Supporting information

Supplemental methods

Current density calculations

The current density was calculated as the current per unit of cross-sectional area (Plonsey & Barr, 2007). The range of current delivered was from ~72 µA (rat with 3 V amplitude) to 400 µA. Note though, given that this was bilateral stimulation with similar electrodes implanted in each side of the brain, the current delivered to each electrode and thus each target site was ~36 µA to 200 µA (~1/2 of the source current). Hence, with an electrode surface area of 0.0044 mm^2, the current density is around 8.1 mA/mm^2 to 45.3 mA/mm^2 per electrode. At 125 µA (our most common current, source current = 250 µA) this is around 28.3 mA/mm^2 per electrode which is less than what Liu and colleagues reported: ~66 mA/mm^2 with a 100 µA current and electrode of tip length 250 µm and surface area = 0.0015 mm^2 (Liu et al., 2012; Wu et al., 2001). Assuming the threshold current density to activate neuronal elements is around 0.2 mA/mm^2 for the cell soma and 0.04 mA/mm^2 for axons (Nowak & Bullier, 1998; Ranck, 1975), at 125 µA, the current density would be around the threshold to activate cell bodies at ~0.2 mm and axons at ~0.5 mm, with volume of activation up to ~0.5 mm^3. For our highest current (200 µA) this would be ~0.3 mm and ~0.6 mm respectively, with volume of activation up to ~0.9 mm^3. For our lowest amplitude (~36 µA), this would be ~0.1 and ~0.3 mm, respectively with volume of activation up to 0.1 mm^3. In Figure 2 (main text), we included grey circles centered on the approximate electrode location for each animal. The radius of each circle was the calculated distance from the center of the electrode tip to the point where, based on the current used, the current density would exceed the

threshold current density to activate axons. Each circle was based on the current used for that given animal. From our mapping studies (Figure S1) we estimated the effective zone to be ~0.4 mm^3

Mapping study

We did a preliminary analysis to map out the effective amygdala zone using four male rats (Sprague-Dawley, ~ 8 – 13 weeks old, 250 g – 400 g, Charles River, US). Each rat underwent the same training paradigm as described in the main text for *Experiment 1* under *Materials and methods.* Rats were then implanted bilaterally with an electrode targeting a different trajectory through the amygdala (refer to *Electrode implantation* under *Experiment 1* of the main text for more information on surgical procedures). Locations were chosen such that all the major divisions of the amygdala would be targeted: extended amygdala (EA), anterior amygdaloid area (AA), medial anterior dorsal nucleus of the amygdala (MeAD), the central nucleus of the amygdala (CeA) – medial and lateral divisions, basomedial amygdala (BMA), and the basolateral amygdala (BLA). Hence, initial target locations were as follows: AP -1.50 mm, ML ±2.98 mm, AP -1.50 mm, ML ±3.25 mm, AP -1.75 mm, ML ±3.79 mm, and AP -2.00 mm, ML ±4.10 mm. All electrode bundles were lowered to a depth just above the amygdala.

After recovery, the rats underwent a mapping study to determine the effective electrode location and minimum effective current that resulted in decreased pellet consumption. For depth testing, the electrodes for each rat were first lowered to 7.5 mm below dura as the starting depth. Rats were tested on an abbreviated version of the limited progressive ratio schedule (Figure 1a, main text). In this case, rats were placed in the chamber for 11 minutes which included a one

minute habituation period at the beginning. Continuous bilateral, monopolar, biphasic stimulation at 130 Hz frequency, 250 µA current, and a pulse width of 100 µs per phase was also turned on at the beginning of the habituation period and remained on for the duration of the session. The number of pellets eaten in the 10 minutes of testing was measured. During testing, rats were observed to determine any effects of stimulation on their behavior. Once the session was finished, the rat was taken out of the chamber, the electrode was lowered by 0.16 mm on both sides, and the test was repeated. This was repeated until the electrode reached the bottom of the amygdala (or the limit of the electrode itself). Note, if stimulation produced motor effects e.g. paw twitching, consistent turning to one side, etc., the test was immediately stopped and the electrode lowered to the next depth. Rats were given a maximum of four test sessions per day with one session always being a no stimulation session. Order of when the rats were run was randomized.

Once the effective depth was determined, two of the rats underwent current sweep tests to determine the threshold current that produced a significant decrease in food consumption without adverse motor impairment or freezing responses. The current sweep tests were similar to the depth testing with the exception that now the depth was fixed and the current was varied. For each rat, the electrode was moved to a depth that was shown to be effective. The animals were then tested on currents of: 0 µA, 20 µA, 60 µA, 100 µA, 150 µA, 200 µA, 225 µA, 250 µA, and 300 µA. For each rat, current was tested on either an ascending or a descending order for one day and the reverse order on the next day. Each rat was given a two minute break between each session.

Supplemental results

Figure S1 shows the results of the depth testing for each rat. For rats 2 and 4, the effective region was between 7.5 mm to 8.3 mm deep (from dura). Rats 1 and 3 showed no consistent effect of stimulation. Rats 2 and 4 were then tested at different currents at the same frequency of 130 Hz. For rat 4, by the end of depth testing, his electrode was no longer in the region that had resulted in decreased consumption with stimulation (Figure S1a). Hence, his electrode was raised back up to a depth of 7.5 mm below dura (i.e., back to a location that had produced decreased consumption with stimulation) before starting the current sweep test. Rat 2's electrode was still in the region that showed decreased consumption with stimulation by the end of depth testing and so his electrode was not moved for the current sweep test. Results showed that the threshold current was 250 μA (Figure S1b).

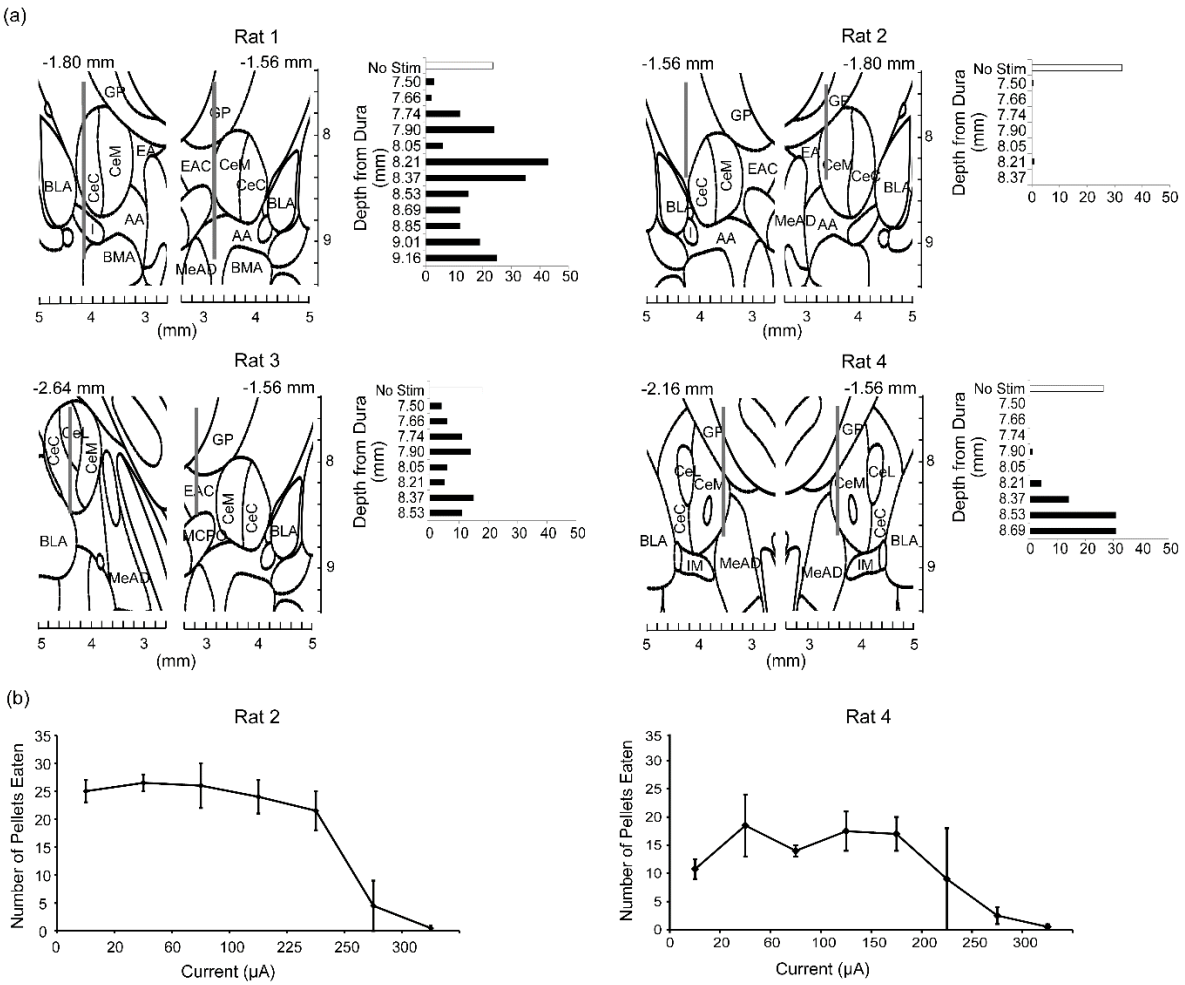

Figure S1 – (a) Mapping study for each rat showing the actual electrode paths (verified with histology) and the number of sucrose pellets consumed in 10 minute tests at different depths. Open bars at the top of each plot show the average pellet consumption when no stimulation (No Stim) was given. Grey lines in the coronal images represent the left and right electrode tracks for each rat and the regions that were stimulated during the test. Coronal images are modified from the Paxinos and Watson atlas, 6th edition (Paxinos & Watson, 2007). (b) Current sweep study done on the two rats that show a marked decreased in pellet consumption from the depth test. For each rat, current sweeps were done at a depth that had shown decreased consumption in the depth test (8.37 mm below dura for rat 2 and 7.5 mm below dura for rat 4).

From the mapping study, it was concluded that the central nucleus of the amygdala was an effective target region to decrease food consumption with deep brain stimulation. The effective zone was ~0.4 mm^3 in volume between AP: -1.5 and -2.2 mm, ML ±3.20 mm and ±3.90 mm, and DV 7.5 mm to 8.3 mm. Effective current began at 250 µA or 125 µA per electrode (though this was subject-dependent as observed in experiments 1 and 2).

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. The clip shows two levers extended into the chamber and the food bowl. The lever on the right is the reward lever and the one on the left is the control lever. Touching the reward lever ten times results in a pellet delivered into the food bowl (via the pellet delivery chute). Touching the control lever produces no pellets. At the beginning of the clip, stimulation is absent and the animal is delivering and eating pellets. About half-way through the clip, 20 Hz stimulation is turned on (the label "Stim On" appears in the video to indicate when the rat is receiving DBS). When stimulation is first turned on, the rat delivers a pellet and puts it in his mouth. However, he then spits it out and stops delivering pellets.