# THE EFFECTS OF DEEP BRAIN STIMULATION IN THE VENTRAL PALLIDUM AND THE CENTRAL NUCLEUS OF THE AMYGDALA ON FOOD CONSUMPTION, MOTIVATION, AND PALATABILITY

by

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## DEDICATION

To my wonderful family who have always given me encouragement and support.

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# PREFACE

The data chapters of this thesis (Chapters 2, 3, and 4) are written in preparation for publication as separate papers. Chapter 1 is an introduction to the thesis. Since the data chapters were meant to stand independently, there is some repetition of introductory information in each. The specific rationale for each chapter's particular experimental goals is unique. Chapter 1 expands the introductory background and also elaborates the overall aims and hypotheses of the dissertation. Chapter 5 is a collated discussion for the thesis as a whole so it will also reiterate some elements of chapter discussions, but also attempt to interpret the overall thesis findings and expand on their significance as a whole. It also outlines potential future work. At the time of this dissertation, Chapters 2, 3, and 4 are being prepared for manuscript submission.

# TABLE OF CONTENTS

DEDICATION .................... ii

ACKNOWLEDGEMENTS .................... iii

PREFACE .................... vi

LIST OF TABLES .................... x

LIST OF FIGURES .................... xi

ABSTRACT .................... xv

CHAPTER 1 INTRODUCTION .................... 1

1.1 Components of Reward .................... 1

1.1.1 Measuring “liking” .................... 2

1.1.2 Measuring “wanting” .................... 4

1.2 Limbic Circuits and Neural Coding of Reward .................... 5

1.2.1 Ventral pallidum .................... 5

1.2.2 Central nucleus of the amygdala .................... 10

1.3 Deep Brain Stimulation Therapy .................... 15

1.3.1 Deep brain stimulation - overview .................... 15

1.3.2 Mechanisms of deep brain stimulation .................... 16

1.3.3 Deep brain stimulation, eating disorders, and addiction .................... 20

1.4 Overall Aims and Hypothesis of this Thesis .................... 21

1.5 Chapter Overviews .................... 22

1.6 List of References .................... 25

CHAPTER 2 DEEP BRAIN STIMULATION IN THE VENTRAL PALLIDUM HAS MIXED EFFECTS ON FOOD CONSUMPTION ........................................................ 44

2.1 Introduction ........................................................................................ 44

2.2 Materials and Methods ........................................................................ 48

2.2.1 Experiment 1: Chocolate Consumption ................................................ 49

2.2.2 Experiment 2: Deep brain stimulation effects on food consumption in a Pavlovian conditioning task ................................................................ 53

2.3 Results ................................................................................................ 61

2.3.1 Experiment 1: M&M consumption ...................................................... 61

2.3.2 Experiment 2: Pavlovian conditioning task .......................................... 63

2.4 Discussion ........................................................................................... 79

2.5 Conclusion ........................................................................................... 83

2.6 List of References ................................................................................ 84

CHAPTER 3 DEEP BRAIN STIMULATION IN THE CENTRAL NUCLEUS OF THE AMYGDALA DECREASES FOOD CONSUMPTION ................................................ 94

3.1 Introduction ........................................................................................ 94

3.2 Material and Methods .......................................................................... 97

3.2.1 Experiment 1: Mapping study ............................................................ 97

3.2.2 Experiment 2: Effects of deep brain stimulation on food consumption .... 101

3.3 Results .............................................................................................. 107

3.3.1 Experiment 1: Stimulation effective area of ~0.4 $mm^3$ was found in the central nucleus of the amygdala ...................................................... 108

3.3.2 Experiment 2: Deep Brain Stimulation decreases reward-seeking behavior in the central nucleus of the amygdala ................................................ 111

3.4 Discussion ......................................................................................... 130

3.5 Conclusion ......................................................................................... 137

3.6 List of References ........ 138

CHAPTER 4 DEEP BRAIN STIMULATION IN THE CENTRAL NUCLEUS OF THE AMYGDALA DECREASES HEDONIC VALUE OF TASTES ........ 145

4.1 Introduction ........ 145

4.2 Material and Methods ........ 148

4.3 Results ........ 156

4.4 Discussion ........ 172

4.5 Conclusion ........ 175

4.6 List of References ........ 176

CHAPTER 5 GENERAL DISCUSSION AND CONCLUSIONS ........ 182

5.1 Summary of Findings ........ 182

5.2 Mechanisms of Action of DBS ........ 186

5.3 Potential Targets for DBS for Reward Dysfunction ........ 193

5.4 Implications for DBS as a Treatment for Reward Dysfunction ........ 195

5.5 Conclusion ........ 196

5.6 Future Work ........ 197

5.7 List of References ........ 200

APPENDIX A ........ 209

A.1 Testing Chamber ........ 209

A.2 Stimulation Artifact Removal Technique ........ 209

A.2.1 Introduction ........ 209

A.2.2 Methods and materials ........ 211

A.2.3 Results of artifact testing ........ 214

A.3 Chamber Set-up for Taste Reactivity Study ........ 218

A.4 List of References ........ 219

## LIST OF TABLES

Table 2.1 – The number of responsive VP units (numerator) relative to total number of VP units (denominator) broken down by test condition and event............................. 71

Table 2.2 – Breakdown of all VP unit responses, all periods for CS+1, CS+2, and UCS. ........................................................................................................................ 76

Table 3.1 - Average time to reach FR 5 and FR 10 schedules....................................... 115

Table 3.2 – The number of responsive CeA units (numerator) relative to total number of CeA units (denominator) broken down by test condition and event ........................ 121

Table 3.3 – Average firing rates of CeA units during stimulation and no stimulation baseline periods.......................................................................................... 122

Table 3.4 – Breakdown of all CeA unit responses, all periods for pellet delivery and active lever responses. ............................................................................... 128

Table 4.1 – The number of responsive CeA units (numerator) relative to total number of CeA units (denominator) broken down by test condition and event. ....................... 162

Table 4.2 – Average firing rates of CeA units during stimulation and no stimulation baseline periods.......................................................................................... 163

Table 4.3 - Breakdown of all CeA unit responses, all periods for sucrose, water, and quinine onset ............................................................................................ 167

Table A.2 – Summary data showing the number of recovered spikes and % recovery after the stimulation artifact has been removed for each session..................................... 216

# LIST OF FIGURES

Figure 1.1 – Orofacial reactions to a sweet taste (top row) and a bitter taste (bottom row) in rodents, primates, and human infants. .................................................................... 3

Figure 1.2 - Sagittal view of a rat brain highlighting major connections of the ventral pallidum and central nucleus of the amygdala.............................................................. 6

Figure 2.1 - Diagram of a typical stimulation artifact and its characteristics.. ................ 48

Figure 2.2 – Experimental timeline of the M&M study. ................................................ 51

Figure 2.3 – (a) Schematic of a Pavlovian trial showing timing of the different events for experiment 2. (b) Experimental paradigm for a given test session showing habituation, pre stimulation period, stimulation period, and post stimulation period. (c) Experimental timeline for experiment 2............................................................... 57

Figure 2.4 - Coronal slices showing electrode placements of rats implanted bilaterally in the ventral pallidum. Filled circles represent the endpoints of electrode locations. 62

Figure 2.5 – Changes in chocolate consumption from control (i.e. no stimulation) with stimulation in the ventral pallidum at different frequencies and pulse widths.. ......... 63

Figure 2.6 - Coronal slices showing electrode placements of rats implanted bilaterally in the ventral pallidum. Filled circles represent the endpoints of electrode locations. 64

Figure 2.7 – Decreased rate of approaches to the food dish with 200 Hz VP stimulation compared to 20 Hz VP stimulation.............................................................................. 66

Figure 2.8 – Decreased rate of approaches to the lever with 200 Hz VP stimulation compared to 20 Hz VP stimulation.............................................................................. 67

Figure 2.9 – No difference in the rate of nose entries into the food dish with VP stimulation............................................................................................................ 68

Figure 2.10 – No effect of stimulation in the VP on latency to retrieve pellet.. .............. 68

Figure 2.11 – Examples of recorded neural traces in the VP before and after stimulation artifacts were removed.......................................................................................... 70

Figure 2.12 – Average firing rates during HFS in the VP were lower compared to pre stimulation and post stimulation. ..................................................................... 72
Figure 2.13– Ventral pallidal units show stimulation-induced patterns of firing. (a – b) Sample PSTHs plots normalized to spikes/s with bins of 0.5 ms and duration of 50 ms for 20 Hz stimulation and 5 ms for 200 Hz stimulation. ........................................... 74
Figure 2.14 – Stimulation did not reduce VP unit responses to events. For each population (LFS and HFS units) the distribution of units that show no response (grey bars), inhibitions (blue bars), or excitations (red bars) are plotted for each behavioral event of interest (CS+1, CS+2, and UCS). ............................................................... 77
Figure 2.15 - Representative PETHs and raster plots for a VP unit tested during a LFS session (top row) and a separate VP unit tested during a HFS session (bottom row).. ........................................................................................................................ 78
Figure 3.1 – (a) Schematic of a limited progressive ratio test for experiment 2 (DBS – deep brain stimulation). (b) Experimental timeline for experiment 2. .................... 103
Figure 3.2 – Results from the 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. ................................................................... 109
Figure 3.3 - Results from the current sweep study done on the two rats that show a marked decreased in pellet consumption from the previous depth test. .................. 109
Figure 3.4 – Coronal Slices showing electrode placements of rats implanted bilaterally in the CeA (circles, n = 8 rats) and in the controls sites (stars, n = 4 rats). ................. 111
Figure 3.5 – DBS in the CeA decreased sucrose consumption...................................... 112
Figure 3.6 - DBS in the CeA decreased approaches to food bowl.. .............................. 113
Figure 3.7 – DBS in the CeA decreases motivation to work for sucrose. (a –b) Average number of active and inactive lever contacts for no stimulation, 20 Hz stimulation, and 130 Hz stimulation. ......................................................................... 114
Figure 3.8 – DBS in the CeA decreases chamber crosses for rats. ................................ 117
Figure 3.9 - DBS in the CeA slightly decreases rearing behavior. ................................ 118
Figure 3.10 – Examples of recorded neural traces from the CeA before and after stimulation artifacts have been removed............................................................... 120

Figure 3.11 – CeA units show stimulation-induced patterns of firing. (a – b) Sample PSTHs plots normalized to spikes/s with bins of 0.5 ms and duration of 50 ms for 20 Hz stimulation and 7.6 ms for 130 Hz stimulation. ................................................ 123
Figure 3.12 - Representative PETHs and raster plots for (a) a no stimulation CeA unit (b) a LFS CeA unit and (c) a HFS CeA unit. ............................................................ 126
Figure 3.13 - Fewer CeA unit responses during stimulation to active lever contacts and pellet delivery. For each population (no stimulation, LFS, and HFS) the distribution of units that show no response (grey bars), inhibitions (blue bars), excitations (red bars), or mixed responses (excitation and inhibitions – purple bars) are plotted. .... 129
Figure 4.1 – (a) Schematic of a taste infusion trial showing timing of the different events. (b) Experimental paradigm for a given test session showing the order of delivery of tastes. (c) Experimental timeline for the taste reactivity experiment...................... 151
Figure 4.2 - Coronal slices showing electrode placements of rats implanted bilaterally in the central nucleus of the amygdala. Circles represent electrode bundle locations with a different color for each rat......................................................................... 157
Figure 4.3 – DBS in the CeA decreased hedonic reactions to sucrose and also increased aversive reactions to all tastes..................................................................... 159
Figure 4.4 – Vacuum aversive reactions are greater with stimulation in the CeA. ........ 160
Figure 4.5 – Examples of recorded neural traces from the CeA before and after stimulation artifacts have been removed................................................................ 161
Figure 4.6 – CeA units show stimulation-induced patterns of firing. (a – b) Sample PSTHs plots normalized to spikes/second with bins of 0.5 ms and duration of 50 ms for 20 Hz stimulation and 7.6 ms for 130 Hz stimulation. ..................................... 165
Figure 4.7 - Fewer responses overall to tastes during stimulation in the CeA. For each population (no stimulation, LFS, and HFS) the distribution of units that show no response (grey bars), inhibitions (blue bars), or excitations (red bars) are plotted... 166
Figure 4.8 – Average taste-evoked firing rates of units in the CeA decreased during deep brain stimulation. (a) – (c) show composite histograms for all units during no stimulation (black), 20 Hz stimulation (orange), and 130 Hz stimulation (blue). .... 169
Figure 4.9 - Representative PETHs and raster plots for (a) a no stimulation CeA unit (b) a LFS CeA unit and (c) a HFS CeA unit. ................................................................ 171

Figure 5.1 - Proposed model of action of DBS in the ventral pallidum. ........................ 188
Figure 5.2 - Proposed model of action of DBS in the central nucleus of the amygdala (CeA)........................................................................................................... 192
Figure A.1 - (a) Picture of testing chamber and (b) Diagram of chamber set-up. Similar set-up used for experiments in Chapters 2 and 3. .................................................... 209
Figure A.2.1 - Diagram of a typical stimulation artifact and its characteristics. ............ 210
Figure A.2.2 – Block diagrams showing the algorithm for the artifact removal program. (a) Shows the steps involved in finding each artifact and zeroing out the sharp transients where the amplifiers are saturated and data is unrecoverable. (b) Shows the steps involved in fitting the exponential decay part of the artifact. .......................... 213
Figure A.2.3 – Snapshot of our artifact removal program. ............................................ 214
Figure A.2.4 – Snapshots of Offline Sorter sessions showing the same session sorted before and after artifact has been removed for the same time period.. ..................... 215
Figure A.2.5 – Peristimulus time line graphs for a sample low frequency stimulation session (a) and high frequency stimulation session (b) before and after removal of stimulation artifacts.......................................................................................... 217
Figure A.3 - Diagram of chamber set-up for the taste reactivity study (Chapter 4). ...... 218

# ABSTRACT

Deep brain stimulation (DBS) has been shown to be an effective treatment for Parkinson's disease and other movement disorders including essential tremor and dystonia. Given its success, DBS is also being investigated as a potential treatment for psychiatric disorders including depression, obsessive compulsive disorder, eating disorders, and addiction. Although the specific therapeutic mechanisms of DBS are not known, studies suggest that this type of electrical stimulation may be causing an entrainment or regularization of firing patterns in neurons, interfering with the information being processed in the underlying neural circuit[1].

The overall goal of this thesis is to evaluate the potential neural interference effects of DBS-like stimulation on motivation and reward consumption. Two structures were targeted: the ventral pallidum (VP), which is thought to be an area of convergence for processing reward and reward-related information[2], and the central nucleus of the amygdala (CeA), which is thought to be especially involved in focusing motivation for particular cues and rewards[3]. The effects on DBS in the VP and CeA on reward-seeking behaviors, food consumption, and hedonic value of tastes were assessed. Results showed that DBS in the VP produced complex patterns of neuronal firing; however, it did not disrupt neural coding of reward and had only minimal effects on food consumption and motivation. DBS in the CeA also resulted in similar complex firing patterns, and additionally (in contrast to the VP) disrupted neural coding of reward. This disruption


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[1] Garcia, L., Audin, J., D'Alessandro, G., Bioulac, B., & Hammond, C. (2003). Dual effect of high-frequency stimulation on subthalamic neuron activity. *Journal of Neuroscience, 23*(25), 8743-8751.
Grill, W. M., Snyder, A. N., & Miocinovic, S. (2004). Deep brain stimulation creates an informational lesion of the stimulated nucleus. *Neuroreport, 15*(7), 1137-1140.

[2] Smith, K. S. (2007). *Reward and motivation in the ventral pallidum: Chemical, physiological, and anatomical mechanisms.* (Ph.D., University of Michigan). *ProQuest Dissertations and Theses,* 1-215. (304849615).

[3] Mahler, S. V. & Berridge, K. C. (2009). Which cue to "want?" central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue. *J Neurosci, 29*(20), 6500-13.

was reflected in altered behavior. DBS in the CeA invoked an immediate and profound decrease in the consumption of ($p < 0.001$) and motivation to work for ($p < 0.001$) sucrose pellets by more than a factor of four. DBS in the CeA also decreased the hedonic value of and increased aversive reactions to sucrose ($p = 0.003$).

Overall results suggest the DBS is modulating neural activity in the underlying target structure, but the location of electrode is very important and this DBS-induced change in neuronal firing may or may not disrupt coding for reward. Data suggests that CeA may be an effective target for blocking food consumption and motivation.

# CHAPTER 1
# INTRODUCTION

Drug addiction, morbid obesity, and other related clinical problems can be viewed as disorders of the brain that lead to excessive consumption of rewards such as drugs and foods (Berridge, Ho, Richard, & DiFeliceantonio, 2010; Robinson & Berridge, 2003). Understanding the underlying brain mechanisms involved in reward behavior and reward-related disorders like drug addiction and morbid obesity has become a vital area of research. One possible therapy that is currently being investigated for reward dysfunction is deep brain stimulation (Chang, 2004; Gubellini, Salin, Kerkerian-Le Goff, & Baunez, 2009; Halpern et al., 2008), which has been shown to be an effective treatment for Parkinson's disease, essential tremor, and dystonia (Gubellini et al., 2009; Schwalb & Hamani, 2008; Weaver et al., 2009). It is also being investigated as a treatment for various neurological and psychiatric disorders including epilepsy, depression, and obsessive compulsive disorder (Chang, 2004; Greenberg et al., 2006; Haber & Brucker, 2009; Halpern, Samadani, Litt, Jaggi, & Baltuch, 2008; Lozano et al., 2008; Mayberg et al., 2005). This thesis investigates the effects of DBS on reward processing in two novel targets: the ventral pallidum and the amygdala.

## 1.1 Components of Reward

One powerful approach, based on the work of Kent Berridge and Terry Robinson, is to parse reward mechanisms into its key components of hedonic quality and motivation, termed by the authors describe as "liking" and "wanting" (Berridge & Robinson, 2003; Berridge, Robinson, & Aldridge, 2009). "Liking" refers to the hedonic impact of the sensory experience of the reward, for example, in the case of a food reward, it is the pleasurable experience of consuming a highly palatable taste, like chocolate. "Wanting" refers to the incentive salience attributed to environmental stimuli associated

with the reward or to the reward itself. For example, cues that predict the availability of a reward – such as the smell of freshly-baked chocolate chip cookies - can become motivational magnets and trigger the desire to seek out and consume the reward (Berridge, 1996; Berridge, 2004; Bindra, 1978; Bolles, 1972; Toates, 1986). In general, these two aspects of reward work together – usually, we "want" what we "like" and we "like" what we "want". However, mounting evidence has shown that these two components of reward are separable and encoded by different brain substrates. In fact, under certain experimental manipulations we can alter one without affecting the other, and even cause them to change in opposite directions (Berridge, 2004). Physiological brain states such as stress, hunger, satiation, and drugs can also affect "liking" or "wanting". For example, extremely salty tastes which are normally "disliked" can become "liked" after the body becomes salt-depleted. Drugs can also boost the motivational potency of reward cues, making them become more "wanted" (Flagel et al., 2011; Smith, Berridge, & Aldridge, 2011).

### 1.1.1 ***Measuring "liking"***

How do we go about measuring "liking"? Humans can provide verbal reports of whether something is pleasurable or not (Cabanac, 1971) but what about animals such as rodents that are unable to verbally report feelings of pleasure? Objective measures of "liking" can be determined from observations of microstructure of animals' behavioral affective reactions. Many animals including humans, rodents, and non-human primates will produce the same characteristic positive (hedonic) or negative (aversive) facial and body reactions to either pleasant or unpleasant tastes, respectively (J. E. Steiner & Glaser, 1984; J. Steiner, Glaser, Hawilo, & Berridge, 2001). Figure 1.1 shows examples of these positive and negative reactions in different species. A taste reactivity test can be used to measure the hedonic impact to pleasant, unpleasant, or neutral tastes (H. J. Grill & Berridge, 1985). Tastes can be directly infused into the animal's mouth and the facial and body responses to these different tastes can be analyzed. Reactions to a pleasant taste, such as sucrose, typically include rhythmic midline tongue protrusions, lateral tongue protrusions and paw licks. These reactions are defined as hedonic. Responses to unpleasant tastes, such as bitter quinine, include gapes, headshakes, forelimb flails, face

washing, and chin rubs (Berridge, 2000). These reactions are defined as aversive. A third category of reactions include rhythmic mouth movements, passive dripping, locomotion, and grooming and mostly occur to tastes that are neither really hedonic nor aversive, like water (Berridge, 2000). These reactions are considered to be neutral.

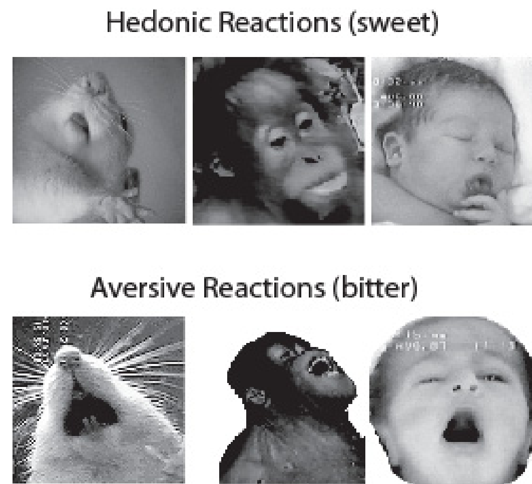


Figure 1.1 – Orofacial reactions to a sweet taste (top row) and a bitter taste (bottom row) in rodents, primates, and human infants. All 3 species are doing a rhythmic tongue protrusion (hedonic reaction) to a sweet taste in the top row, and a gape (aversive reaction) to a bitter taste in the bottom row. Image modified from Berridge 2000 and Kyle Smith's Dissertation (Berridge, 2000; Smith, 2007).

Furthermore, these reactions have been shown to reflect the hedonic valence of the taste rather than the sensory aspect of the taste and can fluctuate depending on physiological, psychological, or pharmacological manipulations (Berridge, 1996). For example, the number of hedonic reactions to sucrose decreases when the animal is satiated on a sucrose solution (Berridge, 1991). If an animal is salt-depleted and the body now requires salt, reactions to an extremely salty taste switch from aversive to hedonic (Berridge, Flynn, Schulkin, & Grill, 1984). This is similar to what occurs in humans. For example, foods are deemed to taste better when one is hungry. Also, in general, there is no specific reaction for a given taste. Animals will do the various positive reactions (described above) to any taste that at the time is deemed to be hedonic and the same goes for negative reactions to aversive tastes. Hence, taste reactivity provides a reliable, objective measure for "liking" and a version of this paradigm was used in Chapter 4 of this thesis to measure the change in the hedonic value of rewards with DBS.

### 1.1.2 ***Measuring "wanting"***

Objective measures of "wanting" in animals presents challenges similar to those of measuring "liking". Neutral stimuli that become associated with rewards (through Pavlovian conditioning) can develop incentive salience properties (Berridge, 2004; Bindra, 1978; Bolles, 1972; Toates, 1986). These conditioned stimuli (CS+ cues) can become motivational magnets and can elicit orienting, approach, and consummatory behaviors (Holland, 1977). For example, when a CS+ is presented, some animals will approach it and even interact with it as they would the reward, such as sniffing or biting, even though interaction with the stimulus has no effect on the delivery of the reward. This is a process known as autoshaping (Brown & Jenkins, 1968; Flagel, Watson, Robinson, & Akil, 2007; Hearst & Jenkins, 1974; Williams & Williams, 1969). Cues that develop incentive salience can also trigger other responses. They can become conditioned reinforcers (animals will actually work for the presentation of just the cue itself). They can maintain operant behaviors even if reward is only being reinforced intermittently (e.g. in second order reinforcement schedules) or increase the amount that the animal is willing to work for a reward (e.g. in progressive-ratio "breakpoint" tests). Cues that develop incentive salience can even facilitate an operant response that had been rewarded previously in separate training sessions, as in the case of Pavlovian-instrumental transfer tests (Di Ciano & Everitt, 2005; Everitt & Robbins, 2000; Hodos, 1961; Wyvell & Berridge, 2000). Cues can also have priming abilities and reinstate behaviors such as food consumption that had ceased due to satiation or extinction (Berridge, 2004).

Rewards also have their own intrinsic stimuli (sight, smell, texture, etc.). Incentive salience can be attributed to these intrinsic properties of the reward and can trigger increased consumption. Hence, food consumption is another measure of "wanting" (Berridge, 1996). Other motivational measures included operant responding in general where the animal has to work for delivery of the reward (Berridge, 1996).

In this thesis, different measures of "wanting" were utilized to assess the effect of DBS on motivated behaviors. These measures are: food consumption, cue-triggered behaviors, and operant responding.

## 1.2 **Limbic Circuits and Neural Coding of Reward**

Several brain structures are involved in the processing of food rewards. These structures form part of the limbic system. Some of these structures include the orbitofrontal cortex, the anterior cingulate cortex, as well as subcortical structures such as ventral pallidum, nucleus, accumbens, and the amygdala, and even brainstem regions like the ventral tegmental area (source of dopamine projections in the mesolimbic system) and parabrachial nucleus of the pons (Haber, 2011; Humphries & Prescott, 2009; Kelley, Baldo, & Pratt, 2005; McGinty et al., 2011). This thesis focuses on two key areas involved in reward processing - the ventral pallidum and the central nucleus of the amygdala.

### 1.2.1 ***Ventral pallidum***

*Anatomical connections*

The ventral pallidum (VP) is anatomically and neurochemically well-placed to act as an integrating system for reward and reward-related information processing in the limbic system (Kelley et al., 2005; Napier & Mickiewicz, 2010; Richard, Castro, Difeliceantonio, Robinson, & Berridge, 2012; Smith, Tindell, Aldridge, & Berridge, 2009); refer to figure 1.2 highlighting key connections with the ventral pallidum. The VP has extensive, reciprocal connections with the nucleus accumbens (Chrobak & Napier, 1993; Churchill & Kalivas, 1994; Phillipson & Griffiths, 1985; Usuda, Tanaka, & Chiba, 1998) and also receives inputs from the amygdala, prefrontal cortex, orbitofrontal cortex, infralimbic cortex, lateral hypothalamus, parabrachial nucleus, and ventral tegmental area (Carnes, Fuller, & Price, 1990; Fuller, Russchen, & Price, 1987; Groenewegen & Berendse, 1990; Grove, 1988; Klitenick, Deutch, Churchill, & Kalivas, 1992; Maurice, Deniau, Menetrey, Glowinski, & Thierry, 1997; Mitrovic & Napier, 1998; Napier, Muench, Maslowski, & Battaglia, 1991; Olive & Maidment, 1998; Reep & Winans, 1982; Saper & Loewy, 1980; Turner, Lavin, Grace, & Napier, 2001; Zaborszky, Gaykema, Swanson, & Cullinan, 1997; Zahm, Zaborszky, Alones, & Heimer, 1985). The VP also projects back to nearly all of its input sources, as well as other structures including preoptic regions, mediodorsal thalamus, subthalamic nucleus, substantia niagra

(Groenewegen, Berendse, & Haber, 1993; Grove, 1988; Haber, Groenewegen, Grove, & Nauta, 1985; Kalivas & Nakamura, 1999; Zahm, 1989), and downstream to the brainstem nuclei (Churchill, Zahm, & Kalivas, 1996; Groenewegen et al., 1993; Grove, 1988; Mogenson, Jones, & Yim, 1980). In addition, the VP participates in a number of cortico-basal ganglia-thalamocortical pathways (Churchill et al., 1996; Gritti, Mainville, Mancia, & Jones, 1997; Groenewegen et al., 1993; Grove, 1988; Pirot, Jay, Glowinski, & Thierry, 1994; Zahm & Heimer, 1987). Given its extensive connections to many regions involved in processing sensory information, reward, and movement, the VP is proposed to be a region that can mediate various aspects of reward (Napier & Mickiewicz, 2010; Richard et al., 2012; Smith et al., 2009) and has also been argued to be involved in translating motivational signals into action (Mogenson et al., 1980; Mogenson & Yang, 1991).

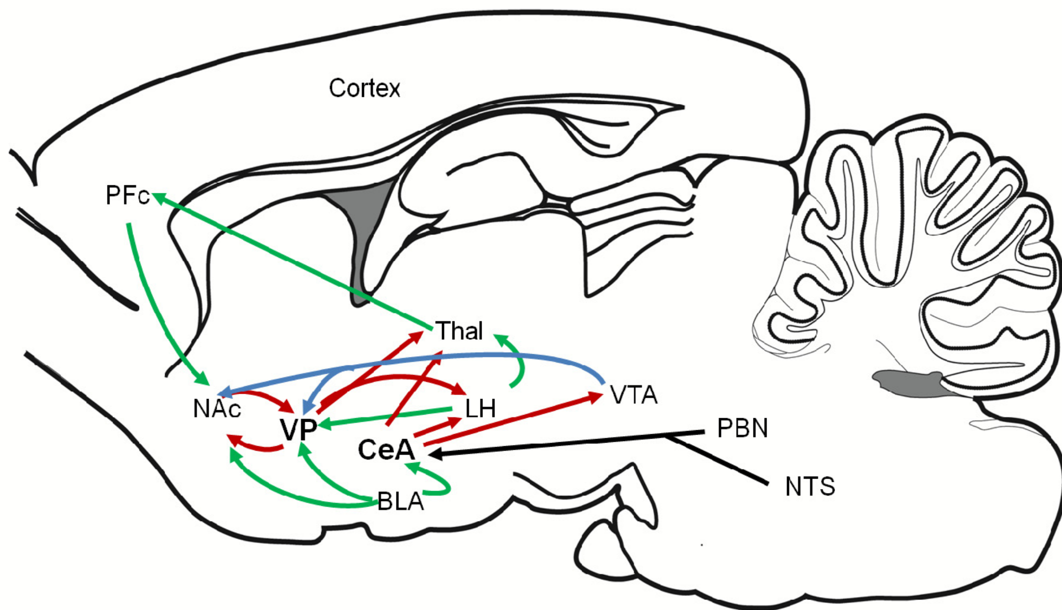


Figure 1.2 - Sagittal view of a rat brain highlighting major connections of the ventral pallidum and central nucleus of the amygdala. Ventral pallidum (VP) receives input from the basolateral amygdala (BLA), nucleus accumbens (NAc), prefrontal cortex (PFC), ventral tegmental area (VTA), and lateral hypothalamus (LH). In turn, VP projects back to NAc, LH, and also projects to thalamus (Thal). The central nucleus of the amygdala (CeA) receives input from BLA, parabrachial nucleus (PBN), and nucleus of the solitary tract (NTS) and sends projections to LH and Thal. Green lines represent glutamatergic projections, red lines represent GABAergic projections, and blue lines represent dopaminergic projections. Image is adapted from Paxinos and Watson atlas, 6th edition (Paxinos & Watson, 2007). Location of structures is approximate, for example CeA and BLA are more lateral than VP and NAc.

*Morphology and electrophysiological properties*

Three main types of neurons have been found in the ventral pallidum. The majority of cells found are the medium spiny projection (MSP) neurons that release γ-Aminobutyric acid-or GABA. These neurons are homologous to medium spiny projection neurons in the globus pallidus. Larger cholinergic cells and smaller non-cholinergic cells are also found. The two main output neuron types are the MSP and cholingergic cells. Medium spiny projection neurons are mostly spontaneously active and cholinergic neurons are typically silent (Bengtson & Osborne, 1999; Bengtson & Osborne, 2000; Lavin & Grace, 1996). The VP has an array of receptors including GABA, glutamate, opioid, serotonin, cholinergic, and dopamine (Bengtson & Osborne, 1999; Mitrovic & Napier, 1998; Napier et al., 1991; Olive & Maidment, 1998; Zahm et al., 1985). Basal firing rates of ventral pallidum neurons are typically around 9 to 14 Hz (Bengtson & Osborne, 2000; Heidenreich, Mailman, Nichols, & Napier, 1995; Tindell, Berridge, & Aldridge, 2004).

The ventral pallidum also receives massive tonic inhibitory GABA inputs from the nucleus accumbens, central amygdala, and other areas. It is proposed that disinhibition of VP neurons via hyperpolarization of nucleus accumbens (and possibly the central amygdala) may be a way that reward and motivation are stimulated (Caille & Parsons, 2004; Caille & Parsons, 2006; Kalivas, 2004; Napier & Mitrovic, 1999). VP also receives cholinergic inputs from the accumbens and the amygdala and in turn sends GABAergic and cholinergic input to these same structures (Bengtson & Osborne, 2000). Dopaminergic neurons also project to the VP (Klitenick et al., 1992; Napier, Simson, & Givens, 1991).

*Functional studies*

Evidence from lesion studies, neuropharmacological studies, and electrophysiological studies has supported the idea that VP mediates both natural (food, sex, etc.) and drug rewards and may be necessary for reward learning, motivational "wanting", and hedonic "liking" of rewards. Lesions of the VP have produced aphagia, i.e. failure to voluntarily eat, (Cromwell & Berridge, 1993; Morgane, 1961) and resulted in increased aversive reactions to palatable tastes (Cromwell & Berridge, 1993). Case

studies in humans with lesions that extend into the VP have also reported anhedonia and disappearance of drug cravings (Miller et al., 2006). Infusions of GABA agonists have also been shown to attenuate intake of saccharine- or quinine- flavored water and result in increased aversive reactions to sweet tastes (Shimura, Imaoka, & Yamamoto, 2006). Lesion and inactivation studies involving the VP have demonstrated impaired reward learning and diminish willingness to work for rewards (Farrar et al., 2008; Harvey et al., 2002; McAlonan, Robbins, & Everitt, 1993; McFarland & Kalivas, 2001; McFarland, Davidge, Lapish, & Kalivas, 2004; Waraczynski, 2006).

The VP is also activated in response to rewards and encodes learning, “wanting”, and “liking” of rewards. Imaging studies have shown that the posterior VP in humans is activated when images of appetizing foods in contrast to bland foods are presented (Beaver et al., 2006). Work in the Aldridge and Berridge labs has shown that VP neurons are responsive to reward and reward-related behavior and that neurobiological applications to the VP can also enhance reward processing. VP neurons encode the predictive and incentive value of reward cues, the reward itself, and hedonic “liking” (Tindell et al., 2004; Tindell, Berridge, Zhang, Pecina, & Aldridge, 2005; Tindell, Smith, Pecina, Berridge, & Aldridge, 2006). Mu-opioid injections into the posterior VP resulted in increased “liking” and “wanting” of rewards, while amphetamine injections into the same region has shown to increase just “wanting” (Smith & Berridge, 2005; Smith & Berridge, 2007; Smith et al., 2011). Other studies have shown enhancement of reward with neurobiological manipulations of the VP. Microinjections of drug into the VP have shown to elicit eating, positive hedonic reactions, locomotion, conditioned place-preference and bar-pressing for delivery of a food reward (Gong, Neill, & Justice, 1996; P. I. Johnson, Stellar, & Paul, 1993). $GABA_A$ receptor blockade (bicuculline) in the VP was shown to cause increased food intake, though interestingly, did not affect hedonic reactions to taste rewards (Shimura et al., 2006; Smith & Berridge, 2005). Electrical self-stimulation in the posterior VP has also shown to be rewarding. Rats would repeatedly press a bar to deliver short bursts of stimulation at threshold frequencies of 20 Hz (Panagis, Miliaressis, Anagnostakis, & Spyraki, 1995).

Pharmacological manipulations in the VP have also shown that “liking” and “wanting” systems can be activated separately. As mentioned earlier, infusions of GABA

antagonists increases food consumption, but do not elevate “liking” reactions (Shimura et al., 2006; Smith & Berridge, 2005). Dopamine agonist infusions into the VP has also shown to enhance “wanting” without “liking” (Smith et al., 2011).

Anatomical subdivisions in the ventral pallidum show differences in reward processing. Extensive research has looked at the differences in the anterior-posterior extent of the VP. Injections of μ-opioids into the posterior VP, specifically into the cubic millimeter region in this area referred to as a “hedonic hotspot”, produced increased “liking” and “wanting”. Injections of amphetamine into the same region produced increased “wanting” (Smith & Berridge, 2005; Smith et al., 2011). In more anterior locations of VP, there is evidence of a “hedonic coldspot”, where μ-opioid injections actually decreased hedonic reactions and consummatory behaviors (Smith & Berridge, 2005). The “hedonic hotspot” overlaps with the area in the VP, which when lesioned, resulted in decreased food consumption and increased aversion to sucrose (Cromwell & Berridge, 1993). Self-stimulation studies also revealed posterior VP to be more effective than the anterior VP (Panagis et al., 1995). Human studies have shown increased activation to pictures of palatable foods in the posterior VP, but increased activation in the anterior VP in response to pictures of disgusting food (Beaver et al., 2006; Calder et al., 2007). Based on this evidence, we choose to target the posterior ventral pallidum as a starting point to investigate the effects of DBS in the VP.

In summary, the ventral pallidum seems to be an important region for processing reward. Studies show that it appears to be necessary for normal “wanting” and “liking”. VP neurons encode reward and elevations of “liking” and “wanting”, and with certain manipulations in the VP, one can modulate “wanting” without affecting “liking”, which shows that these systems are dissociable. Hence, VP may be an important target for DBS for modulation of reward processing; a target which has yet to be investigated and thus, is one goal of this dissertation.

### 1.2.2 ***Central nucleus of the amygdala***

*Anatomical connections*

The amygdala is an almond-shaped region located in the medial temporal lobe that encompasses a heterogeneous group of nuclei involved in a variety of functions including learning, memory, emotions, and attention (Swanson & Petrovich, 1998). The amygdala has many connections to cortical (especially sensory and prefrontal cortex) and sub-cortical regions and has direct output connections to the hypothalamus and brainstem regions that are involved in encoding of autonomic and psychological responses including feeding (Baxter & Murray, 2002; Sah, Faber, Lopez De Armentia, & Power, 2003). The amygdala seems to be a key structure in assigning positive or negative emotions to a given context, possibly by connecting particular autonomic and psychological responses to sensory stimuli (Langevin, 2012; Rogan & LeDoux, 1996).

The central nucleus of the amygdala (CeA) was specifically targeted. The CeA is thought to be closely related to the ventral striatum. In fact, Swanson and Petrovich argue that it should even be considered an extension of the ventral striatum (Swanson & Petrovich, 1998). The CeA functions as the major output nucleus for the amygdala, with direct connections to the hypothalamus and various brainstem nuclei involved in processing autonomic responses. The lateral and basal portions of the amygdala receive emotional sensory stimuli and pass those signals on to the CeA. Emotional integration then largely occurs within the interdivisional circuitry of the CeA (Pitkanen, Savander, & LeDoux, 1997). The CeA receives many inputs from other amygdaloid areas, but sends few out (Sah et al., 2003). The CeA is made up of four main subdivisions: the capsular subdivision (CeC), lateral subdivision (CeL), intermediate subdivision (CeI), and medial subdivision (CeM). These sub regions connect extensively with one another allowing for massive integration of information just within the CeA alone. Inputs to the CeA largely enter the CeC and CeM and the ouput of the CeA is mainly funneled through the CeM. CeM also receives many inputs from CeC and CeL (Jolkkonen & Pitkanen, 1998; McDonald & Augustine, 1993; Sah et al., 2003).

Tracing studies have revealed that systems related to oral sensation, taste, smell, and reward are prominently connected to the CeA. There are several connections

between the CeA and taste pathways from the pons (Norgren, 1976), the parabrachial nucleus (Norgren & Leonard, 1973; Ottersen & Benari, 1978), and the nucleus of the solitary tract (Ricardo & Koh, 1978). The thalamic taste relay, the basal nucleus of the ventromedial complex, also projects to the CeA (Ottersen & Ben-Ari, 1979). Ottersen also found strong projections from the medial frontal cortex including the olfactory cortex related to processing odor information (Ottersen, 1982). There are projections from the allocortical insula, a cortical region thought to relate to disgust (Ottersen, 1982). The parafascicular nucleus (involved in processing behavior flexibility) also projects to the CeA (Brown & Jenkins, 1968; Ottersen & Ben-Ari, 1979). Other studies have shown significant brainstem projections to the CeA including dopaminergic projections from the ventral tegmental area and the substantial niagra (Ottersen & Benari, 1978).

The CeA has widespread connections to various brainstem nuclei (many of which are reciprocal connections), the cortex, and the hypothalamus (Sah et al., 2003). Furthermore, the CeA sends output directly to multiple regions of the hypothalamus, including the ventromedial or "satiety center" and the lateral hypothalamus or "feeding center" (Amaral, Veazey, & Cowan, 1982; J. L. Price & Amaral, 1981). The CeA projects to the dorsal vagal complex, the ventral tegmental area, and the thalamus and has massive connections to the bed nucleus of the stria terminalis (Dong, Petrovich, & Swanson, 2001; J. L. Price & Amaral, 1981). The CeA also has indirect connections to the nucleus accumbens and the ventral pallidum, mostly through the basolateral complex of the amygdala (C. J. Price, Moore, Humphreys, Frackowiak, & Friston, 1996).

The massive number of connections with the CeA from many cortical and subcortical areas involved in processing taste, food consumption, and reward indicates the CeA most likely plays a key role in integrating gustatory information and modulating behavior and autonomic functions based on those inputs, particularly consummatory behavior (Swanson & Petrovich, 1998). This makes it another good candidate for modulating consummatory behaviors with DBS. Figure 1.2 also highlights some important connections with the CeA, especially those involved in reward and consumption.

*Morphology and electrophysiological properties*

The CeA is composed largely of medium-sized spiny GABAergic neurons, similar to the adjacent striatum, with soma sizes typically between 16 and 22 µm in diameter. Connections within the CeA are also mostly GABAergic (Cassell & Gray, 1989; McDonald, 1982; McDonald & Augustine, 1993). Majority of the units, especially in the central medial nucleus are referred to as late firing, meaning in response to current injection, the membrane potential depolarizes slowly on the order of hundreds of milliseconds (Martina, Royer, & Pare, 1999). The CeA receives substantial GABA-ergic innervation (Sun & Cassell, 1993). It is thought that the output of the CeA is largely GABAergic (Swanson & Petrovich, 1998) and that autonomic actions may be produced by activation of intrinsic GABA neurons of the CeA, which in turn, inhibits GABAergic projection neurons of the CeA (Sun & Cassell, 1993). There are also receptors for glutamate, various enkephalins, and corticotrophin releasing factor hormone (Day, Curran, Watson, & Akil, 1999). Neurophysiological investigations have shown that average basal firing rates of CeA neurons are very low, typically 5 Hz or even lower (Collins & Paré, 1999; Duvarci, Popa, & Paré, 2011; Rosenkranz & Grace, 1999; Shabel & Janak, 2009).

*Functional studies*

In the well known Klucy and Bluver experiments in the 1930s, monkeys with bilateral temporal lobotomies (which included the amygdala), among other changes in behavior, became hyperoral and began consuming meat which they normally do not prefer (Kluver, H. & Bucy, P. C., 1938). The ablative lesions in these studies were extensive (Bucy P. C. & Kluver, H., 1955), but gave some of the first evidence of the role of temporal lobe structures, including the amygdala, in food consumption.

Other lesion studies of the CeA offered further support for the idea that the region is involved in food consumption, taste, associative learning, and motivation. Several lesion studies have looked at the role of CeA in tastes. In the late '70s, Kemble reported decreased water and quinine intake in a learned taste aversion paradigm with CeA lesions; though there was no effect on saccharin intake (Kemble, Studelska, & Schmidt, 1979). Other studies have shown that lesions in the CeA resulted in decreased

consumption of need-based (after the animal has been salt-depleted and now has a “salt appetite”) and need-free salt intake, though again there was no effect on sucrose solution intake (Galaverna et al., 1993; Seeley, Galaverna, Schulkin, Epstein, & Grill, 1993). However, others have reported a decrease in the consumption of sweet solutions. Touzani and colleagues saw a decrease in consumption of saccharin solution following CeA lesions (Touzani, Taghzouti, & Velley, 1997).

Inactivation of the CeA also leads to decreased intake of foods. Will and colleagues utilized an experimental paradigm in which opioid activation of the nucleus accumbens dramatically increases the intake of highly palatable substances (M. Zhang & Kelley, 2002). Under these circumstances, simultaneous inactivation of the CeA using muscimol injections decreased both baseline food consumption and DAMGO-increased food consumption (M. J. Will et al., 2009; M. Will, Franzblau, & Kelley, 2004).

The CeA may be particularly involved in translating learning into motivation and focusing motivation for particular cues and rewards (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009; Mahler & Berridge, 2011). Opioid stimulation in the CeA resulted in increased food consumption, increased responses to reward cues, and increased reward-seeking behaviors (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009; Mahler & Berridge, 2011). Inactivation of CeA with muscimol during or after learning suppressed approach and consummatory behaviors directed toward the reward cue and also decreased food intake and time spent eating (Mahler & Berridge, 2009). CeA lesions abolished conditioning or orienting responses to reward cues, though not to the food dish itself (Gallagher, Graham, & Holland, 1990). Lesioning of the CeA also seems to impair autoshaping and in general conditioned motivated responses (Cardinal et al., 2002; Robledo, Robbins, & Everitt, 1996). Lesions of the CeA also impair Pavlovian instrumental transfer learning (Hall, Parkinson, Connor, Dickinson, & Everitt, 2001; Murschall & Hauber, 2006). Petrovich and colleagues found that CeA was critical for control of feeding by aversive learned cues (Petrovich & Gallagher, 2003; Petrovich, 2011; Petrovich, Ross, Mody, Holland, & Gallagher, 2009). They found that lesioning of the CeA prior to acquisition of a conditioned pairing between a cue and a footshock abolished fear-induced cessation of eating (Petrovich et al., 2009).

CeA's role in taste aversion and "liking" in general seems to be mixed. CeA lesions or inactivation had no effect on taste aversion learning (Galaverna et al., 1993; Kemble et al., 1979; Li et al., 2012). Galaverna and colleagues utilized a conditioned taste aversion protocol, pairing a normally palatable taste with lithium chloride or LiCl injections. Lithium chloride injections produce nausea. In order to determine the role of the CeA in the association of a taste with an unpleasant feeling (here, nausea), they created lesions in the CeA prior to training. They found that rats with CeA lesions still produced aversive reactions to normally hedonic tastes now paired with LiCl. Hence, lesions in the CeA did not prevent the animal from forming an association between a particular taste and feeling of nausea. However, they found that CeA-lesionsed rats also produced aversive reactions to non-paired tastes when compared with non-lesioned animals (Galaverna et al., 1993), which could indicate an overall increase in aversion with CeA lesions. Touzani and colleagues found that given a choice between water and different sucrose solutions, rats decreased preference for only the more concentrated sucrose solutions. Also in a two-bottle choice test between quinine and water, these CeA lesioned rats showed decreased preference for quinine compared to control animals (Touzani et al., 1997). Yet, inactivation of the CeA did not affect the expression of a previously learned flavor preference (Dwyer & Iordanova, 2010; Dwyer, 2011). CeA rats showed increase hedonic reactions, similar to sham-lesioned rats, to an extremely salty solution when they were salt-depleted, though as mentioned earlier, it did decrease salt-need intake (Galaverna et al., 1993; Seeley et al., 1993). Lesions in the CeA seem to decrease aversive threshold for sweet, salt, and bitter tastes (Kemble et al., 1979; Li et al., 2012; Touzani et al., 1997). Opioid stimulation decreased hedonic reactions to sucrose solutions, but did not affect reactions to other tastes or changed aversive reactions (Mahler & Berridge, 2011). CeA neurons also respond to taste and oral stimuli (Nishijo, Ono, Uwano, Kondoh, & Torii, 2000; Uwano, Nishijo, Ono, & Tamura, 1995).

Animals will self-stimulate, i.e. press a lever or nose-poke to receive a 500 ms train of stimulation at frequencies ranging from 60 Hz to 200 Hz. This short burst of stimulation is considered to be rewarding and animals will initiate this response. This has been evidenced by previous work (Touzani & Velley, 1998; Waraczynski, 2006; Wurtz & Olds, 1963).

## 1.3 **Deep Brain Stimulation Therapy**

### 1.3.1 ***Deep brain stimulation - overview***

Deep brain stimulation (DBS) is a functional surgical therapy that delivers continuous high frequency stimulation, typically in the range of 130 Hz to 200 Hz, in targeted brain regions (Breit, Schulz, & Benabid, 2004). Stimulation is generated from a pacemaker-like device implanted in the chest and is delivered to specific brain regions via electrodes (Medtronic, 2013). Deep brain stimulation has become a successful treatment for movement disorders including Parkinson's disease, essential tremor and dystonia (Breit et al., 2004; Chang, 2004; Gubellini et al., 2009; Kringelbach, Jenkinson, Owen, & Aziz, 2007; Schwalb & Hamani, 2008; Weaver et al., 2009). There are reports of chronic DBS being used as early as 1960s for the treatment of pain, targeting the thalamus (Hassler, Riechert, Mundinger, Umbach, & Ganglberger, 1960; Ohye, Nagao, Narabayashi, Kubota, & Hongo, 1964). The first use of high frequency stimulation for treatment of Parkinson's disease, in a set-up similar to what is used now, was reported in the late 1980s (Benabid et al., 1991; Benabid et al., 1998). Given its therapeutic benefits, relative low risk of complications (especially compared to ablative studies), and the ability to have finer control and more focused targeted effects compared to drugs (Doshi, 2011; Voges et al., 2006), DBS is being explored as a potential treatment for other neurological and psychiatric disorders (Chang, 2004; Gubellini et al., 2009; Kringelbach et al., 2007; Schwalb & Hamani, 2008). Deep brain stimulation is being used for treatment for epilepsy and chronic pain and clinical trials are ongoing for use in treatment of obsessive compulsive disorder and depression (Chang, 2004; Greenberg et al., 2006; Haber & Brucker, 2009; Halpern et al., 2008; Lozano et al., 2008; Mayberg et al., 2005). It is also being explored as a potential treatment for eating disorders and addiction (Gubellini et al., 2009; Halpern et al., 2008).

Despite its clinical success, there is still a lot not known about how DBS works, nor its potential long-term effects on neuronal circuits (Garcia, D'Alessandro, Bioulac, & Hammond, 2005a; Gubellini et al., 2009; Kringelbach et al., 2007; McIntyre, Savasta, Walter, & Vitek, 2004; Temel et al., 2009). Efficacy of stimulation has been shown to depend on target location and stimulation parameters (Kuncel, Cooper, Wolgamuth, &

Grill, 2007) and, for that matter, the disorder itself. For example, despite being tested for over 50 years, there is still no standard treatment protocol for DBS for chronic pain (Chang, 2004). Target location can also lead to various side effects, such as changes in mood and cognition, especially if electrodes are located more towards the ventral subthalamic nucleus (STN). The dorsal STN is a popular target for DBS for Parkinson's; but the ventral STN has connections with many limbic structures and has been shown to be involved in reward (Breit et al., 2004; Okun, Bakay, DeLong, & Vitek, 2003; Volkmann et al., 2001). In addition, though in many cases effects of DBS can be seem almost immediately, especially for many motor symptoms, some beneficial effects have been reported to take longer to occur and there might be a build-up with peak effects not seen till several days after multiple days of stimulation (Baunez, Christakou, Chudasama, Forni, & Robbins, 2007; Darbaky, Forni, Amalric, & Baunez, 2003). This may be particularly challenging in evaluating effects of DBS on mood and cognition (Mayberg et al., 2005). Despite the challenges, DBS is still a very effective treatment and investigations into new areas of therapy have shown to be promising.

### 1.3.2 ***Mechanisms of deep brain stimulation***

Typical targets for deep brain stimulation for movement disorders (Parkinson's disease, essential tremor, and dystonia) are the subthalamic nucleus (STN), globus pallidus internus (GPi), and thalamus. Given the similarity in therapeutic effects seen with DBS and lesioning in these structures (Aziz, Peggs, Sambrook, & Crossman, 1991; Bergman, Wichmann, & DeLong, 1990; Koller et al., 1997; Kumar, Lozano, Montgomery, & Lang, 1998; Wichmann, Bergman, & DeLong, 1994), it was proposed that DBS was inhibiting the target structure, decreasing output from the stimulated structure and thus mimicking the effects of a physical lesion (Gross et al., 1997; Limousin et al., 1995; Lozano, Lang, Hutchison, & Dostrovsky, 1998). DBS could be inhibiting neurons in the simulated structure by (1) creating a depolarization blockade through inactivation of voltage-dependent ion channels, preventing the neurons from firing (Benazzouz, Piallat, Pollak, & Benabid, 1995; Beurrier, Bioulac, Audin, & Hammond, 2001; Bikson et al., 2001), (2) by depleting neurotransmitter at the synapse (Urbano, Leznik, & Llinas, 2002), or (3) stimulating inhibitory afferents to the target

nucleus and thus shutting it down (Dostrovsky & Lozano, 2002). Further support of the idea of DBS inhibiting structures has come from electrophysiological recordings during or just after DBS. These results showed inhibition in substantia nigra (SNr), STN, and GPi during stimulation (Benazzouz et al., 1995; Boraud, Bezard, Bioulac, & Gross, 1996; Filali, Hutchison, Palter, Lozano, & Dostrovsky, 2004).

More recent evidence has suggested that DBS is not just simply mimicking the effects of an ablation. In some instances opposite effects have been observed with DBS and brain lesions (Krack et al., 1998; Vitek, 2002; J. Y. Zhang, Mewes, Chockkan, & Vitek, 1997). Also, more and more evidence suggests that DBS is not just preventing neurons from firing. Work from modeling studies and electrophysiological recordings *in vitro* and *in vivo* have shown that neurons are still able to fire during DBS. Stimulation has been shown to induce multiphasic patterns of firing in neurons of GPI and even STN (Bar-Gad, Elias, Vaadia, & Bergman, 2004; Cleary et al., 2013; Garcia, Audin, D'Alessandro, Bioulac, & Hammond, 2003; Hashimoto, Elder, Okun, Patrick, & Vitek, 2003; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007; Maurice, Thierry, Glowinski, & Deniau, 2003; McCairn & Turner, 2009). Imaging studies have also reported increased blood flow, local metabolic rates, and blood oxygenation (Ceballos-Baumann et al., 1999; Hershey et al., 2003; Zhao, Sun, Li, & Wang, 2004) in the ipsilateral globus pallidus external (GPe), thalamus, and other brain regions with DBS in the STN. Some of the studies that report stimulation-induced inhibition state that this could be due to activation of afferent projections resulting in the release of GABA which then binds to receptors on the target neurons causing them to be inhibited (Dostrovsky et al., 2000; Lafreniere-Roula et al., 2010; McCracken & Grace, 2007; Vitek, 2002). Studies have also reported glutamate or GABA release (O. Mabrouk, personal communication, June 19 2013; Windels et al., 2003; Windels, Carcenac, Poupard, & Savasta, 2005), increased levels of striatal dopamine in rats (Bruet et al., 2001), or neurochemical by-products such as cyclic GMP (Stefani et al., 2005) in response to DBS.

Other studies have suggested that there could be both inactivation of cell bodies but activation of afferent and/or efferent axons (Anderson, Hu, Iremonger, & Kiss, 2006; Filali et al., 2004; Garcia et al., 2005a; Hashimoto et al., 2003; McIntyre, Grill, Sherman, & Thakor, 2004). This has led to the idea that stimulation could be imposing its own

regular firing pattern; that is, entraining the neurons to fire at a pattern that may no longer be functional meaningful and independent of soma activity (Cleary et al., 2013; Garcia et al., 2005a; Garcia, D'Alessandro, Fernagut, Bioulac, & Hammond, 2005b; W. M. Grill, Snyder, & Miocinovic, 2004; M. D. Johnson & McIntyre, 2008; McIntyre et al., 2004) possibly creating an "informational lesion" (W. M. Grill et al., 2004). However, the story is even more complicated than that. Somatic activity may still provide contributions to the circuit; for instance, cell bodies have been reported to be active during stimulation (Bar-Gad et al., 2004; Garcia et al., 2005b; M. D. Johnson & McIntyre, 2008). Furthermore, antidromic stimulation of afferent axons can influence neural activity within the target structure thus further affecting neural processing within the structure as well as efferent output. Stimulation may also be affecting fibers of passage which could then have effects on structures further downstream. Stimulation of these fibers of passage has been shown to provide therapeutic effects for Parkinson's (Gradinaru, Mogri, Thompson, Henderson, & Deisseroth, 2009; Maks, Butson, Walter, Vitek, & McIntyre, 2009). Hence, the effects of DBS may be due to multiple neuronal mechanisms as well as the type of neural circuit being stimulated and its various connections (Carlson, Cleary, Cetas, Heinricher, & Burchiel, 2010; Cleary et al., 2013; M. D. Johnson & McIntyre, 2008; M. D. Johnson, Zhang, Ghosh, McIntyre, & Vitek, 2012; Okun, 2012).

*Concept of information "lesion"*

As mentioned above, several groups propose that high frequency stimulation could be entraining the neurons to fire at a regular rate. This stimulation could be masking the intrinsic firing rate of the neurons and imposing its own pattern of firing in the circuit (Garcia et al., 2005a; Garcia et al., 2005b; W. M. Grill et al., 2004; M. D. Johnson & McIntyre, 2008; McIntyre et al., 2004). Information in neurons is encoded not only in their firing rate, but also in the pattern of firing, that is, the temporal sequence of intervals in a spike train (Dayan & Abbott, 2001; Gerstein & Kiang, 1960; Moore, Perkel, & Segundo, 1966). Based on information theory, greater variability in this pattern of firing could have the potential to code more information. A more regular pattern with less uncertainty contains less information (Shannon, 1948). Hence, if DBS is imposing a more regular, consistent pattern of firing on the neurons, then these neurons

in a sense are transmitting less information. This has been demonstrated in modeling and experimental studies, which have shown that when the frequency of stimulation is decreased, the irregularity of firing increases, and there is more variation between spikes (Garcia et al., 2003; Garcia et al., 2005b; W. M. Grill et al., 2004; Kuncel et al., 2007).

*High versus low frequency of stimulation*

The idea of high frequency stimulation causing a "blocking" effect of information flow in the neural circuit and low frequency stimulation having a more "activating" effect is supported at least in modeling and experimental studies of DBS for Parkinson's disease, essential tremor, and dyskinesia. Therapeutic effects of DBS typically occur at frequencies greater than 90 Hz. Lower stimulation frequencies (< 50 Hz) were shown to have no therapeutic effect and even worsen symptoms, for example, aggravating tremors (Garcia et al., 2003; Garcia et al., 2005a; Kuncel et al., 2007; Moro et al., 2002; Rizzone et al., 2001; Ushe et al., 2004; Wu, Levy, Ashby, Tasker, & Dostrovsky, 2001). Modeling studies have also shown that frequencies < 100 Hz can superimpose patterns of firing that are more irregular. Increased irregularity of firing has been shown to actual worsen symptoms of Parkinson's disease and essential tremor (W. M. Grill et al., 2004; Kuncel et al., 2007). Even in other structures not associated with Parkinson's disease, there is possibly an activating effect seen with low frequency simulation. For example, low frequency stimulation at 60 Hz in the lateral hypothalamus has shown to ellicit feeding (Berridge & Valenstein, 1991; Mendelson & Chorover, 1965; Valenstein, Cox, & Kakolews, 1968).

This possible "blocking" effect of high frequency stimulation can be beneficial or not depending on the circuit being stimulated and the disorder being treated. In the case of Parkinson's disease, it has been proposed that due to dopamine depletion in the substantia nigra, oscillations develop in the basal ganglia-thalamo-cortical network. These oscillations seem to be correlated with development of tremors (Bergman et al., 1998). Hence, breaking up this synchronized pathological activity with high frequency stimulation has shown to provide a therapeutic effect. Lower frequencies may not be able to disrupt these oscillations resulting in no effect or even increased aggravation of the symptoms. In other circuits though, one may not want to "block", but enhance

information transmission. For example, the ventromedial hypothalamus (VMH) is considered to be the “satiety” center and lesions to this structure have resulted in increased feeding and weight gain (Hetherington & Ranson, 1940; Hetherington & Ranson, 1942). Hence, in order to decrease consumption by targeting the VMH, one would actually want to activate this region instead of blocking it. So in this case, using lower frequency stimulation may be more beneficial. In fact, studies using DBS in the VMH have shown that frequencies < 100 Hz, resulted in decreased consumption (Halpern et al., 2008; Melega, Lacan, Gorgulho, Behnke, & De Salles, 2012).

### 1.3.3 ***Deep brain stimulation, eating disorders, and addiction***

As mentioned above, deep brain stimulation (DBS) is being explored as a potential treatment for several different neurological and psychiatric disorders, including morbid obesity and addiction (Gubellini et al., 2009; Halpern et al., 2008). Studies have shown that DBS could be used to modulate reward processing. Depending on the placement of DBS electrodes, case studies have reported changes in motivation and reward behavior in general. For example, two patients with DBS in the subthalamic nucleus reported a decrease in cravings that had been originally induced due to their L-dopa medication (Witjas et al., 2005). Another patient treated for OCD with DBS in the nucleus accumbens reported decreases in food consumption and smoking (Mantione, van de Brink, Schuurman, & Denys, 2010). Hence, there is evidence that DBS-like stimulation can modulate reward behaviors. Various animal studies and some human studies have looked at the effects of DBS on obesity and addiction (Heldmann et al., 2012; Lacan et al., 2008; Melega et al., 2012; Rouaud et al., 2010; Sani, Jobe, Smith, Kordower, & Bakay, 2007) with promising results.

Some of the major challenges for DBS for eating disorders and addictions include effective target regions and stimulation parameters. (Heldmann et al., 2012; Lacan et al., 2008; Melega et al., 2012; Rouaud et al., 2010; Sani et al., 2007). Also, a lot is still unknown about the underlying brain mechanisms of reward processing. Understanding the causes of reward dysfunction will help steer research towards the most effective target locations and parameters to be used in DBS treatment of these disorders.

Considering the rationale of DBS for morbid obesity, for example, many studies have targeted the lateral hypothalamus or the ventromedial hypothalamus to either "block" feelings of hunger or trigger feelings of satiation, respectively (Lacan et al., 2008; Lehmkuhle, Mayes, & Kipke, 2010; Melega et al., 2012; Sani et al., 2007). However, simply targeting homeostatic control of feeding might not be enough. Sani and coworkers reported that though DBS in the LH between frequencies 185 – 200 Hz resulted in weight loss, it did not affect food intake (Sani et al., 2007). Foods, especially those rich in fats and sugars, are very rewarding. Motivation to seek out and consume rewards beyond physiological need is thought to be a major factor in morbid obesity (Berridge et al., 2010). Brain reward systems of "wanting" and "liking" are proposed to act as "go" systems that can be activated in the presence of cues associated with appetizing foods and the food itself. Satiation can attenuate these "go" systems, but may not be able to generate a strong enough "stop" signal (Berridge et al., 2010) and presence of food cues, for example, can activate these "go" systems again leading to increased consumption beyond satiation (Berridge, 2004; Higgs, Williamson, Rotshtein, & Humphreys, 2008; Rozin, Dow, Moscovitch, & Rajaram, 1998). Hence, targeting excessive motivation to seek out and consume rewards might be more effective than simply targeting appetite (Halpern et al., 2008). Targeting excessive motivation to consume rewards in general would also be applicable for drug addiction treatment (Robinson & Berridge, 2003; Rouaud et al., 2010). Thus, targeting brain regions involved in processing "wanting" of rewards would be a good place to start.

### 1.4 **Overall Aims and Hypothesis of this Thesis**

The overall goal of this thesis was to investigate the effects of deep brain stimulation on the "wanting" of food rewards. Excessive "wanting" even beyond "liking" have been argued to be a potential cause of reward dysfunctions in morbid obesity and addiction (Berridge et al., 2010; Robinson & Berridge, 2003). Hence, an aim of this study was to block "wanting" of rewards without affecting "liking" of the rewards. Another aim of this thesis was to investigate the effects of DBS on the underlying neural circuit. The main hypothesis is as follows:

1. High frequency stimulation (130 Hz – 200 Hz) in brain regions involved in reward processing will create an "information block" and decrease reward consumption and reward-seeking behaviors.

2. Low frequency stimulation (20 Hz) will either have no effect or enhance reward consumption and reward-seeking behaviors.

### 1.5 **Chapter Overviews**

To test this overall hypothesis, the following three main projects (1, 2, and 3) were carried out and were written up in Chapters 2, 3, and 4 of this thesis, respectively:

*Project 1 (Chapter 2) – Deep brain stimulation in the ventral pallidum has mixed effects on food consumption.*

In this project, the effects of deep brain stimulation (DBS) in the ventral pallidum on "wanting" of food rewards were assessed. Two experiments were carried out. The first experiment evaluated different stimulation parameters. For high frequency stimulation, parameters that typically produced therapeutic effects in Parkinson's disease were used as a starting point (Breit et al., 2004; Kuncel & Grill, 2004). Frequencies of 130 Hz and 200 Hz and pulse widths of 60 μs and 100 μs were used. To test the hypothesis of low frequency stimulation, 20 Hz stimulation was chosen, which in the range of low frequencies that have shown to have an "activating" effect is a good mid-point value (W. M. Grill et al., 2004; Kuncel et al., 2007). Pangis and colleagues also showed that 20 Hz was the threshold frequency for producing self-stimulation behavior in the ventral pallidum. Starting at this frequency, rats would press a lever to deliver a short train of electrical stimulation (400 ms in duration) which they found rewarding (Panagis et al., 1995). Effects of DBS were assessed by measuring consumption of a very rewarding food – chocolate M&Ms. In the second experiment, the effects of DBS on another measure of "wanting" - cue-triggered responses - were investigated in addition to food intake of another highly palatable food (sucrose). In this experiment, a Pavlovian conditioning study was done, where two cues were paired with the delivery of sucrose pellets. Responses to the cues and retrieval and consummation of the pellets were assessed with parameters selected from the results of the M&M study. Neural activity

was also recorded and analyzed during stimulation and no stimulation. Results of the experiment yielded only minimal effects of DBS on "wanting". Overall, low frequency stimulation enhanced consumption and motivated behaviors (though results were not significant compared to no stimulation). High frequency stimulation depending on parameters resulted in decreased consumption of chocolate compared to low frequency and a slight decrease overall in cue-triggered responses, but did not significantly decrease food consumption compared to no stimulation. Stimulation induced multiphasic firing patterns, but overall basal firing rates for low frequency stimulation did not change compared to the no stimulation period, and neurons still responded to the reward cues and reward during both high and low frequency stimulation. Hence, high frequency stimulation did not produce a clear "blocking" effect as proposed which was also reflected in neural responses. VP may not be an effective target region for decreasing food consumption as initially proposed. This led to the assessment of another new target for DBS for reward dysregulation – the central nucleus of the amygdala, which is tested in projects 2 and 3.

*Project 2 (Chapter 3) – Deep brain stimulation in the central nucleus of the amygdala decreases food consumption*

In this second project the effects of DBS in the central nucleus of the amygdala (CeA) on food consumption and another "wanting" measure, operant conditioning, were assessed. One concern with the first study was perhaps the responses to the reward cues had become a habit for the animal and stimulation was not able to completely disrupt this behavior. Thus for this study, an operant responding task was used instead, which looked at the animal's motivation to work for a pellet. Neural activity was also recorded. High and low frequency stimulation at 130 Hz and 20 Hz, respectively, were used at a 100 μs pulse width. DBS in the CeA dramatically decreased sucrose pellet consumption and working for pellet delivery. As with project 1, neurons changed firing in response to stimulation, but in addition, were less responsive to the task-related events. Hence, DBS seemed to "disrupt" neural coding of reward and reward behavior. In addition, facial reactions typically associated with an aversive taste occurred sporadically throughout the test session. This result prompted the third project of this dissertation, where the effects

of DBS on hedonic value of different tastes of different valence (hedonic, aversive, and neutral) were directly evaluated.

*Project 3 (Chapter 4) – Deep brain stimulation in the central nucleus of the amygdala decreases hedonic value of tastes*

In project 3, the effects of DBS in the central nucleus of the amygdala (CeA) on "liking" of tastes were measured. The same stimulation parameters as in project 2 were used. The number of taste reactions to a hedonic taste (sucrose), a neutral taste (water), and an aversive taste (quinine) during 20 Hz, 130 Hz, and no stimulation were quanitifed. Again neural activity during stimulation and when there was no stimulation was assessed. DBS increased aversive reactions to all three tastes and decreased hedonic reactions to sucrose. Only 130 Hz stimulation resulted in increased aversive reactions to sucrose, though both stimulation frequencies increased aversive reactions to water and quinine. As in project 2, neurons changed firing in response to stimulation. There were also fewer responses to tastes during stimulation. Hence, DBS seemed to "disrupt" neural coding of reward and reward behavior. Thus DBS in the CeA was very effective at decreasing reward consumption, but also made things more aversive. There was a difference in effect on aversive reactions in stimulation frequencies for sucrose solution, so further manipulation of parameters could be done to find a value that still decreased consumption but causes little or no increase aversion.

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# CHAPTER 2
# DEEP BRAIN STIMULATION IN THE VENTRAL PALLIDUM HAS MIXED EFFECTS ON FOOD CONSUMPTION

## 2.1 Introduction

Deep brain stimulation (DBS) therapy is currently being used to treat various neurological disorders including Parkinson's disease, essential tremor, and dystonia (Gubellini et al., 2009; Schwalb & Hamani, 2008; Weaver et al., 2009). It has been shown to be a very effective treatment and provides a reversible, titratable way to target very specific brain regions with minimal risk of complications (Doshi, 2011; Voges et al., 2006).

The mechanisms of DBS, though, are not well understood (Garcia et al., 2005a; Kringelbach et al., 2007; McIntyre, Savasta, Kerkerian-Le, & Vitek, 2004). Therapeutic benefits of DBS typical occur at stimulation frequencies greater than 100 Hz; however, the effects do depend on amplitude and frequency parameters as well as target location (Dostrovsky & Lozano, 2002; Kuncel & Grill, 2004). Work from modeling and experimental studies suggest that stimulation frequencies greater than 100 Hz may be activating fibers resulting in stimulation-induced modulation of neural activity. High frequency stimulation could be entraining the neurons to fire at a more regular pattern, which "masks" the intrinsic firing pattern in normal circuits and/or interferes with the pathological patterns in the basal ganglia in Parkinson's disease (Garcia et al., 2005a; W. M. Grill et al., 2004; Hashimoto et al., 2003). Low frequency stimulation (< 50 Hz) on the other hand has been shown to have no therapeutic effect and even worsen symptoms (Garcia et al., 2003; Garcia et al., 2005a; Kuncel et al., 2007; Moro et al., 2002; Rizzone et al., 2001; Wu et al., 2001).

Despite the fact that the mechanisms of DBS are not well understood, given its clinical efficacy in treating disorders like Parkinson's disease, DBS is being investigated,

with promising results, as a potential treatment for other neurological and psychiatric disorders including obsessive compulsive disorder, depression, and epilepsy (Chang, 2004; Greenberg et al., 2006; Haber & Brucker, 2009; Halpern et al., 2008; Lozano et al., 2008; Mayberg et al., 2005).

DBS is also being explored as a potential treatment for eating disorders and addiction (Gubellini et al., 2009; Halpern et al., 2008; Heldmann et al., 2012; Lacan et al., 2008; Melega et al., 2012; Rouaud et al., 2010; Sani et al., 2007; van der Plasse, Schrama, van Seters, Vanderschuren, & Westenberg, 2012). Two major challenges for DBS in treatment of reward-dysfunction are target location and effective stimulation parameters. Especially in the case of eating disorders, many of these studies have targeted the lateral hypothalamus ("feeding center") and the ventromedial hypothalamus ("satiety center") which are involved in the homeostatic control of energy consumption (Anand & Brobeck, 1951). However, simply targeting homeostatic control of feeding might not be enough. Motivation to seek out and consume rewards beyond physiological need is thought to be a major factor in morbid obesity (K. C. Berridge et al., 2010). Therefore, it may be more effective to target excessive motivation to seek out and consume rewards (Halpern et al., 2008). Targeting excessive motivation to consume rewards in general would also be applicable for drug addiction treatment (Rouaud et al., 2010).

One candidate structure for neuromodulation of reward behavior is the ventral pallidum (VP). The VP is argued to be a major structure involved in the processing of reward and is thought to be a region of convergence for motivational and hedonic information in the limbic system (Napier & Mickiewicz, 2010; Richard et al., 2012; Smith et al., 2009). The VP has extensive, reciprocal connections with the nucleus accumbens (Chrobak & Napier, 1993; Churchill & Kalivas, 1994; Phillipson & Griffiths, 1985; Usuda et al., 1998) and also receives inputs from cortical and other limbic structures including the lateral hypothalamus, ventral tegmental area, and amygdala (Carnes et al., 1990; Fuller et al., 1987; Groenewegen & Berendse, 1990; Grove, 1988; Klitenick et al., 1992; Maurice et al., 1997; Mitrovic & Napier, 1998; Napier et al., 1991; Olive & Maidment, 1998; Reep & Winans, 1982; Saper & Loewy, 1980; Turner et al., 2001; Zaborszky et al., 1997; Zahm et al., 1985). In turn, the VP projects back to many

of its inputs and sends connections to the cortex via the dorsomedial thalamus (Churchill et al., 1996; Groenewegen et al., 1993; Grove, 1988; Haber et al., 1985; Kalivas & Nakamura, 1999; Mogenson et al., 1980; Zahm, 1989). The VP also has an array of receptors including GABA, glutamate, opioid, enkephalin, and dopamine (Mitrovic & Napier, 1998; Napier et al., 1991; Olive & Maidment, 1998; Zahm et al., 1985) .

Various animal and human studies have demonstrated that the VP mediates both natural (food, sex) and drug rewards and may be necessary for reward learning, motivational "wanting", and hedonic "liking" of rewards. In rats lesions of the VP have produced aphagia, failure to voluntarily eat, (Cromwell & Berridge, 1993; Morgane, 1961) and resulted in increased aversive reactions to palatable foods (Cromwell & Berridge, 1993). Infusions of GABA agonists into the ventral pallidum of rats have also been shown to attenuate intake of saccharine- or quinine- flavored water and result in increased aversive reactions to sweet tastes (Shimura et al., 2006). Case studies in humans with lesions that extend into the VP have also reported anhedonia and disappearance of drug cravings (Miller et al., 2006). Lesions or inactivation of the ventral pallidum in rats have also demonstrated impaired reward learning and diminished willingness to work for rewards (Farrar et al., 2008; Harvey et al., 2002; McAlonan et al., 1993; McFarland & Kalivas, 2001; McFarland et al., 2004).

The ventral pallidum is also activated in response to rewards. Imaging studies have shown that the posterior VP in humans is activated when images of appetizing foods are presented in contrast to bland foods (Beaver et al., 2006). Studies in rats have shown that VP neurons encode the predictive and incentive value of reward cues, the reward itself, and hedonic "liking" (Tindell et al., 2004; Tindell et al., 2005; Tindell et al., 2006). Microinjections of drugs into the VP of rats have also shown to enhance reward including increased food consumption, more hedonic reactions to "liked" tastes, increased locomotion, and strengthened conditioned place-preference and bar-pressing for food rewards (Gong et al., 1996; P. I. Johnson et al., 1993; Smith & Berridge, 2005; Smith & Berridge, 2007; Smith et al., 2011). Finally, electrical self-stimulation in the posterior VP has also shown to be rewarding. Rats would repeatedly press a bar to deliver short bursts of stimulation at threshold frequencies of 20 Hz (Panagis et al., 1995). Therefore,

the ventral pallidum may be an important target for DBS for modulation of reward processing.

The focus of this experiment was to investigate whether the ventral pallidum would be a good target to "block" motivation for food rewards. In addition, another goal was to determine if underlying changes in VP neural activity occurred with stimulation. Hence, neural activity was also recorded during stimulation, which has its own challenges as electrical stimulation induces artifacts unto the recorded neural data. So another part of this study was to develop and utilize an effective method of stimulation artifact removal.

Stimulation artifacts look spike-like in shape, typically with an unrecoverable period during which the recording amplifiers are saturated followed by an exponential decay (see example in figure 2.1). Several factors contribute to these artifacts including the recording electronics and capacitive crosstalk between recording and stimulating leads (McGill et al., 1982; Wagenaar & Potter, 2002). The non-linear behavior of saturated amplifiers and properties of filters make the artifact last much longer than the stimulus pulse-width, many times on the order of milliseconds (Maeda, Robinson, & Kawana, 1995). Neural data cannot be recovered during the period of amplifier saturation, but action potentials can ride on the exponential decay portion of the artifact and we would like to be able to recover them. Furthermore, the shape and duration of artifacts can also vary between channels and there can be small fluctuations in the duration between pulses. Detailed explanations of the artifact removal program that we developed and utilized, as well as, more background information on the different types of artifact removal methods are given in appendix A.2. Briefly we developed a program that found each artifact, zeroed out the period of amplifier saturation (that is the sharp transient period), and then used a local polynomial curve fit of the exponential decay portion. The polynomial curve fitting algorithm was a modified version of the cubic polynomial fit developed by Wagneaar and Potter (Wagenaar & Potter, 2002), though with a higher degree polynomial for improved fit. We were able to successful remove the stimulation artifacts and recover neural data.

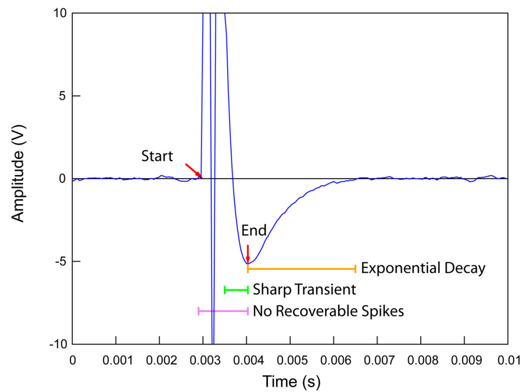


Figure 2.1 - Diagram of a typical stimulation artifact and its characteristics. Zones indicate key regions that are manipulated in the artifact removal program.

In this experiment, we investigated whether the ventral pallidum could be an effective target for modulating reward-seeking behaviors with DBS by measuring the effects of stimulation on free intake of chocolate and in a Pavlovian conditioning task. We compared low frequency stimulation at 20 Hz and high frequency stimulation at 130 and 200 Hz. We hypothesized that low frequency stimulation would either have no effect or an activating effect on the reward circuit resulting in increased eating and motivation. High frequency stimulation would cause a "blocking" effect and result in decreased consumption and motivated behaviors. We also analyzed the effects of DBS on neural activity to glean some more information on the mechanisms of DBS on the local area of stimulation. Our results showed only a slight decrease on motivation and consummatory behaviors of DBS in the VP, and though there were changes in neural response to stimulation, neural encoding of rewards and related-behavior was not disrupted with stimulation.

## 2.2 Materials and Methods

The effect of deep brain stimulation in the ventral pallidum on food consumption was assessed. Animals were tested on two different tasks to measure their motivation for food rewards with and without stimulation. In the first experiment, rats were given free access to a highly palatable food: milk chocolate M&M's candy while receiving stimulation at different parameters. This test was used to assess the efficacy of different stimulation parameters, specifically, frequency and pulse width. In the second

experiment, animals were trained on a Pavlovian conditioning task and the effect of stimulation on animals' responses to reward cues and the reward itself (sucrose pellets) were assessed. Neural responses during stimulation were also quantified.

### *2.2.1 Experiment 1: Chocolate Consumption*

*Subjects*

Ten adult male Sprague-Dawley rats weighing 250 g – 400 g were used in this experiment. Animals were housed individually on a 7:30 AM to 7:30 PM reversed light/dark schedule to ensure that though they were being tested during the day, this time would coincide with their active period. Animals were provided with unrestricted access to standard rat chow and water in their home cages. All of the following experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

*Apparatus*

All training and testing was conducted in a 28 cm x 35 cm x 60 cm plastic chamber with a glass floor. The chamber was illuminated with red light from below. The top of the chamber was open, allowing for the electrode to be connected to a commutator and thus to the stimulator and recording system via a headstage cable. The commutator allowed the rat, though tethered, to still be able to freely move around and explore the chamber. During training and testing, the M&Ms were placed on the floor of the chamber. A software program, MTASK, written in Aldridge lab controlled the timing of the behavioral sessions and onset of stimulation. Stimulation was delivered using the A-M systems model 2300, constant-current digital stimulus isolator stimulator (A-M systems, Carlsborg, WA). Stimulation timing (frequency and pulse-width) was controlled by a program created in the Aldridge Lab using LabVIEW (National Instruments, Austin, TX). Sessions were recorded via a video camera placed underneath the glass floor. Video was recorded at 30 frames per second. Figure A.1 shows a picture of the testing chamber (note, for the M&Ms study, there was no food dish or levers).

*Habituation and M&M training*

**Habituation and exposure to M&M's.** Two to three days prior to the start of training, rats were handled for 10 to 15 minutes per day and then placed in the chamber for 30 minutes in order to become familiar with it. During this time, they were also given 5 pieces of M&Ms in their home cage daily so that they would become familiar with the food.

**M&M Training.** Following a habituation and exposure period, rats were then given access to M&Ms in the testing chamber. For each day over a course of two to four days, rats were handled for 5 to 10 minutes and then placed in the chamber. After 10 minutes in the chamber, 10 M&Ms were placed on the floor. The number of M&Ms remaining in the chamber after 30 minutes was counted. Training was completed when M&M consumption was stable over two days.

*Electrode implantation*

Prior to surgery, rats were weighed and anesthetized with isoflurane gas (induction at 2.5 L/minute and maintenance at 1.5 – 2.0 L/minute). An incision was made in the skin and the skull was exposed. Bilateral holes were drilled in the skull and the electrode was implanted just above the posterior ventral pallidum. The ventral pallidum was targeted bilaterally using the following stereotaxic coordinates: anterior-posterior (AP) at -0.9 mm, medial-lateral (ML) at ±3.0 mm, and dorsal ventral (DV) at 7.6 mm to 8.3 mm below dura. Coordinates were based on of previous studies showing posterior ventral pallidum as a region involved in processing reward (Cromwell & Berridge, 1993; Panagis et al., 1995; Smith & Berridge, 2005; Tindell et al., 2004; Tindell et al., 2005). Electrodes were composed of two bundles of six wires; each individual bundle consisting of two 75 µm stainless steel stimulating wires and four 25 µm tungsten recording wires. In each bundle, one stimulating wire was 0.3 mm higher than the other wire to allow for bipolar stimulation. Each bundle could be lowered or raised independently. The electrode was lowered to a depth just above the ventral pallidum and was later lowered into the ventral pallidum before testing began. Bone screws were implanted into skull to provide anchorage and dental acrylic was used to fix

the electrode in place and seal the wound. Detailed surgical procedures can be found in (Tindell et al., 2004). Rats were given four to five days for recovery.

*M&M testing*

After electrode implantation and recovery, rats were tested on the chocolate consumption test with different stimulation parameters. Prior to testing for that day, each rat was handled for 5 to 10 minutes, weighed, the headstage cable was attached, and then the rat was placed in the chamber. There was a one minute habitation period after which 20 M&Ms were placed on the floor in the chamber. These 20 pieces of chocolate were first weighed before being placed in the chamber. Thirty minutes after the chocolate was placed in the chamber, the session ended and the rat was removed and placed in its home cage. Any pieces of chocolate left in the chamber were collected, counted, and their weight measured. For stimulation days, stimulation was turned on as soon as the rat was placed in the chamber and remained on till the end of the session. Rats were tested with no stimulation and with stimulation at different frequencies and pulse widths as described below under stimulation parameters. Each test condition was repeated though not all rats were tested at the same stimulation parameters. Figure 2.2 shows the experimental timeline. Testing occurred over seven or more days.

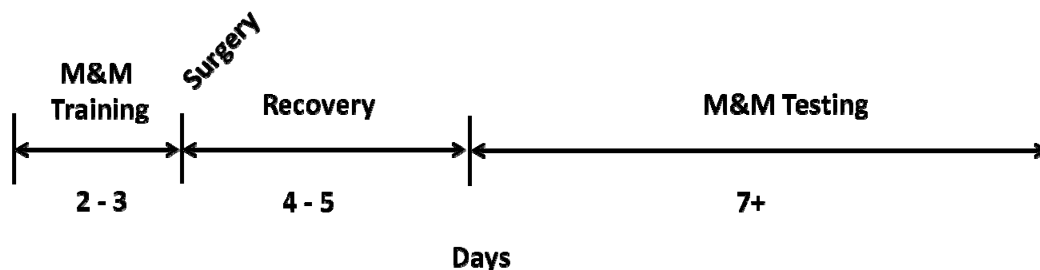


Figure 2.2 – Experimental timeline of the M&M study.

*Histology*

Anatomical localization of electrode sites was done after completion of testing. Rats were anesthetized with isoflurane gas and then a 0.1 mA lesioning DC current was passed for 10 s to mark the electrode location in the brain. Rats were given a drug overdose of pentobarbital and then perfused transcardially with saline and

paraformaldehye. The brains were removed, frozen with dry ice, sliced into 40 μm coronal sections using a microtome, and then stained with cresyl violet. Electrode placement was then confirmed by observing the brain slices under a light microscope.

*Stimulation parameters*

Continuous bipolar, biphasic, cathodal, constant-current stimulation was delivered bilaterally into the posterior ventral pallidum during testing. Bilateral stimulation was used as we were concerned that stimulating only one side would not be sufficient enough to see a significant effect on reward-seeking behaviors. A range of stimulation frequencies and pulse widths was tested based on therapeutic parameters used in DBS studies for Parkinson's disease (Gubellini et al., 2009). We also wanted to test both low and high frequency stimulation to determine if low frequency stimulation would have an "activating" effect (K. C. Berridge & Valenstein, 1991; Kuncel et al., 2007; Panagis et al., 1995). Frequencies of 20 Hz (low), 130 Hz (high), and 200 Hz (high) and pulse widths of 60 μs per phase and 100 μs per phase were tested. Current amplitude was in the range of 100 μA - 500 μA (depending on the rat) corresponding to an approximate charge density per phase of 34 $\mu C/cm^2/ph$ to 170 $\mu C/cm^2/ph$ for a pulse width of 60 μs and 56.6 $\mu C/cm^2/ph$ to 283 $\mu C/cm^2/ph$ for a pulse width of 100 μs.

*Behavioral assessment and data analysis*

For a given testing session, the weight of M&Ms that were left in chamber (if any) after the 30 minute test was subtracted from the initial weight of the 20 M&Ms to give the weight of M&Ms consumed during 30 minutes. Weight cosnumed instead of number of M&Ms consumed was used as the behavioral measure as the rat would leave bits of M&Ms in the chamber and it was difficult to accurately quantify how many M&Ms were left. The weight of M&Ms eaten was then divided by the initial weight of the 20 M&Ms and converted to a percentage to give the % weight of M&Ms consumed. For a given rat and frequency and pulse width combination, this % weight of M&Ms consumed was averaged and then subtracted from the average % weight of M&Ms consumed for no stimulation sessions. This yielded the change in consumption from control (sessions with no stimulation). The difference in % weight of M&Ms consumed

from no stimulation for a given frequency and pulse width was then averaged across rats. ANOVAs and Holm-Sidak *post-hoc* tests were then implemented with the program SigmaPlot (Systat Software Inc., San Jose, CA).

### *2.2.2 Experiment 2: Deep brain stimulation effects on food consumption in a Pavlovian conditioning task*

In this study, 8 adult male Sprague-Dawley rats weighing 250 g – 400 g were used. They were housed individually on a 7:30 AM to 7:30 PM reversed light/dark schedule and given food and water ad libitum. All of the following experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

*Apparatus*

The same experimental chamber was used as in Experiment 1 with the addition of a food dish and a retractable lever (Coulbourn Instruments, Whitehall, PA). Figure A.1 shows a picture of the chamber. The retractable lever was attached to the same wall of the chamber as the food dish. The lever was to the left of the food dish. A white LED was also placed inside the lever chassis just below the lever itself, and would illuminate the lever from below whenever the lever was extended into the chamber. The light and lever extension were used as cues (see description of behavioral paradigm below) to predict delivery of the reward (sucrose pellet). The chamber itself was illuminated with red light from below. As in experiment 1, the MTASK program controlled the delivery of all stimulus and reward presentations and also recorded responses on the lever. Animals were video recorded from below at 30 frames per second. For this experiment, a different stimulator was used. Stimulation was delivered using the Medtronic Test Stimulator model 3625 (Medtronics, Minneapolis, MN). This was a constant-voltage stimulator. Neural activity was also recorded during the test sessions using a program written in the lab, DataTask. This program was written using LabVIEW (National Instruments, Austin, TX). Neural activity was amplified at a gain of 1000 and bandpass filtered between 300 Hz and 6 kHz. We used this relatively low gain to prevent amplifier saturation and consequent lock-out by the large amplitude stimulation artifacts.

Furthermore, it allowed us to record large amplitude signals with greater fidelity without exceeding the voltage limits (±10 V) on the data acquisition board. This increased fidelity facilitated artifact removal and spike recovery (see below). Timestamp clocks for the behavior control program, video recording, and neural recording were all synchronized to enable subsequent analysis of neural activity related to stimulus presentations and behavioral responses.

*Habituation and Pavlovian training*

**Habituation and exposure to sucrose pellets.** For two to three days prior to magazine training, rats were handled for 10 to 15 minutes and then placed in the testing chamber with the lever retracted and the red lights turned on for 45 minutes. Rats were also given five to ten sucrose pellets in their home cage each day after habituation to introduce them to this novel food.

**Magazine training.** Rats then underwent a day of magazine training where they became familiar with the delivery of pellets from the food magazine (pellet dispenser) into the food dish. Rats were handled for 5 to 10 minutes, weighed, and then placed in the chamber with the lever retracted and red lights on. After one minute of exploration, 25 sugar pellets were delivered into the food dish at a variable inter-trial interval of 60 s (30 s – 90 s).

**Pavlovian conditioning.** Pavlovian training followed magazine training. Rats were handled and weighed as described above in magazine training before placing them in the chamber. After 1 minute of habituation, the session started. Rats were given 25 trials of Pavlovian training per session. Each trial began with a variable inter-trial interval of 90 s (30 s – 150 s) and then the lever was extended and illuminated, with a white led, for 8 s. The lever and light served as a combined conditioned stimulus (CS+1). The lever was then retracted, the light turned off and then 500 ms later, the pellet was delivered. The feeder made a clicking sound referred to as a "feederclick" when it delivered a pellet. The rats typically responded to this sound by turning their heads towards the sound before going over to retrieve the pellet. In other instances, the animals would immediately approach the food dish, or if already there, begin poking for the pellet in response to the feederclick. Any animal that did not respond in some way to this

sound was not included in the study. This feederclick acted as another conditioned stimulus (CS+2). Delivery of pellet terminated the trial. See figure 2.3a for the trial design.

Training sessions were approximately 45 to 50 minutes in duration. During the entire session, behavior was recorded on digital video that was synchronized frame by frame to timing of the control computer. Repeated pairings of the lever and light CS with the delivery of a food reward resulted in the acquisition of a Pavlovian conditioned response where some animals approached and interacted with the lever cue before going to the food dish to retrieve the pellet after the lever was retracted (sign-trackers) while others approached the food dish when the lever was extended (goal trackers). Animals were given 6 training sessions and the latency to retrieve the food pellet was recorded. Animals were given extra days of training if they were still not retrieving and eating pellets in less than 10 s after the feederclick for 80% of the trials. If even after an additional 3 days of training, rats had not learned the task, they were excluded from the study. In addition, the number of times the rat approached the lever or the food dish was also recorded for each session and if the rat approached the lever for the majority of time over the last two training sessions, it was classified as a sign tracker and if they approached the food dish for majority of trials over the last two days of training, they were classified as goal trackers (Flagel et al., 2007).

After the rats learnt the Pavlovian paradigm, they were implanted with electrodes and allowed to recover for four to five days after surgery. After recovery, rats were given another day of Pavlovian training to ensure that they still were able to perform the task and get used to being attached to the headstage cable. Again, if they retrieved the pellet in less than 10 s after feederclick for 80% of the trials, they began Pavlovian testing, otherwise they were given more days of training until criterion was reached and was stable across two days.

*Electrode implantation*

The electrode implantation surgery and target location was similar to that described in experiment 1.

*Pavlovian testing*

Rats were handled for 5 - 10 minutes, weighed, and placed in the experimental chamber with the headstage attached. Again, rats were given one minute habituation before the experiment started. For each test session, rats then received 10 Pavlovian trials (same design as in Pavlovian training) with no stimulation, then 10 or 20 trials with either low frequency stimulation at 20 Hz or high frequency stimulation at 200 Hz, and then 10 more trials post stimulation. This allowed for comparison of the same unit in the same recording session while stimulation was on or off. For the first few rats tested in this study they were give only 10 stimulation trials in the session, but the number of stimulation trials per session was increased to 20 with later rats to provide a longer period of stimulation in a test session. The rats were tested on one session per day and were given three consecutive days of low frequency stimulation and three consecutive days of high frequency stimulation. A two day break was given between high and low frequency days to minimize any potential lingering effects of DBS (Gubellini et al., 2009) and the order of which stimulation frequency a rat received first was counter-balanced between rats. All test sessions were video-recorded. Figure 2.3b and 2.3c shows the breakdown for each session and the experimental timeline.

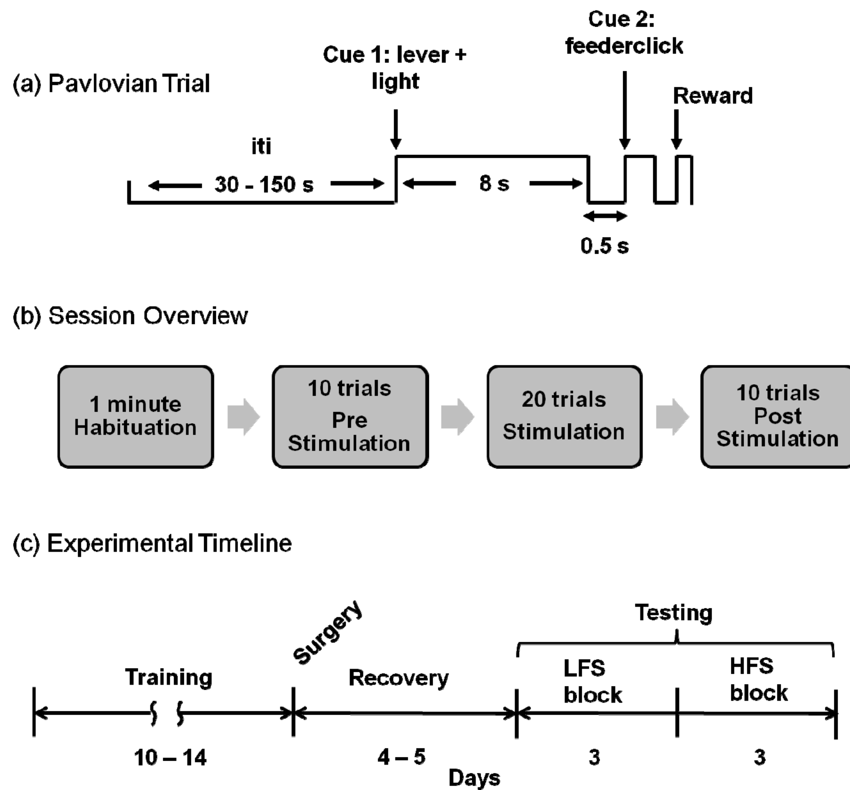


Figure 2.3 – (a) Schematic of a Pavlovian trial showing timing of the different events (iti – inter-trial interval) for experiment 2. (b) Experimental paradigm for a given test session showing habituation, pre stimulation period, stimulation period, and post stimulation period. (c) Experimental timeline for experiment 2 (LFS – low frequency stimulation at 20 Hz, HFS – high frequency stimulation at 200 Hz).

*Histological verification*

Evaluation of electrode location was the same as described in experiment 1.

*Stimulation parameters*

Based on the results of experiment 1, stimulation frequencies of 20 Hz and 200 Hz and pulse width of 60 µs were used. Bipolar, bilateral, cathodal, and monophasic constant voltage at amplitude of 3 V (~ 30 µA to 60 µA depending on electrode impedances) corresponding to an approximate charge density per phase of 10.1 µC/cm$^2$/ph to 20.4 µC/cm$^2$/ph was delivered to the ventral pallidum.

*Behavioral assessment and data analysis*

To measure any changes in the motivation with stimulation, responses to the reward cues and reward consumption was quantified across periods of stimulation and no stimulation. Various behaviors were scored using frame-by-frame video analysis with DataRat software (developed by the Aldridge lab). These behaviors included approaches to the lever, approaches to the food dish, nose entries into the food dish, and time when the sucrose pellet touched the rat's lips. Lever approaches were quantified only during the 8 s period when the lever was extended. Approaches to the food dish and nose entries were measured during the 8 s period just before the lever extended (pre CS period) and during the 8 s when the lever is out (CS period). The time between the delivery of the pellet, i.e. time of the feederclick and when the pellet touched the rat's lips before being eaten was calculated to determine the latency to eat the pellet. The number of pellets consumed was also counted. Responses were averaged across trials, then across sessions, and then rats to compare responses during the pre stimulation period, low and high frequency stimulation periods, and the post stimulation period.

Statistical analyses were performed using ANOVAs and Holm-Sidak *post hoc* tests (unless otherwise specified) and implemented with the program SigmaPlot (Systat Software Inc., San Jose, CA).

*Neural analysis*

**Artifact removal program.** In order to minimize the period of "dead time" when the amplifiers are saturated due to the stimulation pulse, neural data was recorded at a lower gain (x 1,000). The recorded files were further amplified offline by a gain of 5 using a LabVIEW software amplifier program written in the lab. This program also removed any offset from the data files so that the signals were centered at zero. The stimulation artifacts were then removed offline using an artifact removal program developed by Nathan Patel (research assistant in the Aldridge Lab) and myself. The artifact removal program was written in LabVIEW (National Instruments, Austin, TX). Refer to appendix A.2 for detailed information on the artifact removal program.

**Unit discrimination and analysis.** Neural analysis was performed using Epoch builder (a database neural and behavioral analysis program written in the lab),

Neuroexploroer (Nex Technologies, Madison, AL), and Offline Sorter (Plexon, Inc., Dallas, TX). The latter was used to discriminate neural unit spike waveforms from noise and other units. Single units were first identified using a voltage threshold and then further isolated from noise and other units using peak-valley width and principal component analysis. Units were verified by distinct spike waveforms (whose shapes remained consistent throughout the whole recording) and clear refractory periods in their autocorrelation histograms. Units with more than 2 % of spikes within a 1 ms refractory period window were excluded. Cross-correlation analysis (Moore et al., 1966) was performed (using Epoch Builder) to ensure that any unit recorded simultaneously on two electrode sties was counted only once in the analysis. Cross-correlgrams expose the temporal relationship between each spike (reference unit) and spike activity of all other units recorded at the same time. Cross-correlograms had bin widths of 1 ms and a sliding time window of ± 25 ms aligned to each spike of the reference unit. Cross-correlograms with large peaks at time 0 indicate that the two units have high probability of firing at the same time and could be the same unit being recorded on both electrodes. To avoid accidentally counting individual cells twice, the unit with the better signal to noise ratio in Offline Sorter was kept and the other one discarded and not included in further analysis.

Unit population activity was compared and analyzed for different behavioral events of interests and across different conditions (no stimulation, 20 Hz, and 200 Hz stimulation). In a given test session, there were 10 trials of no stimulation, 10 or 20 trials of stimulation, and another 10 trials again with no stimulation (refer to figure 2.3b for the test paradigm). Hence, for a given unit, we were also able to compare changes in firing rates and responses to behavioral events before, during, and after stimulation. It was also verified visually in Offline Sorter that the unit's waveform shape was consistent for the whole session and that the unit was also present throughout the whole session. Any unit that appeared late in the session or did not return during the post stimulation session after stimulation had occurred was not included in the analysis. Absolute firing rates of units during the pre stimulation, stimulation, and post stimulation periods were determined using the 20 s baseline epoch before CS+1 onset. For a given unit, the firing rate during

this epoch was averaged separately across all pre stimulation trials (10), stimulation trials (10 or 20), and post stimulation trials (10).

Firing rates during the stimulation period were corrected to compensate for the artifact dead time (Bar-Gad et al., 2004); that is, firing rates were computed by counting the number of spikes in the sum of times between the ends of the discarded artifact and the onset of the next artifact (artifact periods illustrated by flattened traces along with recovered signal in the zoomed insets of figure 2.11).

Peristimulus time histograms of bin widths of 0.5 ms were computed for units recorded during the 20 Hz and 200 Hz stimulation periods. For each unit, spiking activity was lined up to the onset of each stimulation pulse (for all stimulation pulses in the session) for the period between pulses. For a given test session animals received at most 20 trials of stimulation. Each trial was on average 99 s long (refer to figure 2.3a showing the timing for a given Pavlovian trial); hence, the total length of stimulation period was on average 33 minutes. This resulted in ~39,600 pulses with a period of 50 ms between pulses for 20 Hz stimulation and ~396,000 pulses with a period of 5 ms between pulses for 200 Hz stimulation. A significant decrease or increase in firing was determined if two consecutive bins were greater than ±2 standard deviations from the mean baseline firing rate of the unit computed during the 20 s pre-CS+1 epoch during the pre stimulation period (Carlson et al., 2010). Control PSTH plots were also computed during this pre stimulation period. "Sham stimulation" pulse times were generated at every 50 ms or every 5 ms (for 20 Hz or 200 Hz stimulation, respectively) during the pre stimulation period and spikes were lined up to those "pulses". Assuming again 99 s trials and 10 pre stimulation trials, this resulted in 19,800 sham pulses for "20 Hz stimulation" and 198,000 sham pulses for "200 Hz stimulation".

Perievent time histograms (PETHs) and rasters were analyzed for behavioral events of interest: CS+1 lever extension and light, CS+2 feederclick (pellet delivery), and when the animal made contact with the UCS (sucrose pellet). The 8 s period preceding CS+1 served as a baseline for conditional responses to the above-mentioned events of interests. To determine whether a unit was responsive to CS+1, CS+2, or UCS, firing rates during 0.5 s or 1 s epochs after the stimulus event was compared to the 8 s baseline period (described above). We also looked at the whole 8 s epoch during the CS+1.

Mann-Whitney U tests were used to determine if any of these epochs were significantly different from the 8 s baseline period. Furthermore, for each unit, these responses were analyzed separately for the pre stimulation, stimulation, and post stimulation periods and responses were compared to the corresponding baseline period; that is, responses during the pre stimulation period were compared to 8 s baseline epoch during that same pre stimulation period, responses during the stimulation period were compared to the 8 s baseline epoch during stimulation, and so on.

To determine population responses, each unit was first normalized to the 20 s pre CS+1 baseline period, then for a given period of interest, the relative change in firing in response to a given event from the 8 s pre CS+1 period was determined. Relative changes in firing rates were compared for pre stimulation, 20 Hz, 200 Hz, and post stimulation periods. ANOVAs and Holm-Sidak *post hoc* tests (SigmaPlot, Systat Software Inc., San Jose, CA) were used to determine any statistical significance across conditions.

## 2.3 Results

### *2.3.1 Experiment 1: M&M consumption*

*Electrode verification*

Ten rats were successfully implanted and tested on the chocolate consumption test. Histology confirmed that 7 out of the 10 rats had electrodes in the posterior ventral pallidum (VP) on both sides. Figure 2.4 shows their electrode locations. The three rats whose electrodes were not in the VP were excluded from further analysis.

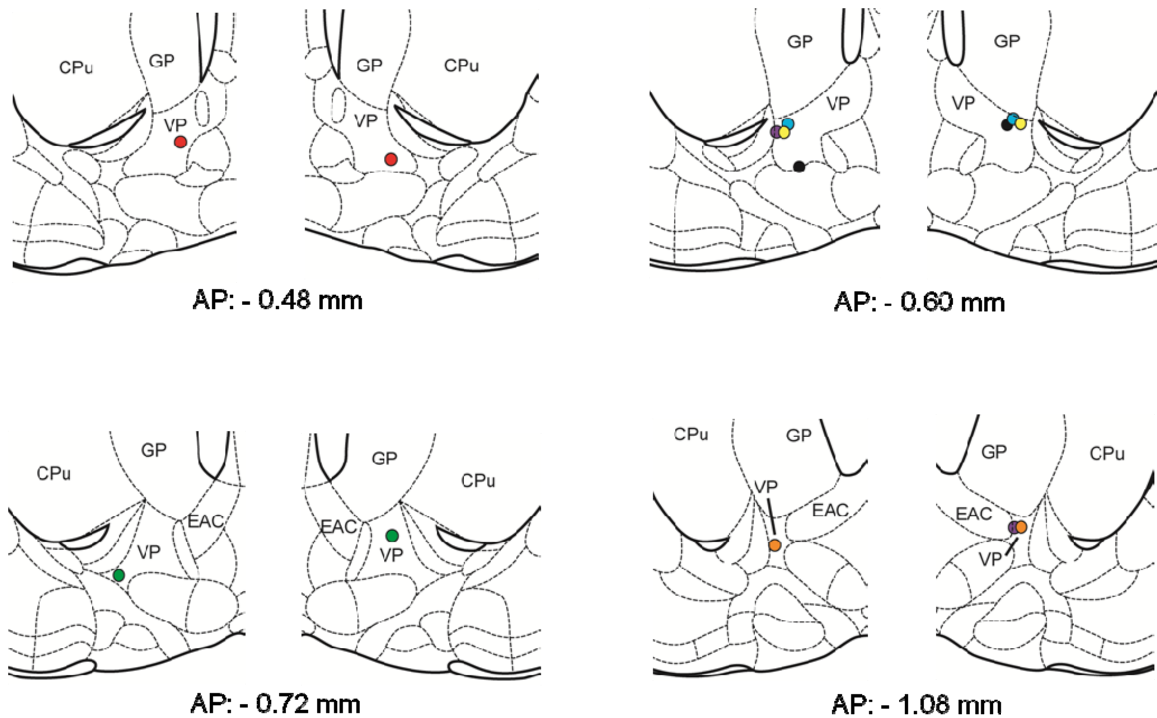


Figure 2.4 - Coronal slices showing electrode placements of rats implanted bilaterally in the ventral pallidum. Filled circles represent the endpoints of electrode locations. Electrode location for each rat is represented by a different color (n = 7 rats). Images modified from the Paxinos and Watson rat atlas, 6th edition (Paxinos & Watson, 2007).

*Stimulation alters consumption of a highly palatable food*

There were differences observed in M&M consumption depending on the stimulation parameters tested. For the smaller pulse width at 60 µs/phase, there was a significant effect of frequency [one-way RM ANOVA; $F(3, 19) = 7.309$, $p = 0.007$] with 20 Hz stimulation showing increased M&M consumption compared to 200 Hz stimulation. In general, rats ate more M&Ms during 20 Hz stimulation compared to no stimulation and less M&Ms compared to no stimulation as shown in figure 2.5. However, statistical analysis showed no significant difference between stimulation and no stimulation. Interestingly, with the longer pulse width of 100 µs/phase, the 200 Hz stimulation now showed an increase in M&M consumption, which was significant compared to 130 Hz stimulation [one-way RM ANOVA; $F(3, 15) = 7.571$, $p = 0.018$]. Again though both 20 Hz and 200 Hz stimulation showed increased consumption and 130 Hz stimulation showed decreased consumption, this was not significant compared to no

stimulation (figure 2.5). Some of the animals were not tested on all combinations, and this could explain some of the variability in the data.

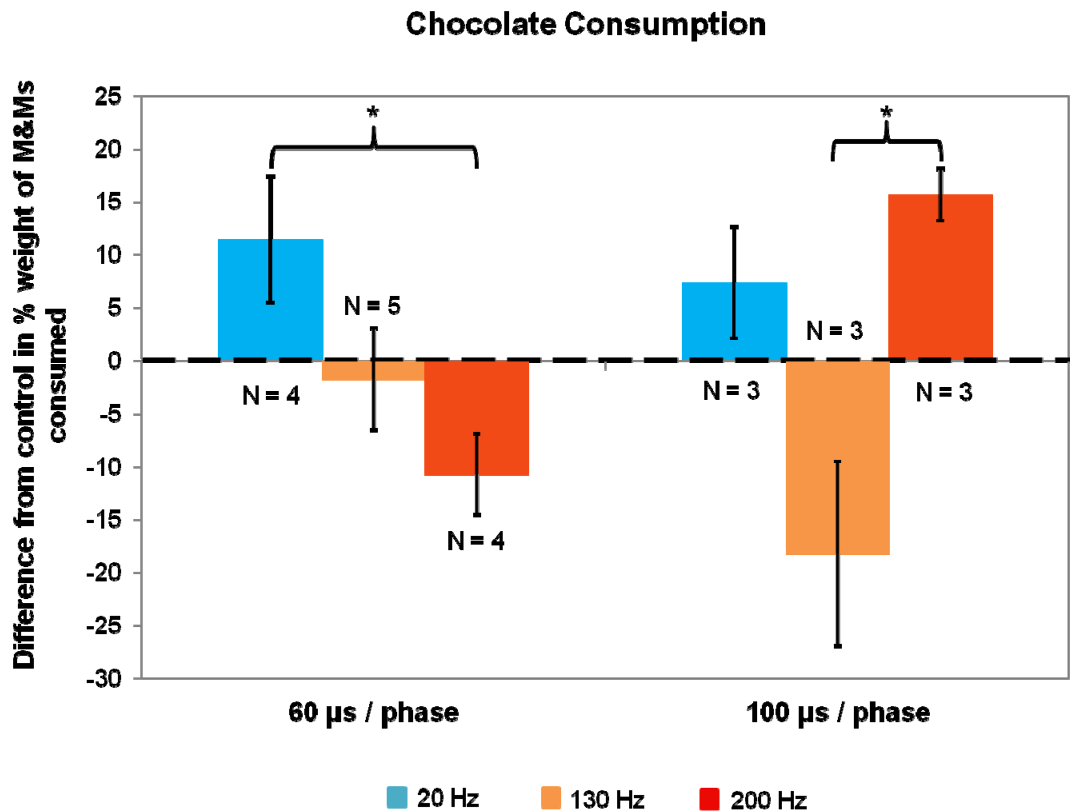


Figure 2.5 – Changes in chocolate consumption from control (i.e. no stimulation) with stimulation in the ventral pallidum at different frequencies and pulse widths. The black dotted line at 0 represents consumption during no stimulation. Anything above the line shows an increase in consumption compared to no stimulation and anything below the line represents a decrease in consumption compared to no stimulation. Y-axis is the difference in the percentage weight of M&Ms consumed compared to no stimulation. The number of rats used for each combination of pulse width and frequency is indicated on the graph, * $p < 0.05$.

### *2.3.2 Experiment 2: Pavlovian conditioning task*

*Electrode verification*

Five out of the eight rats tested were successful implanted bilaterally into the ventral pallidum (VP). Figure 2.6 shows the electrode locations for the 5 rats whose electrodes were in the VP. Only the data from rats whose electrodes were in the VP were analyzed and presented in the results.

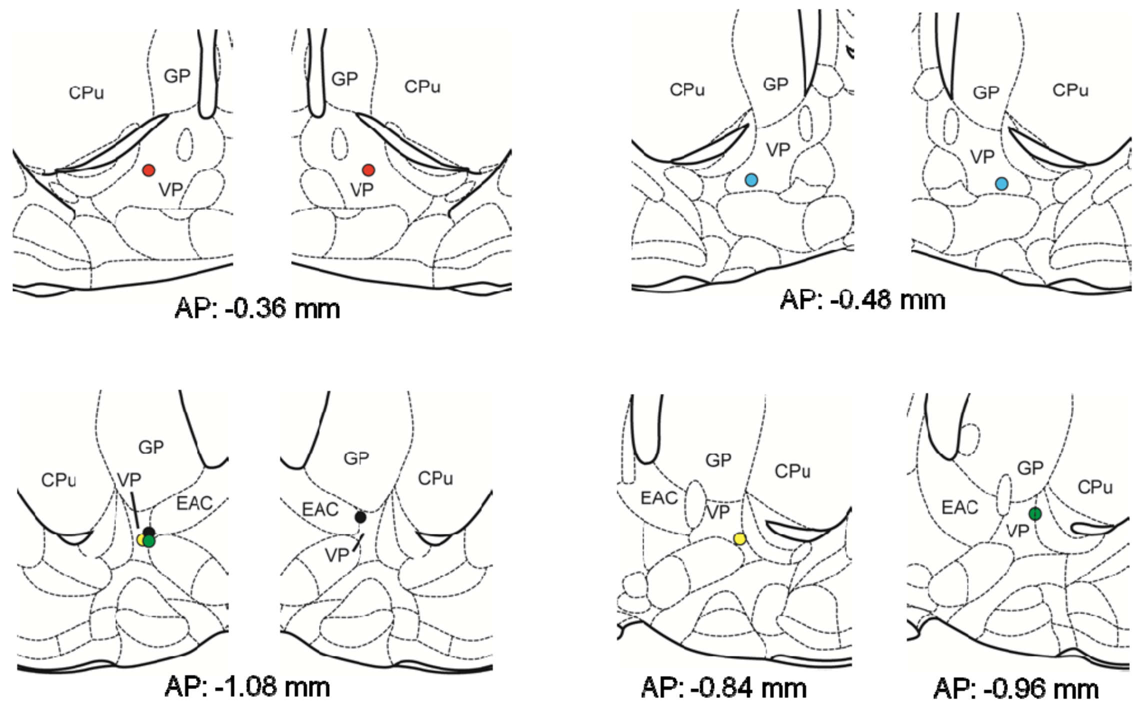


Figure 2.6 - Coronal slices showing electrode placements of rats implanted bilaterally in the ventral pallidum. Filled circles represent the endpoints of electrode locations. Electrode location for each rat is represented by a different color (n = 5 rats). Images modified from the Paxinos and Watson rat atlas, 6th edition (Paxinos & Watson, 2007).

*High frequency stimulation resulted in decreased responses to reward cues compared to low frequency stimulation*

Rats learnt the Pavlovian paradigm - indicated by increased number of approaches to the food dish and/or the lever; as well as, increased nose entries into the food dish during the CS+1 period compared to when there was no cue presented (see results below). In addition, there were no distinct differences in responses seen with the animals initially marked as sign trackers and those marked as goal trackers and hence data for both groups was pooled.

Stimulation did not change consumption of sucrose pellets. Rats always ate all the sucrose pellets delivered in the Pavlovian task. Since overall consumption was not affected, we analyzed whether there were any changes in motivation to seek out and consume rewards or in responses to the reward cues (CS+1 and CS+2) with either low or high frequency stimulation. Recall from figure 2.3b, for a given session, there were ten pre stimulation trials at the beginning of the session and 10 post stimulation trials at the end of the session. For the different behaviors analyzed and reported below, repeated

measures ANOVAs revealed no difference specifically between pre stimulation and post stimulation responses. Hence, data from the pre stimulation and post stimulation periods were pooled together into a no stimulation period to simplify presentation of data. Furthermore, for each behavior measure presented below, ANOVAs were re-run comparing no stimulation, 20 Hz stimulation, and 200 Hz stimulation.

During the CS+1 period when the lever cue was extended and illuminated, there was a significant decrease in approaches during 200 Hz stimulation compared to 20 Hz stimulation [One-Way RM ANOVA on the difference between cue and pre-cue period for different conditions; $F(2, 14) = 7.600$, $p = 0.014$]. Neither frequency of stimulation was significantly different from no stimulation, however. Further analysis comparing pre CS+1 and CS+1 periods for each condition revealed that under no stimulation the difference between pre CS+1 and CS+1 just failed to be significant [$t = -2.762$, $p = 0.051$, $df = 4$] and significant for 20 Hz stimulation [$t = -3.883$, $p = 0.018$, $df = 4$]; but, there was no significant difference between pre CS+1 and CS+1 for 200 Hz stimulation [$t = -2.242$, $p = 0.088$, $df = 4$]. Figure 2.7 shows the averaged number of approaches to the food dish per trial during the 8 s before CS+1 onset and during the 8 s CS+1 period for 20 Hz stimulation and 200 Hz stimulation. To compensate for individual variation in food dish approaches, data was normalized to no stimulation responses. Specifically, the graph shows the differences in approaches compared to the corresponding no stimulation period. Individual variation in approaches was not specific to a given condition. Rats that in general had fewer approaches during no stimulation also had fewer approaches across all conditions compared to other rats in the study; but, all subjects on average had fewer approaches during CS+1 period while receiving 200 Hz stimulation compared to same period during 20 Hz stimulation.

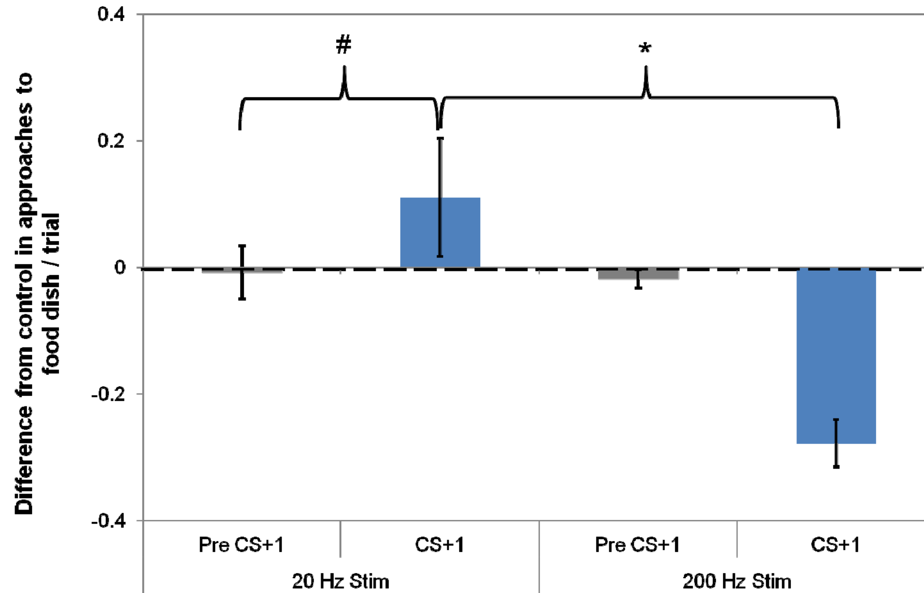


Figure 2.7 – Decreased rate of approaches to the food dish with 200 Hz VP stimulation compared to 20 Hz VP stimulation. Graph shows the difference in average number of approaches per trial from control (i.e. no stimulation) during the 8 s period before CS+1 onset (grey-filled bars) and the 8 s period during CS+1 (blue-filled bars) for 20 Hz stimulation and 200 Hz stimulation. The black dotted line at 0 represents approaches during no stimulation. Anything above the line shows more approaches to the food dish compared to no stimulation and anything below the line represents fewer approaches compared to no stimulation. To account for individual variation, the average number of approaches during the pre CS+1 period for each frequency of stimulation was subtracted from the average number of approaches in that same period for no stimulation. Similarly, the average number of approaches during the CS+1 period for each frequency of stimulation were subtracted from average number of approaches during CS+1 period for no stimulation. Responses during 200 Hz stimulation were significantly fewer compared to 20 Hz stimulation, * $p = 0.005$. There was also a significant decrease in approaches in pre cue period compared to cue period with 20 Hz stimulation, # $p = 0.018$, n = 5 rats. Error bars represent ± standard error.

There were also fewer approaches to the lever cue with 20 Hz compared to 200 Hz stimulation [$F(2, 14) = 7.932$, $p = 0.013$], though not significantly different from when there was no stimulation (figure 2.8). Approaches to the lever were only analyzed when the lever was physically accessible – i.e. just during the CS+1 period. Again, like with approaches to the food dish, there was individual variation among rats across all conditions. Hence, as with figure 2.7, the data presented in figure 2.8 was also normalized to compensate for this variation with the graph showing the difference in approaches relative to no stimulation for both 20 Hz and 200 Hz stimulation.

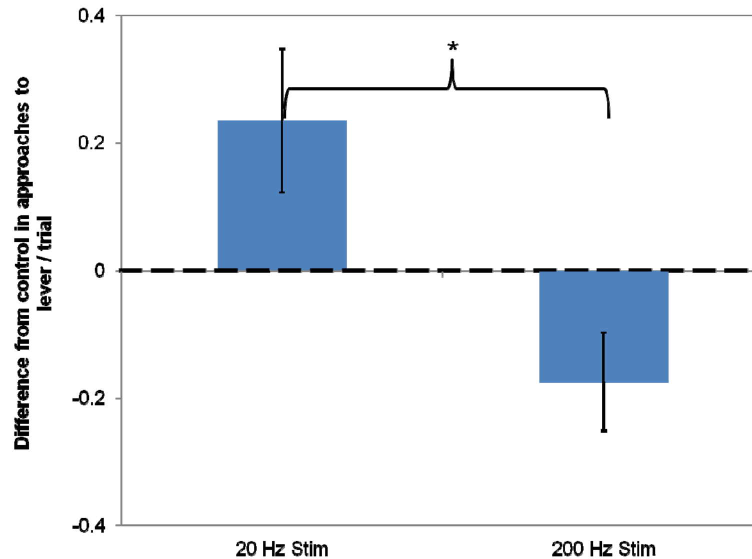


Figure 2.8 – Decreased rate of approaches to the lever with 200 Hz VP stimulation compared to 20 Hz VP stimulation. Graph shows the difference in average number of approaches per trial from control (i.e. no stimulation) during the 8 s CS+1 period for 20 Hz stimulation and 200 Hz stimulation. The black dotted line at 0 represents approaches during no stimulation. Anything above the line shows more approaches to the lever compared to no stimulation and anything below the line represents fewer approaches compared to no stimulation. To account for individual variation, the average number of approaches during the pre CS+1 period for each frequency of stimulation was subtracted from the average number of approaches in that same period for no stimulation. Similarly, the average number of approaches during the CS+1 period for each frequency of stimulation was subtracted from average number of approaches during CS+1 period for no stimulation. Responses during 200 Hz stimulation were significantly fewer compared to 20 Hz stimulation, * $p = 0.013$, n = 5. Error bars represent ± standard error.

In addition to approach behavior, rats also poked their noses into the food dish more often during the CS+1 period compared to pre CS+1 period (figure 2.9). There were no significant differences between no stimulation, 20 Hz stimulation, or 200 Hz stimulation [One-Way RM ANOVA on the difference between cue and pre-cue period for different conditions; $F(2, 14) = 1.315$, $p = 0.321$]. Looking at individual paired T-tests between pre CS+1 and CS+1 periods, there was a significant difference in number of nose entries per trial between the pre CS+1 and CS+1 periods for 20 Hz stimulation [$t = -3.204$, $p = 0.033$, $df = 4$], though not for no stimulation [$t = -2.378$, $p = 0.076$, $df = 4$] or 200 Hz stimulation [$t = -1.493$, $p = 0.210$, $df = 4$].

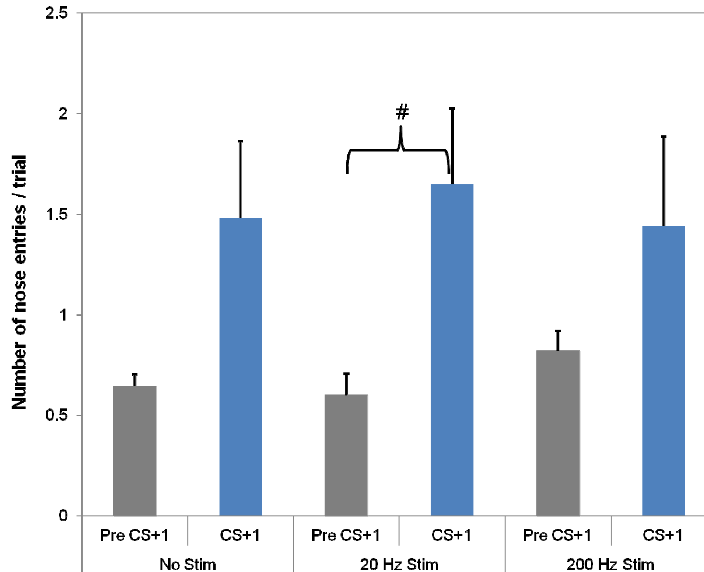


Figure 2.9 – No difference in the rate of nose entries into the food dish with VP stimulation. Graph shows the average number of nose entries per trial during the 8 s period before CS+1 onset (grey-filled bars) and the 8 s period during CS+1 (blue-filled bars) for no stimulation (responses during pre stimulation and post stimulation periods pooled together), 20 Hz stimulation, and 200 Hz stimulation. There was a significant decrease in approaches in pre cue period compared to cue period with 20 Hz stimulation, # $p = 0.033$, n = 5.

Despite slightly fewer approaches and nose entries during high frequency stimulation, the latency to obtain the sucrose pellets was the same across stimulation and no stimulation conditions [$F(2, 14) = 2.733$, $p = 0.125$]. The data does, however, suggest a trend towards increased latency with 200 Hz stimulation compared to no stimulation and 20 Hz stimulation, but the results were not significant (figure 2.10).

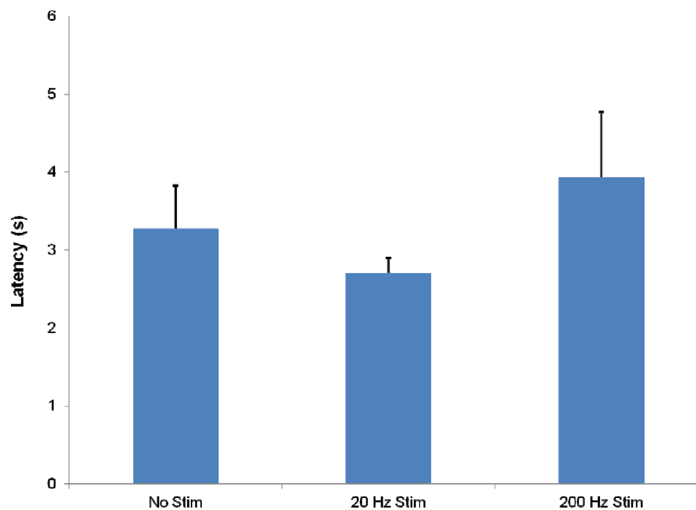


Figure 2.10 – No effect of stimulation in the VP on latency to retrieve pellet. Graphs show the average latency to first contact the pellet after the feederclick for no stimulation (pre stimulation and post stimulation data pooled together), 20 Hz stimulation, and 200 Hz stimulation, n = 5.

*Successful removal of stimulation artifact from neural recordings*

We were able to successfully remove the stimulation artifact from our neural recorded data. Figure 2.11 shows examples of the raw recorded data with the stimulation artifact and the same region after the stimulation artifact has been removed for 20 Hz and 200 Hz stimulation sessions, respectively. Traces show that the units are still firing in between the stimulation pulses. Average dead time for each artifact was 0.3 ± 0.015 ms (± standard error). Again estimating total length of stimulation period to be on average 33 minutes, total dead time due to stimulation artifacts comprises approximately 0.6 % of total recording time during 20 Hz stimulation and 6 % of the recording time during 200 Hz stimulation. (Note, in some of the earlier sessions, rats were given only 10 trials of stimulation, but the total dead time due to the artifacts still compromises the same percentage of recording during the stimulation period.)

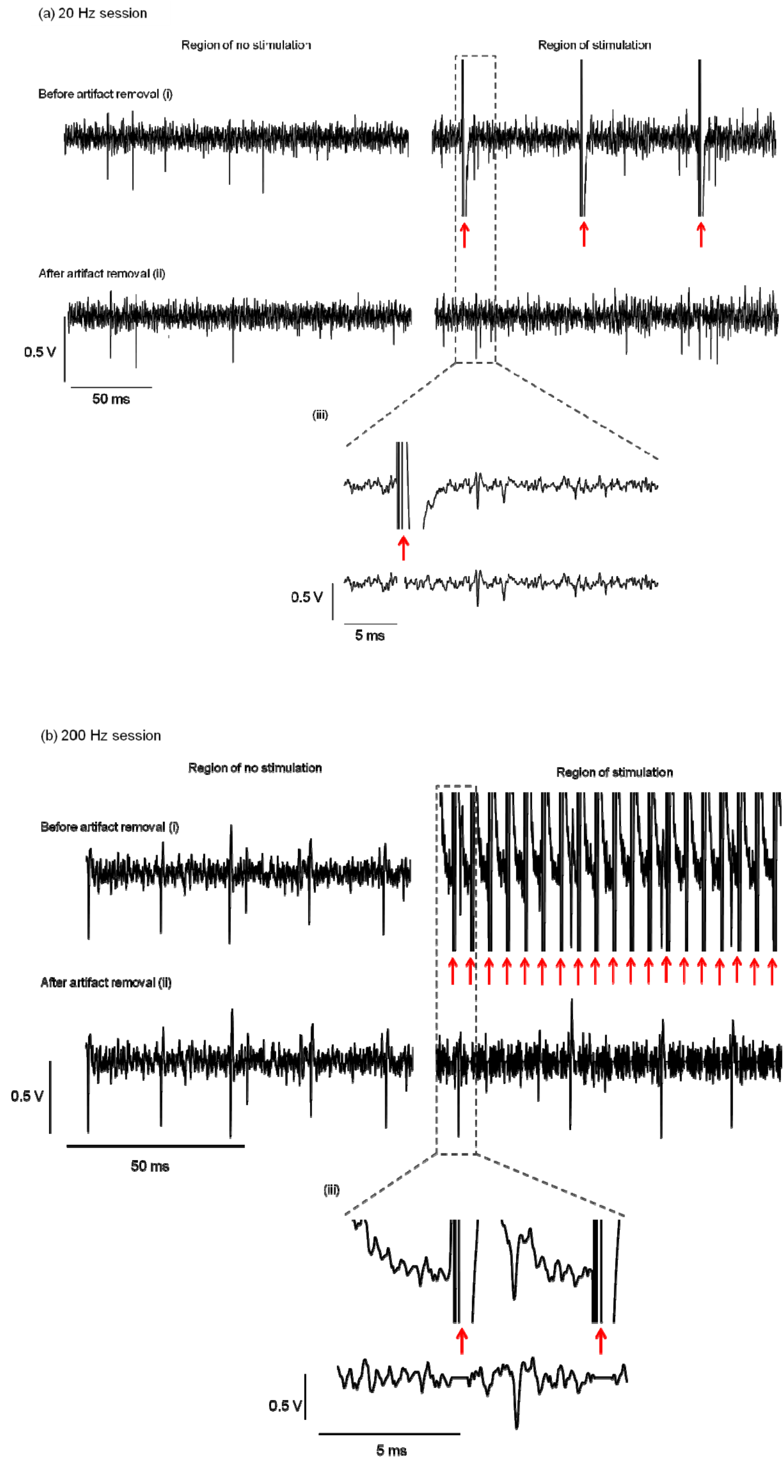


Figure 2.11 – Examples of recorded neural traces in the VP before and after stimulation artifacts were removed. Red arrows indicate the stimulation artifacts. (a) Two hundred milliseconds snippets of recorded neural activity from one electrode channel during a period of no stimulation and 20 Hz stimulation before (i) and after (ii) the artifact has been removed. (iii) Zoomed-in portion of the stimulation trace showing an artifact and units before and after the artifact was removed. The time-expanded region is indicated by a dotted box. (b) (i) and( ii) Similar to (a) but for 200 Hz stimulation session and each sample trace is 100 ms long. (iii) Zoom-in on region indicated by a dotted box showing two artifacts and a spike between.

*Neural overview*

A total of 28 units from 20 sessions and 5 rats were recorded. Cross-correlation analysis ensured that activity was unitary in nature and not an accident of recording a single unit on two nearby electrodes. There were 13 units from the low frequency stimulation (LFS) sessions and 15 units from the high frequency stimulation (HFS) sessions. Overall all units (100%) were responsive either to CS+1, CS+2, UCS, or stimulation itself or a combination of the various events (Table 2.1) and were used in subsequent analyses. The average firing rate of all units in the pre CS+1 period (computed for only the pre stimulation period for all units) was 32.61 ± 5.61 spikes/s.

Table 2.1 – The number of responsive VP units (numerator) relative to total number of VP units (denominator) broken down by test condition and event; % total of responsive units in parenthesis.

| | **CS+1 (Lever Extension + Light)** | **CS+2 (Feederclick)** | **UCS (Sucrose Pellet )** | **Stimulation** |
|---|---|---|---|---|
| **Pre Stimulation+** | 14/28 (50%) | 22/28 (78.6%) | 8/28 (28.6%) | N/A |
| **20 Hz Stimulation** | 6/13 (46.2%) | 9/13 (69.2%) | 7/13 (53.8%) | 10/13 (76.9%) |
| **200 Hz Stimulation** | 6/15 (40%) | 14/15 (93.3%) | 13/15 (86.7%) | 12/15 (80%) |
| **Post Stimulation+** | 15/28 (53.6%) | 18/28 (64.3%) | 16/28 (57.1%) | N/A |

+This is the combined number of units tested in the LFS sessions (total 13 units) and units tested in the HFS sessions (total 15 units)

*Stimulation produced complex firing patterns*

Stimulation at 20 Hz did not significantly alter mean firing rates [$F(2,38) = 1.971$, $p = 0.161$] from pre stimulation or post stimulation periods and there were was also no difference between pre stimulation and post stimulation. However, there was a decrease in firing rates with 200 Hz stimulation compared to the pre stimulation and post

stimulation periods, even after accounting for dead time due to stimulation artifact [$\chi^2$ = 18.533, $p < 0.001$, $df = 2$]. Again, there was no difference between pre stimulation and post stimulation basal rates. Figure 2.12 shows the average firing rates during the 20 s pre CS+1 epoch for LFS and HFS units.

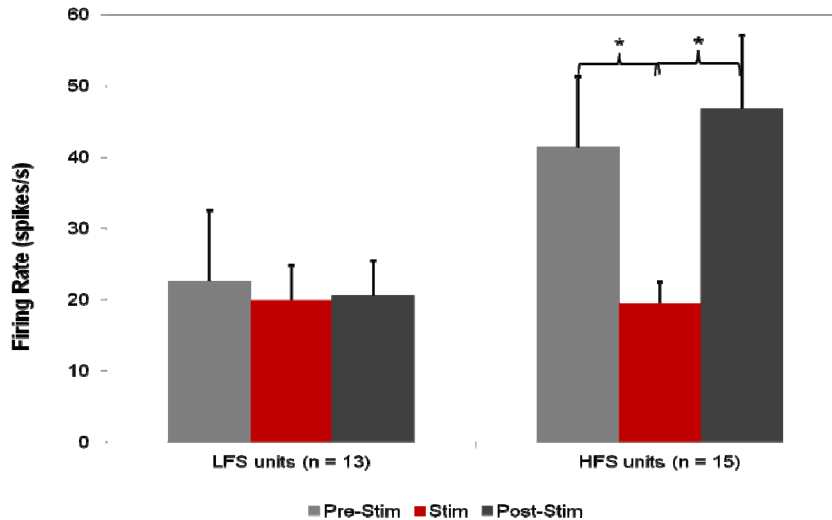


Figure 2.12 – Average firing rates during HFS in the VP were lower compared to pre stimulation and post stimulation. Bar graphs show baseline average firing rates for the LFS (n = 13) and HFS ventral pallidal units (n = 15). Rates were calculated during the 20 s period just before CS+1 onset and were averaged across trials during the pre stimulation period (grey-filled bars), stimulation period at either 20 Hz or 200 Hz (red-filled bars), and post stimulation period (dark grey-filled bars). Units receiving HFS stimulation had decreased basal rates compared to during their pre stimulation and post stimulation periods, * $p < 0.001$, n = 15.

Though overall average rates of firing changed little with 20 Hz stimulation, peristimulus time histograms (PSTHs) plots revealed clear stimulation-induced complex patterns of inhibition and excitation for the majority of the units. Figure 2.13a shows sample PSTHs plots for 20 Hz stimulation during pre-stimulation and stimulation periods.

Responses to 20 Hz stimulation typically showed a multiphasic response with a period of inhibition lasting from as short as 4 ms to as long as 18 ms after stimulation onset, followed by mid-term excitation which was either brief, lasting for 1 to 7 ms or a more sustained excitation lasting for 10 ms or more, and then (in a few cases) another sustained period of inhibition lasting 18 to 22 ms before returning to pre stimulation basal rates. Note, in most cases the first inhibitory period lasted long after the average dead time of 0.3 ms and the recoverable region of the artifact, which was about on average 3

ms long. This initial period of inhibition is not a function of the artifact as we are able to recover spikes during this period. Furthermore, for some units there was actually an immediate excitation following artifact dead time. The majority of units showed some variation of this multiphasic response, with the exception of one unit which did demonstrate a multiphasic response but this was not considered a significant response based on our criteria and two units whose PSTHs plots were flat histograms indicating that the likelihood of a spike firing was independent of stimulation onset (Hashimoto et al., 2003; Maltete et al., 2007; McCairn & Turner, 2009).

With 200 Hz stimulation, 53 % (n = 8) of units showed inhibition throughout the period between stimulation pulses. Two units showed a very brief inhibition lasting about 3 ms after artifact dead time before returning to basal rates. Two other units instead showed a very brief excitation (1- 4 ms long) before returning to basal rates and three units showed a flat response – spike rate remained around the pre stimulation basal rates. Figure 2.13b shows samples PSTH responses during HFS. Note, given how fast the stimulation is (200 Hz), the time between pulses is only 5 ms (compared to the 50 ms for 20 Hz stimulation) and the next stimulation pulse occurs before the unit is able to recover to the extent that another unit can with the slower 20 Hz stimulation.

For each unit (figure 2.13a and b), control PSTHs plots showing neural responses to sham artifact times during the pre stimulation period were also included. These control plots show a flat histogram response indicating that the likelihood of a spike firing was independent of simply lining up spike activity at periodic intervals (Hashimoto et al., 2003; Maltete et al., 2007; McCairn & Turner, 2009).

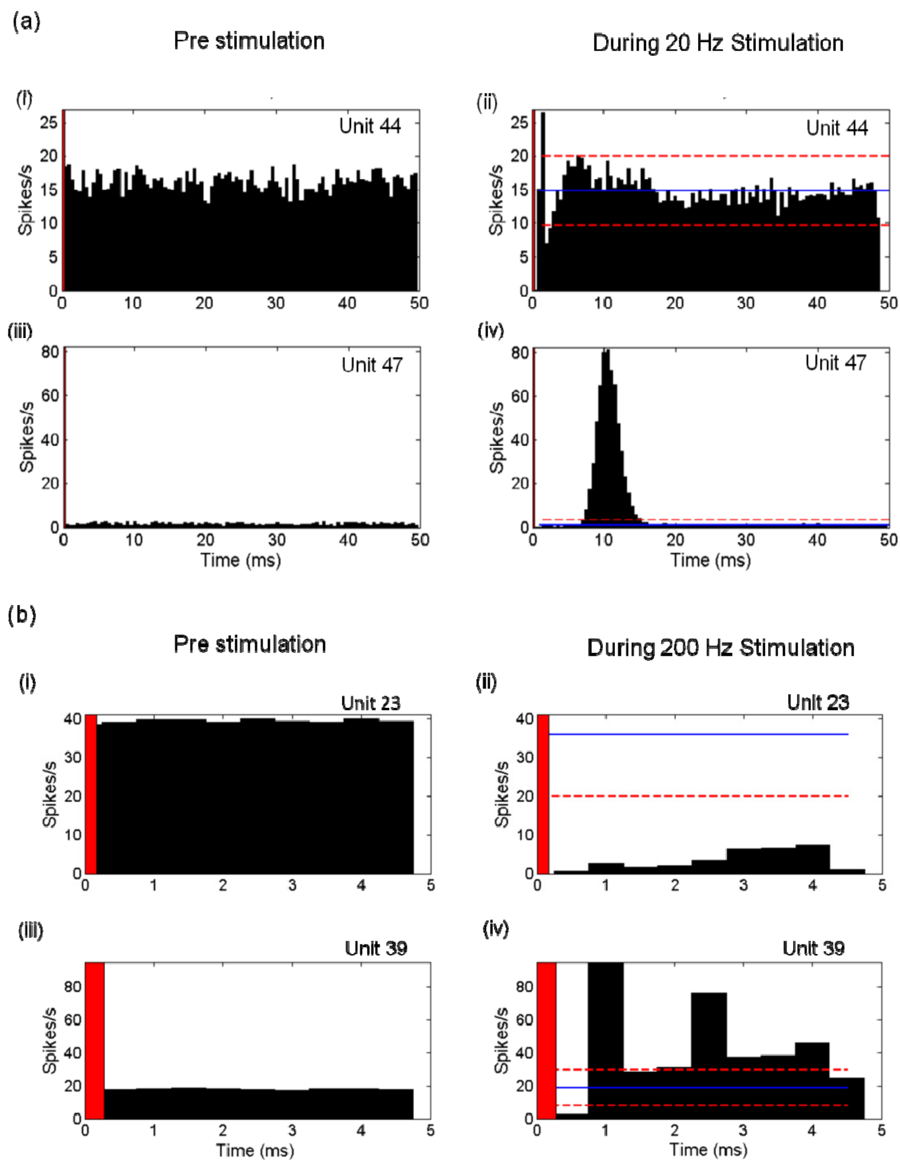


Figure 2.13– Ventral pallidal units show stimulation-induced patterns of firing. (a – b) Sample PSTHs plots normalized to spikes/s with bins of 0.5 ms and duration of 50 ms for 20 Hz stimulation and 5 ms for 200 Hz stimulation. The red box represents the duration of the artifact dead time where no assessment could be made of neural activity. In the control pre stimulation plots the red box only simulates the duration of the dead time. (a) PSTHs for two units recorded during 20 Hz stimulation sessions. Plot (i) is a control PSTH constructed during the 20 s epoch just before CS+1 onset for the 10 trials during the pre stimulation period for a given unit. Spikes are aligned at time = 0 to sham stimulation times occurring every 50 ms. Plot (ii) is the PSTH of the same unit during stimulation. Spikes are aligned to the onset of actual stimulation pulses. The horizontal blue line is the mean firing rate of the unit during the pre stimulation period and the dotted red lines represent ± 2 standard deviations from the mean. Plots (iii) and (iv) are the control and actual PSTH histograms for another unit. Each unit is from a different rat. (b) PSTHs plots for 2 units recorded during 200 Hz stimulation. Explanation for the plots is similar to what was described for (a) except that these are units recorded during HFS and the time scale on the x-axis is different. The duration for each plot is 5 ms which is the period between 200 Hz stimulation pulses. Each unit is from a different rat.

*Ventral pallidal units responded to task related events*

Firing rates during epoch periods of 0.5 s and 8 s after CS+1 onset, and 0.5 s and 1 s after CS+2 and UCS were analyzed and compared to the 8 s pre CS+1 period to determine unit responses. Table 2.2 shows the breakdown of unit responses for all events and epochs for pre stimulation, 20 Hz stimulation, 200 Hz stimulation, and post stimulation periods. All units, with the exception of one, were responsive to either the reward cues (CS+1 and CS+1), the food reward (UCS), or some combination of the three events. Looking at the pre stimulation period for all units, the majority of units responded to a combination of stimuli (56 %; n = 14 units) and only 39 % of units (n = 11) responded uniquely to the CS+1 or CS+2. Furthermore, majority of cue responses were excitatory in nature which is consistent with previous findings (Smith et al., 2011; Tindell et al., 2004; Tindell et al., 2005). Overall, there were fewer responses to the UCS and those responses were typically inhibitory in nature (see table 2.1 and figure 2.14).

*Units still responded to behavioral events during stimulation*

Stimulation did not shut down unit responses to the reward cues and the reward itself. In fact, the majority of units that were responsive to the cues or the UCS during the pre stimulation period also responded to these events during stimulation trials and showed very similar types of responses; for example, a unit that showed excitation during pre stimulation also showed excitation during stimulation. Figure 2.15 shows sample perievent histograms and raster plots for a LFS unit and a HFS unit. Again the majority of units responded to a combination of the stimuli: 73 % (n = 8) during 20 Hz stimulation and 87 % (n = 13) during 200 Hz stimulation. For 20 Hz stimulation, only 2 units responded uniquely to CS+2 and 1 unit responded only to UCS. For 200 Hz stimulation, only 1 unit responded uniquely to CS+2 and one unit responded uniquely to UCS. Responses to these stimuli were mainly excitations. There were also more excitations in response to the UCS. This was especially the case for the population of units recorded in the HFS sessions compared to the pre stimulation and post stimulation periods [$\chi^2$ = 8.927, $p$ 0.012, $df$ = 2]. Refer to table 2.1 for summary of unit responses and figure 2.14 for breakdown of responses for each period and event of interest.

After stimulation, units were still responsive to the cues or the UCS and again the majority of units responded to a combination of the stimuli (63%; n = 17). There were actually more units responding uniquely to UCS (n = 5) compared to pre stimulation (n = 0) and stimulation periods (n = 1). In addition, 2 units responded uniquely to CS+1 and 3 units responded uniquely to the CS+2.

Table 2.2 – Breakdown of all VP unit responses, all periods for CS+1, CS+2, and UCS.

| **Epoch Window** | **Neural Response** | **Pre Stim (n = 28)** | **20 Hz (n = 13)** | **200 Hz (n = 15)** | **Post Stim (n = 28)** |
|---|---|---|---|---|---|
| | | n (% total) | n (% total) | n (% total) | n (% total) |
| **CS+1 (Lever extension + light)** | | | | | |
| 0 - 0.5 s post CS+1 | excitation | 8 (28.6 %) | 3 (23.1 %) | 4 (26.7 %) | 8 (28.6 %) |
| 0 - 0.5 s post CS+1 | inhibition | 2 (7.1 %) | 1 (7.7 %) | 0 (0 %) | 2 (7.1 %) |
| 0 – 8 s post CS+1 | excitation | 7 (25 %) | 3 (23.1 %) | 4 (26.7 %) | 9 (3.2 %) |
| 0 – 8 s post CS+1 | inhibition | 3 (10.7 %) | 1 (7.7 %) | 1 (6.7 %) | 2 (7.1 %) |
| **CS+2 (Feederclick)** | | | | | |
| 0 – 0.5 s post CS+2 | excitation | 15 (53.6 %) | 6 (46.1 %) | 9 (60 %) | 9 (32.1 %) |
| 0 – 0.5 s post CS+2 | inhibition | 3 (10.7 %) | 2 (15.4 %) | 0 (0 %) | 5 (17. 9 %) |
| 0.5 – 1 s post CS+2 | excitation | 12 (42.9 %) | 7 (53.8 %) | 13 (86.7 %) | 12 (42.9 %) |
| 0.5 – 1 s post CS+2 | inhibition | 2 (7.1 %) | 1 (7.7 %) | 0 (0 %) | 5 (17.9 %) |
| 0 – 1 s post CS+2 | excitation | 15 (53.6 %) | 7 (53.8 %) | 14 (93.3 %) | 11 (39.3 %) |
| 0 – 1 s post CS+2 | inhibition | 3 (10.7 %) | 2 (15.4 %) | 0 (0 %) | 4 (14.3 %) |
| **UCS (Sucrose pellet)** | | | | | |
| 0 – 0.5 s post CS+2 | excitation | 2 (7.1 %) | 3 (23.1 %) | 7 (46.7 %) | 6 (21.4 %) |
| 0 – 0.5 s post CS+2 | inhibition | 3 (10.7 %) | 1 (7.7 %) | 0 (0 %) | 3 (10.7 %) |
| 0.5 – 1 s post CS+2 | excitation | 2 (7.1 %) | 5 (38.5 %) | 10 (66.7 %) | 5 (17. 9 %) |
| 0.5 – 1 s post CS+2 | inhibition | 4 (14.3 %) | 2 (15.4 %) | 2 (13.3 %) | 7 (25 %) |
| 0 – 1 s post CS+2 | excitation | 2 (7.1 %) | 4 (14.3 %) | 10 (66.7 %) | 7 (25 %) |
| 0 – 1 s post CS+2 | inhibition | 3 (10.7 %) | 1 (7.7 %) | 0 (0 %) | 6 (21.4 %) |

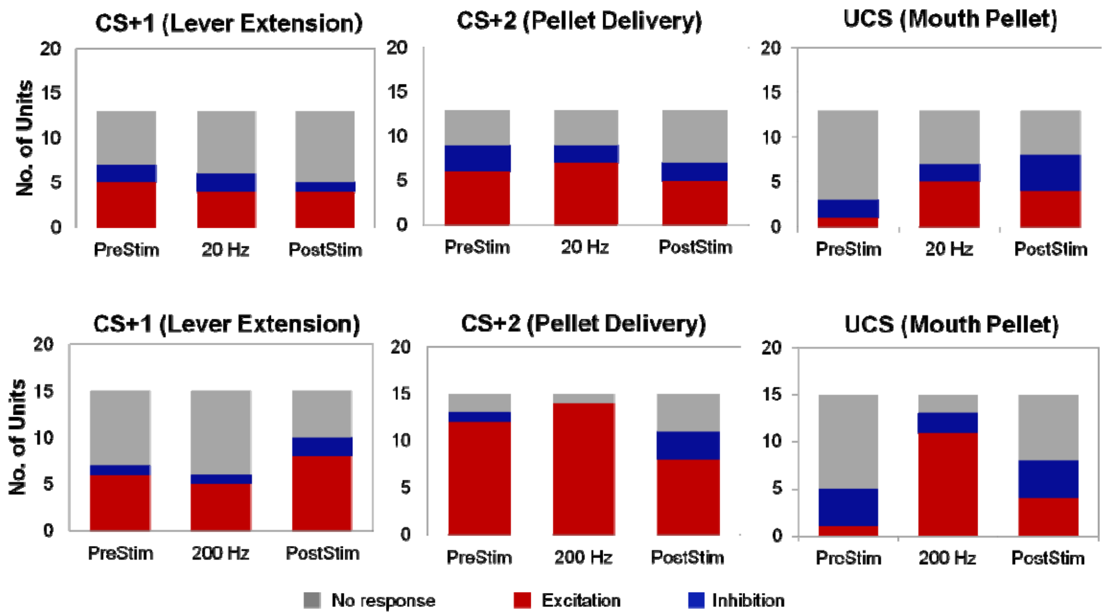


Figure 2.14 – Stimulation did not reduce VP unit responses to events. For each population (LFS and HFS units) the distribution of units that show no response (grey bars), inhibitions (blue bars), or excitations (red bars) are plotted for each behavioral event of interest (CS+1, CS+2, and UCS).

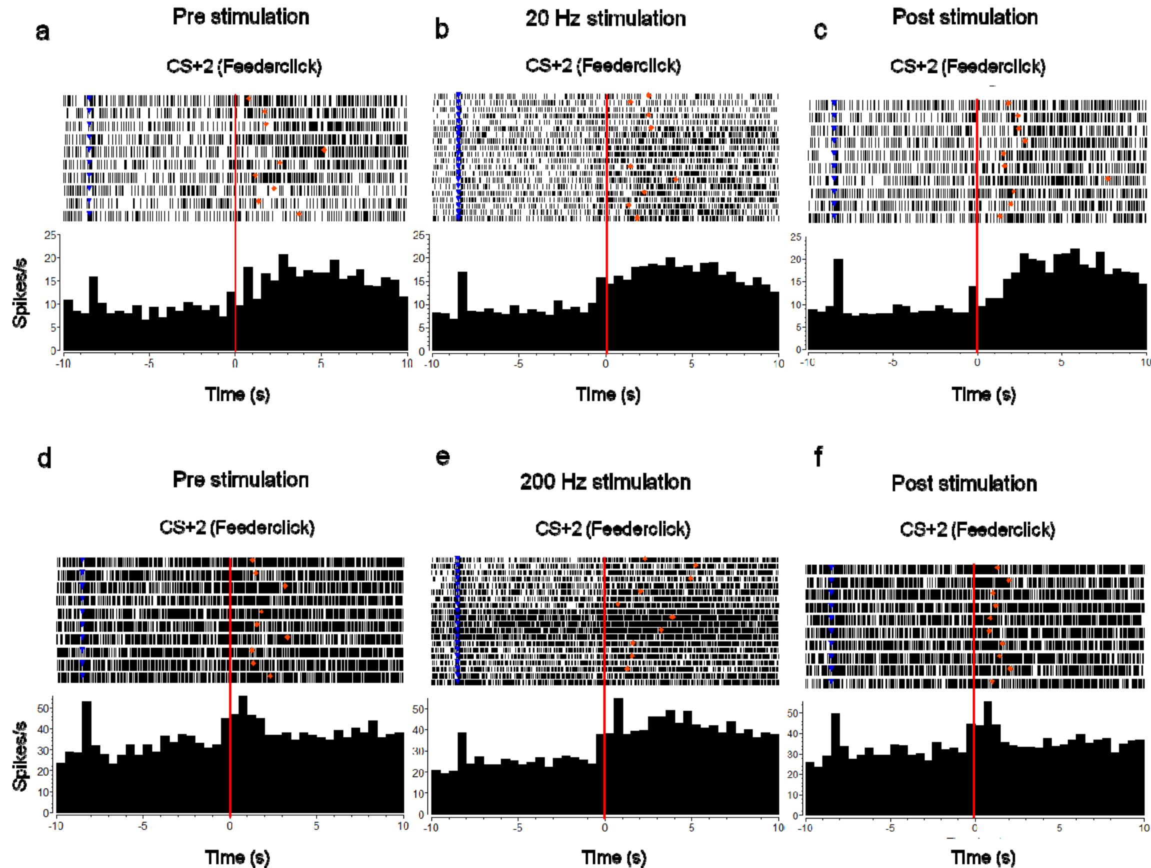


Figure 2.15 - Representative PETHs and raster plots for a VP unit tested during a LFS session (top row) and a separate VP unit tested during a HFS session (bottom row). Plots are lined to CS+2 (feederclick) event and show unit activity up to 10 s before and after CS+2 occurs which is centered at 0 and is indicated by a red line. Each mark in a raster indicates a spike and the each horizontal line is a trial with consecutive trials going from top to bottom. Histograms show the average firing rate across all trials. Bin size for each histogram is 500 ms and the y-axis is in spikes/s. On the raster plots, blue upside-down triangles indicate CS+1 onset and orange diamonds represent the time when the animal's mouth first made contact with the sucrose pellet (UCS). Note, for the stimulation trials, the time of the UCS was only scored for 10 out of the 20 trials, which is why not all trials have the orange markers. Plots (a) and (d) show trials during the pre stimulation period, (b) and (e) show trials during stimulation, and (c) and (f) show trials during the post stimulation period.

*Population unit responses and changes in firing rates*

Normalized firing rates during the 0.5 s and 8 s duration of the CS+1 and 0.5 s and 1 s duration after CS+2 and UCS were compared across pre stimulation, 20 Hz, 200 Hz, and post stimulation periods. Neural rates for stimulation periods were adjusted for artifact dead time. Firing rates did not differ significantly between pre stimulation, LFS, HFS, and post stimulation during the CS+1 for either the duration of the cue [$F(3, 74) = 1.039$; $p = 0.384$] or the 0.5 s duration after cue onset [$F(3, 74) = 1.301$; $p = 0.285$].

Firing rates did not differ significantly between pre stimulation, LFS, HFS, and post stimulation during the 0.5 s epoch after the onset of CS+2 [$F(3, 83) = 2.586$; $p = 0.063$], but there was a significant increase in firing rates during HFS for the 0.5 s to 1 s epoch compared to pre stimulation and post stimulation periods [$F(3, 83) = 3.289$; $p = 0.028$] and for the 0 to 1 s epoch after onset of CS+2 [$F(3, 83) = 3.728$; $p = 0.017$].

For each of the epochs tested after first contact with the UCS, there was a significant increase in firing rates during HFS period for the 0 to 0.5 s epoch [$F(3, 83) = 4.139$; $p = 0.01$], the 0.5 s to 1 s epoch [$F(3, 83) = 4.715$; $p = 0.005$ ], and the 0 to 1 s epoch [$F(3, 83) = 7.507$ ; $p < 0.001$] after UCS contact compared to pre stimulation, LFS, and post stimulation.

## 2.4 Discussion

Overall, high frequency stimulation in the ventral pallidum did not consistently alter consumption of palatable foods or cue-triggered "wanting" as originally hypothesized. From experiment 1 (effects of DBS on M&M consumption) there was a decrease in chocolate consumption during high frequency stimulation at 200 Hz compared to low frequency stimulation at 20 Hz, depending on stimulation pulse width. In experiment 2 (Pavlovian conditioning study), high frequency stimulation at 200 Hz resulted in a decrease in cue-triggered behaviors compared to low frequency stimulation at 20 Hz, but there was no difference compared to no stimulation. In addition, there was no effect of stimulation on sucrose consumption or latency to retrieve the sucrose pellet.

As hypothesized, low frequency stimulation at 20 Hz never resulted in either decreased consumption or motivation, and might even have an "activating" effect though increased consumption was not significant compared to no stimulation. In experiment 1, low frequency stimulation resulted in a significant increase in chocolate consumption compared to high frequency stimulation at 200 Hz depending on pulse width, but was not significant compared to control (no stimulation). There was also no difference between LFS and no stimulation on reward consumption or motivation in the Pavlovian study.

These results were also reflected in the neural data. Though stimulation itself induced changes in firing patterns, overall units were still responsive to the behavioral events during stimulation as compared to no stimulation. In fact, normalized rates in

response to contact with the sucrose pellet were higher during high frequency stimulation compared to low frequency or no stimulation.

*Potential mechanisms of action of DBS in ventral pallidum*

Given that similar therapeutic effects were reported with lesions or deep brain stimulation for the treatment of Parkinson's disease (Aziz et al., 1991; Bergman et al., 1990; Koller et al., 1997; Kumar et al., 1998; Wichmann et al., 1994), it was initially proposed that DBS was inhibiting the target structure, decreasing output from the stimulated structure and thus mimicking the effects of a physical lesion (Gross et al., 1997; Limousin et al., 1995; Lozano et al., 1998). However, other reports have shown that DBS in a given structure does not always have the same effect as physical lesions of that same structure (Krack et al., 1998; Vitek, 2002; J. Y. Zhang et al., 1997). In this study, there also was not a consistent decrease in food consumption and motivated behaviors with high frequency stimulation that has been observed with lesions or inactivation of the VP (Cromwell & Berridge, 1993; Farrar et al., 2008; Harvey et al., 2002; McAlonan et al., 1993; McFarland & Kalivas, 2001; McFarland et al., 2004; Miller et al., 2006; Morgane, 1961; Shimura et al., 2006; Waraczynski, 2006).

Other studies suggest that DBS is instead imposing its own firing pattern, that is, entraining the neurons to fire at a pattern that may no longer be functional meaningful (Cleary et al., 2013; Garcia et al., 2005a; Garcia et al., 2005b; W. M. Grill et al., 2004; M. D. Johnson & McIntyre, 2008; McIntyre et al., 2004). This stimulation-induced modulation of firing could be occurring in afferent and/or efferent axons of the target structure (Anderson et al., 2006; Filali et al., 2004; Garcia et al., 2005a; Hashimoto et al., 2003; McIntyre et al., 2004). Work from modeling studies and electrophysical recordings *in vitro* and *in vivo* have shown that neurons are still able to fire during DBS (Bar-Gad et al., 2004; Cleary et al., 2013; Garcia et al., 2003; Hashimoto et al., 2003; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007; Maurice et al., 2003; McCairn & Turner, 2009; Montgomery E.B., 2005). Our results also show that neurons are able to fire in between stimulation pulses (figure 2.11). Basal firing rates were not significantly different between no stimulation and LFS. For HFS, there was an overall decrease in firing seen with 200 Hz stimulation (after accounting for artifact dead time), but a few

units also showed increases in firing during stimulation. We also observed complex firing patterns of neural activity due to stimulation with periods of inhibition and excitation. This stimulation-induced complex firing patterns have also been reported in other studies looking at activity in the GPi and STN in humans and animals in response to DBS (Bar-Gad et al., 2004; Cleary et al., 2013; Garcia et al., 2003; Hashimoto et al., 2003; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007; Maurice et al., 2003; McCairn & Turner, 2009).

This modulation of neural activity with stimulation has led to the idea that stimulation is creating a "jamming" or interference of information flow in the neural circuit. DBS could be imposing its own pattern in the circuit (Cleary et al., 2013; Garcia et al., 2005a; Garcia et al., 2005b; W. M. Grill et al., 2004; M. D. Johnson & McIntyre, 2008; McIntyre et al., 2004) possibly creating an "informational lesion" (W. M. Grill et al., 2004). However, our findings challenge the simple notion of information blockade by deep brain stimulation. Although the behavioral effect we hypothesized was not observed, there were still some behavioral consequences and physiologically, the evidence of persistent information transfer was strong. During stimulation, units were still responsive to the reward cues and the reward itself. Even at 200 Hz stimulation which resulted in an overall decrease in firing rate, behaviorally related signals "got through" to barrage of stimulation.

It has been argued that the therapeutic effects of DBS may be due to multiple neuronal mechanisms as well as the type of neural circuit being stimulated and its connections (Carlson et al., 2010; Cleary et al., 2013; M. D. Johnson & McIntyre, 2008; M. D. Johnson et al., 2012; Okun, 2012). The ventral pallidum has reciprocal GABAergic connections with the nucleus accumbens (Chrobak & Napier, 1993; Churchill & Kalivas, 1994; Phillipson & Griffiths, 1985; Usuda et al., 1998). When these neurons are excited, GABA is release, inhibiting the target neuron. It has been proposed that reward-related behavior is activated through the disinhibition of VP neurons via hyperpolarization of nucleus accumbens (Caille & Parsons, 2004; Caille & Parsons, 2006; Kalivas, 2004; Napier & Mitrovic, 1999). In our study, stimulation in the ventral pallidum could be activating afferent GABAergic axons coming from the nucleus accumbens which would then release GABA into the VP and thus inhibit it. With the VP

now inhibited, reward-related behavior such as food consumption may be decreased. However, there are also GABAergic inhibitory axons from the VP projecting back to the nucleus accumbens, which if activated can in turn hyperpolarize the nucleus accumbens. Hence, the minimal effect seen in our results could be due to these competing connections, further demonstrating the importance of the target being stimulated. Our behavioral results may also be due to target location. The electrodes may not have been in the right location in the ventral pallidum. This is discussed in more detail below.

*Possible "activating: effect of LFS?*

With low frequency stimulation at 20 Hz, we did not see any decrease in food consumption or motivation. In fact, in experiment 1, chocolate consumption was significantly greater compared to high frequency stimulation. Rats also ate more M&Ms compared to no stimulation (though not significant). This is in line with other studies that suggest that lower frequency stimulation either has no effect or an "activating" effect. In DBS studies for Parkinson's disease, essential tremor, and dyskinesia, low frequency stimulation (typically < 50 Hz) is reported to have no therapeutic effect and in some cases even worsen symptoms (Garcia et al., 2003; Garcia et al., 2005a; Kuncel et al., 2007; Moro et al., 2002; Rizzone et al., 2001; Wu et al., 2001). Low frequency stimulation in the lateral hypothalamus at 60 Hz also increased food consumption (K. C. Berridge & Valenstein, 1991).

*Is the ventral pallidum still a valid candidate?*

Given the results of this study, is the ventral pallidum still a valid candidate for decreasing food consumption and motivation using DBS? We observed slight decreases in consumption and motivated behaviors, though not consistently. We had targeted the posterior ventral pallidum, specifically the hedonic hotspot (about a cubic millimeter region) as this area seemed the most viable candidate. However, the VP is a large heterogeneous region with differences, sometimes even opposite, responses in reward coding depending on location within the structure (Beaver et al., 2006; Calder et al., 2007; Smith & Berridge, 2005). Hence, it is possible that depending on the area being stimulated, there were no significant behavioral effects with DBS. A more careful

mapping of the effects of DBS on different regions of the VP may be needed to determine if there is an effective location in this structure. In addition, other brain circuits may be sufficient to take over and still encode motivated behaviors even if the VP is being affected by DBS. Hence, other next steps would be to explore other brain regions such as the amygdala and the nucleus accumbens which are also heavily involved in reward processing. In the same Pavlovian paradigm, we briefly tested stimulation in the nucleus accumbens with no effect (data not reported), though other studies have reported mixed results with DBS in the accumbens (Heldmann et al., 2012; Knapp, Tozier, Pak, Ciraulo, & Kornetsky, 2009; Liu et al., 2008; Sesia et al., 2008; van der Plasse et al., 2012; Vassoler et al., 2008). Another valid candidate is the amygdala, specifically the central nucleus of the amygdala (CeA), which we tested with promising results. The next two chapters of the thesis discuss these findings in the CeA.

## 2.5 Conclusion

The results of this study suggest that DBS in the posterior ventral pallidum may not be sufficient for decreasing "wanting" of food rewards. Hence this may not be a viable target for DBS for addiction or eating disorders. More testing needs to be done to better evaluate this structure before ruling it out completely, however. In addition, DBS induced changes in firing of ventral pallidal units, but this did not "block" relevant reward information coming through.

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# CHAPTER 3
# DEEP BRAIN STIMULATION IN THE CENTRAL NUCLEUS OF THE AMYGDALA DECREASES FOOD CONSUMPTION

## 3.1 Introduction

Deep brain stimulation (DBS) therapy has become a successful treatment for Parkinson's disease, essential tremor, dystonia, and other movement disorders (Gubellini et al., 2009; Schwalb & Hamani, 2008; Weaver et al., 2009). However, the mechanisms of DBS are not well understood (Garcia et al., 2005a; Kringelbach et al., 2007; McIntyre et al., 2004). Therapeutic benefits of DBS typical occur at stimulation frequencies greater than 100 Hz; though, the effects do depend on amplitude and frequency of the stimualtion as well as target location (Dostrovsky & Lozano, 2002; Kuncel & Grill, 2004). Work from modeling and experimental studies suggest that this high frequency stimulation may be activating fibers resulting in stimulation-induced modulation causing the neurons to fire at a more regular pattern. This regular pattern could be "masking" the intrinsic firing pattern in normal circuits or interfering with the pathological patterns in the basal ganglia in Parkinson's disease (Garcia et al., 2005a; W. M. Grill et al., 2004; Hashimoto et al., 2003).

From a clinical standpoint, DBS therapy provides a reversible way of targeting specific brain regions. Furthermore, given its efficacy in treating neurological disorders like Parkinson's (Doshi, 2011; Voges et al., 2006), it is also being investigated as a potential treatment for other disorders. Clinical trials are underway for DBS treatment of obsessive compulsive disorder, depression, and epilepsy (Chang, 2004; Greenberg et al., 2006; Haber & Brucker, 2009; Halpern et al., 2008; Lozano et al., 2008; Mayberg et al., 2005) with promising results.

DBS is also being explored as a potential treatment for morbid obesity and addiction (Gubellini et al., 2009; Halpern et al., 2008). Depending on the placement of

DBS electrodes, case studies have reported differences in mood and cognition with DBS. For example, two patients with DBS in the subthalamic nucleus reported a decrease in cravings that had been originally induced due to their L-dopa medication (Witjas et al., 2005). Another patient treated for OCD with DBS in the nucleus accumbens reported decreases in food consumption and smoking (Mantione et al., 2010). Various animal studies and some human studies have also looked at the effects of DBS on obesity and addiction (Heldmann et al., 2012; Lacan et al., 2008; Lehmkuhle et al., 2010; Melega et al., 2012; Rouaud et al., 2010; Sani et al., 2007).

In using DBS to modulate reward processing, among other things, questions still remain on the most effective target location and set of parameters. For example, in many of the studies referenced above that are looking at DBS for obesity, they are targeting the lateral hypothalamus or the ventromedial hypothalamus to either "block" feelings of hunger or trigger feelings of satiation respectively. However, simply targeting homeostatic control of feeding might not be enough. Foods, especially those rich in fats and sugars, are themselves very rewarding. Motivation to seek out and consume rewards beyond physiological need is thought to be a major factor in morbid obesity (K. C. Berridge et al., 2010). Hence, targeting excessive motivation to seek out and consume rewards might be more effective than simply targeting appetite (Halpern et al., 2008). Targeting excessive motivation to consume rewards in general would also be applicable for drug addiction treatment (Rouaud et al., 2010).

Results from the previous study of DBS in the ventral pallidum (Chapter 2 of this thesis) showed no conclusive effects of DBS on decreasing consumption of highly palatable foods. However, another potential region could be the amygdala, specifically the central nucleus of the amygdala (CeA). The amygdala is part of the limbic system and has many connections to cortical (especially sensory and prefrontal) and sub-cortical regions. It has direct output connections to the hypothalamus and brainstem regions that are involved in encoding of autonomic and psychological responses (Baxter & Murray, 2002; Sah et al., 2003). Among its many functions, the amygdala plays a central role in emotional learning and memory, including reward learning. It functions as an integrator of emotional events leading to the production of appropriate responses (Langevin, 2012; Rogan & LeDoux, 1996).

The CeA may be particularly involved in translating learning into motivation and specifically focusing motivation for particular cues and rewards (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009; Mahler & Berridge, 2011). The CeA is the major output nucleus of the amygdala with connections to the lateral hypothalamus and brainstem regions including the ventral tegmental area and indirect connections to other limbic regions like the nucleus accumbens and ventral pallidum. Lateral and basal portions of the amygdala receive emotional sensory stimuli and pass those signals on to the CeA. Emotional integration then largely occurs within the interdivisional circuitry of the CeA (Pitkanen et al., 1997). Opioid stimulation in the CeA has been shown to increase food consumption and to increase responses to reward cues, (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009; Mahler & Berridge, 2011). Lesions or muscimol inactivation of the CeA have shown to decrease food consumption (Galaverna et al., 1993; Kemble et al., 1979; Seeley et al., 1993; Touzani et al., 1997; M. J. Will et al., 2009; M. Will et al., 2004) Animals will also self-stimulate, i.e. press a lever or nose-poke to receive a 500 ms train of stimulation at frequencies ranging from 60 Hz to 200 Hz. This short burst of stimulation is considered to be rewarding and animals will initiate this response. This has been evidenced by previous work (Touzani & Velley, 1998; Waraczynski, 2006; Wurtz & Olds, 1963).

Hence, CeA may be a potential area of exploration for neuromodulation of food reward. In this study, we explore the idea of whether DBS in the CeA can "block" the motivation to work for and consume a palatable sucrose pellet. Rats were trained on an operant response paradigm in which they had to touch a lever to receive a sucrose pellet. Responses on the lever and the number of pellets consumed were measured under conditions of no stimulation, low frequency stimulation of 20 Hz, and the typical therapeutic DBS frequency at 130 Hz. We hypothesized that high frequency stimulation in the CeA will decrease the motivation to work for and consume sucrose pellets. Our results showed that both low and high frequency stimulation decreased pellet delivery and consumption. These results demonstrate that DBS in the CeA can effectively decrease consumption of a highly palatable food reward. Hence, CeA could be a potential target for modulating reward processing.

## 3.2 Material and Methods

The effect of deep brain stimulation (DBS) in the amygdala on food consumption was assessed. Animals were tested on an operant responding tasking where they had to work to obtain a food reward. The animals' motivation to work for and consume a highly palatable food (sucrose pellets) was measured. Effective stimulation parameters were also assessed. Two experiments were carried out. Experiment 1 mapped out the effective zone in the amygdala. Experiment 2 compared the effects of low and high frequency stimulation on the motivation to obtain and consume food rewards.

### *3.2.1 Experiment 1: Mapping study*

*Subjects*

Four adult male Sprague-Dawley rats weighing 250 g – 400 g were used in this experiment. Animals were housed individually on a 9:30 AM to 7:30 PM reversed light/dark schedule and provided with unrestricted access to standard rat chow and water in their home cages. Since experiments were run during the day, the reverse light/dark schedule ensured that the rats were tested during their active period. All of the following experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

*Apparatus*

All training and testing were conducted in a 28 cm x 35 cm x 60 cm plastic chamber with a glass floor. A metal grid was placed on top of the glass floor which provided a grounding contact between the animal and the chamber. After electrode implantation, the animal was grounded through the electrode itself, but the metal grid floor remained to maintain consistency in the environment between training and testing. The chamber was illuminated with red light from below. The top of the chamber was open, allowing for the electrode to be connected to a commutator and thus to the stimulator and recording system via a headstage cable. The commutator allowed the rat to be able to freely move around and explore the chamber. Attached to one wall of the chamber were two retractable levers (Coulbourn Instruments, Whitehall, PA) located on

either side of a food dish. One of the levers was designated as “active” and delivered a sucrose pellet to the food dish contingent upon lever pressing. The other lever served as a control for general lever-pressing behavior unrelated to pellet delivery. The location of the active and inactive lever was counter-balanced across rats and remained consistent for each rat throughout the study. The testing chamber was the same as used in the studies done in Chapter 2 (see appendix A.1 for a schematic of the chamber set-up).

Delivery of all stimulus and reward presentations, as well as, recording of responses were all controlled by a software program, MTASK (created in the Aldridge lab). Stimulation timing (frequency and pulse-width) was controlled by a timing program created in the Aldridge Lab using LabVIEW (National Instruments, Austin, TX). Stimulation was delivered using the A-M systems constant current digital stimulus isolator stimulator (A-M systems, Carlsborg, WA). Sessions were recorded at 30 frames per second via a video camera placed underneath the glass floor. The timestamp clocks for the behavioral task control, video recording, and stimulation delivery were synchronized to one another to enable subsequent analysis, in relation to one another, of task events, stimulation, and behavioral events obtained from video analysis or recorded in Mtask.

*Habituation and food self-administration training*

**Habituation and exposure to sucrose pellets.** Three to five days prior to the start of training rats were handled for 10 – 15 minutes per day and also introduced to sucrose pellets in their home cages (between 10 and 15 pellets per day). One day prior to training, rats were placed in the chamber for 30 minutes with the levers retracted to acclimatize them to the testing chamber.

**Fixed-ratio 1 schedule training.** Animals were placed in the chamber with the levers retracted one minute prior to the start of the session in order to acclimatize to the chamber before the session begins. At the start of each session, both levers were extended into the chamber and remained extended throughout the remainder of the session. Sessions were 30 minutes long. Rats were trained on a fixed ratio schedule of 1 (FR 1) where contacting the active lever once resulted in the delivery of 1 sucrose pellet. After the rat delivered a pellet there was a 500ms timeout before he could try and deliver

another pellet. When the rat had delivered pellets more than 10 times during a session and touched the active lever three times more than the inactive lever, he moved on to the next phase of training described below.

**Limited progressive ratio schedule training.** After learning the FR 1 schedule, rats were then trained on a limited progressive ratio. During these sessions, subjects first did a FR 1 schedule for 5 trials. Then they did a FR 5 schedule (1 pellet delivered after 5 lever contacts) for another 5 trials, then a FR 10 schedule (1 pellet delivered after 10 lever contacts) for the remainder of the session. There was a time-out period of 500 ms between contacts and 20 s timeout period before the rat could deliver another pellet after delivering one. Sessions were again 30 minutes in length with a one minute habituation period prior to the start of the session. Once animals demonstrated stable levels of responding across three consecutive days, they were ready for electrode implantation surgery.

*Electrode implantation*

Prior to surgery, rats were weighed and anesthetized with isoflurane gas (induction at 2.5 L/minute and maintenance at 1.5 – 2.0 L/minute). Each rat was bilaterally implanted with an electrode targeting a different trajectory through the amygdala. Electrodes were composed of two bundles of six wires; each individual bundle consisting of two 75 µm stainless steel stimulating wires, as well as four 50 µm tungsten recording wires. Each bundle could be lowered or raised independently. Locations were chosen such that all the major divisions of the amygdala would be targeted. These include: 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. Rats were given four to five days for recovery. Detailed surgical procedures can be found in (Tindell et al., 2004).

*Depth testing and current sweeps*

After rats recovered from electrode implantation surgery they underwent a mapping study to determine the effective electrode location and minimum effective current that resulted in a decrease in pellet consumption. For depth testing, the electrodes for each rat were first lowered to 7.5 mm below dura. This was the starting depth. Rats were tested on an abbreviated version of the limited progressive ratio schedule that they were trained on prior to surgery. 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 and the electrode was lowered on both sides by 0.159 mm and the rat was tested again. 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.

*Histology*

Anatomical localization of electrode sites was done after completion of testing. Rats were anesthetized with isoflurane gas and then a 0.1 mA lesioning DC current was passed for 10 seconds to mark the electrode location in the brain. After euthanizing the rats with a drug overdose of pentobarbital, the brains were removed, frozen in an isopentane and isopropyl alcohol solution, sliced into 40 μm sagittal sections CM 1850 cryostat (Leica Microsystems, Buffalo Grove, IL), and finally stained with cresyl violet. The electrode placement was confirmed by observing the histologic slices under a light microscope.

### *3.2.2 Experiment 2: Effects of deep brain stimulation on food consumption*

In this study, 13 adult male Sprague-Dawley rats weighing 250 g – 400 g were used. Like in experiment 1, they were housed individually on a 9:30 AM to 7:30 PM reversed light/dark schedule and given food and water ad lib. Again, all of the following experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan. Apparatus set-up was the same as in experiment 1 with the addition of recording neural activity during the testing sessions using DataTask, another program written in this lab using LabVIEW (National Instruments, Austin, TX). Neural activity was amplified at a gain of 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. Furthermore, this allowed large amplitude signals to be recorded with greater fidelity without exceeding the voltage limits (±10 V) on the data acquisition board. This increased fidelity facilitated artifact removal and spike recovery (see below). Timestamp clocks for the behavior control program, video recording, neural recording, and stimulation were all synchronized to enable subsequent analysis of neural activity related to task events, stimulation, and behavioral events obtained from video analysis or recorded in Mtask.

*Training*

Habituation, exposure to sucrose pellets, and training on the food self-administration paradigm were the same as described in experiment 1.

*Electrode implantation*

The electrode implantation surgery was similar to that described in experiment 1 with the exception of target location. Eight rats were bilaterally implanted with an electrode 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). Four rats serving as surgical/stimulation controls were implanted in areas outside of the CeA: the ventral pallidum, the extended amygdala, the medial anterior dorsal nucleus of the amygdala, amygdala striatum transitional area, and inferior to the CeA.

*Behavioral testing procedures*

**Screening.** After surgery, rats were screened to determine the effective electrode depth and minimum effective current that resulted in a decrease in pellet consumption. Rats were observed to determine any effects of stimulation on their behavior. If stimulation had no effect on food consumption or instead produced unwanted motor effects (e.g. paw twitching, turning), the electrode was lowered on both sides by 0.159 mm and the rat was tested again. This was repeated until stimulation caused a decrease in food consumption and also caused no adverse motor effects, or if the electrode reached a depth of 8.5 mm below dura with still no observable effects of stimulation. Stimulation current was normally set at 250 μA. However, if the electrode reached approximately half-way through the supposed region of the CeA with no behavioral effect, the cuurent was increased in increments of 50 μA until an effect was seen or amplitude reached 400 μA.

**Limited progressive ratio schedule testing.** After screening, rats were given a day off then began limited progressive ratio testing. During 30-minute sessions, subjects received continuous DBS while performing the original lever-pressing task on an FR 1, FR 5, FR 10 schedule as described in detail under the limited progressive ratio training section in experiment 1. For each rat, the location of the active lever was identical to that

in training. Animals were tested for approximately 9 - 12 days over a two-week span. Testing consisted of three consecutive days of high frequency stimulation (130 Hz), three consecutive days of low frequency stimulation (20 Hz), and three days of no stimulation. A two day break was given between high and low frequency days to minimize any potential lingering effects of DBS (Gubellini et al., 2009). Prior to start of the test session, there was a 10-minute habituation period. After the habituation period, the levers were extended into the chamber indicating the start of the session. On stimulation days, stimulation was turned on five minutes into the habituation period to allow animals to acclimatize to the stimulation and also to provide a period of assessment with stimulation in the absence of the task. On control days stimulation was not delivered. Rats were randomly selected to begin testing with either the high or low frequency stimulation block and control days were interspersed throughout the two-week testing period. Figure 3.1 show schematics of the limited progressive ratio paradigm and the experimental timeline.

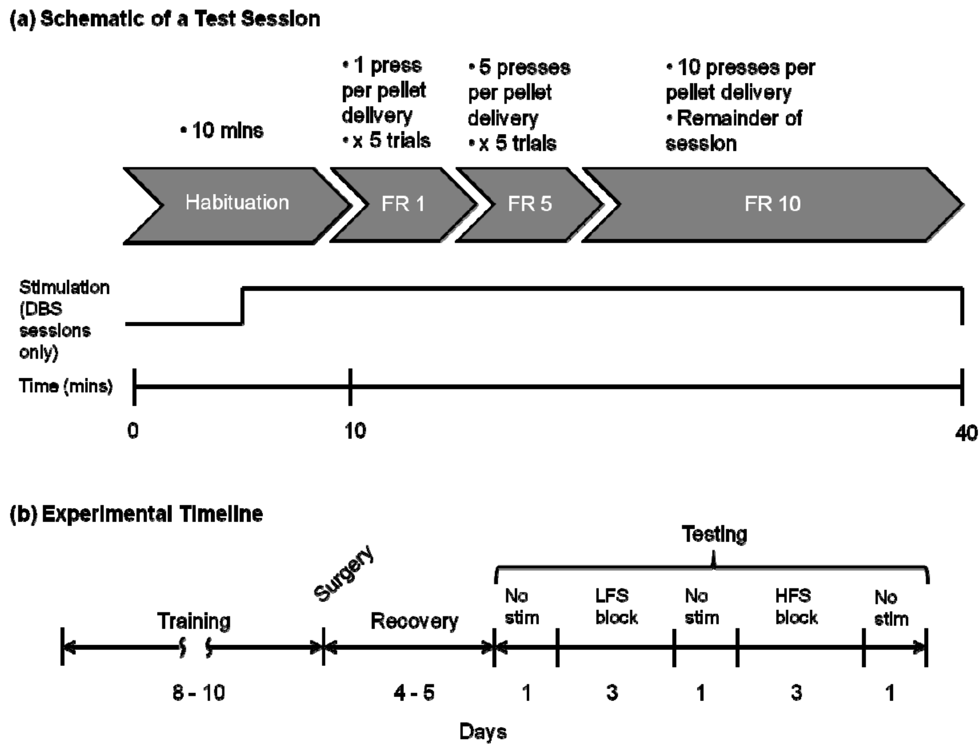


Figure 3.1 – (a) Schematic of a limited progressive ratio test for experiment 2 (DBS – deep brain stimulation). A similar paradigm was used in experiment 1, the only difference was that the habitation period was 1 minute long and stimulation was turned on from the beginning of the habitation period. (b) Experimental timeline for experiment 2 (No Stim – test days where no stimulation was delivered, LFS – low frequency stimulation at 20 Hz, HFS – high frequency stimulation at 130 Hz).

**Frequency sweep.** At the end of formal testing in the limited progressive ratio paradigm and after a break of several days, a brief frequency sweep study was carried out to investigate effects of frequencies even lower than 20 Hz on consummatory and lever-pressing behavior. This was done to determine the threshold frequency that resulted in decreased consumption. This test was done on two rats. The same behavioral paradigm and set-up was done as in the limited progressive ratio test, with the exception that the rats were tested on a range of stimulation frequencies while doing the behavioral task. Each frequency was tested on a separate day and repeated and the number of pellets consumed was measured. The frequencies tested were: 2 Hz, 4 Hz, 10 Hz, and 16 Hz. Rats were also retested on 20 Hz to verify that stimulation at this frequency was still effective and that the effects of stimulation had not change over time.

*Stimulation parameters*

A continuous monopolar and biphasic current (250 - 400 μA), with a pulse width of 100 μs per phase, was delivered bilaterally into the target structure. Bilateral stimulation was used as we were concerned that stimulating only one side would not be sufficient enough to see a significant effect on food consumption. For high frequency stimulation, a frequency of 130 Hz was used. For low frequency stimulation, 20 Hz was used. For the frequency sweep test, frequencies of 2 Hz, 4 Hz, 10 Hz, and 16 Hz were also used. Each rat was tested at their minimum threshold current that caused an effect as determined in the screening test. For the majority of the rats this current was 250 μA. For the range of current from 250 μA to 400 μA, the charge density per phase delivered at the tip of the 75 μm stimulating wire was 141 μC/cm$^2$/ph to 226 μC/cm$^2$/ph.

*Histological verification*

Evaluation of electrode location was the same as described in experiment 1.

*Behavioral assessment and data analysis*

To determine the effects of stimulation on motivation for highly palatable food rewards, the following behavioral measures were assessed: number of lever contacts, number of pellets consumed, and approaches to the food dish. Total number of lever

contacts on both the inactive and active lever was assessed over each 30-minute testing session. The time taken to advance to FR 5 and FR 10 schedules was also evaluated. At the end of each session, the number of pellets left in the chamber was recorded. Hence the number of pellets eaten was calculated as follows: number of pellets delivered minus the number of pellets left in the chamber at the end of testing.

Other behaviors were also assessed and quantified. The number of times the rat crossed over the horizontal and vertical midline markers of the testing chamber (cage-cross) and the number of rears were determined as a measure of general exploratory activity. Rhythmic triangular openings of the mouth typically associated with an aversive taste were also observed in some rats and were also thus quantified. These behaviors and the dish approaches were scored using frame-by-frame video analysis with DataRat software (developed by the Aldridge lab). For each video, the whole of the last five minutes was scored for cage-crossing, rearing, and gaping behavior. During the task itself, approaches, rearing, and gaping were scored for 30 s at intervals of every five minutes. These measurements were all converted to rates for comparison purposes. Statistical analyses were performed using one-way and two-way repeated measures ANOVAs and Holm-Sidak *post hoc* tests (unless otherwise specified) implemented with the program SigmaPlot (Systat Software Inc., San Jose, CA).

*Neural analysis*

Recorded neural files were further amplified by a gain of five and filtered to remove any offsets on the recorded data. This was done offline using a LabVIEW software amplifier program written in the lab (as discussed in Chapter 2 and appendix A.2). Stimulation artifacts were then removed from the neural data that was recorded during the 20 Hz and 130 Hz DBS test sessions using our artifact removal program (appendix A.2). Offline Sorter (Plexon, Inc., Dallas, TX) was then used to discriminate neural unit spike waveforms from noise and other units. Single units were identified using principal component analysis and peak-valley width analysis. Units were verified by distinct spike waveforms (whose shapes remained consistent throughout the whole recording) and clear refractory periods in their autocorrelation histograms. Units with more than 2 % of spikes within a 1 ms refractory period window were excluded. Cross-

correlation analysis was performed using Epoch builder (a database neural and behavioral analysis program written in the lab) to ensure that any unit was being counted only once. Detailed description of the cross-correlation analysis is decribed in Chapter 2. The Epoch builder program and Neuroexploroer (Nex Technologies, Madison, AL) were used to analyze unit acitivty after units were sorted out from noise with Offline Sorter.

Mean firing rates were calculated for each unit during the first five minutes of habituation period and during the later five minutes of the habituation period when the stimulation was turned on (for stimulation test sessions). This provided a baseline firing rate for the units and baseline stimulation firing rates. When making comparisons to baseline rates, firing rates during the stimulation period were normalized to compensate for signal loss due to the artifact dead time; hence, the firing rate during the stimulation period was calculated for the viable duration, excluding the dead time (Bar-Gad et al., 2004).

Peristimulus time histograms were computed for units recorded during the 20 Hz and 130 Hz stimulation test sessions. For each unit, spiking activity was lined up to the onset of each stimulation pulse (for all stimulation pulses in the session) for the period between pulses with bins of 0.5 ms. This resulted in 42,000 pulses with a period of 50 ms between pulses for 20 Hz stimulation and 273,000 pulses with a period of 7.6 ms between pulses for 130 Hz stimulation. A significant decrease or increase in firing was determined if 2 consecutive bins were greater than ± 2 standard deviations from the mean baseline firing rate of the unit computed during the first five minutes of session before stimulation was turned on. Control PSTH plots were also computed for that five minute pre stimulation period. “Sham stimulation” pulse times that occurred every 50 ms or every 7.6 ms (for 20 Hz or 130 Hz stimulation, respectively) were generated during the pre stimulation period and spikes were lined up to those “pulses”. There were 6000 pulses for sham 20 Hz stimulation and 39,000 pulses for sham 130 Hz stimulation during that five minute pre stimulation period.

Perievent time histograms (PETHs) and rasters were analyzed for behavioral events of interest - time of pellet delivery and the first lever contact on the active and inactive lever that the rat made for each trial. The firing rate reference for all unit responses was the average rate during the five seconds period just before the habituation

period ended i.e., before behavioral testing began. CeA firing rates are typically very low (in some cases, less than 1 Hz) (Collins & Paré, 1999; Duvarci et al., 2011; Rosenkranz & Grace, 1999; Shabel & Janak, 2009). For two units, there were no spikes during the last five seconds of the baseline period, so for those units the whole five minute period during the latter half of habituation was used as their baseline period for response analysis.

To determine whether a unit was responsive to pellet delivery, firing rates during 0.5 s or 1 s epochs after the stimulus event was compared to the 5 s baseline period (described above). To evaluate neural activity before and following lever response, a 0 to 0.5 s period just before the event and a 0 to 0.5 s period just after the event were each compared to the 5 s baseline period. Again, neural responses to both active and inactive levers were examined. Mann-Whitney U tests were used to determine if any of these epochs were significantly different from baseline.

To determine population responses, each unit was first normalized to the five minute baseline period during the latter half of the habitation period. Also, given the small number of units to begin with, all units in a given test condition were pooled across rats. To determine any stimulation effects, 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 (pellet delivery, active lever contact, and inactive lever contact). Specific comparisons were made using Holm-Sidak *post hoc* tests. As with the behavioral analysis, all statistical tests were again done using SigmaPlot statistical software.

### 3.3 Results

All animals acquired the limited progressive ratio task and showed consistent, stable responses across three days with the exception of one animal in experiment 2 who failed to learn the FR 1xFR 5xFR 10 training. That animal was excluded from the rest of the experiment.

### *3.3.1 Experiment 1: Stimulation effective area of ~0.4 mm$^3$ was found in the central nucleus of the amygdala*

Figure 3.2 shows the results of the depth testing for each rat. For rats 9 and 11, the effective region was between 7.5 mm to 8.3 mm deep (from dura). Rats 8 and 10 showed no consistent effect of stimulation. Rats 9 and 11 were then tested at different currents at the same frequency of 130 Hz. For rat 11, by the end of depth testing, his electrode was no longer in the region that had resulted in decreased consumption with stimulation (figure 3.2). 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 9'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 3.3).

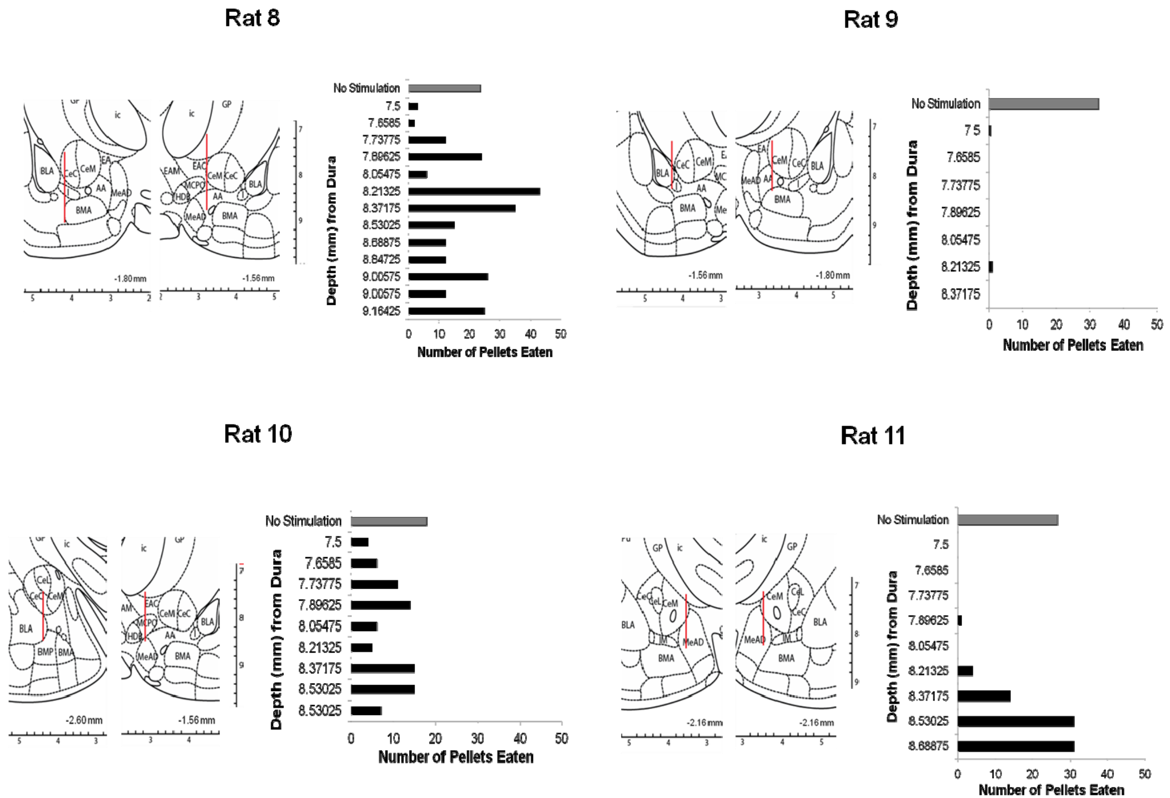


Figure 3.2 – Results from the mapping study for each rat showing the actual electrode paths (verified with histology) and the number of sucrose pellets consumed in 10 minute tests at different depths. Grey bars at the top of each plot show the average pellet consumption when no stimulation was given. Red 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).

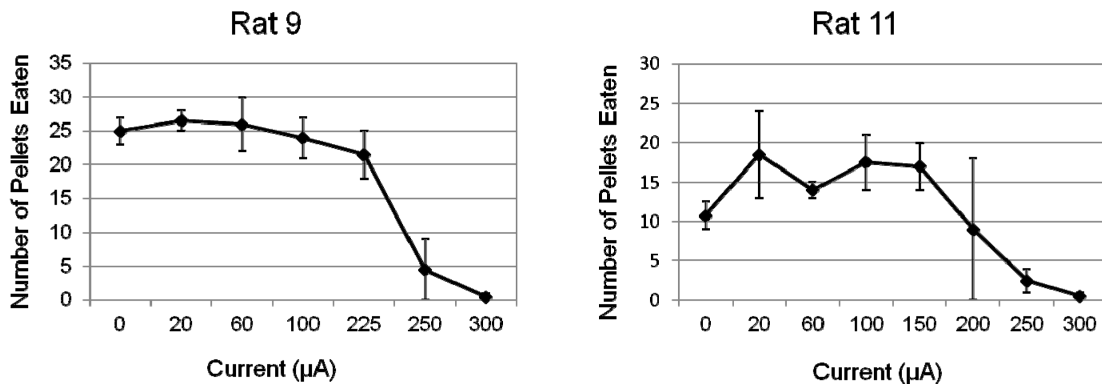


Figure 3.3 - Results from the current sweep study done on the two rats that show a marked decreased in pellet consumption from the previous depth test. For each rat, current sweeps were done at depth that had showed decreased consumption in the depth test (8.37 mm below dura for rat 9 and 7.5 mm below dura for rat 11).

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.

Rats 9 and 11 moved on to additional tests where they were given longer periods of testing and also tested on both 20 Hz and 130 Hz stimulation. However, rat 9 stopped having an effect with stimulation. Histology confirmed that though one of his electrodes was in the central nucleus of the amygdala, the other was in the basolateral amygdala. Also, there was a difference in behavior observed between rats 9 and 11 during the depth tests. Though both rats decreased food consumption with stimulation, rat 9 remained in one corner (the farthest point from the lever) for the whole test; whereas rat 11 moved around in the chamber periodically, groomed, and otherwise looked normal. Hence, with rat 9, it is possible that the stimulation triggered a fear response due to where his electrode was located. Rat 11 went on to be used in experiment 2.

### *3.3.2 Experiment 2: Deep Brain Stimulation decreases reward-seeking behavior in the central nucleus of the amygdala*

*Electrode verification*

Eight rats were successful implanted bilaterally in the central nucleus of the amygdala. One of these rats had undergone a previous mapping test (described above) Four other rats, implanted in surrounding areas, served as surgical/sham controls for the stimulation. Their electrode locations included ventral pallidum, extended amygdala, medial dorsal nucleus of the amygdala, intercalated amygdaloid nucleus, and the amygdala striatum transitional area. Figure 3.4 shows electrode placement for all 12 rats implanted.

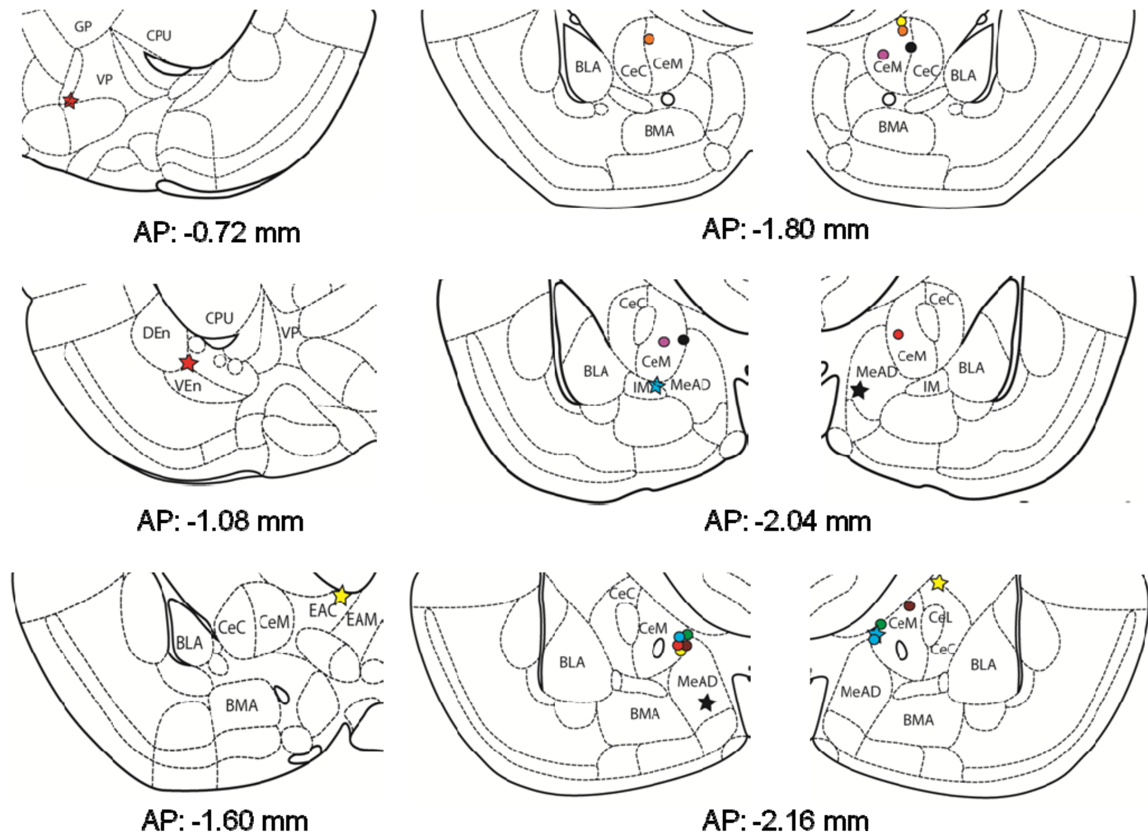


Figure 3.4 – Coronal Slices showing electrode placements of rats implanted bilaterally in the CeA (circles, n = 8 rats) and in the controls sites (stars, n = 4 rats). Within each group – CeA rats or controls rats - electrode location for each rat is represented by a different color. Images modified from the Paxinos and Watson rat atlas, 6th edition (Paxinos & Watson, 2007).

Rats implanted bilaterally in the central nucleus of the amygdala decreased pellet consumption with stimulation during depth testing. The results of the depth test also

determined the minimum effective current for each rat, which depending on the rat, was from 250 μA to 400 μA. This effective current for each rat was the one used during subsequent tests. One rat implanted in the CeA developed a seizure from the screening test and was excluded from the remainder of the experiment. Seizures can be induced in the amygdala via a process called kindling by electrically stimulating repeatedly in short bursts at frequencies of 60 Hz or lower (Goddard, 1969). Rats whose electrodes were located outside of the central nucleus of the amygdala (see figure 3.4) failed to show any decrease in pellet consumption even at currents up to 400 μA during the screening test.

*DBS in the CeA decreases consumption of sucrose pellets*

Deep brain stimulation in the central nucleus of the amygdala decreased consumption of sucrose pellets by more than a factor of six [one way RM ANOVA, $F(2, 20) = 41.61$, $p < 0.001$]. Holm-Sidak *post hoc* analysis showed that sucrose consumption during both 20 Hz and 130 Hz stimulation were significantly lower compared to when stimulation was not present; however, there was no effect of frequency (figure 3.5a). Conversely, rats stimulated outside of the CeA showed no difference in pellet consumption between no stimulation, 20 Hz stimulation, and 130 Hz stimulation [one way RM ANOVA, $F(2, 11) = 3.818$, $p = 0.085$] (figure 3.5b).

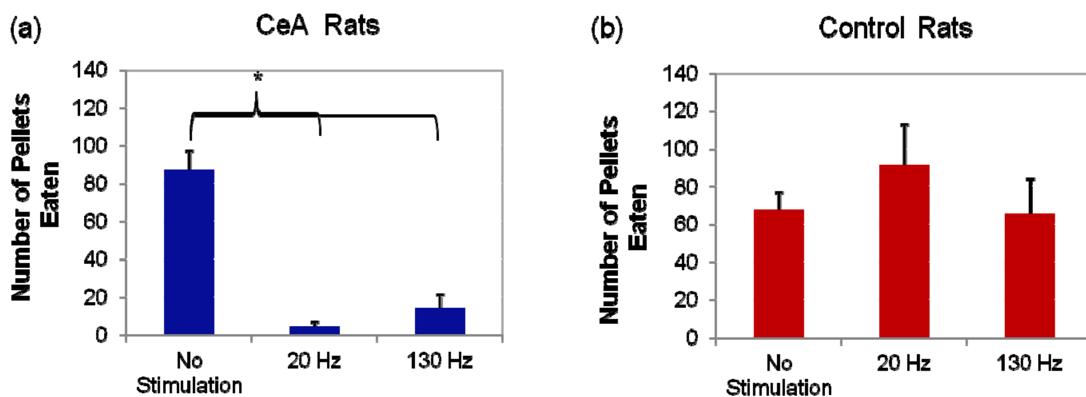


Figure 3.5 – DBS in the CeA decreased sucrose consumption. (a – b) Graphs show average number of sucrose pellets consumed in 30 minutes during no stimulation, 20 Hz stimulation, and 130 Hz stimulation. Error bars represent standard error. (a) Stimulation decreased pellet consumption for rats implanted bilaterally in the CeA (n = 7), * p < 0.001. (b) Results for surgical controls (n = 4). There was no difference in pellet consumption across all three conditions (p = 0.085).

Interestingly, sometimes during these stimulation test sessions, the CeA rats would still occasionally approach the food bowl, sniff the pellets, and even put the pellets in their mouths at times and then spit them out. Sometimes, they would even nibble on the pellets and as figure 3.5a shows, consume some.

Though rats stimulated in the CeA approached the food bowl at times, the rate of bowl approaches did significantly decrease with both low and high frequency stimulation as shown in figure 3.6a [one way RM ANOVA, $F(2, 20) = 42.33$, $p < 0.001$]. Again, Holm-Sidak *post hoc* tests revealed that there was an effect of stimulation, but not frequency. On the other hand, as figure 3.6b shows, stimulation in the control sites, showed no significant effect of stimulation on approaches to the food bowl [one-way repeated measures ANOVA; $F(2,11) = 1.93$, $p = 0.23$)].

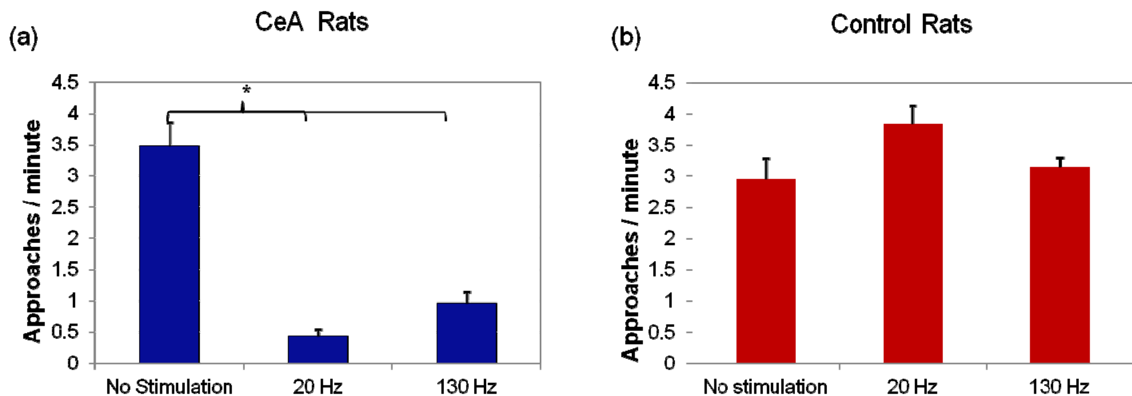


Figure 3.6 - DBS in the CeA decreased approaches to food bowl. (a - b) Average number of approaches to the food bowl per minute during no stimulation, 20 Hz stimulation, and 130 Hz stimulation. Error bars represent standard error (+SEM). (a) Stimulation decreased rate of approaches for rats implanted bilaterally in the CeA (n = 7), * p < 0.001. (b) There was no significant effect of stimulation for control rats (n = 4), p = 0.23.

*DBS in the CeA decreased working for sucrose pellets*

Rats implanted in the CeA not only consumed less pellets with stimulation, but also delivered far fewer pellets due to decrease engagement with the active lever (figure 3.7a). Results of a one-way RM ANOVA on the difference of lever contacts between active and inactive levers for each test condition showed a significant effect of stimulation [$F(2, 20) = 43.85$, $p < 0.001$]. There was no difference between low frequency and high frequency stimulation (Holm-Sidak *post hoc* analysis). When

stimulation was absent, rats delivered pellets throughout the whole 30 minute test while pretty much ignoring the inactive lever [Mann-Whitney Rank Sum Test, $T(1) = 77.000$, $p < 0.001$]. However, when stimulation was present (either 20 Hz or 130 Hz), contacts on the active lever, like the inactive lever, were very low.

In contrasts, rats implanted outside the CeA (in control sites) continued to deliver pellets throughout the 30 minute test regardless of stimulation or not (figure 3.7b). There was no effect of stimulation in the difference in contacts with the active versus inactive lever [One way RM ANOVA; $F(2, 11) = 4.399$, $p = 0.067$]. Specifically, for all conditions (no stimulation, 20 Hz, and 130 Hz) inactive lever contacts were significantly lower than inactive lever contacts [Mann-Whitney Rank Sum Tests; $T(1) = 78.000$, $p < 0.001$].

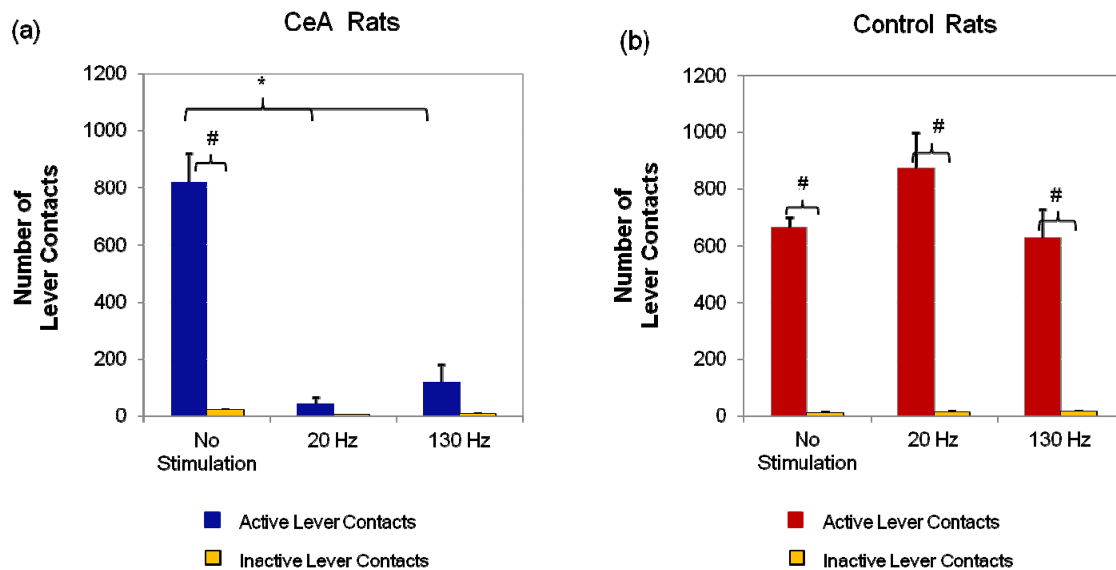


Figure 3.7 – DBS in the CeA decreases motivation to work for sucrose. (a –b) Average number of active and inactive lever contacts for no stimulation, 20 Hz stimulation, and 130 Hz stimulation. Error bars represent standard error (+SEM). (a) For rats implanted in the CeA (n = 7). Blue bars represent active lever contacts and orange bars represent inactive lever contacts. Difference in contacts between active and inactive lever was significantly reduced with stimulation, * p < 0.001. There was also a significant decrease in inactive lever contacts compared to active lever contacts for no stimulation, # p < 0.001. (b) For rats implanted in areas outside of the CeA (n = 4). Red bars represent active lever contacts and orange bars represent inactive lever contacts. Difference in contacts between the active and inactive lever was the same across all conditions, p = 0.067. There was a significant decrease in inactive lever contacts compared to active lever contacts for each test condition, # p < 0.001.

DBS in the CeA seemed to decrease the motivation to work for and obtain a food reward. During DBS, rats contacted the active lever less frequently and took a much

longer time to reach the FR 5 and FR 10 schedules compared to when they were not being stimulated. In many instances, the rats did not even reach FR 10 with stimulation. Rats took less than a minute to reach FR 5 and less than two minutes to reach FR 10 during no stimulation. During 20 Hz stimulation, they took over 12 minutes to reach FR 5 and over 23 minutes to reach FR 10. During 130 Hz stimulation, they took over 8 minutes to reach FR 5 and over 20 minutes to reach FR 10. See Table 3.1 for data. Rats significantly took a longer time to reach FR 5 during 20 Hz stimulation compared to no stimulation [one-way RM ANOVA; $F(2, 20) = 4.533$, $p = 0.034$]. Rats also significantly took a longer time to reach FR 10 (when they did) with 20 Hz and 130 Hz stimulation compared to no stimulation [one-way RM ANVOA; $F(2, 20) = 11.836$, $p = 0.001$].

Rats with stimulation sites outside the boundaries of CeA i.e., control rats, were still highly motivated to deliver sucrose pellets. They took less than 1 minute to reach FR 5 for all conditions. A one-way RM ANOVA on the time it took to reach FR 5 showed no significant difference between conditions [$F(2, 11) = 0.628$, $p = 0.565$]. These rats took less than two minutes to reach FR 10 again for all conditions [one-way RM ANOVA, $F(2, 11) = 1.251$, $p = 0.352$], see Table 3.1.

Table 3.1 - Average time to reach FR 5 and FR 10 schedules. Numbers reported in mean ± standard error

| Group | Time to Fixed Ratio (minutes) | No Stimulation | 20 Hz | 130 Hz |
|---|---|---|---|---|
| **CeA** | Time to FR 5 | 0.63 ± 0.11 | 12.34 ± 4.12 | 8.38 ± 2.95 |
| | Time to FR 10 | 1.29 ± 0.14 | 23.95 ± 3.34 | 20.44 ± 4.25 |
| **Surgical Controls** | Time to FR 5 | 0.61 ± 0.10 | 0.75 ± 0.11 | 0.75 ± 0.25 |
| | Time to FR 10 | 1.31 ± 0.24 | 1.62 ± 0.18 | 1.54 ± 0.29 |

*DBS in the CeA also produced spontaneous aversive taste reactions*

While observing the animals during the experiments, we noticed that occasionally, CeA rats periodically produced gapes throughout the time during stimulation. This was noted even during the habituation period before the levers or sucrose pellets were present. There were no gapes produced in sessions when stimulation was not present or for rats whose electrodes were in the control sites. Frame-by-frame

video analysis of the last 5 minutes of the habitation period for each test session revealed that on average rats with electrodes implanted in the CeA did 2.85 ± 1.14 gapes/minute during 20 Hz stimulation and almost doubled their aversive reactions during 130 Hz stimulation to 4.50 ± 1.38 gapes/minute. There was a significant difference in number of gapes/minute between no stimulation and 130 Hz stimulation [one way RM ANOVA, $F(2, 20) = 7.023$, $p = 0.010$]. *Post hoc* analysis showed a trend toward a difference between no stimulation and 20 Hz.

Gapes were also observed periodically with stimulation during the task itself. In general, gapes were not observed when there was no stimulation with the exception of one rat. Average gapes/minute was 0.083 ± 0.083 with no stimulation, 0.76 ± 0.42 with 20 Hz stimulation, and 1.04 ± 0.27 with 130 Hz stimulation. A one-way RM ANOVA on ranks (Friedman's test) was performed and revealed that the data just failed to show significance between stimulation and no stimulation [$\chi^2(2) = 6.000$, $p = 0.051$]. Rats stimulated in the control sites did not produce any gapes.

*General exploratory behavior also diminished with stimulation*

Locomotor activity in the form of chamber crosses and rearing was also analyzed. The average number of chamber crosses during the last five minutes of habituation did decrease significantly with stimulation [one way RM ANOVA, $F(2, 20) = 6.496$, $p = 0.012$] for rats implanted in the CeA. *Post hoc* analysis using the Holm-Sidak test showed that there was significant decrease in chamber crosses during both 20 Hz and 130 Hz stimulation compared to no stimulation (figure 3.8a). Again, there was no frequency effect. Rats in the control sites did not show a significant difference in cage-crossing behavior during the last five minutes of habituation (figure 3.8b); one-way repeated measures ANOVA; $F(2,11) = 1.776$, $p = 0.248$) demonstrating that stimulation in general is not causing a motor impairment.

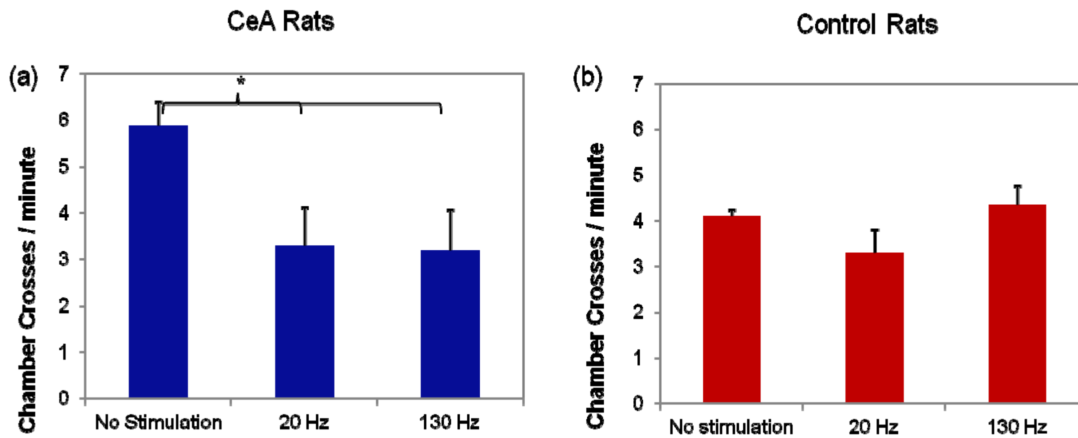


Figure 3.8 – DBS in the CeA decreases chamber crosses for rats. (a - b) Average number of chamber crosses per minute for no stimulation, 20 Hz stimulation, and 130 Hz stimulation. The chamber was divided in four quadrants and a cross was counted anytime the rat crossed from one quadrant to the other. Error bars represent standard error (+SEM). (a) For CeA rats (n = 7) stimulation was significantly different compared to no stimulation,* p = 0.012. (b) There was no difference in chamber crosses between conditions for control rats (n = 4), p = 0.248.

During the habituation period, the number of rears per minute also decreased significantly with stimulation compared to no stimulation [one way RM ANOVA, $F(2, 20) = 8.53$, $p = 0.005$] for rats implanted in the CeA (figure 3.9a). Holm-Sidak *post hoc* tests showed that both stimulation frequencies were significantly different from no stimulation and that there was no frequency effect. When comparing rearing behavior between no stimulation and stimulation conditions during the actual operant responding task, a one-way repeated measures ANOVA showed no significant differences [$F(2, 20) = 1.35$, $p = 0.296$]. Figure 3.9b shows the rears per minute during the operant responding task for rats implanted in the CeA. There was no significant difference either during the habituation period or during the FR task itself for the controls. (Figure 3.9c; one-way RM ANOVA; $F(2,11) = 3.592$, $p = 0.094$; figure 3.9d; one-way RM ANOVA $F(2,11) = 4.500$, $p = 0.064$).

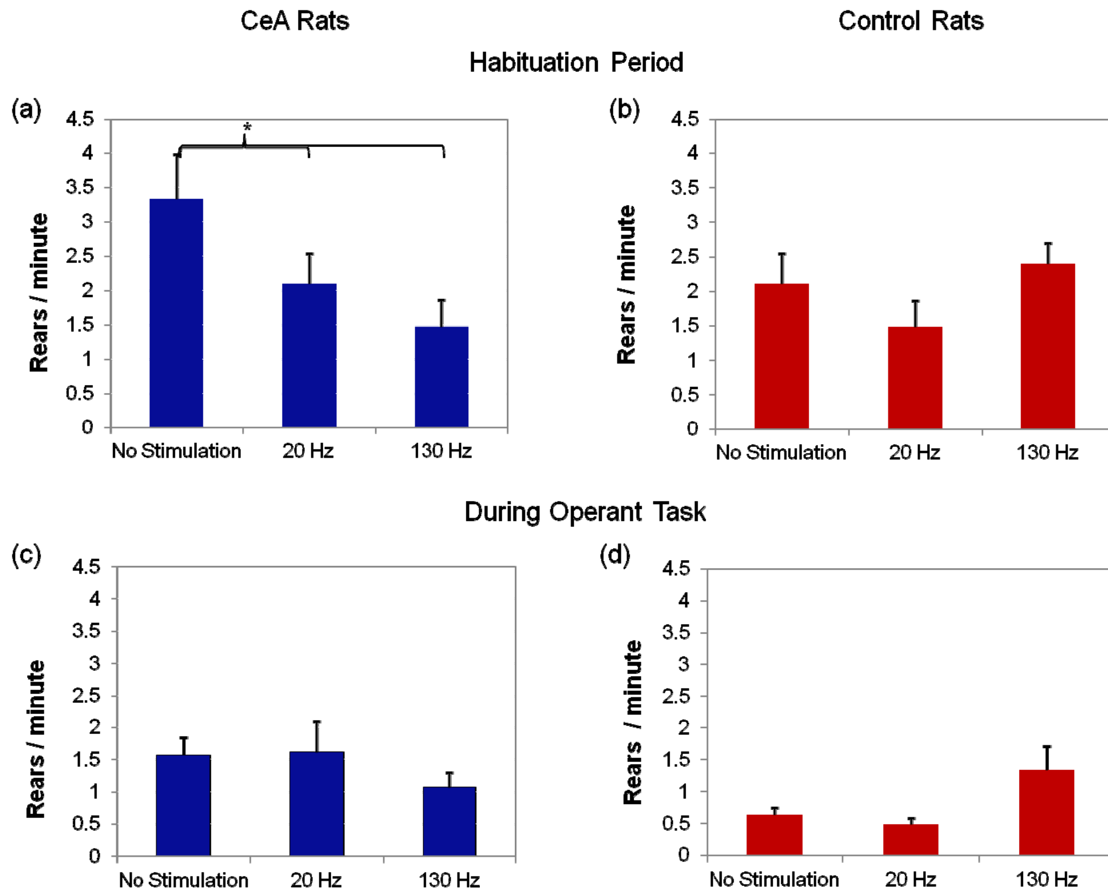


Figure 3.9 - DBS in the CeA slightly decreases rearing behavior. (a - b) Average number of rears per minute during the last five minutes of habituation for no stimulation, 20 Hz stimulation, and 130 Hz stimulation. Error bars represent standard error (+SEM). (a) Stimulation was significantly different compared to no stimulation for rats implanted in the CeA (n = 7), * p = 0.005. (b) There was no significant difference between stimulation and no stimulation for control rats, p = 0.094. (c - d) Same as (a - b) but for rears counted during the operant task. (c) There was no difference due to stimulation for rats implanted in the CeA (n = 7), p = 0.296. (d) There was no significant difference between stimulation and no stimulation for control rats, p = 0.064.

Though there was decreased locomotor activity in rats receiving stimulation in the CeA, general observations of the rats' behavior during testing showed that animals were not simply frozen in place. Animals would move around the chamber periodically and/or sit and move their head back and forth as if surveying the area. Some would also engage in the task periodically. Animals also groomed during stimulation, though overall there was very little grooming observed with or without stimulation.

From the frequency sweep pilot study, stimulation at 16 Hz also resulted in decreased pellet consumption and the values were comparable to 20 Hz and 130 Hz

stimulation. Stimulation at 10 Hz and lower frequencies yielded the same results as no stimulation. Rats were also tested again at 20 Hz stimulation to verify that any changes seen (or not seen) with these lower frequencies was not just due to loss of effect of stimulation. So this brief frequency sweep suggests that somewhere between 10 and 16 Hz, stimulation is producing a "blocking" effect and decreasing food consumption. Also lower frequencies (at least as low as 2 Hz) did not show an "activating" effect and did not increase pellet consumption above no stimulation conditions.

*Neural overview*

Stimulation artifacts were successfully removed from neural data recorded during stimulation test sessions using our artifact removal technique described in detail in appendix A.2. Figure 3.10 shows examples of the raw recorded data with the stimulation artifact and the same region after the stimulation artifact has been removed for 20 Hz and 130 Hz stimulation sessions, respectively. Traces show the units are still firing in between the stimulation pulses. Given that in this study, biphasic stimulation with a pulse width of 100 μs per phase was used, the average dead time for each artifact was 2 ms ± 0.11 ms (± standard error). Hence, total dead time due to artifact comprises approximately 4% of total recording time during 20 Hz stimulation and 26% of the recording time during 130 Hz stimulation.

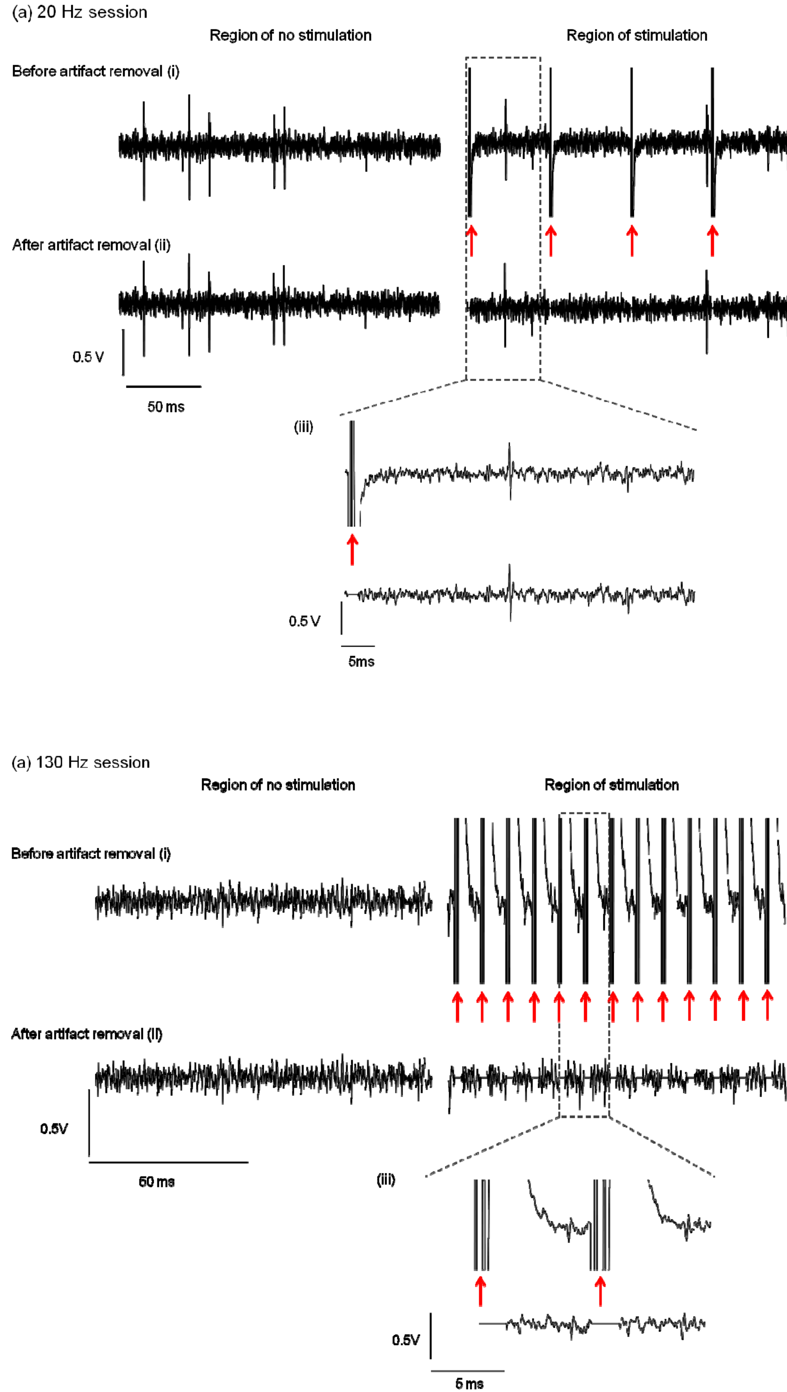


Figure 3.10 – Examples of recorded neural traces from the CeA before and after stimulation artifacts have been removed. Red arrows indicate the stimulation artifacts. (a) Two hundred milliseconds snippets of recorded neural activity from one electrode channel during a period of no stimulation and 20 Hz stimulation before (i) and after (ii) the artifact has been removed. (iii) Zoomed-in portion of the stimulation trace showing an artifact and two units before and after the artifact has been removed. This time-expanded region is indicated by a dotted box. (b) (i) and (ii) Similar to (a) but for 130 Hz stimulation session and each sample trace is 100 ms long. (iii) Zoom-in on region indicated by a dotted box showing two artifacts and the spikes between.

A total of 15 units were recorded from a total of 12 sessions and four rats. Cross-correlation analysis ensured that activity was unitary in nature and not an accident of recording a single unit on two nearby electrodes (Moore et al., 1966). Specifically, if the cross-correlation between two units showed a large peak at time 0, then the unit with the better signal to noise ratio in Offline Sorter was kept and the other unit was discarded from further analysis. There were 8 units from the no stimulation tests, 5 units from 20 Hz stimulation tests, and 2 units from 130 Hz stimulation tests. Overall all 15 units (100%) were responsive either to the pellet delivery (feederclick), operant response (active lever touch), or to the stimulation itself (Table 3.2) and were used in subsequent analyses. The average firing rate of all units in the first five minutes of habitation (baseline period) was 8.18 ± 2.81 spikes/s. The majority of units had basal firing rates of 5 Hz or less which is similar to what is reported in the literature (Collins & Paré, 1999; Duvarci et al., 2011; Rosenkranz & Grace, 1999; Shabel & Janak, 2009), though 4 units had firing rates of over 10 Hz.

Table 3.2 – The number of responsive CeA units (numerator) relative to total number of CeA units (denominator) broken down by test condition and event

| | **Feederclick** | **Pre Lever Response** | **Post Lever Response** | **Stimulation** |
|---|---|---|---|---|
| **No Stim Units** | 8/8 | 7/8 | 8/8 | N/A |
| **LFS Units** | 1/5 | 1/5 | 0/5 | 5/5 |
| **HFS Units** | 1/2 | 0/2 | 0/2 | 2/2 |
| **Total Units** | 10/15 | 8/15 | 8/15 | 7/7 (just counting LFS & HFS units) |

*Stimulation produced complex firing patterns*

Stimulation at either 20 Hz or 130 Hz did not significantly alter mean firing rates. A one-way ANOVA on the change in firing rates during the later five minutes of habituation when stimulation was on compared to first five minutes of habituation when there was no stimulation showed no significant effect of stimulation [$F(2,14) = 1.6$, $p$ =

0.242]. Table 3.3 shows the basal firing rates for no stimulation, LFS, and HFS units during the first 5 minutes of habituation and the change in firing rates during the later five minutes of habituation. Interestingly, though the results are not significant as mentioned above, the majority of units had a small decrease in firing rate during stimulation. However, with only 15 total units, the statistical power is limited for discerning differences. For the no stimulation units, there was never any stimulation turned on, but for these units, the two time periods were still compared to ensure that any changes during the second half of the habituation period were not just due to drifts in neuronal firing rates over time. Note that these comparisons corrected for the time lost due to artifact removal.

Table 3.3 – Average firing rates of CeA units during stimulation and no stimulation baseline periods.

| **Type of Unit** | **Baseline Rate (spikes/s)$^{+}$** | **Overall Change from Baseline$^{++}$** | **Decrease from Baseline (n units)** | **Increase from Baseline (n units)** |
|---|---|---|---|---|
| **20 Hz Units (n = 5)** | 5.11 ± 1.13 | - 2.26 ± 0.89 | - 2.26 ± 0.89 (n = 5) | none |
| **130 Hz Units (n = 2)** | 10.38 ± 0.375 | - 5.23 ± 4.11 | - 9.35 (n = 1) | + 1.12 (n = 1) |
| **No Stim Units$^{+++}$ (n = 8)** | 9.55 ± 5.28 | + 1.50 ± 0.78 | - 0.55 ± 0.52 (n = 2) | + 1.82 ± 1.02 (n = 6) |

$^{+}$This is the first 5 minutes of habituation, no stimulation delivered.
$^{++}$ Change in firing rate in last 5 minutes of habituation when stimulation was on.
$^{+++}$For no stimulation units there was never any stimulation on, just comparing the first half of the habituation period with the second half of the habituation period.

Though overall average rates of firing changed little, peristimulus time histograms (PSTHs) plots revealed clear stimulation-induced complex patterns of inhibitions and excitations within a unit. Figure 3.11a and b show sample PSTHs plots for 20 Hz and 130 Hz stimulation, respectively during pre stimulation and stimulation periods.

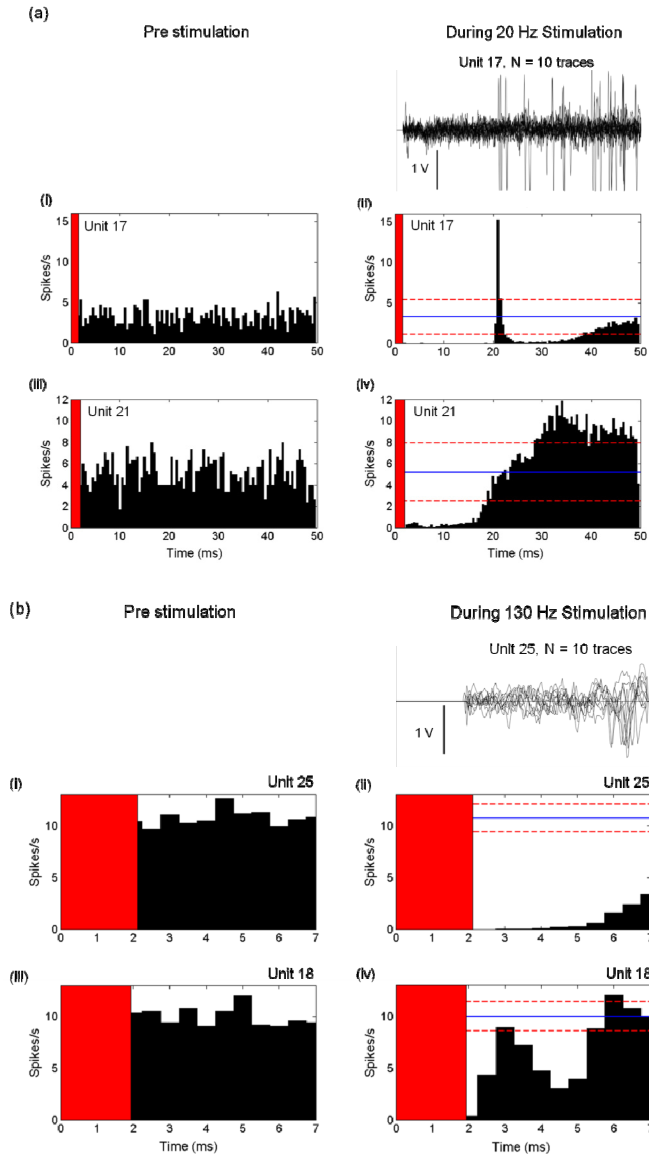


Figure 3.11 – CeA units show stimulation-induced patterns of firing. (a – b) Sample PSTHs plots normalized to spikes/s with bins of 0.5 ms and duration of 50 ms for 20 Hz stimulation and 7.6 ms for 130 Hz stimulation. The red box represents the duration of the artifact dead time where no assessment could be made of neural activity. In the control pre stimulation plots the red box only simulates the duration of the dead time. (a) PSTHs plots for 2 units recorded during 20 Hz stimulation sessions. Plot (i) is a control PSTH constructed during the first 5 minutes of habituation when there was no stimulation for a particular unit. Spikes are aligned at time = 0 to sham stimulation times occurring every 50 ms. Plot (ii) is the PSTH of the same unit during stimulation. Spikes are aligned to the onset of actual stimulation pulses. The horizontal blue line is the mean firing rate of the unit during the pre stimulation period and the dotted red lines represent ± 2 standard deviations from the mean. Above the histogram are 10 raw traces each lined up to a stimulation pulse overlaid on each other showing the spiking pattern. Plots (iii) and (iv) are the control and actual PSTH histograms for another unit. (b) PSTHs plots for 2 units recorded during 130 Hz stimulation. Explanation for the plots is similar to what was described for (a) except that these are units recorded during HFS and the time scale on the x-axis is different. The duration for each plot is 7.6 ms, the period between stimulation pulses at a frequency of 130 Hz. Note, unit 17 and 18 are from the same rat and unit 21 and 25 are from another rat.

Responses to 20 Hz stimulation typically showed a multiphasic response with a period of inhibition lasting 16 - 24 ms after stimulation onset, followed by mid-term excitation which was either very brief lasting for 1 – 2 ms or a more sustained excitation lasting for 6 – 10 ms, and then another period of inhibition lasting for 10 – 20 ms after which the unit's firing rate returned to basal values. Note that the first inhibitory period lasted long after the average dead time of 2 ms and the recoverable region of the artifact (3 to 5 ms long). This initial period of inhibition is not a function of the artifact as we are able to recover spikes during this period. All units showed at least one of the phases described above. Two out of the 5 units had the same response pattern as described above. One unit had a very similar pattern though its mid-term response never became an excitation but instead returned to basal rate following the first inhibitory period. Another unit had a more sustained mid-term excitation. Finally, the last unit only had a period of long inhibition followed by a sustained excitation that started about 24 ms from stimulation onset and remained till the start of the next pulse. With 130 Hz stimulation, one unit was inhibited throughout the period between stimulation pulses. The other unit also had a period of inhibition lasting 5 ms followed by a brief excitation of 1 ms. It should be emphasized that with 130 Hz stimulation even though the onset inhibition is similar to the 20 Hz stimulation period, the next stimulation pulse arrives before the neuron is able to recover to the extent it is allowed with slower 20 Hz stimulation.

Variations in unit responses, for example unit 17 versus 21 in figure 3.11a, could be due to difference in channel membrane properties of the different units, difference in stimulation amplitude and thus current spread, or even location of both stimulating and recording electrodes (Bar-Gad et al., 2004; Cleary et al., 2013; M. D. Johnson & McIntyre, 2008). Units 17 and 21 are from two different rats, with slightly different locations in the CeA and receiving different amplitudes. Unit 17 was recorded from a rat receiving 350 μA stimulation in the CeA. Unit 21 was from a rat receiving 250 μA stimulation and whose electrode was located more medial in the CeA closer to the boundary between CeA and the medial anterior dorsal nucleus of the amygdala. Amplitude has been shown to have an effect on the extent of time-locking of neural firing to stimulation. Johnson and colleagues reported greater time locking of globus pallidus

internus (GPi) units to higher amplitude local stimulation also in the GPi (M. D. Johnson & McIntyre, 2008).

For each unit (figure 3.11), control PSTHs plots were also created showing neural responses to sham artifact times during the first five minutes of the session when there was no stimulation. The plots show a flat histogram response indicating that the likelihood of a spike firing was independent of simply lining up spike activity at periodic intervals (Hashimoto et al., 2003; Maltete et al., 2007; McCairn & Turner, 2009).

*CeA units under no stimulation conditions responded to food delivery, lever contacts*

All the CeA units recorded in the no stimulation test sessions (8 out of 8 units) responded to the delivery of the food reward compared to a 5 s baseline period. Eighty percent of those units showed inhibitions to pellet delivery, while 1 unit showed an excitation and another unit had a period of inhibition in the first 500 ms after pellet delivery followed by period of excitation (see figure 3.12a for sample perievent time histograms and rasters for individual units). The majority of the units responded within the first 500 ms of pellet delivery. One unit had a delayed inhibition starting 500 ms after pellet delivery. Ninety percent of the units that responded to pellet delivery also showed inhibition 500 ms before the first active lever contact in a given trial. This pre-lever response could represent an anticipation to or preparation for making a lever contact. In addition, all units (8 out of 8) had inhibitions during 500 ms period after lever contact. Table 3.4 shows the breakdown of all the different responses over the different periods analyzed.

Responses to the first inactive lever contact compared to 5 s baseline period were also determined. There were only 4 units that had enough trials (greater than 5) to determine responses to the inactive lever contacts. Of the 4 units, 2 out of 4 did show inhibitions during the 500 ms window just before inactive lever response relative to baseline and 4 out of 4 units showed inhibitions during 500 ms post inactive response relative to baseline.

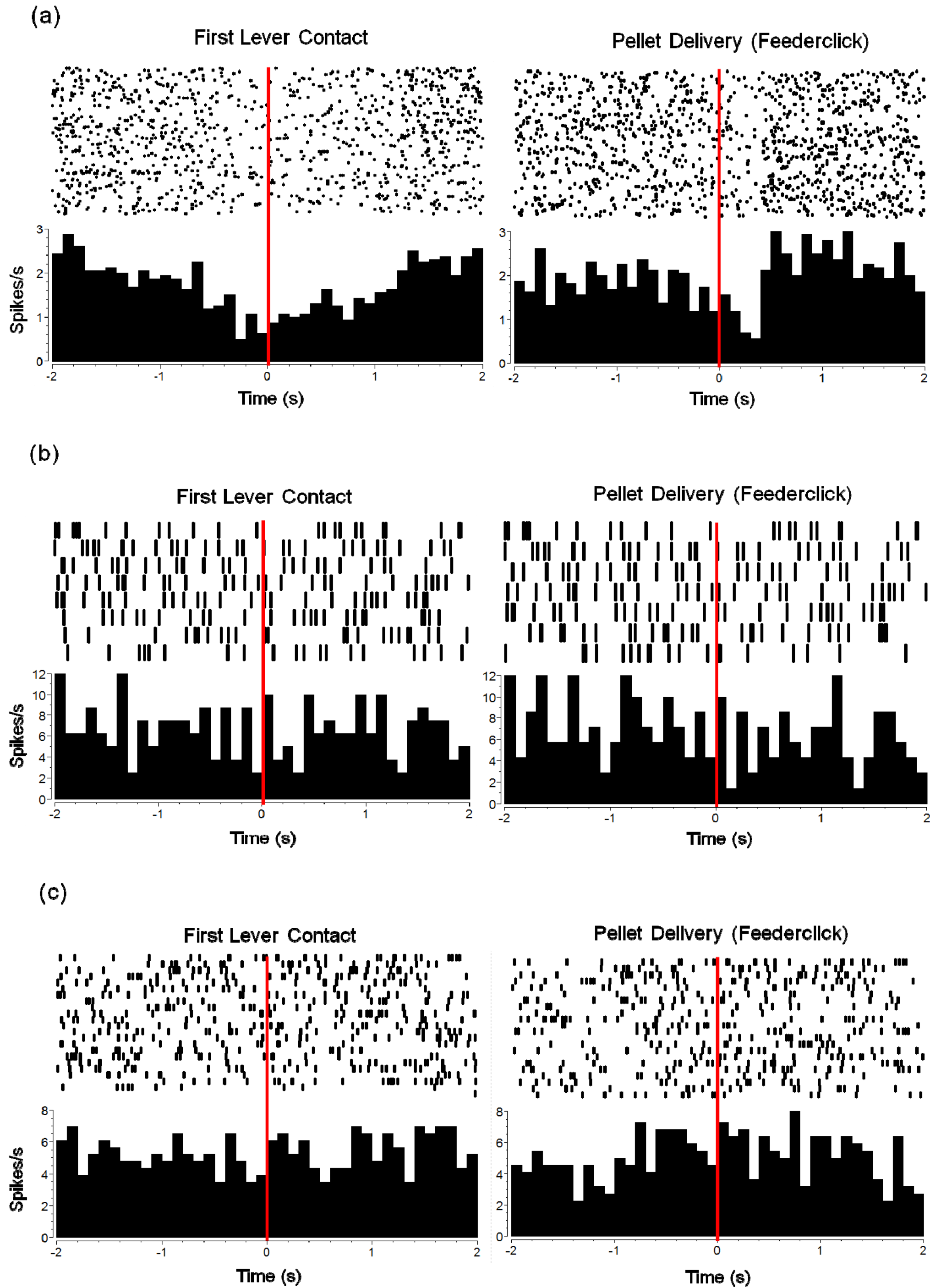


Figure 3.12 - Representative PETHs and raster plots for (a) a no stimulation CeA unit (b) a LFS CeA unit and (c) a HFS CeA unit. Plots on the left show responses to the first active lever contact for each trial and plots on the right show responses to the pellet delivery. Plots show unit activity up to 2 s before and after the event of interest which is centered at 0. The red line indicates when the event occurred. 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.

In general with the stimulation on, rats made very few lever contacts (or none at all) in the task. Thus, there were not enough trials to measure responses to task events for three out of the five units during LFS testing even though the units were responsive to the stimulation itself. Of the other two LFS units, one unit showed an excitation to the pellet delivery (lasting up to 1 s after the event) and to the 500 ms period just before the first lever contact in a trial. The second unit showed no response to either the pellet delivery or lever contacts. Inactive levers, which were rarely contacted even without stimulation, never had adequate numbers to assess responses. For the two HFS units, one unit showed an inhibition to the pellet delivery. Neither unit responded to lever contacts. See figure 3.12b and c for sample PETH plots for LFS and HFS units, respectively and table 3.4 for breakdown of unit responses. None of the units had enough trials to determine if any neurons were responsive to inactive lever contacts.

Table 3.4 – Breakdown of all CeA unit responses, all periods for pellet delivery and active lever responses.

| Epoch Window | Neural Response+ | No Stim Units (n = 8) | LFS Units (n = 5) | HFS Units (n = 2) |
|---|---|---|---|---|
| | | n (% total) | n (% total) | n (% total) |
| **Pellet Delivery (Feederclick):** | | | | |
| 0 - 0.5 s post feederclick | excitation | 1 (12.5%) | 1 (20%) | 0 (0%) |
| 0 - 0.5 s post feederclick | inhibition | 6 (75%) | 0 (0%) | 1 (50%) |
| 0.5 – 1 s post feederclick | excitation | 1 (12.5%) | 1 (20%) | 0 (0%) |
| 0.5 – 1 s post feederclick | inhibition | 6 (75%) | 0 (0%) | 0 (0%) |
| 1 – 1.5 s post feederclick | excitation | 1 (12.5%) | 0 (0%) | 0 (0%) |
| 1 – 1.5 s post feederclick | inhibition | 6 (75%) | 1 (20%) | 1 (50%) |
| 0.5 – 1.5 s post feederclick | excitation | 2 (25%) | 0 (0%) | 0 (0%) |
| 0.5 – 1.5 s post feederclick | inhibition | 6 (75%) | 0 (0%) | 0 (0%) |
| **First Lever Contact:** | | | | |
| 0 - 0.5 s pre lever | excitation | 0 (0%) | 1 (20%) | 0 (0%) |
| 0 - 0.5 s pre lever | inhibition | 7 (87.5%) | 0 (0%) | 0 (0%) |
| 0 - 0.5 s post lever | excitation | 0 (0%) | 0 (0%) | 0 (0%) |
| 0 - 0.5 s post lever | inhibition | 8 (100%) | 0 (0%) | 0 (0%) |

+Responses compared to 5 s baseline Mann Whintey U Tests, $p < 0.05$ as responsive unit

*Population unit responses and changes in firing rates*

For population responses, units had very few responses to reward-related events (see figure 3.13) during periods of stimulation compared to periods of no stimulation.

Specifically, there was a significant effect of stimulation frequency on unit responses to pellet delivery and lever contacts depending on the period of interest. A two way ANOVA comparing baseline to the 1 s to 1.5 s epoch after pellet delivery showed a significant effect of frequency with firing rates lower during 130 Hz compared to 20 Hz [$F(2, 23) = 4.009$, $p = 0.036$].

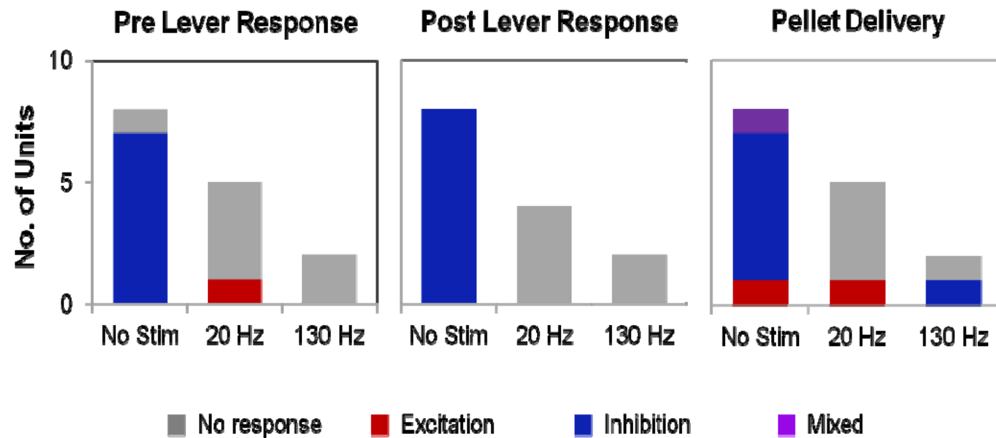


Figure 3.13 - Fewer CeA unit responses during stimulation to active lever contacts and pellet delivery. For each population (no stimulation, LFS, and HFS) the distribution of units that show no response (grey bars), inhibitions (blue bars), excitations (red bars), or mixed responses (excitation and inhibitions – purple bars) are plotted.

There was also a significant effect of frequency and interaction between epoch and frequency for the pre-lever response [two-way ANOVA; $F(2, 23) = 7.885$, $p = 0.003$; $F(2, 23) = 7.238$, $p = 0.005$; respectively]. *Post hoc* tests revealed that overall firing rates during 20 Hz was greater compared to that of both 0 Hz and 130 Hz ($p = 0.001$, $p = 0.004$, respectively). Specifically, within the 500 ms period just before the first lever contact, firing rates during 20 Hz stimulation was greater compared to both 0 Hz and 130 Hz ($p < 0.001$, $p = 0.003$, respectively). Also compared to baseline, there was a significant decrease in firing rate during the 500 ms pre lever epoch for both 0 Hz and 20 Hz ($p = 0.022$, $p = 0.011$, respectively).

There was also a significant effect of frequency and interaction between epoch and frequency for responses following the first lever contact [two-way ANOVA; $F(2, 23) = 9.123$, $p = 0.002$; $F(2, 23) = 6.318$, $p = 0.008$; respectively]. Holm-Sidak *post hoc* tests again revealed a significant difference between 0 Hz and 20 Hz ($p = 0.002$) and between 20 Hz and 130 Hz ($p <0.001$). Firing rates during the 500 ms period following lever

contact were significantly greater during 20 Hz stimulation compared to 0 Hz ($p$ <0.001) and compared to 130 Hz ($p$ <0.001). For no stimulation, there was a significant decrease in firing rate in the 500 ms period following lever contact and baseline ($p$ = 0.003). Looking within 20 Hz, there was also a significant decrease in firing rate in the 500 ms period following lever contact and baseline ($p$ = 0.042).

In comparing pre-lever responses to post-lever responses for the different stimulation conditions, again there was an effect of frequency overall [two-way ANOVA; $F(2, 23) = 13.289$, $p < 0.001$]. Holm-Sidak *post hoc* tests revealed that overall firing rates during 20 Hz and 130 Hz stimulation were greater compared to during no stimulation ($p < 0.001$ and $p = 0.003$, respectively). Specifically, within the 500 ms pre lever contact period, firing rates were greater during 20 Hz stimulation compared to no stimulation ($p = 0.004$) and also greater during 130 Hz stimulation compared to no stimulation ($p = 0.006$). Also during the 500 ms post lever contact period, firing rates during 20 Hz stimulation was again greater than no stimulation ($p = 0.007$).

**3.4 Discussion**

Both 20 Hz and 130 Hz stimulation in the central nucleus of the amygdala (CeA) significantly decreased delivery and consumption of sucrose pellets by more than half. The effects of stimulation were reversible. Within a few seconds after the stimulation was turned off, rats immediately went to the food bowl, ate pellets there that had accumulated in the food dish (if any), and then proceeded to deliver and eat more pellets at around the same rate as they did in no stimulation test sessions; that is, responses after stimulation was turned off returned to normal.

Correct targeting is also very important with mapping studies from Experiment 1 revealing an effective region of ~0.4 $mm^3$ which was mostly in the medial aspect of the central nucleus of the amygdala (CeM). However, the lateral aspect of the central nucleus of the amygdala (CeL) cannot be completely ruled out as. In rats, at least, parts of the CeL is surrounded on the top and bottom by the CeM (Paxinos & Watson, 2007) and the boundaries between the two are difficult to distinguish using standard cresyl violet staining techniques that were employed for target verification. In addition, some electrodes were also located on the boundary between the CeM and the capsular

subdivision of the central nucleus of the amygdala or CeC. Nevertheless, the effective site is small and focused primarily in the medial aspect of the central nucleus of the amygdala. More extensive mapping studies and current spread measurements are needed to better strictly define the boundaries of the effective zone within the CeA. Other regions near to and surrounding the CeA were tested and stimulation in control sites did not have any significant effects. These control regions included: the ventral pallidum, medial anterior dorsal region of the amygdala, amygdaloid area, extended amygdala and basal medial amygdala, and basal lateral amygdala. A temporary decrease in consumption was seen in one rat whose electrode was implanted on one side in the central nucleus and in the basal lateral amygdala on the other side during experiment 1, but when the rat was tested on longer 30 minute test sessions, stimulation no longer had an effect on lever-pressing behavior or consumption. The basal lateral amygdala has been proposed to be another possible area for targeting for DBS and reward (Baxter & Murray, 2002; Fontanini, Grossman, Figueroa, & Katz, 2009; Rolls & Rolls, 1973; M. Will et al., 2004); however, at least in our study, the rat's behavior suggested more of a fear response as the animal remained frozen in the corner (farthest away from his active lever and the food dish) unlike our other animals stimulated bilaterally in the CeA and after a few days of testing, the animal performed the task similarly with and without stimulation. Hence, more testing would be needed to determine if the BLA could also be a viable area as well.

Stimulation also produced complex firing patterns in the majority of units (86 %, n = 7 total) of the central nucleus of the amygdala, though overall causing a slight decrease in firing rates. This decrease is not simply a function of stimulation artifact but a real decrease compared to periods of no stimulation. These stimulation-induced patterns consisted of multiphasic responses of inhibitions and excitations to both 20 Hz and 130 Hz stimulation, though one unit only showed inhibition to 130 Hz stimulation.

As seen in figure 3.11 and discussed briefly in the results, there were some variations in these multiphasic responses across units. This could be due to differences in properties of the neurons themselves. Majority of units in the CeA are of one type – GABAergic medium spiny projection neuron (McDonald, 1982), but there could still be variations in channel membrane properties and in other physiological properties that

could explain these differences. A modeling study of GABAergic globus pallidus interna or GPi neurons revealed that the strength of the time-locked neural response observed with DBS in the GPi depended on relative conductance of $Na^+$ and $K^+$ channels (M. D. Johnson & McIntyre, 2008). Stimulation amplitude and current spread could also explain these variations. The same modeling study discussed above showed that higher voltages resulted in greater time-locked excitations whereas lower voltages produced a greater percentage of inhibitions or inhibitions with mild locking (M. D. Johnson & McIntyre, 2008). Recordings of human GPi neurons during GPi stimulation also support the effect of amplitude, though the findings in this study show that a greater number of units were entrained with high amplitude stimulation at distal sites or low voltage stimulation at local sites (Cleary et al., 2013). Differences in the results between these studies could be due to different methods - modeling versus *in vivo* recording - but both studies argue the contribution of amplitude on entrainment of neurons as well as others (Hashimoto et al., 2003). Exact location of the stimulating and recording electrode and their positions relative to one another could also influence these multiphasic patterns and the percentage of entrainment seen due to stimulation (Bar-Gad et al., 2004; Cleary et al., 2013; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007).

All units responded to stimulation, but there were much fewer responses to the reward-related behavioral events (operant responses and pellet delivery) in our study. This was not due to the fact that CeA units, in general, do not respond to these events as majority of units did in fact respond to either operant response or pellet delivery during no stimulation. Note though that we were only able to obtain a few units so any conclusions on DBS effects of neural activity in the CeA are preliminary. Also we recorded separate populations of units for 20 Hz, 130 Hz, and no stimulation (though from the same rats just on separate days). More testing is required to obtain a larger sample size of unit data in order to make any strong conclusions.

*Potential mechanisms of action of DBS on food consumption in CeA*

Our results show that DBS in the CeA decreases food consumption and reward-seeking behaviors such as approaches to the food dish and lever-pressing to deliver the reward. How could DBS be affecting the underlying circuit to cause this effect? Results

from the literature and some of our work on stimulation effects on neuronal firing may present some possible answers. The results from our study show that stimulation of the CeA produces a similar behavioral effect as seen with lesioning or inactivation of the CeA. Lesion or inactivation of the CeA has shown to decrease food consumption and reward-seeking behaviors (Galaverna et al., 1993; Kemble et al., 1979; Seeley et al., 1993; Touzani et al., 1997; M. J. Will et al., 2009). Hence, it is possible that DBS could be inactivating the CeA, in a sense creating a functional lesion of the structure.

Earlier work in DBS studies for the treatment of Parkinson's disease have reported similar therapeutic effects seen with lesions or inactivation of the subthalamic nucleus (STN), internal segment of the globus pallidus (GPi), and thalamus and DBS of these structures (Aziz et al., 1991; Bergman et al., 1990; Koller et al., 1997; Kumar et al., 1998; Wichmann et al., 1994). This has led to the idea that DBS is inhibiting the stimulated structure resulting in a decrease in the structure's output. DBS could be shutting down the neurons perhaps by creating a depolarization blockade thus preventing the neurons from firing (Benazzouz et al., 1995; Beurrier et al., 2001; Bikson et al., 2001) or depleting neurotransmitter at the synapse (Urbano et al., 2002). Neural recordings have also shown inhibitions in substantia niagra (SNr), STN, and GPi during stimulation (Benazzouz et al., 1995; Boraud et al., 1996; Filali et al., 2004).

However, more recent evidence has suggested that DBS is not just shutting down neurons in the stimulated regions. Work from modeling studies and electrophysical recordings *in vitro* and *in vivo* have shown that neurons are still able to fire during DBS. Some of the studies that reported stimulation-induced inhibitions report that this could be due to activation of afferent projections resulting in the release of GABA and thus inhibiting target neurons (Dostrovsky et al., 2000; Lafreniere-Roula et al., 2010; McCracken & Grace, 2007; Vitek, 2002). We also observed unit activity during stimulation pulses in our study.

Other studies have suggested that there could be both inactivation of cell bodies but activation of axons (Anderson et al., 2006; Filali et al., 2004; Garcia et al., 2005a; Hashimoto et al., 2003; McIntyre et al., 2004). Cell bodies, though can also be active as well (Bar-Gad et al., 2004; Garcia et al., 2005b; M. D. Johnson & McIntyre, 2008). This has led to the proposal that stimulation is creating a "jamming" or interference of

information flow in the neural circuit. DBS could be imposing its own pattern in the circuit (Cleary et al., 2013; Garcia et al., 2005a; Garcia et al., 2005b; W. M. Grill et al., 2004; M. D. Johnson & McIntyre, 2008; McIntyre et al., 2004) possibly creating an "informational lesion" (W. M. Grill et al., 2004). Our neural data also suggests this may be happening. Our results show that stimulation in the CeA induced multiphasic patterns of firing (inhibitions and excitations). These patterns represent loose entrainment of unit activity to stimulation. These patterns are not observed in the control PSTHs during no stimulation periods. Furthermore, raw traces in figure 3.11 show that units were more likely to fire at specific latencies from stimulation pulse onset consistent across stimulation pulses for each unit, suggesting a form of entrainment. These types of patterns has been reported in neurons of GPi, globus pallidus externa (GPe), SNr, and STN in both human and animal studies (Bar-Gad et al., 2004; Cleary et al., 2013; Hashimoto et al., 2003; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007; McCairn & Turner, 2009). 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; M. D. Johnson & McIntyre, 2008) and also at distal targets (Hashimoto et al., 2003; Maltete et al., 2007; McCairn & Turner, 2009).

Studies argue that by causing this loose entrainment of neural firing, stimulation could be imposing its own information which may no longer be meaningful to the underlying circuit (Cleary et al., 2013; Garcia et al., 2005a; Garcia et al., 2005b; W. M. Grill et al., 2004; M. D. Johnson & McIntyre, 2008; McIntyre et al., 2004) and thus disrupting information flow in the circuit whatever that information may be – normal or pathological (in case of Parkinson's disease). Therefore, we were interested in seeing if normal coding for reward-related events in the CeA were affected with DBS. This seems to be the case. There were fewer responses to pellet delivery and lever contacts during stimulation; whereas, in the absence of stimulation, all units responded to one or both of these events. Thus, stimulation does seem to be disrupting neural coding for reward-related events. With very few units, it is difficult to make any strong conclusions and more units would need to be analyzed to determine if this is indeed the case.

This stimulation-induced disruption could be due to the activation and/or inhibition of inputs and outputs of the CeA. The CeA consists of many subdivisions

including lateral and medial aspects (CeL and CeM, respectively). Neurons in these sub regions are mostly GABAergic (Sah et al., 2003; Swanson & Petrovich, 1998) and the CeL projects to the CeM resulting in a feed-forward inhibition. Hence if stimulating CeM could antidromically activate CeL GABAergic neurons, this could result in an evoked release of GABA causing the initial inhibition of CeM neurons. There are also direct glutamergic projections from the basolateral amygdala (BLA) to the CeM. Increased firing also seen in the CeM could be due to activation of axons from the BLA causing increased firing of the CeM. The CeM outputs to the lateral hypothalamus (LH) and other brainstem regions included ventral tegmental area (VTA), which sends dopaminergic neurons to the nucleus accumbens. This change in pattern of firing in CeM could disrupt responses in the LH and VTA resulting in decreased motivation to obtain and consume food rewards.

*LFS at 20 Hz as effective as HFS at 130 Hz*

We initially hypothesized that high frequency stimulation of 130 Hz would have a "blocking" effect, but low frequency stimulation of 20 Hz would have an activating effect or no effect on information flow in the circuit and thus behavior. Previous studies have shown that there is a frequency effect with therapeutic DBS for Parkinson's disease, essential tremor, and dyskinesia being effective at frequencies greater than 90 Hz and lower frequencies (< 50 Hz) actually having no therapeutic effect or even worsening symptoms such as increased tremor activation (Garcia et al., 2003; Garcia et al., 2005a; Kuncel et al., 2007; Moro et al., 2002; Rizzone et al., 2001; Wu et al., 2001). Modeling studies have also shown that frequencies < 100 Hz can superimpose patterns of firing that is more irregular. Increased irregularity of firing has been shown to actual worsen symptoms of Parkinson's disease and tremors (W. M. Grill et al., 2004; Kuncel et al., 2007).

In Chapter 2 of this dissertation, the results of the 20 Hz stimulation supported our hypothesis (stated at the beginning of the above paragraph); but, in this current study both 20 Hz and 130 Hz stimulation resulted in decreased consumption. CeA units also have very low firing rates typically less than 5 Hz (Collins & Paré, 1999; Duvarci et al., 2011; Rosenkranz & Grace, 1999; Shabel & Janak, 2009). Hence, for this structure, 20 Hz

might still be a "high frequency" overwhelming the very low intrinsic frequency of CeA neurons. It has been reported that frequency effects depend on the type of structure being stimulated (Dostrovsky & Lozano, 2002). Also, from modeling studies, the critical frequency above which a "blocking effect" of DBS is seen is the intrinsic frequency of the neuron (W. M. Grill et al., 2004). Therefore, we did a small study looking at pellet consumption at frequencies lower than 20 Hz. There was no effect of DBS at frequencies 10 Hz and lower; hence, perhaps somewhere between 10 Hz and 16 Hz is this critical frequency above which the stimulation frequency is "fast" enough to overwhelm the circuit, and below which normal neural responses to reward events can pass through.

*Is this a decrease in "wanting" only?*

Our results showed that DBS decreased food consumption and the number of operant responses on the active lever. Furthermore, the rats showed fewer approaches to the food dish. However, is this decrease in consumption only due to a decrease in motivation to seek out and consume the food or also due to a change in palatability of the food. Some rats do put the pellets in their mouths and then spit them out which could suggest they still "want" the pellet but it is no longer palatable. Also, other rats remain by the food dish or the lever and even occasionally deliver more pellets. But there are individual differences and some rats do completely ignore the lever and food dish while stimulation is on. Histology did not reveal a difference in electrode locations that could account for these individual differences, but it is possible that differences in underlying tissue and current spread, etc. could result in different neuronal components being activated and thus the slight differences in behavior of the rats.

More interestingly, we found that rats would sometimes produce aversive gaping reactions similar to what is observed when they receive a bad taste in their mouths (K. C. Berridge, 1996; H. J. Grill & Berridge, 1985). Hence, the stimulation could be creating a general aversive state or leaving the impression of a bad taste in the mouth. Certainly there are mixed results on whether stimulation could be affecting taste. Mahler and colleagues have shown that opioid stimulation increases food consumption but decreased hedonic reactions to sucrose and has no effect on aversive reactions to quinine (Mahler &

Berridge, 2011). They have argued that the CeA could be a structure that codes "wanting" without "liking" (Richard et al., 2012).

Some studies have also shown that lesions of the CeA do not affect aversive responses to bad tastes nor taste aversion learning (Galaverna et al., 1993; Kemble et al., 1979; Seeley et al., 1993). In addition, CeA lesioned rats showed increased "liking" reactions to salt in a sodium-deplete state similar to intact rats, (Seeley et al., 1993). However, another study showed increased aversive reactions to quinine with CeA lesions (Touzani et al., 1997). Furthermore, CeA does have connections to taste pathways. It has reciprocal projections to the nucleus of the solitary tract and the parabrachial nucleus and has connections to the gustatory and insular cortex (Norgren, 1976; Sah et al., 2003; Swanson & Petrovich, 1998). All of this has prompted us to look at whether stimulation could indeed be making things unpalatable resulting in decrease consumption. This issue is further addressed in the fourth chapter of this thesis.

**3.5 Conclusion**

Our results demonstrate that the central nucleus of the amygdala could be a potential target for deep brain stimulation in the treatment of morbid obesity and addiction. Both 20 Hz and 130 Hz resulted in significant decreases in food consumption. There were stimulation induced changes in neural firing and stimulation also disrupted neural responses to behavioral events. For the CeA at least, it is possible that stimulation is "blocking" encoding of reward-related information which also translates in a decrease in food consumption and motivated behaviors. However, the data also suggests that this decrease in consumption could be due to a concomitant decrease in "liking" of the food (evidenced by the aversive reactions seen during stimulation). This has prompted the study in the subsequent chapter of this thesis (Chapter 4), which looks at the effects of DBS on the "liking" of rewards.

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# CHAPTER 4
# DEEP BRAIN STIMULATION IN THE CENTRAL NUCLEUS OF THE AMYGDALA DECREASES HEDONIC VALUE OF TASTES

## 4.1 Introduction

Deep brain stimulation (DBS) is being increasingly used to treat movement disorders such as Parkinson's disease, essential tremor, and dystonia (Gubellini et al., 2009; Schwalb & Hamani, 2008; Weaver et al., 2009). The mechanisms of DBS are still not well understood (Garcia et al., 2005a; Kringelbach et al., 2007; McIntyre et al., 2004), though growing evidence from modeling and experimental studies (in humans and animals) suggest that stimulation could be activating fibers and/or inhibiting cell bodies and imposing its own firing pattern on the circuit being stimulated (Garcia et al., 2003; Garcia et al., 2005b; Hashimoto et al., 2003; M. D. Johnson & McIntyre, 2008; M. D. Johnson et al., 2012; McIntyre & Grill, 2000). In doing so, stimulation could be disrupting the information being processed in the circuit, creating a form of "informational lesion" (W. M. Grill et al., 2004). Therapeutic benefits of DBS typical occur at stimulation frequencies greater than 100 Hz; however, the effects do depend on amplitude and frequency parameters as well as target location (Dostrovsky & Lozano, 2002; Kuncel & Grill, 2004).

Given its clinical efficacy in the treatment of Parkinson's and other motor disorders (Doshi, 2011; Voges et al., 2006), DBS is also being investigated as a potential treatment for other neurological and psychiatric disorders, including obsessive compulsive disorder, depression, epilepsy, and addiction (Chang, 2004; Greenberg et al., 2006; Gubellini et al., 2009; Haber & Brucker, 2009; Halpern et al., 2008; Lozano et al., 2008; Mayberg et al., 2005).

In investigating DBS for eating disorders and addictions, major questions still remain on the most effective target region, and stimulation parameters (Heldmann et al.,

2012; Lacan et al., 2008; Melega et al., 2012; Rouaud et al., 2010; Sani et al., 2007). Understanding the underlying causes of these disorders will help steer us towards the most effective target locations and parameters to use. For example, many studies on DBS for morbid obesity have targeted the lateral hypothalamus or the ventromedial hypothalamus to either "block" feeling of hunger or trigger feelings of satiation, respectively (Lacan et al., 2008; Lehmkuhle et al., 2010; Melega et al., 2012; Sani et al., 2007). However, simply targeting homeostatic control of feeding might not be enough. Foods, especially those rich in fats and sugars, are themselves very rewarding. Motivation to seek out and consume rewards beyond physiological need is thought to be a major factor in morbid obesity (K. C. Berridge et al., 2010). Hence, targeting excessive motivation to seek out and consume rewards might be more effective than simply targeting appetite (Halpern et al., 2008).

Reward can be broken down into two components: the hedonic impact of the reward or "liking" and the incentive salience or motivational magnet attributed to the reward or "wanting". Experimental manipulations have shown that these two systems are dissociable and maybe encode by different brain substrates (K. C. Berridge, 1996). Ideally, we would like to target areas involved in modulation of "wanting" without affecting "liking".

The central nucleus of the amygdala looks to be a good candidate for this. Evidence suggests that this region of the amygdala seems to be particularly involved in translating learning into motivation and specifically focusing motivation for particular cues and rewards (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009; Mahler & Berridge, 2011). The CeA is the major output nucleus of the amygdala and has many connections to other limbic regions including the lateral hypothalamus (Sah et al., 2003; Swanson & Petrovich, 1998).

Neurobiological manipulations of the CeA have suggested that this may be a region involved in "wanting" without "liking" (Richard et al., 2012). Opioid stimulation in the CeA has been shown to increase food consumption and reward-seeking behaviors (DiFeliceantonio & Berridge, 2012; Mahler & Berridge, 2009; Mahler & Berridge, 2011), but did not cause increased "liking" (Mahler & Berridge, 2011). Lesions or muscimol inactivation of the CeA have shown to cause decreases in food consumption,

but did not affect “liking” or “disliking reactions in conditioned taste aversion or sodium depletion paradigms (Galaverna et al., 1993; Kemble et al., 1979; Li et al., 2012; Seeley et al., 1993).

In the previous study investigating the effects of DBS in the CeA on food consumption (Chapter 3 of this thesis), we observed that DBS produced orofacial movements typically associated with a bad taste. This finding suggests that DBS could also be affecting the palatability of the reward, causing it to become “disliked” and thus no longer appetizing to the animal. There is some evidence that supports this. CeA receives gustatory input from brainstem nuclei including the nucleus of the solitary tract, parabrachial nucleus and pons (Norgren, 1976; Ottersen & Benari, 1978; Sah et al., 2003), thalamus (Ottersen, 1982), and insular cortex (Norgren, 1976; Ottersen & Ben-Ari, 1979). Other studies have shown evidence that CeA neurons encode taste palatability with preferential encoding to aversive stimuli (Nishijo et al., 2000). Touzani et al reported that lesions of the CeA also increased aversive reactions to quinine (Touzani et al., 1997). Hence, we would like to further investigate the effect of palatability with CeA electrical stimulation by directly assessing tastes of different hedonic value (positive, negative, and neutral).

To test hedonic value in general, a taste reactivity test can be used. This type of test has been shown to be sensitive to the palatability of food and dissociable from sensory processes (H. J. Grill & Berridge, 1985). Tastes can be directly infused into the animal’s mouth and the facial and body responses to these tastes can be analyzed. Typically, characteristic reactions are done to a “liked” taste (positive reactions) and a “disliked” taste (aversive reactions). Positive hedonic responses include rhythmic midline tongue protrusions, lateral tongue protrusions and paw licking (K. C. Berridge, 2000). Aversive responses include gapes, headshakes, forelimb flails, face washing and chin rubs. In addition, neutral reactions can also occur. These include rhythmic mouth movements, passive dripping, locomotion, and grooming. These responses mostly occur to tastes that are neither really hedonic nor aversive, like water (K. C. Berridge, 2000). These responses have been shown to be uniform across different species including non-human primates and human infants (J. Steiner et al., 2001). Also, these behaviors have been shown to reflect affective properties of the tastes, rather than sensory properties (K.

C. Berridge, 2000). We utilized this taste reactivity paradigm to determine if DBS alters the affective value of rewards.

CeA may be a potential area of exploration for neuromodulation of food reward. However, the question remains whether this decrease is due to a decrease in “liking” of the food. To test this, rats were given intraoral infusions of a highly palatable taste (sucrose), a neutral taste (water), and an aversive, bitter taste (quinine) under conditions of 20 Hz stimulation, 130 Hz stimulation, and no stimulation. Our objective was to test the hypothesis that stimulation decreases the hedonic value of sucrose. We found that stimulation decreased the hedonic value of sucrose, but also increased aversive reactions to all three tastes (each taste having a different valence - hedonic, aversive, and neutral). These findings suggest that stimulating the CeA makes tastes more aversive in general.

## 4.2 Material and Methods

*Subjects*

Nine adult male Sprague-Dawley rats weighing 250 g – 400 g were used in this experiment. Animals were housed individually on a 9:30 AM to 7:30 PM reversed light/dark schedule ensuring that animals were being tested during their active period, since experiments were carried out during the day. Animals were provided with unrestricted access to standard rat chow and water in their home cages. All experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

*Apparatus*

All training and testing were conducted in a clear plastic test cylinder of diameter 25 cm which was placed inside of a 28 cm x 35 cm x 60 cm clear plastic chamber with a glass floor. The chamber was illuminated with white light from below. The use of white light provided better illumination of the rat’s mouth and tongue which was necessary for taste reactivity video scoring (see detailed description of behavioral analysis below). The top of the cylinder and chamber was open, allowing for plastic tubing connections from the oral cannulae to the syringe pump that delivered the tastes and also connections from

the electrode to the commutator via a headstage cable. See appendix A.3 for a schematic diagram of the chamber set-up.

Delivery of tastes and stimulation was controlled by a software program, MTASK (created in the Aldridge lab). Stimulation timing (frequency and pulse-width) was controlled by another program created in the Aldridge Lab using LabVIEW (National Instruments, Austin, TX). Stimulation was delivered using the A-M systems constant current digital stimulus isolator stimulator (A-M systems, Carlsborg, WA). Note, three of the rats were tested with the Medtronic Test Stimulator model 3625 (Medtronics, Minneapolis, MN) instead. Neural activity was recorded during the testing sessions using DataTask (also written in the Aldridge lab using LabVIEW (National Instruments, Austin, TX). Neural activity was amplified at a gain of 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, thus reducing lost recording time due to amplifier saturation. This also allowed us to record large amplitude signals with greater fidelity without exceeding the voltage limits (±10 V) on the data acquisition board. This increased fidelity facilitated stimulation artifact removal and spike recovery (see below). Sessions were recorded at 30 frames a second via a video camera placed underneath the glass floor. Timestamp clocks for the behavior control program, video recording, neural recording, and stimulation were all synchronized to enable subsequent analysis of neural activity related to task events, stimulation, and behavioral events obtained from video analysis or recorded in Mtask.

*Habituation and exposure to sucrose solutions*

Prior to surgery, rats were handled for 10-15 minutes and then given daily access to 20-ml 17 % sucrose solution in their home cage. The amount of liquid consumed was quantified for four days. Rats who did not consume at least 15 ml of sucrose solution by day 4 were not included in the study. In addition, for two days just before surgery, rats were also introduced to the chamber. They were placed in the chamber for 10 minutes to acclimatize to the experimental set-up. Rats then underwent oral cannulae and electrode implantation as described below under “Surgical procedures”.

*Surgical procedures*

Rats were weighed and anesthetized with ketamine (100mg/kg) and xylazine (10mg/kg) injected intraperitoneally. On each side of the mouth, an intraoral cannula was inserted in the mouth lateral to the first molar and exited the head near the skull screws. A stainless steel 19 G guide cannula was then attached to each intraoral cannula where it exited at the top of the head.

In the same surgery, rats were then implanted with a recording and stimulating electrode targeting the central nucleus of the amygdala (AP -2mm, ML ±3.5mm, DV 7.5-8.5mm) on each side of the brain. Electrodes were composed of two bundles of six wires; each individual bundle consisting of two 75 μm stainless steel stimulating wires, as well as four 50 μm tungsten recording wires. Each bundle could be lowered or raised independently. Rats were given six to seven days for recovery. Detailed surgical procedures can be found in (Tindell et al., 2004; Tindell et al., 2006).

*Taste reactivity training*

After surgical recovery, rats underwent three days of taste-infusion training. Animals were placed in the testing chamber which was illuminated from underneath with white light. Sessions consisted of a two-minute habituation period, followed by trials of intraoral infusions. Rats were tested in blocks of 10 trials of the same solution: 17 % sucrose solution, tap water, and 0.01% quinine solution. For each session, they were given all three tastes starting with a block of sucrose, then water, and then quinine (figure 4.1b). Quinine was always presented last as it left a lingering bitter taste in the mouth. Between blocks of taste, an extra infusion of distilled water was given in order to rinse the mouth between tastes. For each trial, 0.1 ml of the tastant was infused directly into the rat's mouth using a 3 ml syringe attached to a computer-controlled pump and connected to hollow tubing (PE-50 connected to a PE-10 delivery nozzle) that was attached to a single oral cannula. The taste was infused over a period of 1 s with a variable interval of 40 s to 60 s separating infusions (figure 4.1a).

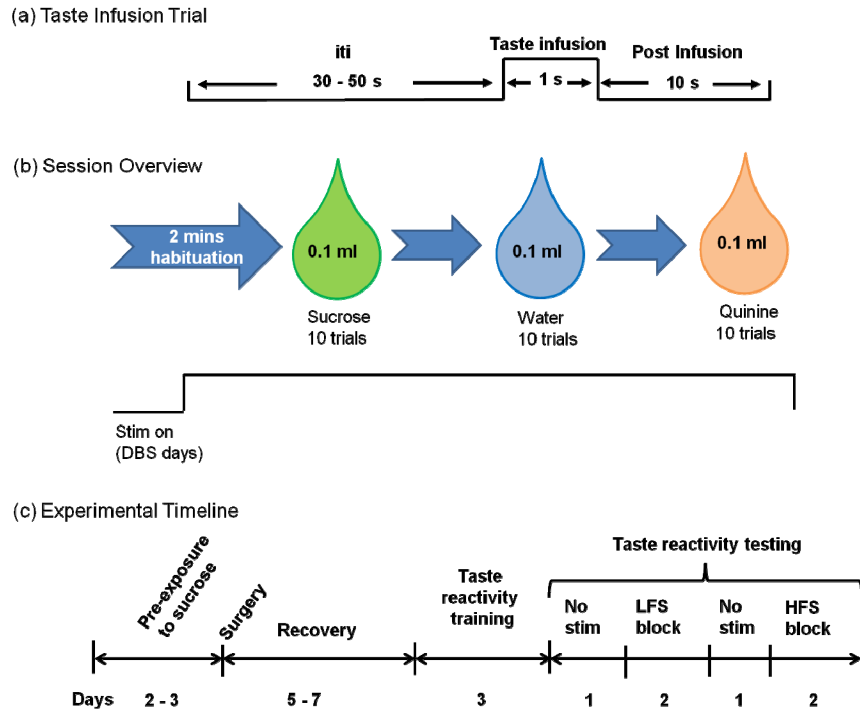



Figure 4.1 – (a) Schematic of a taste infusion trial showing timing of the different events (iti – inter-trial interval). (b) Experimental paradigm for a given test session showing the order of delivery of tastes. For stimulation test sessions, the stimulation was turned on 1 minute into the habituation period and remained on for the rest of the session. (c) Experimental timeline for the taste reactivity experiment (LFS – low frequency stimulation at 20 Hz, HFS – high frequency stimulation at 130 Hz).

*Screening*

After taste reactivity training but before testing, rats were first given a screening test to determine effective electrode depth and minimum effective current that produce the sporadic mouth movements and to verify that stimulation had no unwanted side effects (such as motor impairments). Rats were placed in the chamber and the current was turned on starting at 250 μA for 5 minutes. Their behavior was observed during stimulation. If stimulation had no desired effect or instead produced unwanted motor effects (e.g. paw twitching, turning), the electrode was lowered on both sides by 0.159 mm and the rat was tested again. This was repeated until stimulation produced gapes or other taste reactivity movements and no adverse motor effects, or if the electrode reached a depth of 8.5 mm below dura with still no observable effects of stimulation. Stimulation current began at 250 μA and, if the electrode reached approximately half-way through the supposed region of the CeA with no effect, current was increased in increments of 50 μA

until an effect was seen or current reached 400 μA. In some cases, when unwanted side effects were seen along with the desired effects, the current was turned down until the unwanted side effects were not observed.

*Taste reactivity testing*

Rats underwent six days of testing. Video recordings of taste reactions were made with the camera zoomed in and focused on the mouth and forelimb region to visualize and record facial and body reactions for video scoring analysis later. Testing conditions were similar to training conditions with the exception that neural activity was now being recorded and that rats received stimulation of four out of the six testing days. For stimulation sessions, the stimulation was turned on one minute into the habitation period and remained on till the end of the session. Stimulation consisted of either low frequency at 20 Hz or high frequency at 130 Hz. Rats were tested first with a no stimulation session, followed by two days of either high or low frequency, then another day of no stimulation, followed by two more days of the other frequency of stimulation. The order of stimulation was counter-balanced across rats with some rats receiving 130 Hz stimulation first, while the others received 20 Hz stimulation first. To further randomize the order of tests, with some rats, the second no stimulation test day was given after both blocks of stimulation were tested. Figure 4.1c shows the overall experimental timeline.

*Histology*

Anatomical localization of electrode sites was done after completion of testing. Rats were anesthetized with isoflurane gas and then a 0.1 mA lesioning DC current was passed for 10 s to mark the electrode location in the brain. After euthanizing the rats with a drug overdose of pentobarbital, the brains were removed, frozen in an isopentane and isopropyl alcohol solution, sliced into 40 μm sagittal sections CM 1850 cryostat (Leica Microsystems, Buffalo Grove, IL), and finally stained with cresyl violet. The electrode placement was confirmed by observing the brain slices under a light microscope.

*Stimulation parameters*

A continuous monopolar and biphasic current (180 - 250 μA), with a pulse width of 100 μs per phase, was delivered bilaterally into the target structure. Bilateral over unilateral stimulation was chosen as we were concerned that stimulating only one side may not be sufficient enough to see a behavioral effect and also to be consistent with the previous two studies (Chapters 2 and 3). For high frequency stimulation, a frequency of 130 Hz was used. For low frequency stimulation, 20 Hz was used. Each rat was tested at their minimum threshold current that caused an effect as determined in the screening test (described above). For the range of current from 180 μA to 250 μA, the charge density per phase delivered at the tip of the 75 μm stimulating wire was 101.9 $\mu C/cm^2/ph$ to 141.5 $\mu C/cm^2/ph$. For three of the rats, the Medtronic Test Stimulator model 3625 (Medtronics, Minneapolis, MN) was used instead with a range of amplitude of 3 - 5 V (~100 - 170 μA based on electrode impedances).

*Behavioral assessment and data analysis*

Taste reactions were scored offline using frame-by-frame video analysis and the Datarat scoring program (developed by the Aldridge lab). Three 10 s time periods within a trial were examined for five out of ten trials for each taste. Facial and body reactions to the taste were assessed in a 10 s period beginning at the onset of the infusion. Reactions (if any) during the 10 s period just before taste infusion and during another 10 s period beginning 30 s before the infusion during the inter-trial interval were also scored. In later analysis, the two background time periods were combined into one, creating one background period 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). If this is the case, it could influence the interpretation of reactions seen in response to a taste during stimulation. Positive (hedonic) reactions included lateral tongue protrusions, rhythmic tongue protrusions, and paw licking. Neutral reactions included mouth movements and or just letting the liquid slow drip out of the mouth. Aversive responses consisted of gapes, forelimb flails, chin rubs, defensive treading, and headshakes. Detailed behavioral scoring procedures are described in (K. C. Berridge, 2000), but briefly, events like lateral tongue protrusions,

passive drip, gapes, forelimb flails, chin rubs, and headshakes typically occurred as discrete events and each instance was counted as one. The onset time of each of these events was also recorded. Other reactions - rhythmic tongue protrusions, paw licking, mouth movements, and defensive treading - always occurred in bouts. The onset and offset time of these bouts were recorded and the bout duration was converted into counts with 1 s equaling one count of occurrence. For each trial within a given period of analysis of interest, the total number of hedonic, neutral, and aversive counts was summed. Then, the average count of hedonic, neutral, and aversive reactions per trial for each taste was calculated for each session. Reactions were averaged across sessions and rats to provide overall hedonic, neutral, and aversive responses for each taste to each test condition.

Statistical analyses were performed using one-way and two-way repeated measures ANOVAs and Holm-Sidak *post hoc* tests (unless otherwise specified) implemented with the program SigmaPlot (Systat Software Inc., San Jose, CA).

*Neural analysis*

Recorded neural signals were amplified offline by a gain of 5 using a LabVIEW software amplifier program written in the lab. Stimulation artifacts were removed from the neural data that was recorded during the 20 Hz and 130 Hz DBS test sessions using our artifact removal program (described in detail in appendix A.2). Offline Sorter (Plexon, Inc., Dallas, TX) was then used to discriminate neural unit spike waveforms from noise and other units. Single units were identified using principal component analysis tools of Offline Sorter. Units were verified by distinct spike waveforms (whose shapes remained consistent throughout the whole recording) and clear refractory periods in their autocorrelation histograms. Units with more than 2 % of spikes within a 1 ms refractory period window were excluded. Cross-correlation analysis was performed using Epoch builder (a database neural and behavioral analysis program written in the lab) to ensure that any unit was being counted only once. Detailed description of the cross-correlation analysis is described in Chapter 2. In general, unit analysis was performed using Epoch builder and Neuroexploroer (Nex Technologies, Madison, AL).

Mean firing rates were calculated for each unit during the first minute of habituation period and the later minute of the habituation period when the stimulation was on (for stimulation test sessions). This provided a baseline firing rate and a baseline stimulation firing rate for units. When making comparisons to baseline rates, firing rates during the stimulation period were normalized to compensate for signal loss due to the artifact dead time; hence, the firing rate during the stimulation period was calculated for the viable duration, excluding the dead time (Bar-Gad et al., 2004). The average length of dead time for each stimulation unit was computed by averaging the dead time for all artifacts in a 5 s period randomly selected in the baseline stimulation period. The artifact dead times for all units were then averaged together to get the final mean dead time.

Peristimulus time histograms of bin size 0.5 ms were computed for units recorded during the 20 Hz and 130 Hz stimulation test sessions. For each unit, spiking activity was lined up to the onset of each stimulation pulse (for all stimulation pulses in the session) for the period between pulses. For a given test session animals received 10 trials per taste and estimating a minute per trial, plus the additional one minute during the habituation period, total recorded time during stimulation was on average 31 minutes (see figure 4.1a for trial timing). This resulted in ~37,200 pulses with a period of 50 ms between pulses for 20 Hz stimulation and ~241,800 pulses with a period of 7.6 ms between pulses for 130 Hz stimulation. Since the intertrial intervals are variable, the period of stimulation for the session could be a little longer or shorter than 31 minutes and thus there may be slightly fewer or greater pulses than the numbers reported above. A significant decrease or increase in firing was determined if two consecutive bins were greater than ± 2 standard deviations from the mean baseline firing rate of the unit computed during the first one minute of session before stimulation was turned on. Control PSTH plots were also computed for that minute pre stimulation period. "Sham stimulation" pulse times that occurred every 50 ms or every 7.6 ms (for 20 Hz or 130 Hz stimulation, respectively) were generated during the pre stimulation period and spikes were lined up to those "pulses". For "20 Hz stimulation" the number of sham pulses was 1200 and for "130 Hz stimulation" the number of sham pulses was 7000.

Perievent time histograms (PETHs) and rasters were analyzed for time of onset of each taste (sucrose, water, or quinine). The firing rate reference for all unit responses

was the average rate during the 1 s period just before taste infusion. To determine whether a unit was responsive to taste onset, firing rates during 0.5 s or 1 s epochs after the stimulus event was compared to the 1 s baseline period (described above). Mann-Whitney U tests were used to determine if any of these epochs were significantly different from baseline.

To determine population responses, each unit was first normalized to the one minute baseline period during the latter half of the habitation period. Also, given the small number of units to begin with, all units in a given test condition were pooled across rats. To determine any stimulation effects, two-way ANOVAs were carried out comparing different epochs and stimulation conditions (0 Hz, 20 Hz, 130 Hz) for taste onset. Within each test condition (i.e. no stimulation, 20 Hz, or 130 Hz stimulation) ANOVAs were carried out to compare responses to the different tastes within a population of units. Specific comparisons were made using Holm-Sidak *post hoc* tests. As with the behavioral analysis, all statistical tests were again done using SigmaPlot statistical software.

**4.3 Results**

Histology confirmed that six out of the nine rats tested had electrodes bilaterally implanted in the central nucleus of the amygdala (CeA). Figure 4.2 shows the target locations for the rats in the CeA. The data presented is from the rats whose electrodes were in the CeA.

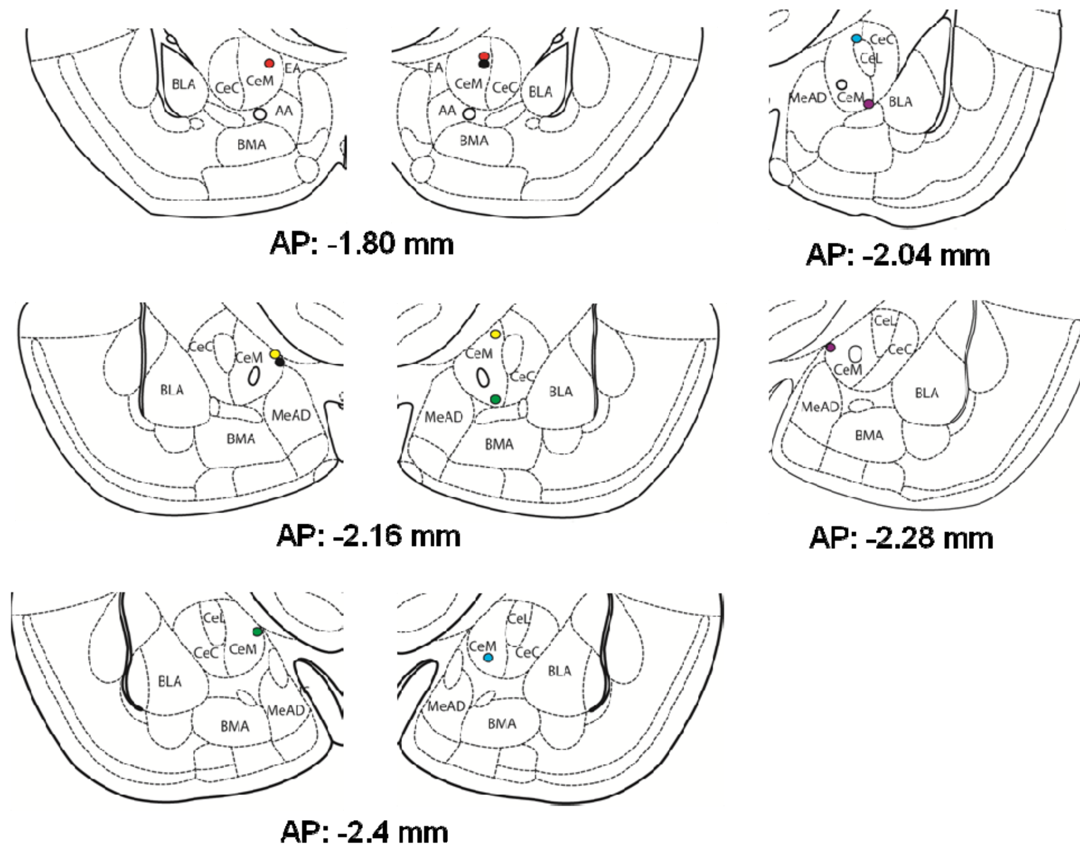



Figure 4.2 - Coronal slices showing electrode placements of rats implanted bilaterally in the central nucleus of the amygdala. Circles represent electrode bundle locations with a different color for each rat (n = 6 rats). Images modified from the Paxinos and Watson rat atlas, 6th edition (Paxinos & Watson, 2007).

*Under no stimulation conditions, sucrose was "liked" and bitter quinine was "disliked"*

As expected, with no stimulation, there were significantly more hedonic (positive) taste reactions to the highly palatable sucrose solution compared to the more neutral water ($p$ <0.001), and bitter quinine ($p$ < 0.001) [effect of taste: $F(2, 20) = 9.934$, $p$ = 0.004; two way RM ANOVA on hedonic reactions comparing different tastes and stimulation]. On the flipside, quinine elicited significantly more aversive reactions compared to sucrose ($p$ <0.001) and water ($p$ = 0.02) [effect of taste: $F(2, 20) = 32.245$, $p$ <0.001; two way RM ANOVA on aversive reactions comparing different tastes and stimulation]. There was no difference in neutral reactions across all tastes during no stimulation, though as with the other reactions, there was an effect depending on stimulation (see below). Hence, as expected the palatable taste, sucrose, was generally "liked" and the aversive taste, quinine, was "disliked" (H. J. Grill & Norgren, 1978).

Figure 4.3 shows the averaged hedonic, neutral, and aversive reactions to the three tastes under no stimulation (control) conditions.

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

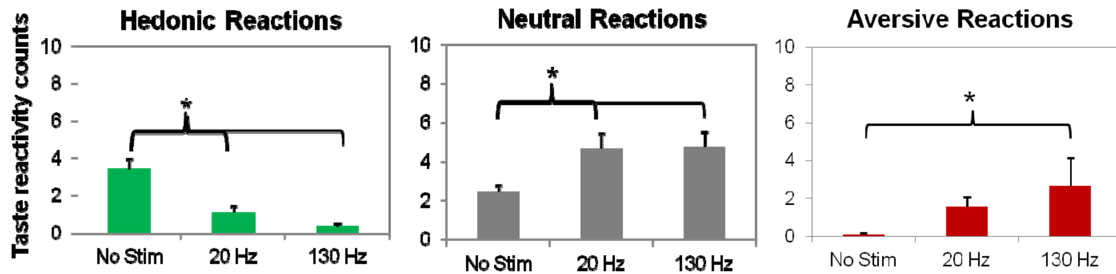
For sucrose, there was a significant effect of valence (hedonic, neutral, aversive) [$F(2, 20) = 7.407$, $p = 0.011$] and an effect of stimulation condition depending on the type of valence [$F(4, 20) = 5.725$, $p = 0.003$] using a two-way RM ANOVA test. With both 20 Hz and 130 Hz stimulation, rats now produced significantly fewer hedonic reactions to sucrose ($p = 0.013$ and $p = 0.002$, respectively) and increased neutral reactions ($p = 0.016$ and $p = 0.011$, respectively). Also, high frequency stimulation not only made sucrose less hedonic, but also resulted in increased aversive reactions to sucrose (p = 0.007). Figure 4.3a also shows taste reactions to sucrose during 20 Hz and 130 Hz stimulation. For sucrose, under no stimulation conditions, hedonic reactions were significantly greater than aversive reactions ($p = 0.005$); with 20 Hz stimulation, neutral reactions were significantly greater than hedonic reactions ($p = 0.003$) and aversive reactions ($p = 0.009$). Finally, with 130 Hz stimulation, neutral reactions were significantly greater than hedonic reactions ($p < 0.001$).

Responses to water were also significantly different depending on stimulation condition [effect of valence: $F(2, 20) = 4.351$, $p = 0.044$ and an effect of stimulation depending on the type of valence: $F(4, 20) = 3.999$, $p = 0.015$]. Water, which normally produced few aversive reactions, elicited significantly more aversive reactions with both 20 Hz ($p < 0.001$) and 130 Hz stimulation ($p = 0.012$). Also, during 20 Hz stimulation, aversive reactions were significantly greater than hedonic reactions ($p < 0.001$) and neutral reactions ($p = 0.005$). Figure 4.3b shows the number of taste reactions to water for all three test conditions.

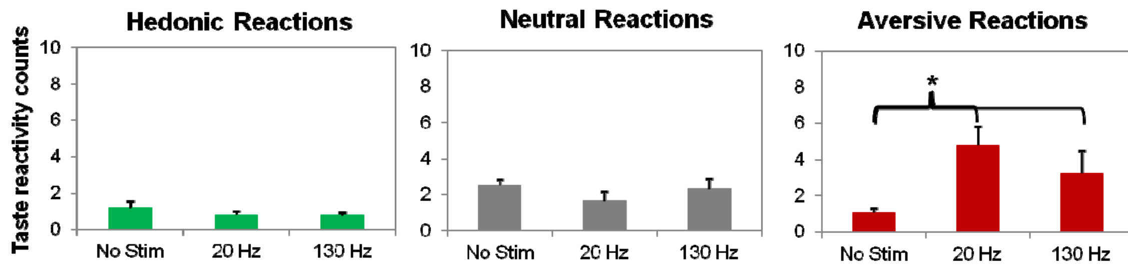
Quinine infusions also produced different reactions depending on type of stimulation received [effect of valence: $F(2, 20) = 16.748$, $p < 0.001$) and an effect of stimulation depending on the type of valence: $F(4, 20) = 3.877$, $p = 0.017$]. Quinine is normally aversive and both low and high frequency stimulation produced significantly more aversive reactions to quinine infusions ($p = 0.004$, $p < 0.001$, respectively). *Post hoc* tests also revealed that aversive reactions to quinine were significantly greater than

hedonic reactions with no stimulation ($p$ = 0.016). With both 20 Hz and 130 Hz stimulation, aversive reactions were still significantly greater than hedonic reactions ($p$ <0.001 and $p$ <0.001, respectively) and neutral reactions ($p$ <0.001 and $p$ <0.001, respectively). See figure 4.3c for the number of taste reactions to quinine for the different stimulation conditions.

(a) Sucrose




(b) Water




(c) Quinine

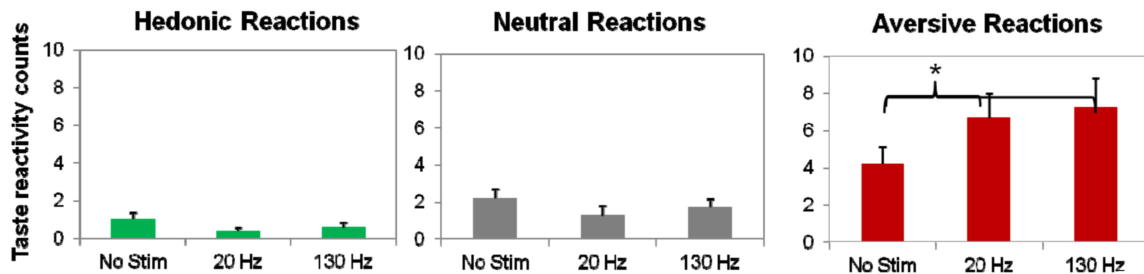



Figure 4.3 – DBS in the CeA decreased hedonic reactions to sucrose and also increased aversive reactions to all tastes. Plots show the average taste reactivity counts per trial during the 10 s post infusion period. Error bars represent standard error. (a) Hedonic, neutral, and aversive reactions to sucrose solution for each test condition (no stimulation, 20 Hz stimulation, and 130 Hz stimulation). Plots in (b) and (c) show the hedonic, neutral, and aversive reactions to water and quinine, respectively; * $p < 0.05$, n = 6 rats.

*Overall there were more "vacuum" reactions during the background period with stimulation.*

Rats at times produced taste reactions in the absence of the actual taste, referred to as vacuum tastes (K. C. Berridge & Valenstein, 1991). Though there were fewer vacuum reactions compared to the number of actual taste reactions, there was an effect of stimulation [$F(2, 20) = 4.201$, $p = 0.047$] and a valence effect [$F(4, 20) = 3.048$, $p = 0.041$]. For 20 Hz stimulation, there were significantly more aversive reactions during the inter-trial interval compared to hedonic reactions ($p = 0.003$). There was also more vacuum aversive reactions during 20 Hz and 130 Hz stimulation sessions compared to no stimulation ($p < 0.001$ and $p = 0.023$, respectively). Figure 4.4 shows the number of hedonic, neutral, and aversive vacuum reactions per trial for no stimulation, 20 Hz stimulation, and 130 Hz stimulation. Note that these reactions were observed during the inter-trial period and not during the actual taste infusions.

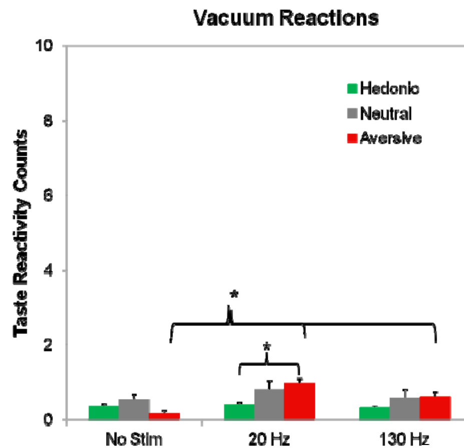



Figure 4.4 – Vacuum aversive reactions are greater with stimulation in the CeA. Plot shows the average taste reactivity counts per trial (for all trials) during the 10 s background period between infusions (i.e. when no taste was delivered) for each test condition (no stimulation, 20 Hz stimulation, and 130 Hz stimulation); * $p < 0.05$, n = 6 rats. The same y-axis range was used as that for figure 4.3 in order to compare the number of taste reactions to that observed during actual taste infusions.

*Neural overview*

The simulation artifact was successfully removed from the recorded neural data using the stimulation artifact removal program described in detail in appendix A.2. Figure 4.5 shows examples of the raw neural recorded data with the stimulation artifact and the same region after the stimulation artifact has been removed for 20 Hz and 130 Hz

stimulation sessions. The average dead time for each artifact was 1.84 ms ± 0.23. Again estimating total stimulation time for a given session to be on average 31 minutes, the total dead time due to stimulation artifacts comprised about 3.68 % of total recording time during 20 Hz stimulation and about 24.9 % of the recording time during 130 Hz stimulation.

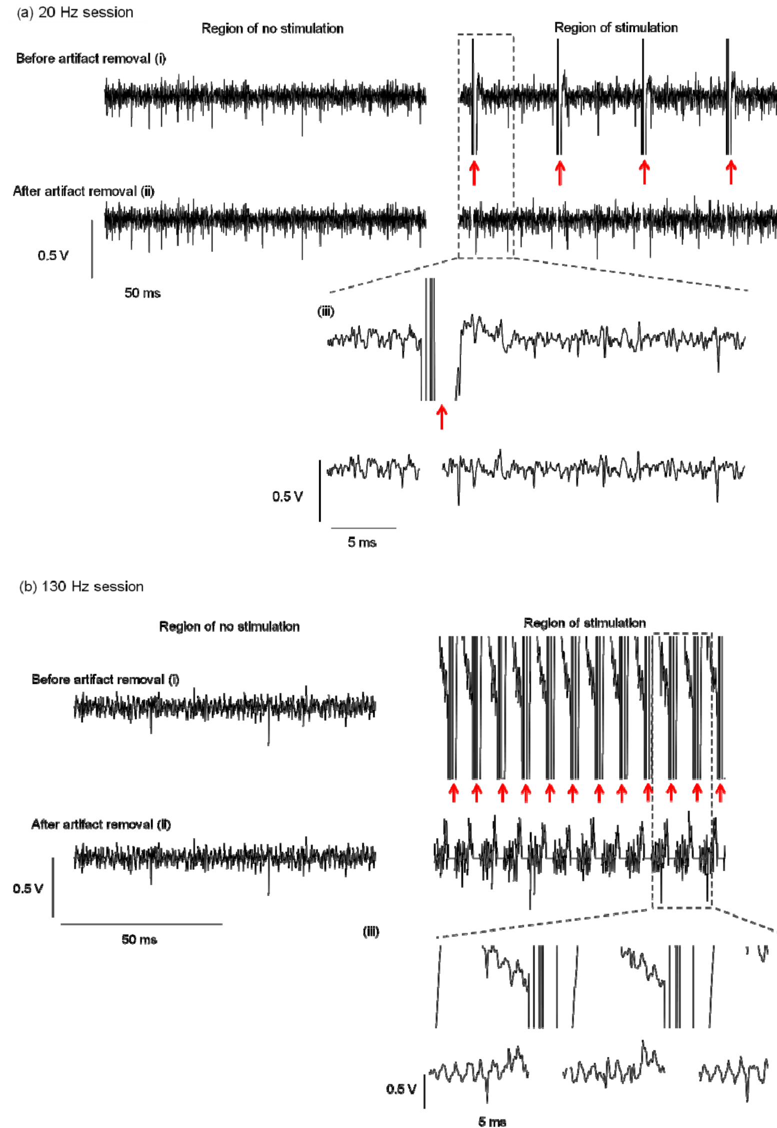



Figure 4.5 – Examples of recorded neural traces from the CeA before and after stimulation artifacts have been removed. Red arrows indicate the stimulation artifacts. (a) Two hundred milliseconds snippets of recorded neural activity from one electrode channel during a period of no stimulation and 20 Hz stimulation before (i) and after (ii) the artifact has been removed. (iii) Zoomed-in portion of the stimulation trace showing an artifact and units. This time-expanded region is indicated by a dotted box. One of the units was on the exponential decay part of the artifact and would not have sorted out, but after artifact removal, the unit is recovered. (b) (i) and( ii) similar to (a) but for 130 Hz stimulation session and each sample trace is 100 m s long. (iii) Zoom-in on region indicated by a dotted box showing two artifacts and the spikes between.

A total of 20 units from 14 sessions and five rats were recorded. Cross-correlation analysis verified that no unit (in a given session) was counted more than once. There were eight units from the no stimulation tests, eight units from 20 Hz stimulation tests, and four units from 130 Hz stimulation tests. Ninety-five percent of units (n = 19) were responsive either to the taste onset, or stimulation itself (table 4.1). The average firing rate of all units in the first minute of habitation (baseline period) was 11.08 ± 2.52 spikes/s. Majority of units had basal firing rates of 5 Hz or less which is similar to what is reported in the literature (Collins & Paré, 1999; Duvarci et al., 2011; Rosenkranz & Grace, 1999; Shabel & Janak, 2009), though six units did have firing rates of over 10 Hz.

Table 4.1 – The number of responsive CeA units (numerator) relative to total number of CeA units (denominator) broken down by test condition and event; % total of responsive units in parenthesis.

| | **Sucrose Infusion** | **Water Infusion** | **Quinine Infusion** | **Stimulation** |
|---|---|---|---|---|
| **No Stim Units** | 6/7 (85.7 %) | 5/7 (71.4 %) | 3/7 (42.9 %) | N/A |
| **20 Hz Units** | 1/8 (12.5 %) | 2/8 (25 %) | 1/8 (12.5 %) | 6/8 (75 %) |
| **130 Hz Units** | 0/4 (0 %) | 0/4 (0 %) | 0/4 (0 %) | 4/4 (100 %) |

*Stimulation produced complex firing patterns, though overall did not alter basal rates*

Stimulation at either 20 Hz or 130 Hz did not cause a significant decrease in the average firing rates of units (after accounting for lost time due to the stimulation artifacts). Comparing the change in firing rates during the later minute of habituation when stimulation was on, compared to first minute of habituation when there was no stimulation, there was no significant effect of stimulation [$H = 3.796$, $p = 0.15$, $df = 2$]. Table 4.2 shows the basal firing rates for no stimulation, LFS, and HFS units during the first minute of habituation and the change in firing rates during the later minute of habituation. Though the overall rates were not different between stimulation and no stimulation, the majority of units showed an increase during stimulation compared to the pre stimulation period, but with a small number of units, the statistical power is limited for determining any differences. Note, for the no stimulation units, there was never any

stimulation turned on, but the same two time periods for these units were compared as with the stimulation units to ensure that any changes during the second half of the habituation period were not just due to drifts in neuronal firing rates over time. Again, comparisons are corrected for the time lost due to artifact removal.

Table 4.2 – Average firing rates of CeA units during stimulation and no stimulation baseline periods.

| Type of Units (n units) | Baseline Rate (spikes/s)[+] | Overall Change from Baseline[++] | Decrease from Baseline (n units) | Increase from Baseline (n units) |
|---|---|---|---|---|
| **No Stim Units[+++] (n = 8)** | 9.85 ± 2.84 | 2.67 ± 1.27 | -0.42 ± 0.21 (n = 4 ) | 4.92 ± 2.03 (n = 4) |
| **20 Hz Units (n = 8 )** | 11.02 ± 4.75 | 18.02 ± 9.25 | -1.60 (n = 1 ) | 20.36 ± 10.33 (n = 7) |
| **130 Hz Units (n = 4 )** | 10.92 ± 6.83 | 6.34 ± 4.40 | -19.41 (n = 1 ) | 1.99 ± 0.88 (n = 3) |

[+]This is the first minute of habituation, no stimulation delivered.
[++] Change in firing rate in the second half of the habituation period when stimulation was on.
[+++]For no stimulation units there was never any stimulation on, just comparing the first half of the habituation period with the second half of the habituation period.

Though average firing rates changed little overall, peristimulus time histograms (PSTHs) revealed stimulation-induced complex patterns of inhibitions and excitations within a unit as seen in previous chapters of this thesis for both ventral pallidum units (Chapter 2) and CeA units (Chapter 3) and reported in the literature (Bar-Gad et al., 2004; Cleary et al., 2013; Hashimoto et al., 2003; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007; McCairn & Turner, 2009). Figure 4.6 shows sample PSTHs plots for 20 Hz and 130 Hz stimulation, respectively during pre stimulation and stimulation periods.

Responses to 20 Hz stimulation typically showed a multiphasic response with periods of excitations and inhibitions. Four units showed a period of excitation starting

almost immediately after the artifact dead time and lasting 3 – 6 ms, followed by a brief period of inhibition lasting 2 – 4 ms, then a sustained excitation starting around 9 – 12 ms from stimulus onset and lasting till the start of the next pulse. Two units showed a brief immediate excitation 2 – 4 ms and then the response returned to basal rates. Finally, two other units showed a period of inhibition after dead time followed by a gradual return to basal rates. For the two units that showed this initial inhibitory response, one unit just had a brief inhibition of ~2ms. The other had a longer inhibition of about 25 ms (though this inhibition was not significant based on our criteria of being below 2 standard deviations from the mean basal rate).

PSTH plots in response to 130 Hz stimulation, showed a mixture of responses. Two units demonstrated increased activity in response to stimulation about 1 to 2 ms after artifact dead time. Another unit was inhibited, and the fourth unit had a brief period of inhibition of a little less than 2 ms in duration following dead time before returning quickly to basal rates. Figure 4.6 shows PSTH plots for both 20 Hz and 130 Hz stimulation for representative units.

For controls, PSTHs plots were constructed with neural activity aligned to sham artifact times during the first minute of the session when there was no stimulation. The histogram responses in these plots were flat, indicating that the likelihood of a spike firing was independent of any alignment with regular intervals of the "sham" stimulation (Hashimoto et al., 2003; Maltete et al., 2007; McCairn & Turner, 2009). Control PSTH plots for the units shown in figure 4.6 are also included.

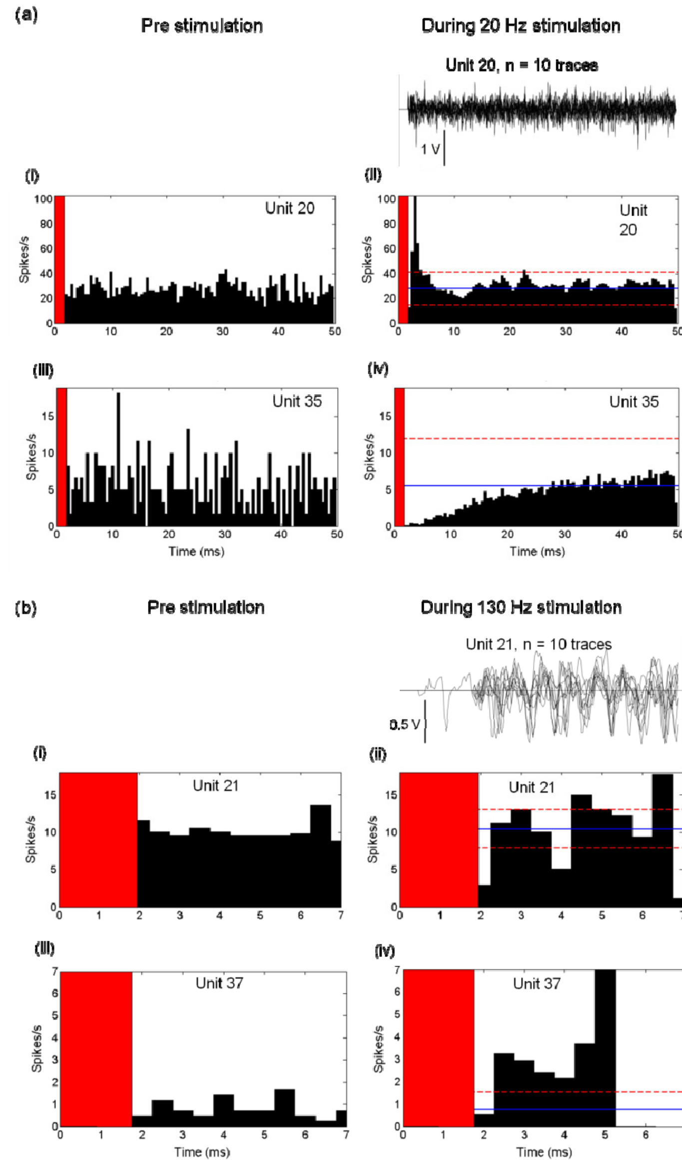



Figure 4.6 – CeA units show stimulation-induced patterns of firing. (a – b) Sample PSTHs plots normalized to spikes/second with bins of 0.5 ms and duration of 50 ms for 20 Hz stimulation and 7.6 ms for 130 Hz stimulation. The red box represents the duration of the artifact dead time where no assessment could be made of neural activity. In the control pre stimulation plots the red box only simulates the duration of the dead time. (a) PSTHs plots for 2 units recorded during 20 Hz stimulation sessions. Plot (i) is a control PSTH constructed during the first minute of habituation when there was no stimulation for a particular unit. Spikes are aligned at time = 0 to sham stimulation times occurring every 50 ms. Plot (ii) is the PSTH of the same unit during stimulation. Spikes are aligned to the onset of actual stimulation pulses. The horizontal blue line is the mean firing rate of the unit during the pre stimulation period and the dotted red lines represent ± 2 standard deviations from the mean. Above the histogram are 10 raw traces each lined up to a stimulation pulse overlaid on each other showing the spiking pattern. Plots (iii) and (iv) are the control and actual PSTH histograms for another unit. (b) Same as (a) except these are units recorded during a 130 Hz stimulation session. Note the shorter time scales in the histograms of higher frequency stimulation, which at 130 Hz has a stimulus pulse every 7.6 ms. Unit 20 and 37 are from the same rat. Unit 21 and 35 are each from different rats.

*CeA units responsive to tastes, though fewer responses in general to quinine*

Firing rates during 0.5 s and 1 s epochs after taste onset were analyzed and compared to the 1 s pre taste period to determine unit responses. Out of the 8 units recorded during no stimulation tests, one did not respond to anything and was discarded from the remainder of the analysis. For the remainder of units, the majority responded to sucrose (6/7) and water (5/7), and a smaller number responded to quinine (3/7). Most of the units also responded to at least two tastes (typically to sucrose and water). Only two units responded to just a single taste. Refer back to table 4.1 for the breakdown of responsive units to the different tastes. As figure 4.7 and table 4.3 shows, overwhelming the responses to the tastes were excitations (Tindell et al., 2006); there was only one unit that was inhibited with onset of quinine (though it showed an excitation to sucrose).

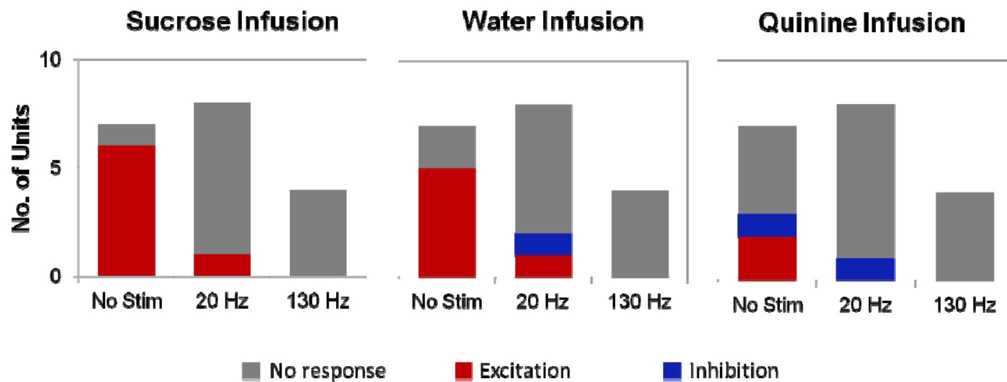



Figure 4.7 - Fewer responses overall to tastes during stimulation in the CeA. For each population (no stimulation, LFS, and HFS) the distribution of units that show no response (grey bars), inhibitions (blue bars), or excitations (red bars) are plotted.

Table 4.3 - Breakdown of all CeA unit responses, all periods for sucrose, water, and quinine onset

| Epoch Window | Neural Response | No Stim (n = 7) | 20 Hz (n = 8) | 130 Hz (n = 4) |
|---|---|---|---|---|
| | | n (% total) | n (% total) | n (% total) |
| **Sucrose Onset** | | | | |
| 0 - 0.5 s post taste onset | Excitation | 6 (85.7 %) | 1 (12.5 %) | 0 (0 %) |
| 0 - 0.5 s post taste onset | Inhibition | 0 (0 %) | 0 (0 %) | 0 (0 %) |
| 0 – 1 s post taste onset | Excitation | 6 (85.7 %) | 0 (0 %) | 0 (0 %) |
| 0 – 1 s post taste onset | inhibition | 0 (0 %) | 0 (0 %) | 0 (0 %) |
| **Water Onset** | | | | |
| 0 - 0.5 s post taste onset | Excitation | 2 (28.6 %) | 1 (12.5 %) | 0 (0 %) |
| 0 - 0.5 s post taste onset | Inhibition | 0 (0 %) | 0 (0 %) | 0 (0 %) |
| 0 – 1 s post taste onset | Excitation | 5 (71.4 %) | 0 (0 %) | 0 (0 %) |
| 0 – 1 s post taste onset | Inhibition | 0 (0 %) | 1 (12.5 %) | 0 (0 %) |
| **Quinine Onset** | | | | |
| 0 - 0.5 s post taste onset | Excitation | 1 (14.3 %) | 0 (0 %) | 0 (0 %) |
| 0 - 0.5 s post taste onset | Inhibition | 1 (14.3 %) | 1 (12.5 %) | 0 (0 %) |
| 0 – 1 s post taste onset | Excitation | 2 (66.7 %) | 0 (0 %) | 0 (0 %) |
| 0 – 1 s post taste onset | Inhibition | 1 (14.3 %) | 1 (12.5 %) | 0 (0 %) |

*Fewer responses to taste with stimulation*

Like in Chapter 3, the data also suggests that stimulation in the CeA results in fewer responsive units to tastes. With 20 Hz stimulation, only one out of eight units responded to sucrose and quinine. Only two units responded to water. Also, unlike with the no stimulation, units responded to only a single taste. With 130 Hz stimulation, no units were responsive to any of the tastes (figure 4.7). There were much fewer responses to sucrose during stimulation compared to no stimulation ($\chi^2 = 11.556$: $p = 0.003$, $df = 2$).

There was also significantly fewer responses to water with stimulation compared to no stimulation ($\chi^2 = 6.414$, $p = 0.04$, $df = 2$).

*Decreased neural firing to sucrose with stimulation*

Firing rates during the 0.5 s and 1 s period following taste onset were compared to 1 s period just before taste infusion. Firing to sucrose was significantly reduced with both 20 Hz and 130 Hz stimulation [$F(2,40) = 17.956$, $p < 0.001$]. *Post hoc* tests showed that there was a decrease in firing rate during the 0.5 s and 1 s epochs following taste infusion with 20 Hz stimulation ($p < 0.001$ for both epochs) and 130 Hz stimulation ($p = 0.008$ and $p = 0.012$, respectively) compared to no stimulation. Figure 4.8a also shows that the elevated firing to sucrose under no stimulation conditions is reduced with stimulation.

In response to water, there was no difference in firing rates with no stimulation, 20 Hz stimulation, or 130 Hz stimulation during the 0.5 s post taste infusion period [ANOVA on ranks; $H = 0.493$, $p = 0.174$, $df = 2$] or during 1 s post taste epoch [ANOVA on ranks; $H = 5.296$, $p = 0.071$, $df = 2$], though there seems to be a lower firing rate during 20 Hz stimulation compared to no stimulation. With more units, this may reveal to be significant. Likewise, no changes in firing rate in response to quinine with stimulation compared to no stimulation for either 0.5 s [ANOVA on ranks; $H = 2.796$, $p = 0.247$, $df = 2$] or 1 s [$F(2, 19) = 0.794$, $p = 0.468$] post taste epochs.

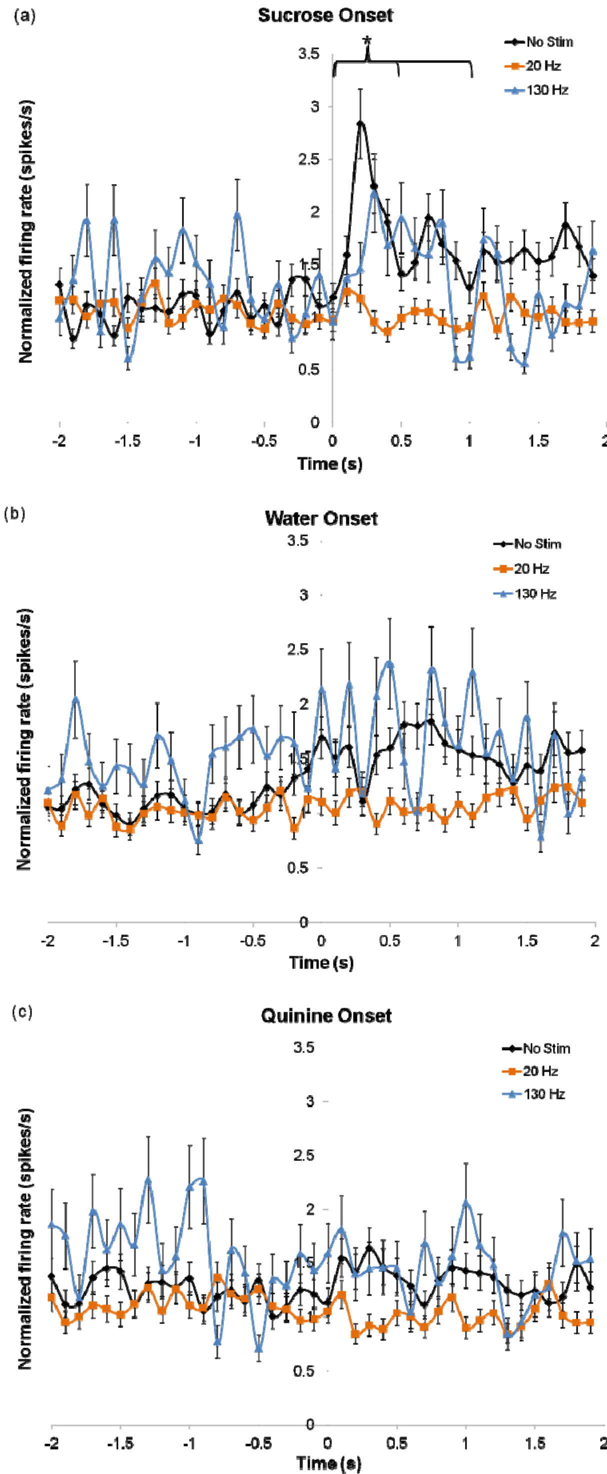
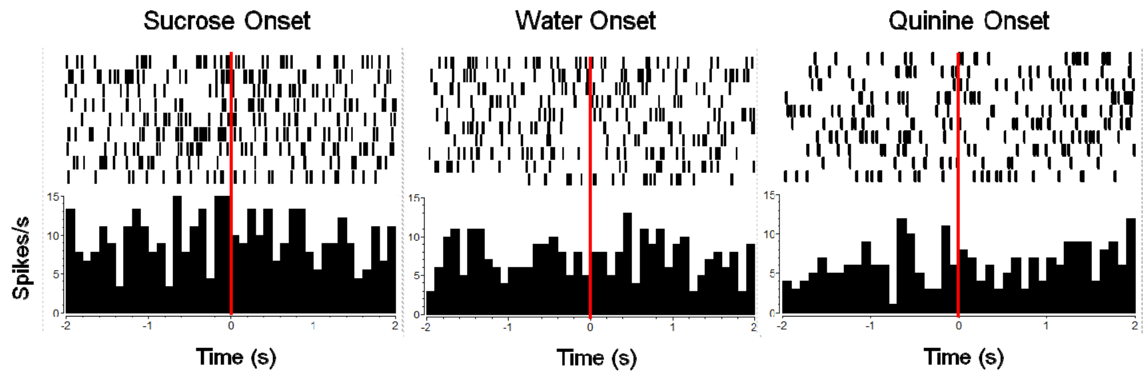



Figure 4.8 – Average taste-evoked firing rates of units in the CeA decreased during deep brain stimulation. (a) – (c) show composite histograms for all units during no stimulation (black), 20 Hz stimulation (orange), and 130 Hz stimulation (blue). Error bars represent standard error. Histograms are lined to (a) sucrose onset, (b) water onset, and (c) quinine onset showing 2 s before and after the taste infusion. All units are normalized to their respective mean firing rates during the first minute of habituation when no stimulation occurred. Y-axis is the normalized firing rate; *$p < 0.05$. Error bars represent ± standard error.

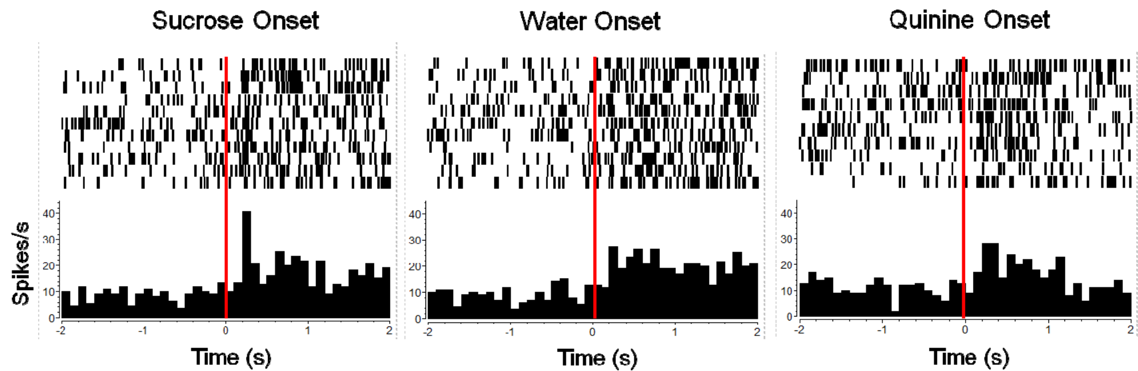
Elevated firing rates during sucrose infusion could be encoding hedonic value of sucrose compared to a more neutral taste or aversive taste (Tindell et al., 2006; Uwano et al., 1995). Comparing the different post taste epochs for no stimulation units revealed a significant effect of taste [$F(5, 47) = 5.535$, $p < 0.001$]. Firing rates during 0.5 s post sucrose window were greater than 0.5 s post water ($p = 0.003$), 0.5 s post quinine ($p < 0.001$), and 1 s post quinine ($p < 0.001$). Also, firing during 1 s post sucrose was also elevated compared to 1 s post quinine ($p = 0.003$).

During the 20 Hz stimulation, there was no difference in taste-evoked firing rates during either the 0.5 s or 1 s post taste infusion period for any of the tastes [$F(5, 47) = 2.029$, $p = 0.099$]. The same finding was found with 130 Hz stimulation [$F(5, 23) = 1.503$, $p = 0.247$]. This finding could be reflecting the overall decreased hedonic value and increased aversive value of tastes with stimulation. More units need to be tested to determine if this indeed could be the case. Figure 4.9 shows perievent histograms (PETHs) and rasters for a representative no stimulation unit, LFS unit, and a HFS unit lined to taste onset for sucrose, water, and quinine.

(a) No Stimulation




(b) 20 Hz Stimulation




(c) 130 Hz Stimulation

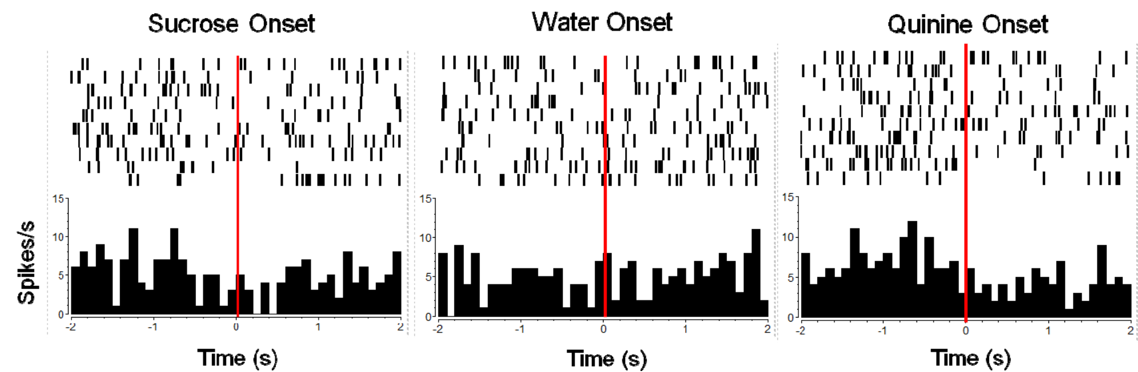



Figure 4.9 - Representative PETHs and raster plots for (a) a no stimulation CeA unit (b) a LFS CeA unit and (c) a HFS CeA unit. For each row, the plot on the left shows responses to sucrose onset for each trial, the plot in the middle shows responses to water, and the plot to the right shows responses to quinine. Plots show unit activity up to 2 s before and after the event of interest which is centered at 0. The red line indicates when the event occurred. Each mark in a raster indicates a spike and the each horizontal line is a trial with consecutive trials going from top to bottom. Histograms show the average firing rate across all trials. Bin size of each histogram is 100 ms.

### 4.4 Discussion

Deep brain stimulation at frequencies of both 20 Hz (low) and 130 Hz (high) decreased hedonic reactions to the normally "liked" sucrose solution. High frequency stimulation also caused increased aversive reactions to sucrose. In addition, both high and low frequency stimulation increased aversive reactions to a neutral taste, water, and to an already "disliked" taste, quinine. Hence, stimulation may be changing the hedonic value of rewarding tastes, like sucrose, making it less "liked" and even "disliked".

*Is DBS affecting "liking", "wanting", or both?*

We found in our previous study, which examined the effects of DBS on sucrose pellet consumption in the amygdala (Chapter 3 of this thesis), that both 20 Hz and 130 Hz stimulation resulted in decreased consumption of sucrose. This decrease in consumption could be due to a decrease in the hedonic value of the sucrose pellet with stimulation. A question that remains to be answered is if DBS is separately directly affecting both the "liking" and "wanting" aspects of food rewards, or is it only directly affecting the hedonic value of rewards and in turn making the animals less motivated to obtain these rewards.

Experimental manipulations have shown that "wanting" and "liking" systems can be manipulated separately and even be affected in opposite ways (K. C. Berridge & Valenstein, 1991; K. C. Berridge, 1996; Galaverna et al., 1993; Mahler & Berridge, 2011; Pecina, Cagniard, Berridge, Aldridge, & Zhuang, 2003; Seeley et al., 1993). During salt depletion state, lesions of the CeA diminished need-induced salt intake, but still resulted in increased hedonic reactions and fewer aversive reactions to salt. CeA lesions also decreased salt consumption in normal state, i.e. when salt was not physiologically needed (Galaverna et al., 1993; Seeley et al., 1993). Opioid manipulations in the CeA increased food consumption, but showed decrease hedonic reactions to sucrose (and no effect on water or quinine tastes). This has led to the argument that CeA could be a region that encodes "wanting" without "liking" (Richard et al., 2012). However, our data shows that both food consumption and taste palatability are affected with stimulation. Hence, we could be influencing both of these systems. The relationship between "wanting" and "liking" circuits are complex and more research is needed to determine if both systems

are being activated or if one is directly impacting the other (for example, are animals not eating because the food is no longer palatable or does the animal neither “like” nor “want” the food).

*Is DBS simply modulating sensory properties of taste?*

The CeA does receive input from taste pathways from brainstem nuclei, thalamus, and gustatory cortex (Ottersen & Benari, 1978; Ottersen & Ben-Ari, 1979; Ottersen, 1982; Sah et al., 2003; Swanson & Petrovich, 1998). Electrical stimulation in the CeA has been shown to antidromically activate pontine gustatory neurons (Norgren, 1976). So could DBS be simply changing sensory properties of taste? We do not think this is the case. CeA neurons have been reported to encode taste palatability (Nishijo et al., 2000); though compared to our results, they reported preferential encoding to aversive stimuli like quinine. Our results showed the greatest neural response to sucrose and least to quinine. They were looking at different areas of the amygdala and did not report specific coordinates, though they claim most of the units were from the central nucleus of the amygdala. However, a previous study by the same group showed greater firing rates to sucrose solution compared to water and salt, which our data supports. Their coordinates in this older study overlapped with ours (Uwano et al., 1995).

Our neural data showed that most units responded to sucrose and water with little response to quinine. Also, most of the responses were excitations, with normalized rates to sucrose being the greatest. With stimulation, fewer units responded to the tastes, and firing rates in response to infusions of any of the three tastes were decreased. This was reflected behaviorally in increased aversive reactions to all tastes.

*Alternate explanations?*

Taste reactions in the absence of actual tastes were also noted. These are referred to as vacuum responses (Berridge & Valenstein, 1991) and there were more aversive vacuum reactions with stimulation. One could ask if stimulation might just be generating a motor or sensorimotor response which has nothing to do with the hedonic value of the tastes. Though we do see more of these aversive reactions during stimulation, the reactions overall are still very few compared to reactions produce to tastes during

stimulation. Also these reactions are not just limited to a particular type of motor response, but like normal responses to tastes, range from forelimb flails, gapes, defensive treading, to headshakes, etc. All of these represent aversive reactions.

The data shows that stimulation is not just affecting palatable tastes. It is also making a neutral and even a bitter taste more aversive. Hence, stimulation could be making things in general aversive, not just food or tastes. In a presence of a more negative state, food may no longer be rewarding. This aversive state could be the result of activating feelings of disgust and/or sensation of a bad taste, which become amplified in the presence of an actual taste. As mentioned earlier, taste information does project to the CeA and also information of disgust (Ottersen, 1982). Disgust produces orofacial reactions similar seen in response to a bad taste. For example, pairing a normally hedonic taste with something that makes the rat nauseous (example, lithium chloride) produces aversive reactions to the hedonic taste after this pairing (K. Berridge, Grill, & Norgren, 1981). Some studies suggest that lesions in the CeA may be decreasing the aversive threshold for sweet, salt, and bitter tastes (Kemble et al., 1979; Li et al., 2012; Touzani et al., 1997). Further testing would be needed to tease apart whether this general aversive state could be due to feelings of disgust or the sensation of a bad taste. Also, future studies should investigate whether this aversion affects other reward behaviors like sex and drugs, or is this is just limited to food rewards.

*What does this mean for the mechanism of DBS?*

DBS in the CeA decreases food consumption and also increases aversive reactions to tastes. As discussed in Chapter 3 of this thesis, DBS could be activating inputs to the CeA. The CeA is composed largely of medium-sized spiny neurons, similar to the adjacent striatum (McDonald & Augustine, 1993) and receives substantial GABAergic innervation and also is thought to send out mostly GABAergic projections (Sah et al., 2003; Swanson & Petrovich, 1998). DBS could be activating inputs containing information about taste and taste palatability, which through opioid activation and/or other mechanisms, could be increasing aversion to tastes (Mahler & Berridge, 2011; Ricardo & Koh, 1978)

Also, as with other DBS studies (Bar-Gad et al., 2004; Garcia et al., 2005b; Hashimoto et al., 2003; Jaeger & Kita, 2011; Montgomery E.B. & Baker, 2000; Montgomery E.B., 2005), our stimulation produced complex mutltiphasic patterns of response in our CeA neurons. Furthermore, counting for artifact dead time, overall average firing rates did not change with stimulation, in fact, many of the units showed overall increased firing during stimulation. This argues against DBS simply shutting down the structure. Like in the previous study (Chapter 3), neural coding of tastes seems to be disrupted with stimulation. After accounting for artifact dead time, there were fewer responses to tastes and also decreases in normalized firing rates to taste. Hence, it is possible that stimulation is inducing its own pattern of firing and thus disrupting neural coding for tastes (especially a hedonic taste).

**4.5 Conclusion**

Our data suggests that beep brain stimulation is making things more aversive which could explain why it had significantly decreased sucrose consumption in the previous study (Chapter 3 of this thesis). Questions remain whether stimulation is independently affecting both "wanting" and "liking" circuits, or only directly modulating "liking" circuits which in turn is affecting the motivation to work for and consume rewards. Furthermore, if stimulation is creating a general aversive state one wonders if this extends to other rewards. DBS in the CeA is very effective at decreasing food consumption and CeA could still be a potential target for treating addictive disorders, though more experiments are needed to determine if we can modulate this strong aversive response. Perhaps low frequency might be useful as at least for sucrose tastes, as this frequency of stimulation did not produce a significant number of aversive reactions. Further adjustments of stimulation parameters might produce fewer aversive responses and still have clinically useful applications.

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# CHAPTER 5
# GENERAL DISCUSSION AND CONCLUSIONS

## 5.1 Summary of Findings

The main goal of this dissertation was to determine if deep brain stimulation (DBS) would decrease motivation for and consumption of food rewards. Another important objective was to assess the feasibility of DBS for treatment for reward dysfunction disorders such as morbid obesity. Part of this goal was to also assess and compare the viability of ventral pallidum and the central nucleus of the amygdala as therapeutic targets. As far as we know, neither of these brain regions has been tested before as potential targets for DBS for reward-related disorders. Another major goal of this dissertation was to record neural activity during stimulation and to analyze the effects of stimulation on this neural data. Part of that goal was to develop an artifact removal program in order to recover neural data distorted by the stimulation artifact. Finally, we wanted to investigate the effects of different frequencies on neural activity and also on behavior to determine if indeed high frequency stimulation disrupted behavior and low frequency stimulation excited or had no effect on behavior. Our overall hypothesis was that high frequency stimulation (> 100 Hz) would "block" reward-seeking behaviors and that low frequency stimulation (< 100 Hz) would either have no effect or increase reward-seeking behaviors.

We found that DBS in the ventral pallidum did not cause a stable decrease in food consumption or motivation. In contrast, stimulation in the central nucleus of the amygdala affected a dramatic decrease in both consumption and motivation. This decrease in consumption in amygdala could be due to a concomitant decrease in "liking" of rewards as the last study (Chapter 4) showed that DBS increased aversive reactions overall. For all three main projects, stimulation produced complex patterns of firing, which were reported in other studies looking at stimulation on neural firing *in vivo* and *in*

*vitro* in other basal ganglia structures in humans and animals (Bar-Gad et al., 2004; Cleary et al., 2013; Garcia et al., 2003; Hashimoto et al., 2003; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007; Maurice et al., 2003; McCairn & Turner, 2009).

Despite this modulation of firing, VP neurons were still responsive to reward-related events, but CeA neurons were not. Remarkably, our findings suggest that despite the intense interference of firing with stimulation, information content related to behavior was still able to pass through the circuit, that is, neurons especially in VP may be able carry both signals. Thus, VP neurons seem to be able to multiplex information from several sources including the intense activation of deep brain stimulation. This ability to multiplex differs among neuron types and this may contribute in part to the therapeutic efficacy of DBS targets. Below is a breakdown of the summary by chapter, followed by a discussion of the data as a whole.

*Project 1 (Chapter 2) – Deep brain stimulation in the ventral pallidum has mixed effects on food consumption.*

Overall, high frequency stimulation in the ventral pallidum did not consistently decrease consumption of palatable foods or cue-triggered "wanting" as we originally hypothesized. From the M&Ms® study, depending on stimulation pulse width, high frequency stimulation (200 Hz) resulted in a decrease in chocolate consumption compared to low frequency stimulation (20 Hz). In the Pavlovian conditioning study, high frequency stimulation (200 Hz) resulted in a decrease in cue-triggered behaviors – compared to low frequency stimulation (20 Hz), but there was no difference in sucrose consumption or latency to retrieve the sucrose pellet. Neither study showed significant differences between 200 Hz stimulation and no stimulation. We also hypothesized that low frequency stimulation would either have no effect or maybe an "activating" effect. This hypothesis was supported as 20 Hz stimulation never resulted in either decreased consumption or motivation, and there was a trend towards increased consumption though this was not significant compared to no stimulation. Specifically, in the M&M study, low frequency stimulation resulted in a quantitative increase in chocolate consumption and depending on the stimulation pulse width, was significantly increased compared to high frequency stimulation. In the Pavlovian study, there was no difference in pellet

consumption or motivated behaviors between low frequency stimulation and no stimulation.

Ventral pallidal units also showed complex responses to stimulation. During 20 Hz stimulation, most units showed a three phase response to stimulation pulse onset with typically a period of inhibition (even after accounting for artifact dead time), a period of excitation, followed by another period of inhibition or return to baseline firing rate. Also, accounting for artifact dead time, there was no difference in average firing rates during the stimulation period compared to the no stimulation period. During 200 Hz stimulation, majority of units showed a decreased likelihood of firing between stimulation pulses, though a small percentage of units showed a period of inhibition followed by excitation. With 200 Hz stimulation, there was only 5 ms between pulses, so units might not have had enough time to recover from this period of inhibition before the next pulse came along. Also, overall firing rates (after accounting for artifact dead time) were lower compared to no stimulation. These patterns of response were not seen when the units were lined up to sham stimulation pulses during the pre stimulation period demonstrating that changes in unit response seen during stimulation was most likely due to the stimulation itself. Thus as reported in other studies, our findings suggest that stimulation produces a loose entrainment of neural firing with units showing stimulation-induced firing patterns. However, this entrainment of neural activity to stimulation did not prevent neurons from responding to the cues and rewards in a manner similar to when there was no stimulation. In many instances, neurons exhibited the same responses to the task events as those observed when there was no stimulation. At least for ventral pallidal units, these results challenge the simple notion of information blockade by high frequency stimulation. Despite the overall decrease in firing rates during 200 Hz stimulation, behaviorally-related signals were still able to "get through".

*Project 2 (Chapter 3) – Deep brain stimulation in the central nucleus of the amygdala decreases food consumption*

Unlike in the ventral pallidum study, both low frequency stimulation (20 Hz) and high frequency stimulation (130 Hz) in the central nucleus of the amygdala (CeA)

significantly decreased the motivation to work for and consume sucrose pellets. Based on the mapping study, the effective zone of stimulation was ~0.4 $mm^3$ which was mostly in the medial aspect of the central nucleus of the amygdala (CeM). Over the course of the stimulation sessions, rats would also sometimes go towards the food dish or lever and even deliver a few more pellets and even eat one or two. Sometimes, the rats would even put pellets in their mouths, but then spit them out. The effects of stimulation were reversible. Within a few seconds after the stimulation was turned off, rats immediately went to the food bowl, ate pellets there that had accumulated in the bowl (if any), and then proceeded to deliver and eat more pellets at the same rate as they did in no stimulation test sessions. Though high frequency stimulation followed our hypothesis, low frequency stimulation also resulted in decrease consumption, unlike what we had predicted and what had occurred in the ventral pallidum. This difference may be due to the differences in circuitry or neuronal properties of the two different structures. Units in the CeA also fire at basal rates that are at least half the value of VP basal rates (Bengtson & Osborne, 2000; Collins & Paré, 1999; Duvarci et al., 2011; Heidenreich et al., 1995; Shabel & Janak, 2009). Hence, for CeA units, 20 Hz frequency may still be of a sufficient "high" frequency to block information content and thus food consumption (more discussion on this below).

Like in the case of the ventral pallidum units, stimulation of the central nucleus of the amygdala produced complex firing patterns in its neurons. For 20 Hz stimulation, a three phase response was observed with typically a period of inhibition followed by a period of excitation, and then another period of inhibition and/or return to basal rates. This pattern was similar to that observed in VP. Perhaps an interesting piece to the puzzle of the difference in low frequency stimulation effect for VP and CeA is that the initial inhibitory period for CeA units to stimulation onset was approximately two times longer than the VP units in response to 20 Hz stimulation. Hence, low frequency stimulation resulted in a longer inhibitory phase before units began to fire again. Though, this was not always the case with CeA units as shown in Chapter 4. For 130 Hz stimulation, there were only two units with one showing a period of inhibition followed by excitation and the other showing just inhibition. Unlike with the VP units, these stimulation-induced patterns also seem to disrupt neural responses to behavioral events.

To understand these differences, further experimental work would be necessary and more unit data needs to be collected.

*Project 3 (Chapter 4) – Deep brain stimulation in the central nucleus of the amygdala decreases hedonic value of tastes*

Along with a decrease in consumption, deep brain stimulation at both 20 Hz and 130 Hz decreased hedonic reactions to the normally "liked" sucrose solution and increased aversive reactions to all three tastes – sucrose (sweet), water (neutral), and quinine (bitter). Thus, stimulation could be making things more aversive. This effect on "liking" could be separate from the decrease in "wanting" as these systems have been shown to be dissociable (K. C. Berridge, 1996; Mahler & Berridge, 2011; Pecina et al., 2003; Smith et al., 2011; Wyvell & Berridge, 2000). Perhaps by making things more aversive, consumption decreased. More testing needs to be done to determine if this increased aversion also blocks "wanting" for other rewards, like drugs, to determine if this a general effect on reward or specific to food and taste modalities.

Responses to stimulation itself were similar to the previous two projects, though in some cases, the three phase response to 20 Hz stimulation consisted of two excitations interspersed with an inhibition. Some units in responses to 130 Hz stimulation also showed excitations instead of inhibitions. Hence, CeA unit response to stimulation did not always begin with an inhibitory phase. As in project 2 (Chapter 3), coding of taste onset was also disrupted. Units were less responsive to tastes during stimulation compared to no stimulation and normalized firing rates to the taste infusions were also much less compared to units during no stimulation tests.

## 5.2 Mechanisms of Action of DBS

What do the results from both these studies tell us about possible mechanisms of deep brain stimulation (DBS)? Like all brain regions, the two circuits studied here are very complex with many inputs and outputs and several reciprocal connections (Haber, 2011; Sah et al., 2003; Smith et al., 2009; Swanson & Petrovich, 1998). These two structures receive information from and send information to cortical and subcortical areas. Both structures are made up of mostly GABAergic medium spiny projection

neurons and receive significant GABAergic inputs and their output is also primarily GABAergic (Bengtson & Osborne, 1999; Bengtson & Osborne, 2000; Lavin & Grace, 1996; McDonald, 1982; McDonald & Augustine, 1993; Sah et al., 2003; Swanson & Petrovich, 1998). Hence, both have this feed forward inhibition. However, the CeA is thought to be similar to the striatum of the basal ganglia with units that have much lower firing rates compared to VP units (Bengtson & Osborne, 2000; Collins & Paré, 1999; Duvarci et al., 2011; Heidenreich et al., 1995; Shabel & Janak, 2009). The CeA could possibly be viewed as a structure closer to the beginning of the limbic pathway receiving sensory input from brainstem structure and integrating that information before sending it to nucleus accumbens, ventral tegmental area, and indirectly to the ventral pallidum. The VP is proposed to be "a final common pathway for reward" (Smith, 2007) and perhaps represent more the output of the limbic system.

The results from stimulation in the VP and CeA demonstrate that DBS did not simply act like a lesion with complete cessation of firing of the stimulated units. Instead, stimulation induced multiphasic responses in the neurons. Low frequency stimulation at 20 Hz produced periods of inhibitions and excitations in many cases either two periods of inhibitions interspersed with an excitation or vice versa. High frequency stimulation produced either inhibitions or excitations, or multiphasic responses typical consisting of inhibitions followed by excitations. There were variations in these responses which could be due to electrode location, stimulation amplitude, and/or differences in physiological properties of the neurons being recorded (Bar-Gad et al., 2004; Cleary et al., 2013; M. D. Johnson & McIntyre, 2008). These results correspond to similar responses seen in other studies of DBS in the subthalamic nucleus (STN), internal and external segments of globus pallidus (GPi and GPe), and the substantia niagra (SNr) (Bar-Gad et al., 2004; Cleary et al., 2013; Garcia et al., 2003; Hashimoto et al., 2003; M. D. Johnson & McIntyre, 2008; Maltete et al., 2007; Maurice et al., 2003; McCairn & Turner, 2009). It has been proposed that this stimulation-induced firing pattern could result in "jamming" of information flow in the circuit (Cleary et al., 2013; Garcia et al., 2005a; Garcia et al., 2005b; W. M. Grill et al., 2004; M. D. Johnson & McIntyre, 2008; McIntyre et al., 2004). In the case of Parkinson's disease, this entrainment of neural activity to stimulation could be imposing its own pattern in the circuit and disrupting the

normal pathological patterns present (Carlson et al., 2010; Garcia et al., 2005a; Montgomery E.B. & Baker, 2000). In a normal circuit (i.e. not Parkinsonian) this stimulation-imposed activity could still be interfering with normal information flow and introducing activity that may or may not be functionally meaningful depending on the circuit being stimulated.

*Proposed mechanisms of DBS in the ventral pallidum*

What does this stimulation-induced modulation of firing mean for our studies? How does this translate to changes (or not) in reward-seeking behaviors? In the ventral pallidum study, though we saw no clear, consistent decrease in reward behaviors with high frequency stimulation, there were still changes in firing in response to stimulation. Yet neurons were still able to respond to the reward cues and the reward itself as they did when stimulation was not present. Figure 5.1a shows a very simplified schematic of the VP showing important connections with the nucleus accumbens and ventral tegmental area.

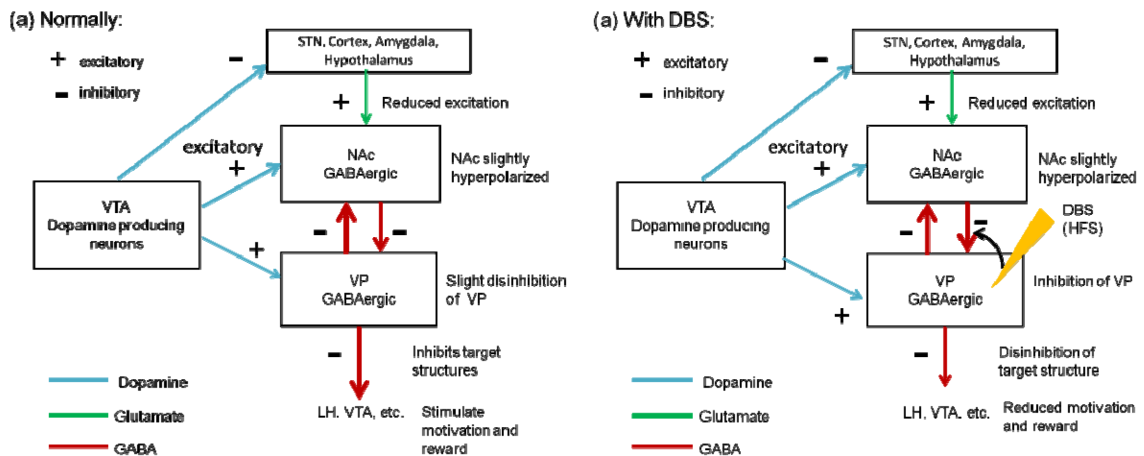


Figure 5.1 - Proposed model of action of DBS in the ventral pallidum. Ventral tegmental area (VTA) sends dopaminergic projections to nucleus accumbens (NAc) and ventral pallidum (VP). VTA also sends dopaminergic projections to the subthalamic nucleus (STN), cortex, amygdala, and hypothalamus, which in turn sends glutamergic input to the NAc. NAc sends GABAergic projections to the VP, which in turn sends GABAergic projections to many structures including the lateral hypothalamus (LH) and back to the VTA. Green arrows represent glutamatergic projections, red arrows represent GABAergic projections, and blue arrows represent dopaminergic projections. Pluses represent excitatory effects on the target structure and minuses represent inhibitory effects on the target structure. (a) Under normal conditions, it is proposed that slight hyperpolarizarion of the NAc results in slight disinhibition of the VP causing the VP to fire more and inhibit downstream structures, triggering reward behaviors. (b) DBS in the VP could be activating axons projecting from the NAc, causing GABA to be released into the VP and thus inhibiting the structure. This results in disinhibition of targets like LH which ultimately results in decrease reward-related behaviors like food consumption.

It has been proposed that disinhibition of VP neurons via hyperpolarization of nucleus accumbens may be a way that reward and motivation are stimulated (Caille & Parsons, 2004; Caille & Parsons, 2006; Kalivas, 2004; Napier & Mitrovic, 1999) Normally, the nucleus accumbens could be slightly hyperpolarized resulting in slight disinhibition of ventral pallidum, this slight disinhibition of VP allows the GABAergic inhibitory neurons of the VP to fire, resulting in inhibition of target structures such as the lateral hypothalamus and the ventral tegmental area and ultimately a decrease in motivation and reward circuits in general. This has been supported by studies that have shown that injection of GABA agonists, glutamate antagonists, opioid agonists, or cannabinoid agonists into the nucleus accumbens seem to cause local inhibitions of the nucleus accumbens which then disinhibits the ventral pallidum and stimulate increased consumption and pursuit of drugs (Bakshi & Kelley, 1993; Mahler, Smith, & Berridge, 2007; Pecina, Smith, & Berridge, 2006; Reynolds & Berridge, 2001; Reynolds & Berridge, 2002; Torregrossa, Tang, & Kalivas, 2008).

Other studies have also shown that GABA antagonists into the ventral pallidum (which would prevent VP neurons from being inhibited thus allowing them to fire and inhibit downstream structures) increased eating behavior (Shimura et al., 2006; Smith & Berridge, 2005; Stratford, Kelley, & Simansky, 1999). On the flipside, GABA agonists (which would inhibit the VP neurons and prevent them from firing) decreased consumption (Shimura et al., 2006; Smith & Berridge, 2005). Lesions of the VP, which would result in disinhibition of downstream structures, also caused aphagia (Cromwell & Berridge, 1993; Morgane, 1961) and impaired reward learning and diminish willingness to work for rewards (Farrar et al., 2008; Harvey et al., 2002; McAlonan et al., 1993; McFarland & Kalivas, 2001; McFarland et al., 2004; Waraczynski, 2006).

If DBS results in entrainment of firing, especially through antidromic activation of axons projecting to that region (Anderson et al., 2006; Gradinaru et al., 2009), stimulation in the ventral pallidum may most likely activate axons from the nucleus accumbens (NAc), especially since it receives numerous projections from the NAc (Chrobak & Napier, 1993; Churchill & Kalivas, 1994; Phillipson & Griffiths, 1985; Usuda et al., 1998). Figure 5.1b shows a proposed mechanism of action of DBS in the ventral pallidum. Stimulation could ultimately be activating GABAergic axons from the

nucleus accumbens to the ventral pallidum. This could trigger release of GABA into the ventral pallidum, causing hyperpolarization of the ventral pallidum which would then disinhibit (remove the brakes) from the downstream structures, resulting in decreased consumption. During 200 Hz stimulation, there was overall decreased firing of ventral pallidal units, which supports the idea that afferent axons are being activated releasing GABA into the ventral pallidum and thus inhibiting VP neurons. However, the results from our study showed that VP neurons still encoded reward and the rats still ate the sucrose pellets – only a modest decrease in motivation was seen.

DBS has also been proposed to activate efferent axons and also possibly, fibers of passage though the target structure (Carlson et al., 2010; Cleary et al., 2013; Filali et al., 2004; Garcia et al., 2005b; M. D. Johnson & McIntyre, 2008; M. D. Johnson et al., 2012; Okun, 2012). The ventral pallidum projects back heavily to the nucleus accumbens. If efferent axons were also being excited these could be releasing GABA and slightly inhibiting the nucleus accumbens, which could then no longer be inhibiting the VP as much. VP also projects back to the dopamine producing cell bodies of the ventral tegmental area (VTA). Hence, given these various connections, information relevant to reward coding may still be able to pass though. Also as discussed earlier, the properties of VP neurons may be such that these neurons are able to multiplex information from several sources including the intense activation of high frequency deep brain stimulation.

Results also may strongly depend on target location. Our electrodes may not have been in the best location to activate the right fibers and/or cell bodies to have an effect. DBS studies have shown that even within the effective structure, location within that structure can result in difference in effects. For example, it is proposed that placing DBS electrodes near or just outside the dorsal border of the subthalamic nucleus may be an optimal location for DBS for Parkinson's disease (Maks et al., 2009; Plaha, Ben-Shlomo, Patel, & Gill, 2006).

*Proposed mechanisms of DBS in the central nucleus of the amygdala*

What about the possible means of action of DBS in the CeA? As mentioned in Chapter 1, the CeA is divided into four main structures: the capsular subdivision (CeC), lateral subdivision (CeL), intermediate subdivision (CeI), and medial subdivision (CeM).

These subregions connect extensively with one another, with input to the CeA largely entering the CeC and CeM. The output of the CeA is mainly funneled through the CeM. CeM also receives a lot of inputs from CeC and CeL (Jolkkonen & Pitkanen, 1998; McDonald & Augustine, 1993; Sah et al., 2003). These interconnections are mostly GABAergic and the output of the CeA is also GABAergic (McDonald, 1982; McDonald & Augustine, 1993; Sah et al., 2003; Swanson & Petrovich, 1998). Histological verification from our study showed that the majority of electrodes were in the CeM (though some bundles passed though CeL and a few were on the border of CeM and CeC). Figure 5.2a show a simplified schematic of the CeA, highlighting the CeM connections specifically. Some of the primary connections to the CeM are from the CeL (which is GABAergic) and the basolateral amygdala, BLA (glutamatergic). Increased firing of the CeL would release GABA into the CeM and thus inhibit it. However, excitation of BLA releases glutamate into the CeM which would thus cause the CeM neurons to fire more. BLA also directly projects to the CeL and will cause it to fire. Response in the CeM could ultimately be due to the ratio of activation from BLA and inhibition from the CeL which could then result in increased firing of the CeM neurons thus inhibiting target structures like the lateral hypothalamus and ventral tegmental area resulting in food consumption and other reward-related responses (like in the ventral pallidum). This also depends on whether BLA or CeL has the greater influence on CeM activity. As in the ventral pallidum, lesions or inactivation of the CeA results in decreased consumption of reward (Galaverna et al., 1993; Kemble et al., 1979; Seeley et al., 1993; Touzani et al., 1997; M. J. Will et al., 2009; M. Will et al., 2004).

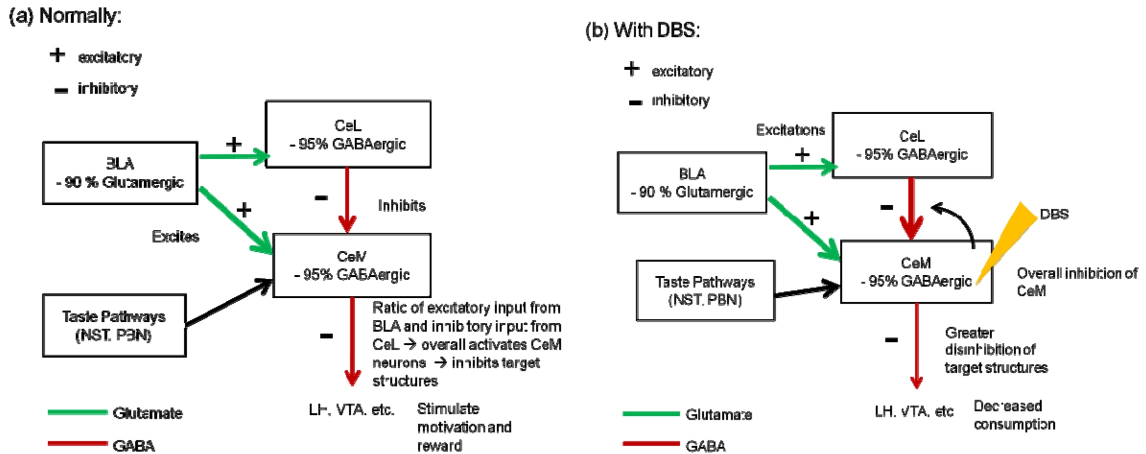


Figure 5.2 - Proposed model of action of DBS in the central nucleus of the amygdala (CeA). The basolateral amygdala sends glutamatergic projections to the central lateral nucleus of the amygdala (CeL) and the central medial nucleus of the amygdala (CeM). Both CeM and CeL are a part of the CeA. Our electrode locations were mostly in the CeM. CeL also sends GABAergic projections to the CeM which then sends GABAergic projections out to various structures involved in reward processing including the lateral hypothalamus (LH) and the ventral tegmental area (VTA). There are also projections from taste pathways to the CeM. Green arrows represent glutamatergic projections, red arrows represent GABAergic projections, and blue arrows represent dopaminergic projections. Pluses represent excitatory effects on the target structure and minuses represent inhibitory effects on the target structure. (a) Under normal conditions, it is possible that the CeM is activated by BLA but partially inhibited by the CeL, the ratio between activation and inhibition could ultimately result in CeM overall being activated. This will in turn inhibit target structures, triggering reward behaviors. (b) DBS in the CeM could be activating axons projecting from the CeL, pushing the ratio of activation from BLA and inhibition from CeL to overall inhibition of the CeM. This results in disinhibition of targets like LH which ultimately could decrease reward-related behaviors like food consumption.

Figure 5.2b now shows a proposed effect of deep brain stimulation. DBS could again result in activation of fibers including afferent axons projecting into the CeM. If the fibers from the CeL are more likely to be activated, more GABA would be released into the CeM, shifting the overall ratio to inhibition of CeM resulting in disinhibition of downstream structures ultimately decreasing food consumption and motivation. Taste pathways entering into the CeA could also be activated ultimately resulting in increased aversion possibly though opioid activation (Mahler & Berridge, 2011; Ricardo & Koh, 1978). Again this could represent a mechanism by which both “wanting” and “liking” systems are being modulated in the CeA, but through different brain mechanisms. However, there is still the question of the role of BLA input. Are both BLA and CeL projections into the CeM being activated? Though overall firing showed decreased firing rates during stimulation (after accounting for artifact lost time), the perstimulus time

histograms showed both excitations and inhibitions to stimulation, which suggests both inhibition of some of the CeM units as well activation of others. Even with 20 Hz stimulation, some units had very long inhibitory periods (over twenty milliseconds) before firing, whereas others showed excitation within a few milliseconds after stimulation pulse onset. Hence, units showing initial inhibitory responses to stimulation may be due to activation of CeL GABAergic inputs to the CeM. Units showing immediate excitation to stimulation may be due to instead activation of BLA glutamatergic inputs. More targeted stimulation such as optogenic stimulation (Gradinaru et al., 2009) could used to shed more light on these different unit responses.

*Summary of mechisms of DBS in both structures*

Regardless, for both the VP and the CeA, results are showing modulation of neuronal firing due to stimulation, but this seems to only affect neural coding in the CeA, not the VP. This shows that loss of information content due to stimulation modulation may not always be the case and it may depend on the types of neurons being simulated as well as the neural circuit being targeted. It has been proposed that therapeutic effects of DBS may be due to combined effects of multiple neuronal elements (M. D. Johnson & McIntyre, 2008; M. D. Johnson et al., 2012; Okun, 2012). Hence, depending on the target structure, neuronal properties, and pathways, there may not be an effect of DBS. Nevertheless, our findings do indicate though that DBS is not just silencing neurons altogether. Further elucidation of specific mechanism of action of DBS in the VP and CeA may be better determined by using more specific tools like optogenetics (Gradinaru et al., 2009) to selectively activate or inhibit certain pathways to see which connections at least result in decreased consumption with stimulation.

## 5.3 Potential Targets for DBS for Reward Dysfunction

Ultimately, what do the results of these studies mean for the feasibility of using deep brain stimulation for the treatment of reward dysfunction? Stimulation in the central nucleus of the amygdala produced a dramatic decrease in food consumption and working for delivery of the reward pellets. Both 20 Hz and 130 Hz stimulation were effective in this. Hence, DBS successfully decreased "wanting" of food rewards which

was the overall aim of this thesis, but it also resulted in increased aversive reactions to a hedonic, neutral, and aversive taste and decreased the hedonic value of a sweet taste. Hence, this decrease in "wanting" was also accompanied by an increase in disliking of tastes. This brings in to question the feasibility of using this location as a target for treatment of eating disorders or addictions, especially in the case of morbid obesity if it is making things more aversive in general. DBS in the CeA is not just making palatable tastes like sucrose less "liked", but is also affecting neutral and even aversive tastes. Hence, DBS in the CeA may not only be blocking motivation for rewarding foods, but perhaps for all foods. Also, the fact that DBS is making things more aversive, may mean that this increased negative state of the animal with stimulation could extend to other rewards – not just food. Hence, one needs to look at the effects of DBS on other rewards. The CeA, however, could still be a viable target depending on the stimulation parameters. At least for taste reactions to sucrose, there was a frequency difference, with 20 Hz stimulation decreasing hedonic reactions to sucrose but not significantly increasing aversive reactions. Hence, with further exploration of stimulation frequencies and even currents, there may be a combination of parameters that causes this decrease in "wanting" without increasing aversion.

In some ways, the VP could still be a better target. DBS in the VP did not induce any aversive reactions as it did in the amygdala. Furthermore, the slight decrease in motivated behaviors with high frequency stimulation in the VP, though small and inconsistent, was at least promising. A more extensive search on finding the effective location and parameters may yet yield an effective zone for DBS in the VP. Though we did not see any aversive reactions with stimulation, a direct measure of "liking" using the taste reactivity paradigm described in Chapter 1 and Chapter 4 of this thesis should be carried out to rule out that DBS in the VP does not affect "liking".

These results do reflect the feasibility of "blocking" reward-related behaviors, though they also demonstrate the complexity of reward mechanisms in general. Other groups have also proposed other targets to treat DBS for reward dysfunction including the ventral subthalamic nucleus (Baunez et al., 2007; Gubellini et al., 2009; Rouaud et al., 2010; Temel et al., 2009; Witjas et al., 2005) and the nucleus accumbens (Heldmann et al., 2012; Knapp et al., 2009; Liu et al., 2008; Sesia et al., 2008; van der Plasse et al.,

2012; Vassoler et al., 2008); though both targets have their own challenges. Correct targeting in the subthalamic nucleus is key as going too dorsal can affect motor behavior (dorsal STN is a very common target for DBS for Parkinson's disease). Furthermore, studies have suggested that DBS in the STN may not be applicable for treating eating disorders, though it could be a viable location for treating addictive disorders. Lesions of the STN did not affect food consumption and was also shown to actual increase motivated behaviors for food rewards (Baunez, Amalric, & Robbins, 2002; Baunez, Dias, Cador, & Amalric, 2005; Lardeux & Baunez, 2008). Lesions of the STN also showed mixed results on drug intake - not affecting overall consumption of alcohol or cocaine, but causing reduced responses for cocaine (Baunez et al., 2005; Lardeux & Baunez, 2008). The results from DBS in the nucleus accumbens have also been mixed. For example, one study showed that DBS in the nucleus accumbens shell did not decrease food consumption or sucrose preference, but did decrease operant responding for sucrose pellets (van der Plasse et al., 2012). These results demonstrate the challenges for DBS in reward-dysfunction. The effects of DBS in all these different studies (including those in this thesis) need to be carefully assessed to determine the best location and parameters with minimal side effects.

### 5.4 Implications for DBS as a Treatment for Reward Dysfunction

The present study and other work have shown that DBS can be used to modulate reward and affect both "liking" and "wanting" of rewards whether food or drugs (Halpern et al., 2008; Heldmann et al., 2012; Knapp et al., 2009; Kuhn et al., 2007; Lacan et al., 2008; Lehmkuhle et al., 2010; Liu et al., 2008; Mantione et al., 2010; Melega et al., 2012; Rouaud et al., 2010; Sani et al., 2007; Vassoler et al., 2008). However, these studies also demonstrate the challenges of modulating reward-related behaviors with many showing mixed effects of DBS and/or very subtle changes. For example, van der Plasse and colleagues have shown that DBS in the nucleus accumbens shell decreases operant responding for sucrose but does not affect food consumption or sucrose preference (van der Plasse et al., 2012). DBS in lateral hypothalamus did not affect food consumption, but did affect weight loss possibly due to changes in metabolic rate (Sani et al., 2007). This was also observed with DBS in the ventromedial hypothalamus (Lehmkuhle et al.,

2010). Rouaud and colleagues also demonstrated differences in motivated behaviors for food versus cocaine with DBS in the subthalamic nucleus (Rouaud et al., 2010). Results from this thesis showed dramatic decreases in food consumption with DBS in the CeA, but also aversive reactions. Questions of target location and stimulation parameters are still being debated for all DBS investigations.

Despite the the progress in reward studies, more work needs to be done to learn how the brain processes rewards and how drugs of abuse usurp these natural reward mechanisms. Many brain regions and neurotransmitiers are involved in reward processing (Haber, 2011; Humphries & Prescott, 2009; Kelley et al., 2005; McGinty et al., 2011; Richard et al., 2012) making the search for the "best" brain target and stimulation parameters especially difficult. This challenge is magnified by the fact that our ideal goal will be to preferentially diminish only excessive "wanting" of food rewards in case of morbid obesity and drug rewards in the case of addiction. Furthermore, in the case of morbid obesity, how do we target "excess" consumption without making all foods unpalatable and potentially stopping consumption altogether? Further investigations of the brain reward mechanisms rewards are needed to choose the "best" target.

Other questions arise with the idea of using DBS to treat morbid obesity or addiction. What would prevent patients from turning the stimulation off when he/she wants to eat something appetizing? Patients treated with DBS for movement disorders typically have the ability to turn the stimulation on or off and it is likely that the same controls would be ethically required for patients with consumption disorders as well. The ideal goal will be to find a "sweet spot" where DBS will only affect excessive "wanting" of food rewards. This goal is very challenging and will take dedication and patience by caregivers and patients.

### 5.5 Conclusion

The overall results of this thesis demonstrate the feasibility of modulating reward in specific targets in the brain. Deep brain stimulation (DBS) in the central nucleus of the amygdala (CeA) resulted in significant decreases in both sucrose consumption and willingness to work for sucrose pellet delivery. However, our results suggest that DBS in the CeA may also be creating an overall aversive state as DBS in the CeA increased the

numbers of aversive reactions, though this may depend on frequency of stimulation. More experiments are needed before ruling out this structure as a potential target. DBS in the ventral pallidum (VP), unlike CeA, did not seem to make things more aversive overall; however, motivation and consumption were only altered modestly. Again more testing is needed to assess the effects of DBS in the VP on food consumption.

DBS in both the VP and CeA did not silence neurons, but instead modulated neural activity with excitations and inhibitions in response to the stimulation. These results support findings in other studies (Bar-Gad et al., 2004; Cleary et al., 2013; Garcia et al., 2003; Hashimoto et al., 2003; Johnson & McIntyre, 2008; Maltete et al., 2007; Maurice et al., 2003; McCairn & Turner, 2009). This modulation of firing corresponded with disruption of reward coding in the CeA, but interestingly, not in the VP. These results show that simply creating these stimulation-induced patterns does not necessarily disrupt information content in the circuit. Effects of DBS depend on target location, parameters, properties of the neurons themselves, and the connectivity of the circuit of interest (Johnson & McIntyre, 2008; Johnson et al., 2012; Okun, 2012).

### 5.6 Future Work

Results from this dissertation have demonstrated that deep brain stimulation (DBS) has potential for treating reward dysfunction and given some insight on how DBS may be exerting its effects. Both the ventral pallidum and the central nucleus of the amygdala present scientific and translational challenges. More detailed testing is needed for each structure before ruling either one out. The effects of deep brain stimulation on less palatable foods like standard chow need to be assessed in both structures, though the effects of DBS in the CeA on water and quinine suggests that DBS in the CeA will likely decrease consumption of standard chow as well. “Wanting” and “liking” behaviors have been shown to depend on the current physiological state (such as hunger or salt balance) (K. C. Berridge, 2004; J. Zhang, Berridge, Tindell, Smith, & Aldridge, 2009). This means that the efficacy of DBS modulation may also depend on these states. How this might impact potential therapeutic function is unknown. It must also be pointed out that stimulation in this study was delivered for at most one hour. Typically, DBS treatments

consist of continuous stimulation. Future studies would be needed to assess potential changes that might arise from these differences.

In the central nucleus of the amygdala and the ventral pallidum, neural activity changed in response to stimulation, but there was either less or no change in the encoding of task-related information depending on the target structure. Ventral pallidal units remained remarkably responsive to the reward cues and the reward itself during stimulation; whereas, units in the central nucleus of the amygdala seemed to be less responsive to reward-related behavior. In the latter case it should be noted, that we were able to sample from only a small population of units. Future studies will be needed to verify this pattern from larger unit populations.

Although weaker, the study of Chapter 2 on the ventral pallidum still suggests a DBS effect. The ventral pallidum is a large structure with differences in reward processing seen along the anterior/posterior aspect and possible differences in medial/lateral and dorsal/ventral region (Beaver et al., 2006; Calder et al., 2007; Cromwell & Berridge, 1993; Panagis, Miliaressis, Anagnostakis, & Spyraki, 1995; Smith & Berridge, 2005; Smith et al., 2011). A more rigorous study assessing the effective location and stimulation parameters should be done before ruling out the efficacy of DBS in VP.

DBS in the central nucleus of the amygdala was extremely effective; pellet consumption and lever responding dropped dramatically (Chapter 3). Unfortunately, DBS also decreased hedonic value of sucrose and increased aversive reactions to all tastes making neutral tastes aversive and even making aversive tastes more aversive. These results suggest that DBS is so strong in creating taste aversion that already "disliked foods" would be disliked even more (Chapter 4).

Whether DBS in CeA should be entirely discounted is not clear. Our findings hint that different stimulation patterns such as, different rates of stimulation frequency, might be effectively "tuned" to achieve a desired effect. Twenty hertz stimulation did not increase aversive reactions to sucrose significantly. Investigating slightly lower frequencies, such as 16 Hz which we found to also decrease consumption (Chapter 3) and further tuning of other parameters, such as, pulse width and current levels may allow one to block "wanting" circuits while minimizing the aversive side effects of stimulation.

Finally, the CeA also has several projections from odor and disgust centers (Ottersen, 1982). Hence, another study could look at the effects of DBS on smell to also see if DBS could be creating this aversion by triggering feelings of disgust in general or producing the sensation of an unpleasant odor.

As mentioned above in section 5.2, further elucidation of specific mechanisms of action of DBS in the VP and CeA may be better determined by using more specific tools like optogenetics (Gradinaru et al., 2009) to selectively activate or inhibit certain pathways to see which connections result in decreased consumption with stimulation and/or aversive reactions (in the case of the CeA). Detailed modelling studies of typical cell types in the CeA and VP could also be done to further investigate the effects of DBS on neural components in these structures.

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# APPENDIX A

## A.1 Testing Chamber

(a)

(b)

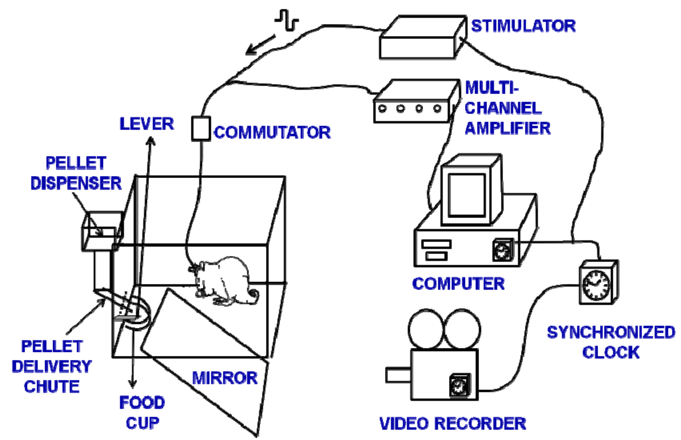



Figure A.1 - (a) Picture of testing chamber and (b) Diagram of chamber set-up. Similar set-up used for experiments in Chapters 2 and 3.

## A.2 Stimulation Artifact Removal Technique

### A.2.1 *Introduction*

Electrical stimulation induces artifacts unto the neural recording. These artifacts typically have an unrecoverable period during which the recording amplifiers are saturated followed by period of exponential decay (see example in figure A.2.1 which shows a schematic of a typical artifact). Several factors contribute to these artifacts including the recording electronics and capacitive crosstalk between recording and stimulating leads (McGill et al., 1982; Wagenaar & Potter, 2002). The non-linear behavior of saturated amplifiers and properties of filters also make the artifact last much longer than the stimulus pulse width, many times on the order of milliseconds (Maeda et al., 1995). The shape and duration of artifacts can also vary between channels and there

can be small fluctuations in the duration between pulses which makes artifact removal challenging.

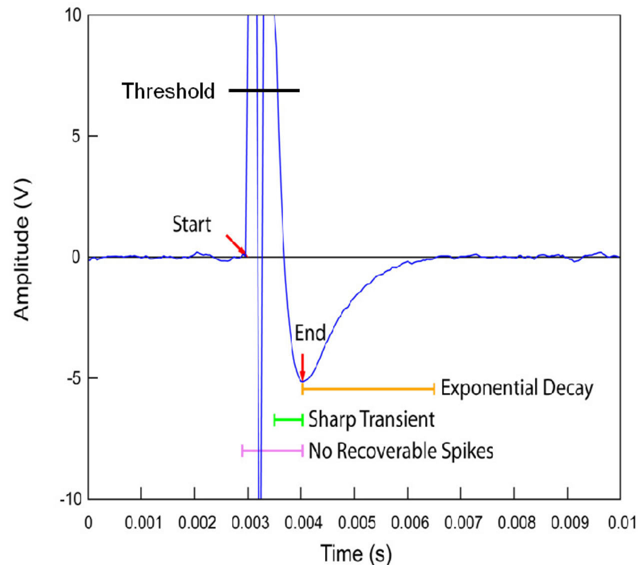



Figure A.2.1 - Diagram of a typical stimulation artifact and its characteristics. Zones indicate key regions that are manipulated in the artifact removal program.

Various hardware and software strategies have been utilized to reduce or even prevent artifacts from entering the system in the first place. However, there are limitations of these various methods especially depending on the needs of the experiment itself. For example, one strategy is to minimize the artifact by having the recording and stimulating site separated by several hundred microns apart (Grumet, Wyatt, & Rizzo, 2000). Other strategies include hardware blanking (Knaflitz & Merletti, 1988; Roby & Lettich, 1975), such as the sample and hold method in which the hardware circuits are held during the artifact period and do not sample any of the input signal at this time (Babb et al., 1978; Freeman, 1971; Jimbo, Tateno, & Robinson, 1999; Novak & Wheeler, 1988). However, these blanking techniques depend on a fixed interval between artifacts, and the duration of stimulus artifacts do vary even on the same channel (O'Keeffe, Lyons, Donnelly, & Byrne, 2001). Furthermore, mixed reports have been published about the effectiveness of these techniques (O'Keeffe et al., 2001; Wagenaar & Potter, 2002). Other techniques utilize software methods such as template subtraction, in which stimulation artifacts are averaged to create a template artifact which is then subtracted

from all the artifacts during the recorded period (Hashimoto, Elder, & Vitek, 2002; Montgomery E.B., Gale, & Huang, 2005; Wichmann, 2000). However, again this assumes that the artifact shape and duration remains constant across all trials and channels which is not always the case. Digital blanking, in which the artifact is set to zero, can also be utilized (O'Keeffe et al., 2001) but any action potentials occurring on the exponential decay signal is then also lost.

An effective technique to recover the exponential decay and address the issue on non-uniformity of artifact shapes is to perform a local polynomial curve fit at each artifact from the beginning of the exponential decay to a fixed point later in time. Wagenaar and Potter developed an algorithm that uses an asymmetric cubic polynomial fit to remove the sharp transient and exponential decay separately. This algorithm is called “Subtraction of Artifacts by Local Polynomial Approximation” or SALPA (Wagenaar & Potter, 2002). While this works during real time application due to decreased processing time, a more effective fit would be achieved by a single polynomial fit of only the exponential decay. Hence, in our artifact removal algorithm, we first find each sharp transient and remove it from the neural data then implement a full polynomial fit on the exponential decay region. We also utilize a higher degree of polynomial fit ($7^{th}$ degree compared to the $3^{rd}$ degree fit for SALPA) that improves the accuracy of the fit. With the algorithm that was developed, we were able to successful remove the stimulation artifacts and recover neural data.

### A.2.2 ***Methods and materials***

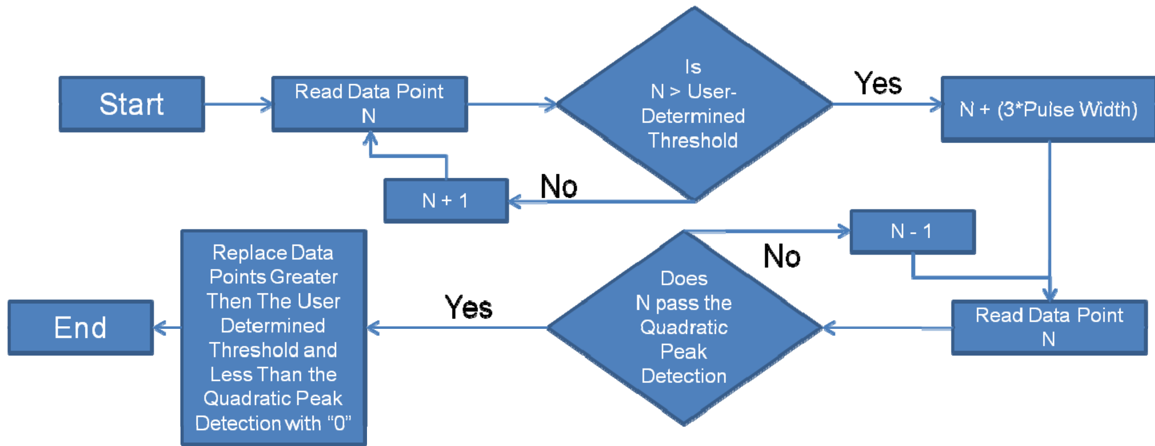
As mentioned earlier, there are slight perturbations in the timing between artifacts, for example, in Chapter 2’s study during 200 Hz stimulation in the ventral pallidum, each artifact pulse did not occur exactly 5 ms after the preceding artifact. Hence, we wanted to be able to scan through the neural data and find each artifact independently. To do so, we used a user-defined threshold (see black horizontal line indicated in figure A.2.1). Whenever the signal crosses this threshold, the program marks the time this happens as the start of the artifact (indicated by “Start” in figure A.2.1). This threshold value should be of large enough amplitude that it will be triggered by the artifact but not any spikes. Once the start of an artifact is marked, the program then

advances ahead by an interval three times the duration of the stimulus pulse width, which effectively moves the pointer beyond the exponential decay of the artifact.

From the end of the exponential decay of each stimulation artifact, the signal is scanned in reverse for the last major peak or valley, which is determined by fitting a quadratic function to a group of data points (chosen based on the rate of exponential decay). The vertex of each quadratic fit is tested against the user-determined threshold for detecting a peak or valley to make sure that the peak or valley is above the level of background noise. Once the last major peak or valley has been determined, that data point marks the end of the sharp transient period of the artifact (figure A.2.1: "End") and the beginning of the exponential decay. Every data point between the start of the artifact and the start of the exponential decay is set to 0 (i.e. the region delineated by the "Start" and "End" in figure A.2.1). This removes the section of the artifact that has a steep slope or has saturated the amplifier, during which time no recoverable data is available. A block diagram describing this part of the artifact removal algorithm is shown in figure A.2.2a.

The next phase is to fit the exponential decay region and bring it back to baseline. We implement a local polynomial fitting algorithm similar to SALPA (Wagenaar & Potter, 2002), though a $7^{th}$ degree polynomial was used to individually fit each artifact on all channels. This higher degree polynomial yielded a smoother fit than a lower degree polynomial. In addition, an odd number degree yields an extra degree of freedom (Wagenaar & Potter, 2002). The number of data points used to calculate this polynomial is 1 ms longer than the user-determined region of exponential decay. The best fit of the $7^{th}$ degree polynomial is optimized using Given's rotation algorithm (Halleck, 2007) to solve the linear least squares optimization through QR decomposition (Bretscher, 2005). The fitted waveform that is 1 ms longer than the user-determined region of the polynomial fit is then truncated to contain the length of the user-determined region of fit. The truncated fitted waveform is subtracted from the original waveform to yield the processed signal. The integer values for each data point of the processed signal are averaged. This average is then subtracted from each data point to give a y-axis shift of the processed signal towards zero. See figure A.2.2b for the block diagram of the second stage of the artifact removal process.

(a) Part One: Artifact Saturation Removal




(b) Part Two: Artifact Decay Attenuation

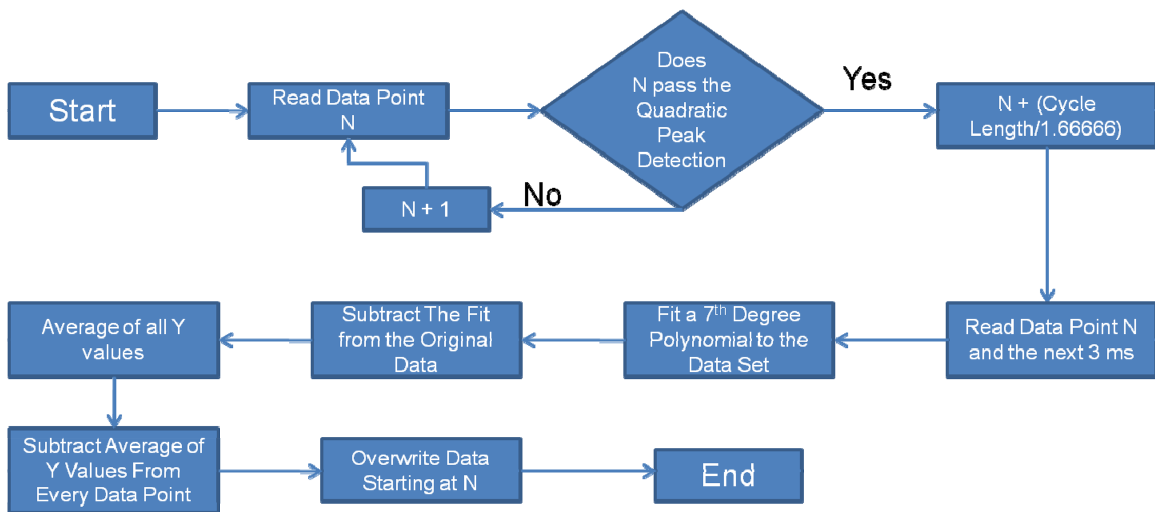



Figure A.2.2 – Block diagrams showing the algorithm for the artifact removal program. (a) Shows the steps involved in finding each artifact and zeroing out the sharp transients where the amplifiers are saturated and data is unrecoverable. (b) Shows the steps involved in fitting the exponential decay part of the artifact.

This algorithm was implemented in LabVIEW (National Instruments, Austin, TX). Figure A.2.3 shows the front panel of the artifact removal program. User-defined values such as the threshold to detect the start of the artifact, the threshold and number of data points used to detect a peak or valley, the type of fit to use, length of fit, degree of

polynomial, etc. can all be entered. Also, the artifact removal process can be viewed real time to assess how well the algorithm is working and whether parameters need to be adjusted.

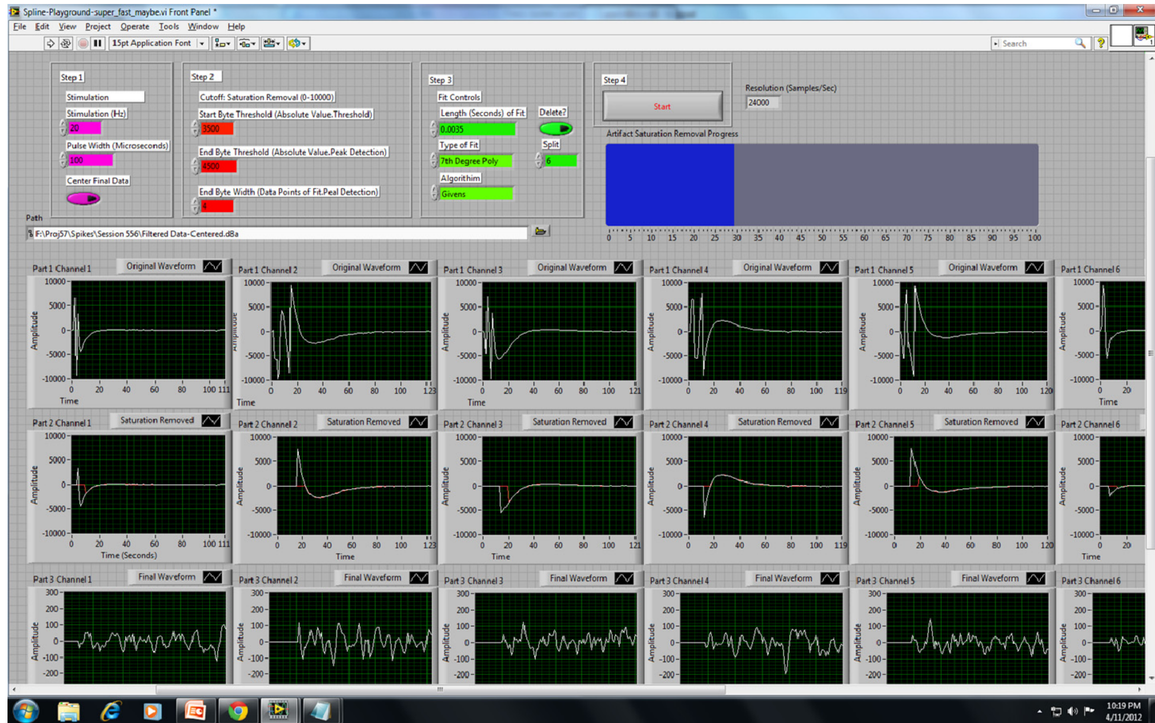



Figure A.2.3 – Snapshot of our artifact removal program. Various parameters can be entered in by the user. The progress of the artifact removal can also be viewed real time for each channel of recorded neural data.

To assess the quality of the artifact removal program, the number of spikes recovered after the amplifier saturated period before and after artifact removal was compared. Two high frequency (200 Hz) stimulation sessions and two low frequency (20 Hz) stimulation sessions from the ventral pallidum Pavlovian study done in Chapter 2 were used. The same user carefully sorted each of these sessions before and after the artifact was removed using the Offline Sorter program (Plexon, Inc., Dallas, TX). The number of spikes between artifact pulses and the latency from artifact onset to when spikes occurred were compared for each session before and after it was sorted.

### A.2.3 *Results of artifact testing*

We were able to successfully recover spike data and also recover spikes much earlier after artifact dead time after our sessions went through the artifact removal program. Figure A.2.4 shows snippets of a low frequency session and a high frequency

session after the artifact was removed. The pictures show action potentials that were riding on the exponential decay region, being successfully recovered after running through the artifact removal program.

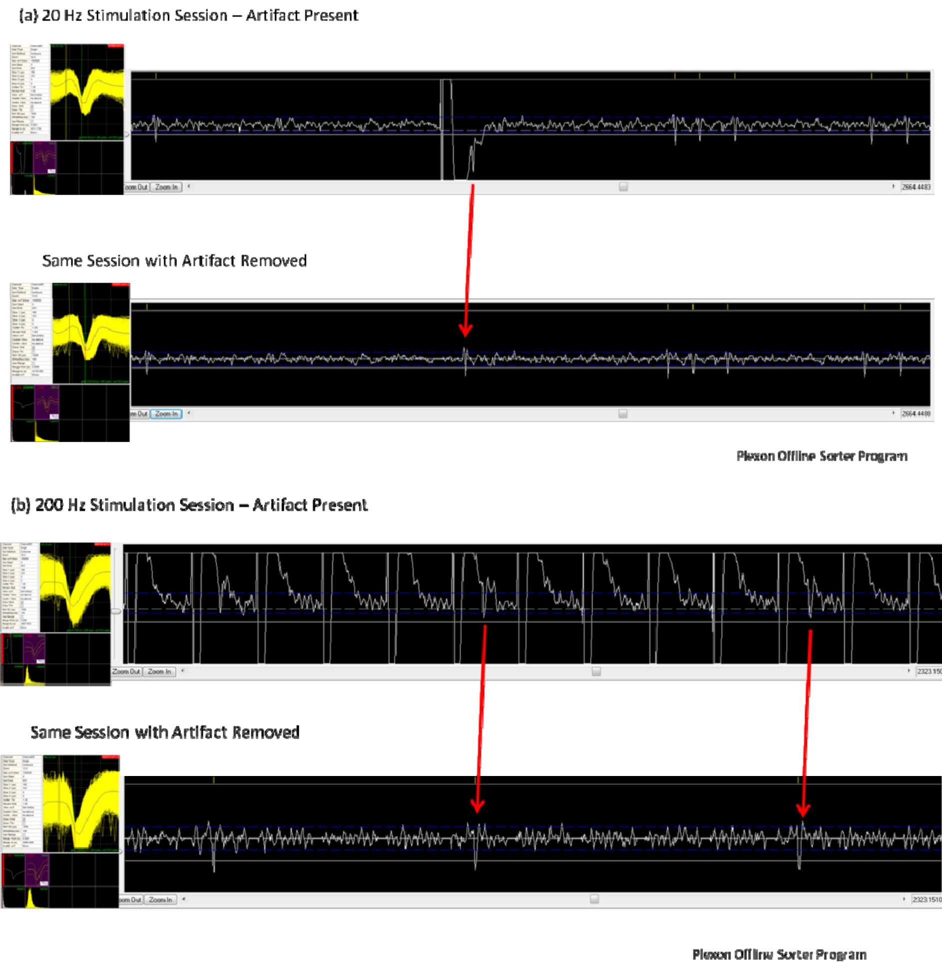



Figure A.2.4 – Snapshots of Offline Sorter sessions showing the same session sorted before and after artifact has been removed for the same time period. Each red arrow points from a given action potential in the session where the artifact was present to the same waveform after the artifact was removed. (a) 20 Hz stimulation session and (b) 200 Hz stimulation session.

Table A.2 summarizes the number of spikes recovered for each session after the stimulation artifact was removed. The table shows the total length of stimulation used in the analysis, the total number of spikes found, the total number of spikes recovered, and the percentage of recovery. Given that 200 Hz stimulation had much more pulses compared to 20 Hz, overall there was more time lost due to the stimulation artifact. Hence, the percentage of spikes recovered was greater for the 200 Hz sessions.

Table A.1 – Summary data showing the number of recovered spikes and % recovery after the stimulation artifact has been removed for each session.

| Rat ID | Stimulation | Stim Duration | Session | Total Found | Recovered | % Recovered |
|---|---|---|---|---|---|---|
| 51 | LFS | 1771 s | 442 | 7190 | 238 | 3.31% |
| 62 | LFS | 2099 s | 556 | 50035 | 3247 | 6.49% |
| 61 | HFS | 1945 s | 554 | 6486 | 2185 | 33.69% |
| 62 | HFS | 1952 s | 576 | 74168 | 36479 | 49.18% |

Figure A.2.5 shows the peristimulus time line graphs for the same session before and after stimulation artifacts were removed. Neural activity is lined up to each stimulation pulse onset for the duration of the time between each pulse. On the same plot is the peristimulus time graph for the original session and the same session after the stimulation artifact was removed. After running through the stimulation artifact program, more spikes were recovered and were recovered earlier after the artifact dead time for a given session. Spikes were recovered as early as 0.6 ms after stimulation pulse onset. Figure A.2.5a shows the peristimulus time graph for a sample low frequency session and figure A.2.5b shows the peristimulus time graph for a high frequency session.

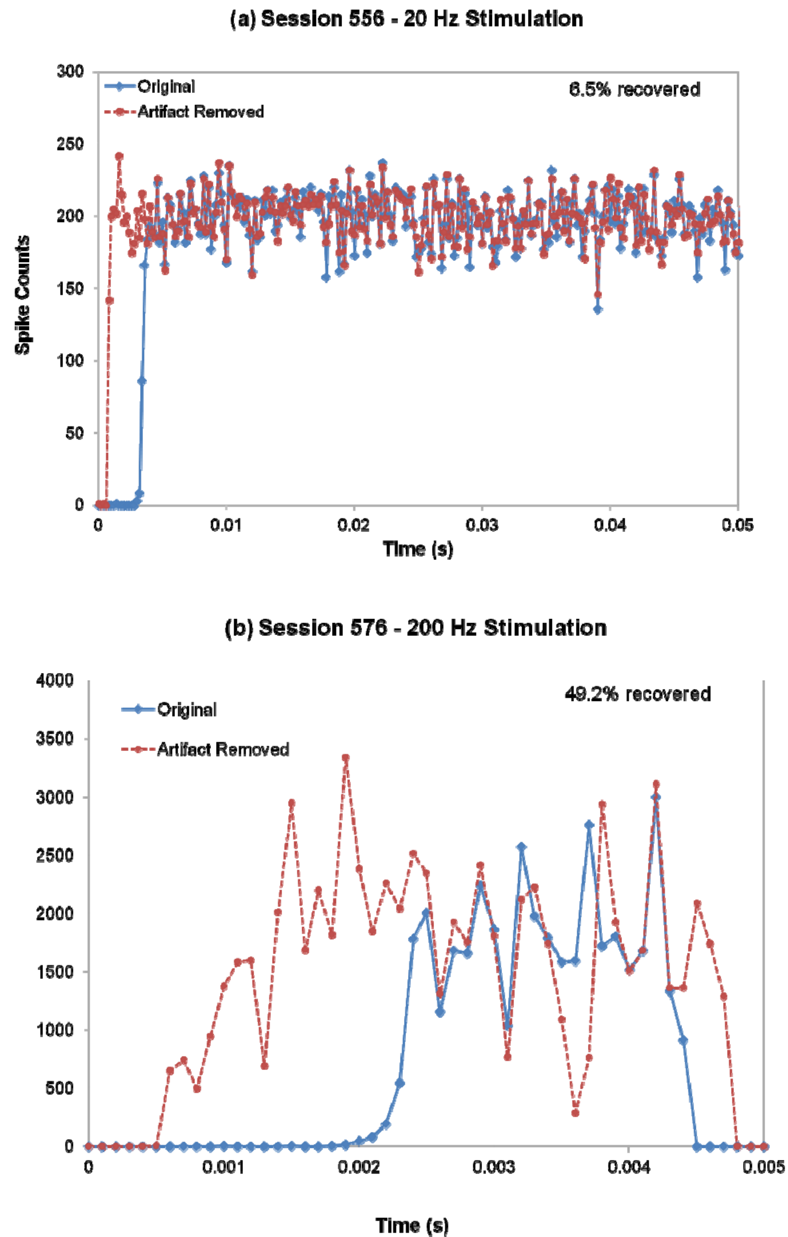



Figure A.2.5 – Peristimulus time line graphs for a sample low frequency stimulation session (a) and high frequency stimulation session (b) before and after removal of stimulation artifacts. Spike activity is lined to each stimulation pulse onset for the time between pulses. Y-axis is the number of spikes and x-axis is the time between pulses (this is 50 ms for 20 Hz stimulation and 5 ms for 200 Hz stimulation). The blue line is the original recorded session with the stimulation artifact present and the red graph is the same session after the stimulation artifact has been removed using our program.

We were able to successfully recover neural data after the stimulation artifact was removed. Our artifact removal program did not distort spike waveforms.

## A.3 **Chamber Set-up for Taste Reactivity Study**

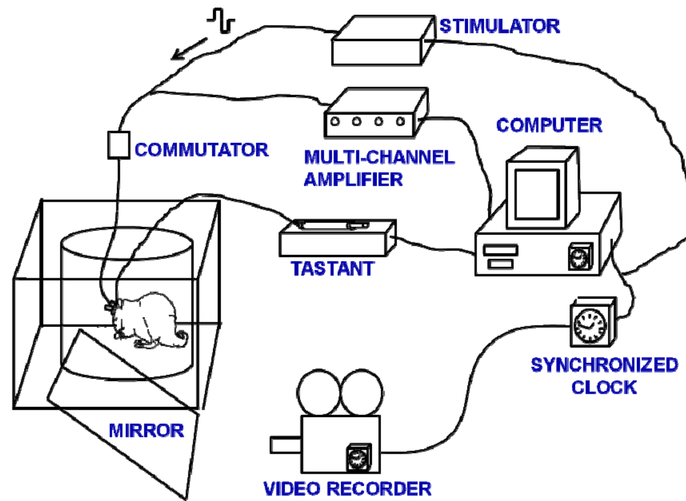



Figure A.3 - Diagram of chamber set-up for the taste reactivity study (Chapter 4). Animals were tested in the same chamber as from Chapters 2 and 3, but the levers and food dish were removed and a clear plastic tube was placed in the chamber. The rat was placed inside the tube. Also, a syringe connected to a computer- controlled pump delivered tastes to the rat via a plastic tubing that connected to an oral cannula on the rat's head.

A.4 **List of References**

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