (unfilled bars) contacts for different frequencies of stimulation and no stimulation; $^{\#}P < 0.001$ reward vs. control lever; $^{*}P < 0.01$ compared to 0 Hz, 2 Hz, 4 Hz, 10 Hz; $n = 2$. (b) Average number of pellet consumption for same range of frequencies; $^{*}P < 0.01$ compared to 0 Hz, 2 Hz, 4 Hz, 10 Hz; $n = 2$. (c) Animals implanted in the CeA and control sites all acquired self-stimulation with significant more nose-pokes into the reward nose-poke hole (black bars) compared to the control nose-poke hole (unfilled bars), $^{\#}P < 0.01$ at 130 Hz frequency, $n = 11$. (d) When comparing responses at different frequencies, higher frequencies produced more robust responses ($^{*}P < 0.001$ for 130 Hz compared to 20 Hz and 60 Hz; $^{\$}P < 0.001$ for 20 Hz vs. 60 Hz, $n = 11$); and at all 3 frequencies, animals did more nose-pokes in the reward hole (black bars) compared to the control hole ($^{\#}P < 0.001$, unfilled bars), NP – nose-poke. Error bars represent standard error.

decreased ($P < 0.001$, ES = 1.36, ES = 1.78) while neutral reactions increased ($P < 0.001$, ES = 1.3 for both frequencies) [frequency \times taste reaction \times period: $F_{4,192} = 7.70$, $P < 0.001$]. Overall, aversive reactions outnumbered hedonic reactions ($P = 0.007$, ES = 1.15). Stimulation at 20 Hz had a similar effect with more neutral reactions compared to hedonic reactions ($P > 0.001$, ES = 2.08) and more aversive reactions ($P < 0.001$, ES = 1.80) (Fig. 5a). Even water, which normally produces few aversive reactions, elicited significantly more aversive reactions with 20-Hz ($P < 0.001$, ES = 2.92) and 130-Hz stimulation ($P < 0.001$, ES = 1.35) (Fig. 5b). Not surprisingly, quinine tastes evoked even more aversive reactions than normal (Fig. 5c) ($P < 0.001$, ES = 1.7 for both frequencies) [frequency \times taste reaction: $F_{4,192} = 4.42$, $P = 0.002$; taste reaction \times period: $F_{2,192} = 53.60$, $P < 0.001$].

'Vacuum' reactions. During the inter-trial interval when no taste was present, rats exhibited 'vacuum' reactions, that is, facial reactions in the absence of the actual taste (Berridge & Valenstein, 1991). These reactions in total were two or less per trial and did not differ significantly between stimulation and no stimulation (Fig. 5d) (hedonic reactions: 0 vs. 20: $P = 0.87$, ES = 0.09; 0 vs. 130: $P = 0.90$, ES = 0.07; neutral reactions: 0 vs. 20: $P = 0.48$, ES = 0.22; 0 vs. 130: $P = 0.98$, ES = 0.006; aversive reactions: 0 vs. 20: $P = 0.19$, ES = 0.32; 0 vs. 130: $P = 0.45$, ES = 0.23).

Stimulation produced multiphasic firing patterns

As a preliminary study, we studied a small number of units when possible in experiment 1 (no stimulation: 8 units, LFS: 5 units, HFS: 2 units, total 15 units) and experiment 2 (no stim: 8 units, LFS: 7 units, HFS: 4 units, total 19 units). Neurons were not silenced by stimulation which others have also shown in different brain regions (Hashimoto *et al.*, 2003; Bar-Gad *et al.*, 2004; Maltete *et al.*, 2007; McCairn & Turner, 2009; Cleary *et al.*, 2013). Indeed, after a brief inactivation period (2–25 ms) some units actually fired faster than baseline (Fig. 6). Firing rates were assessed in the habituation period after stimulation artifacts were removed and recording time was compensated for dead time removal (1.83 ± 0.06 ms of dead time after each pulse; $n = 18$ units). There was no significant rate difference during stimulation compared to the period before stimulation for either experiment 1 [$F_{2,14} = 1.6$, $P = 0.242$, $n = 7$ units] or experiment 2 [$F_{2,18} = 2.627$, $P = 0.103$, $n = 11$ units]. In experiment 1, average firing rates during the first 5 min of habituation (before stimulation was turned on) was 5.11 ± 1.13 spikes/s for the LFS units and dropped to 2.84 ± 1.04 spikes/s during the latter 5 min of the habituation period when stimulation was on. For the HFS units these rates were 10.38 ± 0.38 spikes/s and 6.27 ± 4.86 spikes/s respectively. In experiment 2, comparing the mean firing rates during the first minute of the habituation period

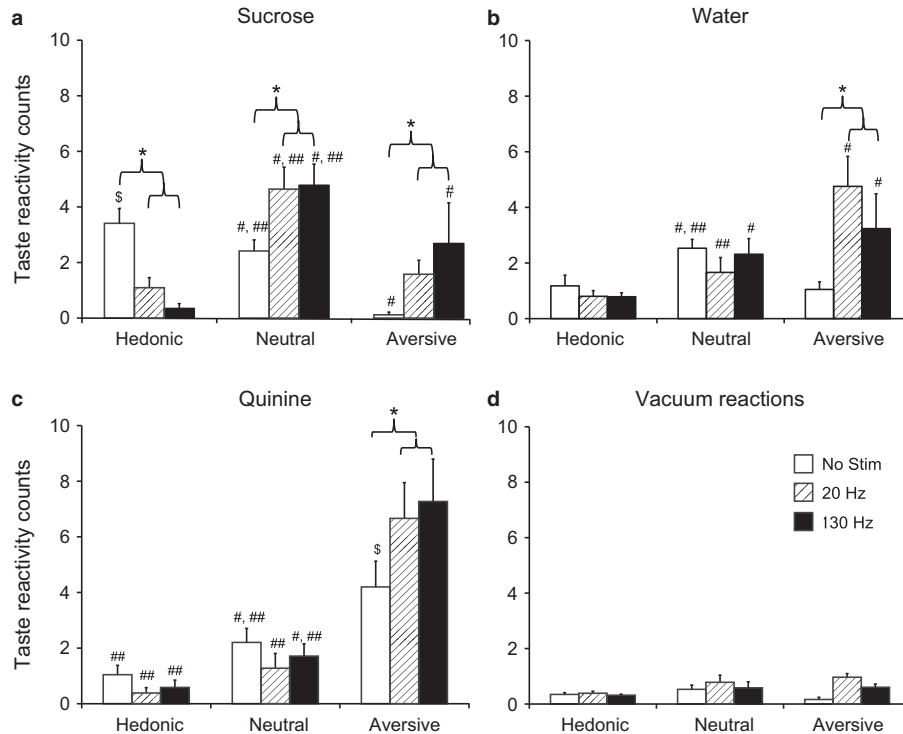


FIG. 5. DBS in the CeA decreased hedonic reactions to sucrose and also increased aversive reactions to all tastes. Plots (a–c) show the average taste reactivity counts per trial during the 10 s post infusion period (a) Hedonic, neutral and aversive reactions to sucrose solution for each test condition (no stimulation, 20-Hz stimulation and 130-Hz stimulation). Plots in (b) and (c) show hedonic, neutral and aversive reactions to water and quinine respectively. During no stimulation there were more hedonic reactions to sucrose compared to water and quinine and more aversive reactions to quinine compared to water and sucrose ($^{\$}P < 0.001$). For each taste: $^*P < 0.05$ compared to no stimulation within a given taste reaction, $^{\#}P < 0.05$ compared to hedonic reactions within a given stimulation frequency, and $^{\#\#}P < 0.05$ compared to aversive reactions within a given stimulation frequency. (d) Plot shows the average taste reactivity counts per trial during the 10 s background period between infusions (i.e. when no taste was delivered) for each test condition. These reactions were significantly fewer compared to reactions during taste ($P < 0.03$). Error bars represent standard error, $n = 6$ rats.

and the second minute when stimulation was on, LFS units had 12.59 ± 5.18 spikes/s and 21.67 ± 6.28 spikes/s respectively. For HFS units, this went from 10.92 ± 6.83 to 7.56 ± 2.85 spikes/s.

Deep brain stimulation at 20 Hz evoked multiphasic patterns of excitations and inhibitions (Fig. 6a). Most responses began with a period of inhibition. Five units were inhibited before returning to mean pre-stimulation rates (i.e. baseline) during the period between stimulation pulses (e.g. Unit 4, Fig. 6a). One unit had a period of excitation following inhibition (Unit 2, Fig. 6a), and two units had two periods of inhibition with either an excitation (Unit 1, Fig. 6a) or a return to baseline (Unit 5, Fig. 6a) in between. Inhibition started from ~ 2 ms after stimulation artifact and ranged from 2 ms to 22 ms before returning to baseline. A smaller number of units started with a period of excitation before returning to basal rates (e.g. Units 3 and 6, Fig. 6a, $n = 4$). At 130 Hz DBS rates, responses were split with three units being inhibited (e.g. Unit 7, Fig. 6b) and two showing excitations followed by inhibitions (e.g. Unit 10, Fig. 6b). Inhibition was not due to stimulus artifact interference as spikes could recover between 2 and 3 ms after the stimulus (e.g. Units 8 and 10, Fig. 6b). There were no inherent periodic patterns evident in the control 'PSTHs' (Fig. 6 insets) indicating that the likelihood of a spike firing was independent of simply lining up spike activity at periodic intervals (Hashimoto *et al.*, 2003).

Neural-behavioral activation

Neural units in CeA from experiment 1 were activated in association with behavioral events such as lever contact and feederclick, that is,

reward delivery (Fig. 7a, first row, $n = 6/8$). However, as animals rarely touched the control lever or consumed pellets with DBS on, it is challenging to assess whether neurons might still be coding behavior. Of seven units examined during stimulation, only two were, at best, weakly task responsive (Fig. 7a, second and third row).

In experiment 2, with stimulation absent, most neurons responded to sucrose (6/8) and water (5/8). A smaller number responded to quinine (3/8) (Fig. 7b, first row). Because taste reactivity testing is passive, we were able to test taste reactions while DBS was on. In contrast to high proportions of responses without stimulation, only one out of seven units responded to sucrose and quinine during 20 Hz stimulation and no units responded during 130 Hz stimulation (Fig. 7b, second and third row respectively). Deep brain stimulation at either 20 Hz or 130 Hz significantly reduced neural firing to sucrose [main effect comparing 1-s periods before and after taste onset: $F_{2,37} = 16.280$, $P < 0.001$]. There was no effect of stimulation on neural firing to tastes of water [$F_{2,18} = 2.698$, $P = 0.098$] or quinine [$F_{2,18} = 0.687$, $P = 0.518$]. With no stimulation, firing rates to sucrose tastes were higher than both water and quinine [main effect: $F_{5,47} = 5.535$, $P < 0.001$]; however, with stimulation at either frequency these differences dissipated [20 Hz: $F_{5,41} = 1.108$, $P = 0.374$; 130 Hz: $F_{5,23} = 1.503$, $P = 0.247$].

Discussion

Deep brain stimulation (DBS) of the central nucleus of the amygdala (CeA) caused animals to stop lever pressing for and

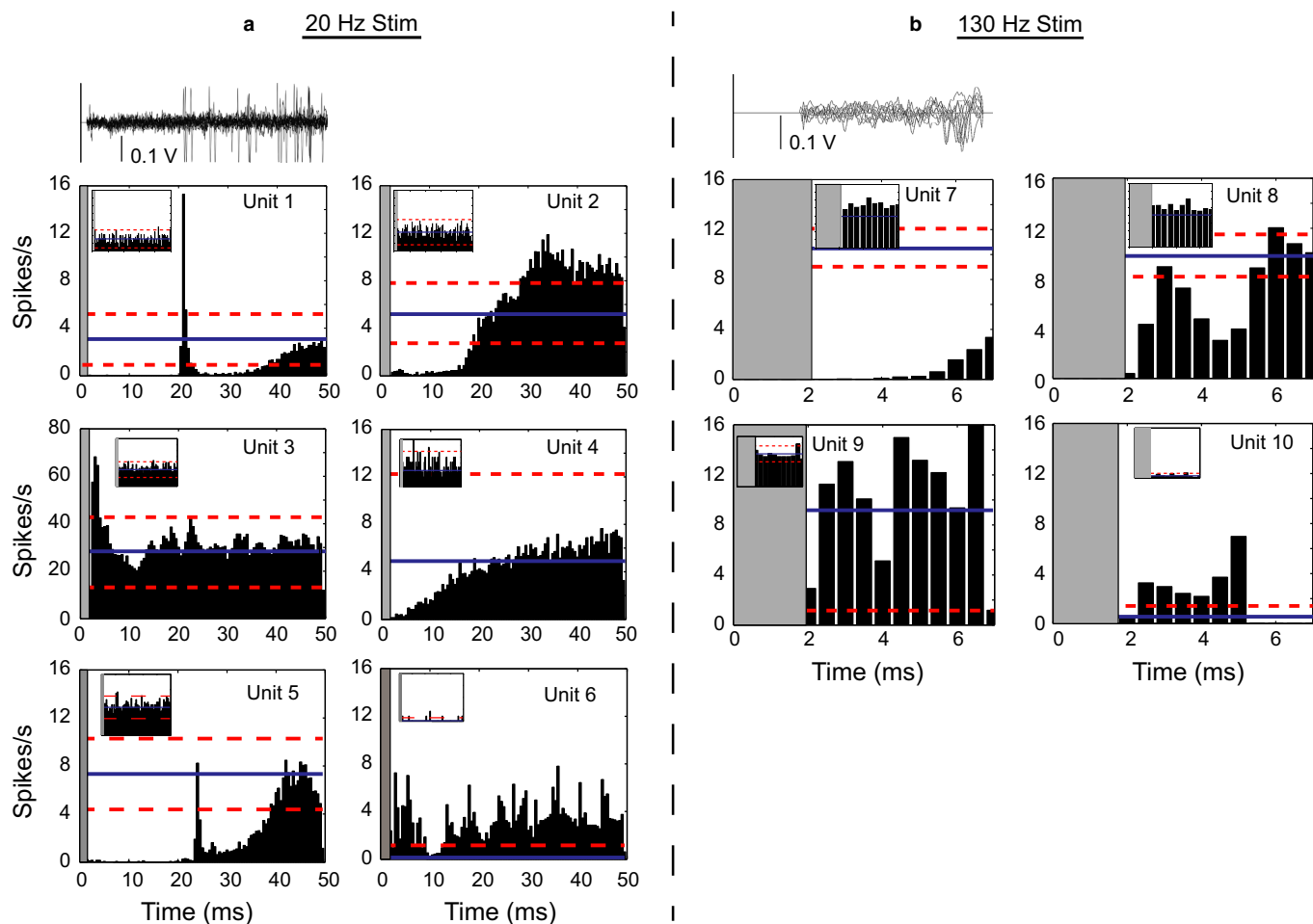


FIG. 6. Common patterns of spike activation evoked by stimulation pulses at 20 Hz (a) and 130 Hz (b) for CeA units from experiment 1 (units: 1, 2, 5, 7 and 8) and experiment 2 (units: 3, 4, 6, 9 and 10). Number of stimulation pulses were 42 000 for units 1, 2 and 5; 273 000 for units 7 and 8; 37 200 for units 3, 4 and 6; and 241 800 for units 9 and 10. (a, b, Top row) 10 superimposed raw waveform sweeps from the unit in the averaged histogram shown immediately below. All histograms are aligned to the stimulus at time = 0 for the period between stimulation pulses with 0.5 ms bins. The mean firing rate (solid line) of the unit and 2 standard deviations from the mean (dashed lines) are indicated on the histogram plots. The gray areas beginning on the left of each histogram mark the period of stimulus artifact dead time during which no spikes could be detected. The inset histograms computed by alignment to 'sham' stimulation pulses in the period before stimulation (in the same units) show no inherent background spike activity pattern (for experiment 1: 6000 stimulation pulses for 20 Hz and 39 000 for 130 Hz; experiment 2: 1200 for 20 Hz and 7000 for 130 Hz). At 130 Hz, the inter-stimulus interval is 7.6 ms; hence, by necessity x-axes for the right graphs are shorter. [Color version of figure available online].

consuming sucrose pellet rewards. Taste reactivity tests revealed increased 'disliking' reactions to palatable, neutral, and aversive tastes. Surprisingly, DBS presented in brief pulses in the same location under the animals' control resulted in vigorous self-stimulation. Overall, neural recordings during stimulation showed smaller proportions of responsive neurons to reward-related events.

LFS at 20 Hz as effective as HFS at 130 Hz

Therapeutic effects of DBS for Parkinson's disease, essential tremor and dyskinesia typically occur at frequencies greater than 90 Hz. Conversely, lower frequencies (< 50 Hz) have been reported to have no therapeutic benefit or even worsen symptoms (Rizzone *et al.*, 2001; Moro *et al.*, 2002; Kuncel *et al.*, 2007). Thus, we anticipated that stimulation at 130 Hz would 'block' while 20 Hz stimulation might 'activate' the circuit and behavior. Surprisingly though, both frequencies decreased pellet delivery and consumption and also increased aversive reactions to tastes.

CeA units have very low firing rates, typically less than 5 Hz (Collins & Paré, 1999; Rosenkranz & Grace, 1999). So, for CeA, 20 Hz might effectively be a 'high' frequency that overwhelms the very low intrinsic frequency of CeA neurons. Frequency effects depend on the type of structure (Dostrovsky & Lozano, 2002) and the intrinsic frequency of neurons being stimulated (Grill *et al.*, 2004). Our frequency sweep study did show that there was no effect of DBS at frequencies 10 Hz and lower (Fig. 4a and b) with a surprising drop off between 10 and 16 Hz. We did not test frequencies between 10 Hz and 16 Hz so it is possible that between those values there are frequencies that might produce an intermediate effect, although others have reported abrupt transitions with DBS (Wu *et al.*, 2001). This threshold effect we observed could be due to an intrinsic oscillation frequency between 10 Hz and 16 Hz above which the stimulation frequency is 'fast' enough to overwhelm the circuit and below which normal neural responses to reward events can pass through. Most neurons in the medial nucleus of the CeA are referred to as 'late firing', that is, in response to current injection the membrane potential depolarizes slowly over hundreds of

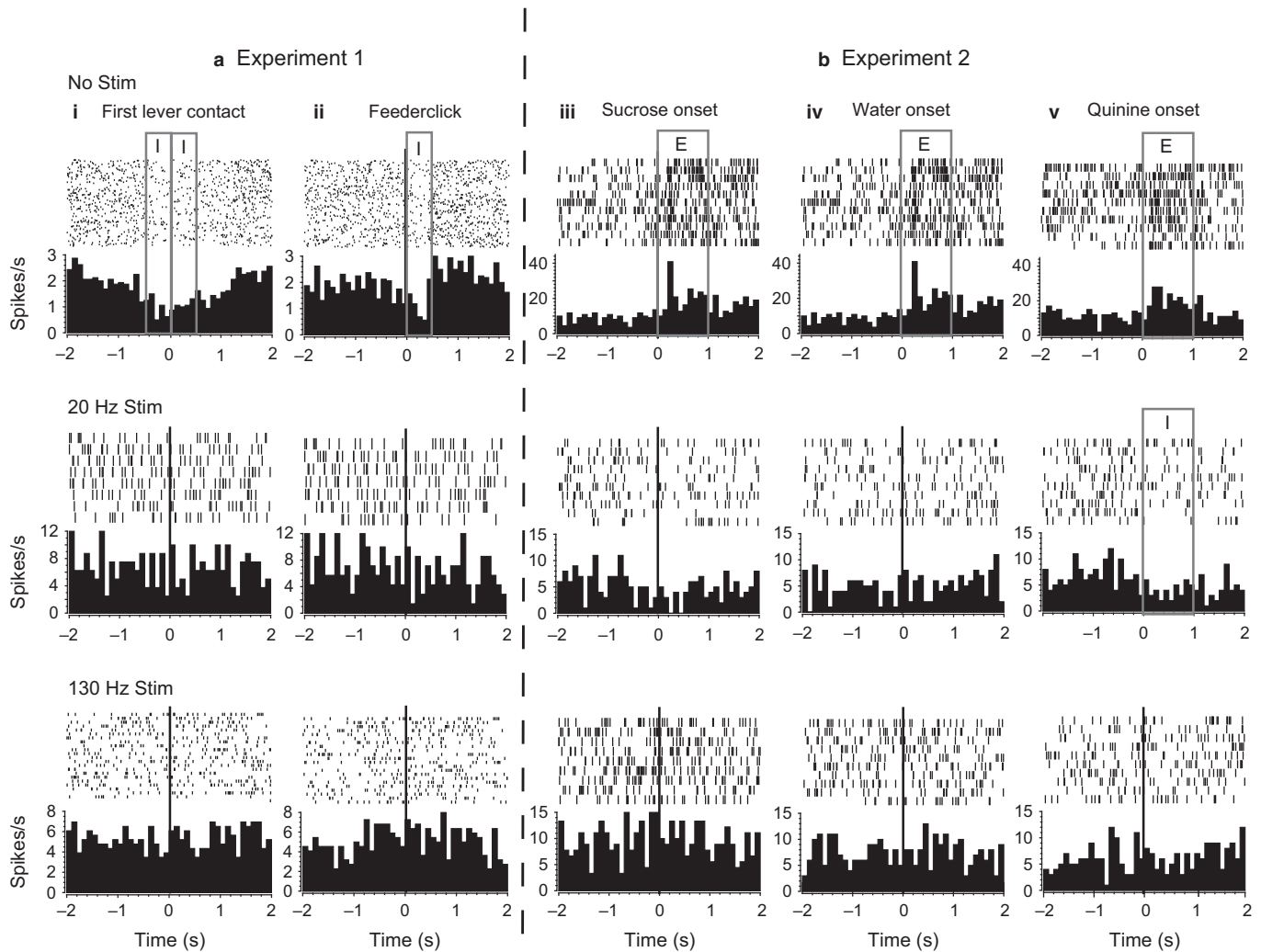


FIG. 7. Fewer CeA unit responses during stimulation to task events. Representative PETHs and raster plots for (a) experiment 1 and (b) experiment 2 during no stimulation (top row), 20 Hz stimulation (middle row), and 130 Hz stimulation (bottom row) for different events (columns i–v). Plots show unit activity up to 2 s before and after the event of interest which is centered at 0. Each mark in a raster indicates a spike and each horizontal line is a trial, with consecutive trials going from top to bottom. Histograms (bin width = 100 ms) show the average firing rate across all trials. Inhibition (I) and excitation (E) responses to particular events are highlighted by gray rectangles. Responses are with respect to the 5-s period just before the lever extended for experiment 1 (a) and the 1-s period before the taste onset for experiment 2 (b).

milliseconds (Martina *et al.*, 1999). These membrane dynamics could contribute to the frequency dependence and threshold effects for DBS-like stimulation.

Is this a decrease in 'wanting' only?

Our results showed that DBS decreased food consumption and the number of operant responses on the reward lever. But is this decrease in pellet delivery and consumption only due to a decrease in motivation or also due to a change in palatability of the food? During DBS some rats would put the pellets in their mouths and then spit them out, suggesting they still 'wanted' the pellets but no longer found them palatable. Other rats remained by the food bowl or the lever and even occasionally delivered more pellets, while some completely ignored the lever and food bowl. Histology did not reveal a difference in electrode locations that could account for these individual differences; but, the CeA is a heterogeneous nucleus (Swanson & Petrovich, 1998) and it is possible that differences in the underlying tissue and current spread could result in different neuronal components being activated. Furthermore, stimulation

could be impacting both upstream and downstream structures by activating afferent and efferent axons as well as fibers of passage (Okun, 2012). Hence, all of these factors could account for these slight differences in behavior.

CeA stimulation not only decreased 'wanting' of food rewards, but also affected 'liking' responses. In our taste reactivity study, stimulation resulted in decreased 'liking' and increased 'disliking' reactions to tastes. Riley & King (2013) also reported increases in aversive reactions to tastes with CeA stimulation. In addition, we observed slightly more neutral and aversive reactions in the absence of tastes (although not significant). Even in our operant response experiment, rats would sometimes produce gaping reactions. Hence, DBS in the CeA could be creating a general aversive state. Studies have reported increases in aversive reactions to quinine (Touzani *et al.*, 1997) and reduction in quinine consumption (Kemble *et al.*, 1979) with CeA lesions and it has been proposed that CeA may play a role in modulating food intake based on taste palatability (Kemble *et al.*, 1979; Touzani *et al.*, 1997; Li *et al.*, 2012). However, others have reported no change in aversive reactions to disliked tastes with CeA manipulations (Galaverna *et al.*, 1993; Seeley

et al., 1993; Mahler & Berridge, 2011; Robinson *et al.*, 2014). These differences could be due to different types of manipulations used and the heterogeneity of the structure itself. A recent study by Cai *et al.* (2014) has identified populations of neurons in the lateral subdivision of the CeA that play important, but opposite roles in influencing feeding.

The CeA is a site that supports self-stimulation (Wurtz & Olds, 1963). We also found that animals would self-stimulate if the same stimulation parameters were used in short bursts (0.5 s) of stimulation. Both 20 and 130 Hz stimulation were effective although the higher frequency was more robust as other studies have shown (Arvanitogiannis & Shizgal, 2008). Interestingly, although animals would vigorously engage in CeA self-stimulation they would sometimes produce 'aversive' gapes similar to that was observed in the operant responding and taste reactivity experiments, which may again suggest stimulation is also triggering an aversive response. It is also possible that this short burst of stimulation could be triggering motivational circuits and hence be 'wanted' although not necessarily 'liked'. It should be noted that Robinson *et al.* (2014) showed that animals would not self-stimulate with optogenetic activation of CeA. These differences could be due in part to the duration of the stimulation train used (8 s vs. 0.5 s) and the type of stimulation (optogenetic vs. electrical) with electrical stimulation likely activating axons and cells in more widespread regions than optogenetic stimulation. Finally, the intrinsic membrane properties of CeA neurons mentioned above (Martina *et al.*, 1999) with regard to thresholds may also confer different consequences for short and long bursts of stimulation.

Perhaps it is not a change in palatability in the food *per se* that is impacting food consumption. Feeding behavior and motivational mechanisms behind feeding can be modulated by many signals (Saper *et al.*, 2002; Sternson, 2013). Need-based states and neurons and/or chemicals that signal these states can impact motivation to seek out and consume food. This motivation could stem from the desire to reduce the negative valence due to hunger (Sternson, 2013). Agouti related protein (AGRP)-expressing neurons in the hypothalamus are activated by energy deficit resulting in increased feeding behavior. However, continuous photo-stimulation of these neurons decreased instrumental responding for food over the course of days (Betley *et al.*, 2015). Betley *et al.* hypothesized that as these neurons kept signaling a negative state, the animals stopped responding as their actions failed to reduce this state. We also saw decreased instrumental responding with DBS, so it is possible that something similar could be occurring here, although we saw decreased responding within seconds and minutes, compared to over a day in their study. Testing could be done to determine if these neurons are being activated with the type of stimulation used in our study. Again, given the differences in optogenetic vs. electrical stimulation, DBS may be decreasing consumption by different mechanisms. Perhaps DBS is inhibiting motivational or hunger circuits. Further discussion on this possible inhibitory mechanism of DBS is discussed below.

More directly, within the CeA itself, in the lateral subdivision, there is a subpopulation of neurons (PKC- δ^+) that are activated by multiple anorexigenic signals. Unpalatable food, but also other anorexigenic signals that trigger satiation or visceral malaise, can lead to decrease in food consumption. Optogenetic activation of these neurons resulted in decreased food consumption in both food-deprived and fed mice (Cai *et al.*, 2014). Hence, we could be also be activating those neurons resulting in decreased motivation for food rewards and increased aversive responses. (Note, this does not rule out a decrease in perceived palatability as these neurons respond to multiple signals including bitter tastants).

Inactivation of the CeA has also been shown to decrease dopamine baseline levels and dopamine efflux in the nucleus accumbens (Ahn & Phillips, 2002). DBS could be inactivating the CeA, resulting in a similar mechanism. Future studies could look at the effects of DBS in the CeA with 6-OHDA lesions to tease out the role of dopamine in this (something similar could be done for opioid antagonists). Howland and colleagues (2002) did observe no significant change in dopamine levels during a short burst of stimulation delivered to the CeA (10 s, 300 μ A). However, they did indicate that their microdialysis technique may be too slow to record faster changes in dopamine. Interestingly, at higher amplitudes (up to 800 μ A), they reported chewing behavior which could be similar to the increased mouth movements we observed with stimulation (Howland *et al.*, 2002).

Our data show that both 'wanting' and 'liking' of food rewards are affected with stimulation, although more research is needed to determine if both systems are being activated or if one is directly impacting the other (i.e. are animals not eating because the food is no longer palatable or does the animal neither 'like' nor 'want' the food). Further testing is needed to determine whether it is a change in palatability or a more physiological reason for this decrease in 'wanting'. As part of that, it also should be determined if DBS in the CeA is creating a general aversive state, by exploring DBS's effects on other rewards such as sex and drugs.

Is this decreased feeding due to fear and anxiety?

Our results could possibly be due to an increase in fear and anxiety. The CeA does play a role in fear and anxiety (Maren & Fanselow, 1996; Pitkanen *et al.*, 1997; Tye *et al.*, 2011). Although we cannot completely rule this out, there is some evidence that suggests this is not the case. In experiment 1, rats receiving stimulation in the CeA would still cross the center of the chamber, rear, groom, and interact with the food bowl and levers (even sniffing and occasionally nibbling at the bowl). We did not see any significant differences in chamber crossing and rearing between rats being stimulated in the CeA vs. in our control sites. Some of the animals receiving stimulation in the CeA did deliver and eat pellets throughout the session (although significantly less than when stimulation was off). In both experiments, we observed gapes and forelimb flails and occasional defensive treading during stimulation, but we did not see any obvious signs of freezing. We also did not observe any obvious changes in autonomic systems such as changes in respiration rate (though this was not directly measured). Optogenetic excitation of the CeA has also been reported to have anxiolytic effects (Tye *et al.*, 2011; Cai *et al.*, 2014). However, more tests should be carried out before we can rule this out completely.

Mechanism of DBS?

We found that DBS in the CeA produced similar behavioral effects (decrease in food and liquid consumption and increased aversive reactions) as seen with lesioning or inactivation of the CeA. The various manipulations included the use of fiber-sparing lesioning agents such as kainic acid (Hajnal *et al.*, 1992) and ibotenic lesions (Touzani *et al.*, 1997); as well as, lidocaine (Ahn & Phillips, 2002), muscimol (Will *et al.*, 2009) and cauterization of tissue (Kemble *et al.*, 1979; Galaverna *et al.*, 1993). Ahn & Phillips (2002) also noted that their CeA-lesioned rats would put food in their mouths but then discard the partially eaten food, which was a behavior we observed in some of our rats during stimulation. However, our findings with neural recordings indicate that DBS did not just silence

neurons. We found that stimulation in the CeA induced multiphasic patterns of firing that could represent loose entrainment of unit activity to stimulation. Units were more likely to fire at specific latencies from stimulation pulse onset (Fig. 6), suggesting a form of entrainment. These responses have been seen in units recorded near the site of stimulation (Bar-Gad *et al.*, 2004; Carlson *et al.*, 2010; Cleary *et al.*, 2013) and at distant targets (Hashimoto *et al.*, 2003; Maltete *et al.*, 2007; McCairn & Turner, 2009). Stimulation also seems to be disrupting CeA encoding of reward. Even after accounting for artifact dead time, we saw fewer and weaker responses to reward-related events in both studies.

It is argued that DBS could be imposing its own pattern that is no longer meaningful to the underlying circuit (McIntyre *et al.*, 2004; Garcia *et al.*, 2005a,b; Johnson & McIntyre, 2008). In a sense, creating an 'information lesion' (Grill *et al.*, 2004) and disrupting information flow (whether normal or pathological) in the circuit. Therefore, stimulation could be inducing its own pattern of firing in the CeA and disrupting neural coding for food rewards and taste palatability, making things more aversive.

Histological verification from our study (Fig. 2) showed that the majority of electrodes were in the medial division of the CeA (CeM). Some of the primary connections to the CeM are from the lateral subdivision of the CeA (CeL) which is GABAergic. The output of the CeM is also primarily GABAergic (Swanson & Petrovich, 1998). Given some of the similar responses between lesion and activating studies and our results and the likelihood of DBS activating axons, it is possible that the stimulation could be activating axons from the CeL releasing GABA into the CeM and thus inhibiting it. This, in turn could lead to disinhibition of downstream structures ultimately decreasing food consumption and motivation. For example, axons from the CeA have been proposed to synapse onto a local GABAergic interneuron network in the ventral tegmental area (VTA). Disinhibition of these interneurons could result in increased inhibition of the VTA and thus inhibition of dopaminergic neurons projecting to the nucleus accumbens (Howland *et al.*, 2002). Taste pathways entering into the CeA could also be activated ultimately resulting in increased aversion possibly although opioid activation (Ricardo & Koh, 1978; Mahler & Berridge, 2011). More targeted stimulation such as optogenetic stimulation (Gradinaru *et al.*, 2009) could be used to shed more light on these different unit responses.

Conclusion

Our results demonstrate that the CeA is a very effective target for blocking 'wanting' and 'liking' of food rewards. Both 20 Hz and 130 Hz stimulation resulted in significant decreases in working for the delivery of a food reward, consumption of that reward but also increased aversive responses to sweet, neutral, and bitter tastes. Stimulation produced multiphasic patterns in CeA units and resulted in fewer responsive units to reward-related events. Questions remain whether stimulation is independently affecting both 'wanting' and 'liking' circuits, or only directly modulating 'liking' circuits which in turn is affecting 'wanting'. Furthermore, if stimulation is creating a general aversive state one wonders if this extends to other rewards and it presents obstacles to the potential clinical utility of DBS for blocking reward dysfunction. Although DBS in the CeA was very effective at decreasing motivation for food rewards and thus food consumption, there were accompanying aversive reactions. Fewer aversive reactions were seen with 20-Hz stimulation, so it is possible that stimulation parameters could be optimized. Further testing is needed to assess possible side effects as well as long term effects of DBS in the CeA.

Conflict of interests

The authors declare no competing financial interests.

Supporting Information

Additional supporting information can be found in the online version of this article:

Data S1. Current density calculations and volume of tissue activated due to stimulation.

Fig. S1. Mapping study for each rat showing the actual electrode paths (verified with histology) and the number of sucrose pellets consumed in 10 min tests at different depths.

Movie S1. Clip of a rat (video-recorded from below) implanted in the CeA performing the operant responding task from experiment 1, first in the absence of stimulation and then during 20-Hz stimulation.

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Abbreviations

AA, anterior amygdaloid area; AST, amygdaloatrial transition area; BLA, basolateral amygdaloid nucleus; BMA, basomedial amygdaloid nucleus; CeA, central nucleus of the amygdala; CeC, central nucleus of the amygdala, capsular part; CeL, central nucleus of the amygdala, lateral division; CeM, central nucleus of the amygdala, medial division; CPu, caudate putamen; DBS, deep brain stimulation; DEN, dorsal endopiriform nucleus; EA, extended amygdala; FR, fixed ratio; GP, globus pallidus; HFS, high-frequency stimulation; I, intercalated nuclei of the amygdala; IM, intercalated amygdaloid nucleus main part; LFS, low frequency stimulation; MCPO, magnocellular preoptic nucleus; MeAD, medial amygdaloid nucleus, anterior dorsal; MePD, medial amygdaloid nucleus posterodorsal part; VEn, ventral endopiriform nucleus; VP, ventral pallidum.

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