NEURAL REPRESENTATION OF HEDONIC TASTES IN THE VENTRAL PALLIDUM

by

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PREFACE

Chapter 1 is a general introduction to the thesis. Background information that is relevant to more than one chapter was presented in Chapter 1, but not duplicated in the introductions of Chapters 2, 3, and 4 to minimize repetition. The introductions of Chapters 2, 3, and 4 contain background information and goals specific each chapter. Chapter 5 is an overall discussion for the thesis so it will have some components from previous chapter discussions, but will also attempt to compare findings from the different chapters and outlines potential future work. Chapters 2, 3, and 4 are being prepared for manuscript submission.

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ABSTRACT

The overall goal of this thesis was to investigate how the ventral pallidum tracks experimentally-induced changes in the hedonic value of tastes. Taste reactions allows us to measure how 'liked' or 'disliked' a taste is by a rat. These reactions are not purely reflexive as they are altered when circumstances change: hunger can increase and satiety can decrease the tastiness of foods (Berridge 1996) and a salt appetite can make a normally 'disliked' salt solution 'liked' (Berridge et al. 1984, Schulkin 1991). Therefore taste reactions reflect the palatability of tastes. Understanding how the reward pathway tracks changes in the hedonic value of tastes may elucidate how neural coding is affected in disorders such as drug addiction and obesity.

The first project investigated how neurons in the ventral pallidum (VP) track changes in hedonic value of a sweet taste by a conditioned taste aversion. When a taste changed from 'liked' to 'disliked', the predominant VP response also changed: taste responsive VP cells tastes typically increase firing rate in response to 'liked' tastes whereas the hedonically devalued taste typically triggered a decrease in firing rate. The second project compared the effects of dopamine and opioid modulation of 'liked', relatively neutral, and 'disliked' taste stimuli. We found that opioid modulation enhanced the hedonic value of quinine (which is normally 'disliked'), but that only morphine resulted in a VP population response that mirrored the hedonic value of the tastes. The third project tested the effects of dopamine and opioid modulation on the

hedonic value of a pair of cues (water infusions) and reward (a sucrose infusion).

Overall, the cues did increase in hedonic value via association with reward, and the VP

reflected the similarity in hedonic value between the cues and reward by responding

similarly to cues and reward, under most drug conditions. Amphetamine decreased the

proportion of units that responded to the first cue with an increase in firing rate, relative

to vehicle control. In summary, the VP tracked changes in hedonic value under a variety

of experimental manipulations, supporting evidence of its role in the neural coding of

hedonia.

CHAPTER 1
INTRODUCTION

Overview

The hedonic value of a taste, or how much a taste is 'liked', can be altered by experimental manipulations. Changes in motivation and learned associations can make a taste that was once 'liked' become 'disliked, and vice versa. Furthermore, the hedonic impact of natural rewards, such as pleasant tastes, can be acquired by cues that predict the delivery of such rewards. Altered neural coding of the hedonic value of tastes or cues for tastes might contribute to disorders like obesity.

Behavioral measures of 'liking'

'Liking' is generally experienced as a subjective emotion. While we can ask adult humans to describe or rate their feelings, as experimenters we need a clear behavioral expression to measure hedonics in non-verbal animals such as rats. We are able to measure the motivational value of a stimulus to an animal by quantifying approach, interaction, pursuit, etc. However, measuring purely the hedonic value, and not the motivational value, of most stimuli in a rat is not easy. We do not have reliable ways to measure the 'liking' of visual, auditory, tactile, or olfactory stimuli that do not include some aspect of motivational value. However, many species, including human infants, old and new world primates, and rodents exhibit stereotyped hedonic and aversive facial reactions to pleasant and unpleasant tastes (Figure 1.1).

Adult humans generally learn to exhibit control over reactions to tastes, but an infant will lick its lips in a rhythmic manner (this behavior has been categorized as "tongue protrusions") when a given a drop of sugar water on its tongue, even before their first feeding, just hours after birth, before any postnatal learning about tastes can occur (Steiner, 1973; Steiner and Glaser, 1995). In other words, these taste reactions are instinctual rather than acquired. Very different behaviors are seen in response to a bitter taste like quinine, such as a gaping of the mouth or shaking of the head, which is an indication of an aversive taste reaction. Other mammalian species show similar 'liking' reactions to sweet tastes and 'disliking' to bitter tastes (Steiner and Glaser, 1995; Steiner et al., 2001). Rats, like human infants, display tongue protrusions when presented with a sweet taste in the mouth, although the speed of taste reactions is roughly inversely proportional to the size of the animal. Primates and human infants have a slower rhythm than rodents, but if you slow a video of rodent reactions down, the behaviors are remarkably similar. Additional hedonic reactions in rats include paw licking and slower non-rhythmic licks that go further to the side than the tongue protrusions (lateral licks). Rats, like infants, display aversive gaping reactions and head shakes in response to an aversive bitter taste. Rats also show face washing, forelimb flails, and chin rubbing in response to 'disliked' tastes (Grill and Norgren 1978, Grill and Berridge 1985, Berridge 2000).

While these reactions do not need to be learned, they are at the same time not purely reflexive. These affective taste reactions are altered when circumstances change. Hunger or satiety can increase or decrease the hedonic value of a sweet taste (Berridge 1996). A salt solution that is triple the concentration of sea water is aversive to humans

and rats, but if a rat is physiologically depleted of salt by hormone injections, the ensuing 'salt appetite' elicits hedonic reactions to the same salty taste (Berridge et al. 1984, Schulkin 1991). Additionally, microinjections of drugs into various brain areas, lesions, and psychological manipulations (taste associations or learned taste aversions) are able to manipulate palatability (Peciña et al. 2006, Grill and Norgren 1978, Cromwell and Berridge 1993, Pfaffmann et al. 1977, Berridge 2000). Therefore tastes reactions reflect more than mere sensory properties. Rather, they reflect the palatability of tastes, i.e. whether the taste is 'liked' or 'disliked'.

Neural representation of 'liking'

Researchers can record neural activity sites involved in 'liking' to investigate how the brain encodes information about the hedonic value of tastes. Many aspects of neuronal activity could contribute to a neurophysiological code for 'liking'. Historically, one of the most common measures of neural responses is the frequency of action potentials (neuronal firing rates) evoked by a stimulus. 'Liking' might be represented as an increase or decrease in firing rate of single neurons compared to a pre-stimulus baseline period. Or it could be represented as a population response, via the percentage of neurons recorded that respond with a change in firing rate or the proportions of different type of responses. There could be differences in the duration of the firing rate response (phasic vs. tonic responses) or the latency of the firing response (how quickly a neuron responds to stimulus). Furthermore, there could be a difference in the pattern of firing (tonic vs. bursty). Neurons might encode 'liking' information in ways other than firing rates, such as local field potential oscillations or action potential waveform shapes. Electrophysiology in awake behaving animals was used in Chapters 2 through 4, in

conjunction with intra-oral delivery of liquid tastants, to assess how single neurons

encode 'liking'.   In Chapter 2, the psychological manipulation of taste aversion learning

is used to investigate how the ventral pallidum (VP) encodes a shift in hedonic value. In

Chapters 3 and 4, we investigated the effect of dopamine and opioid modulation on

'liking'. In Chapter 4 we used classical conditioning to investigate how reward

association alters the 'liking' of cues. Following prior studies, firing rate is used as the

principal measure for neural encoding of reward throughout these experiments.

The Ventral Pallidum (VP)

A site of convergence for the reward pathway

The ventral pallidum (VP) integrates and processes reward information flowing

through the limbic system (Kelley et al. 2005, Napier and Mickiewicz 2010, Richard et

al. 2012, Smith et al. 2009). Figure 1.2 diagrams the main VP connections while Figure

1.3 diagrams the taste pathway inputs to the VP. There are reciprocal connections to the

nucleus accumbens (NAcc), the ventral tegmental area, prefrontal cortex, orbital frontal

cortex, infralimbic cortex, amygdala, lateral hypothalamus, and parabrachial nucleus

(Bourdelais and Kalivas 1990, Carnes et al. 1990, Chrobak and Napier 1993, Churchill

and Kalivas 1994, Fuller et al.1987, Groenewegen and Berendse 1990, Groenewegen et

al. 1993, Grove 1988 a and b, Haber et al. 1985, Johnson and Napier 1997, Kalivas and

Nakamura 1999, Klitenick at al. 1992, Maurice et al. 1997, Mitrovic and Napier 1998,

Napier et al. 1991, Olive and Maidment 1998 a and b, Phillipson and Griffiths 1985,

Reep and Winans 1982, Saper and Loewy 1980, Turner et al. 2001, Usuda et al. 1998,

Zaborszky et al.1997, Zahm 1989, Zahm et al. 1985 and 1996). The NAcc invervates the

VP heavily with GABAergic and enkephalinergic outputs. The VP in turn sends

GABAergic efferents back to the NAcc, creating a negative feedback loop (Churchill and Kalivas 1994, Hakan et al. 1992, Hakan and Eyl 1995, Heimer and Wilson 1975, Napier and Mitrovic 1999, Phillipson and Griffiths 1985, Usuda et al. 1998, Zahm et al. 1985, Zahm 2000).

VP projections additionally target preoptic regions, the mediodorsal nucleus of the thalamus to connect to limbic cortex and cortico-basal ganglia-thalamocortical loops , basal ganglia nuclei such as the subthalamic nucleus and substantia nigra , and brainstem motor nuclei like pedunculopontine nucleus to translate reward signals into behavioral action (Churchill et al. 1996, Groenewegen et al. 1993, Grove 1988 a and b, Mogenson et al. 1980). 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). In sum, the VP is in an anatomical position to be a principle player for reward functions and evidence suggests that the VP is necessary for normal 'liking' (Crowell and Berridge 1993).

Hedonia and motivation in the VP

Previous studies indicate that VP neurons encode and represent 'liking' and 'wanting' (motivation) by tracking changes in the hedonic values of tastes or the incentive salience of Pavlovian cues (Tindell et al. 2004, 2005 a and b, and 2006; Smith and Berridge 2005 and 2007). VP neurons respond to taste rewards and cues that predict delivery of such rewards with an average increase in firing rate compared to baseline

activity (Tindell et al., 2004), providing evidence that they might encode 'liking' and 'wanting'. VP neurons fired in response to an orally infused sucrose taste that was 'liked', but did not respond to an intensely salty (3X seawater concentration) taste (Tindell et al., 2006). However, after animals were salt depleted, the hedonic value of the salt solution flipped from 'disliked' to 'liked' and the salt solution now evoked a large firing rate increase, equal to the sucrose response. In other words, the VP response represented the hedonic value rather than the identity of the taste. Another study looked at the VP coding of Pavlovian cues that predicted salt or sucrose (Tindell et al. 2005a). Once a tone was associated with sucrose delivery, it triggered an increase in firing rate in VP units. The tone associated with salt had almost no VP response while the rat 'disliked' salt. After sodium depletion, when both salt and sucrose were 'liked', the salt and sucrose tones now equally evoked a firing rate response from VP neurons. The neurons thus tracked the change in incentive salience of the salt cue as salt became rewarding as well as the hedonic impact of a taste ('liking'). A recent human study has shown that bilateral VP activity was correlated with the subject's rating of the inferred pleasantness food images (Simmons et al. 2013). Beyond natural rewards, rats will develop a place preference for intra-VP cocaine infusions (Gong et al. 1996). Animals will also work to electrically stimulate the VP (Murray and Shizgal 1996, Panagis et al. 1995).

Summary of Aims and Hypotheses

The overall goal of this thesis was to investigate how the ventral pallidum tracks experimentally-induced changes in the hedonic value of tastes. The following three main

projects were carried out and were written up in Chapters 2, 3, and 4 of this thesis, respectively:

Project 1 (Chapter 2) – Ventral Pallidal Coding of Taste Aversion Learning

In this project we examined the neural activity and behavioral effect of a learned taste aversion, induced by pairing nausea-inducing LiCl injections with sweet tastes that are normally pleasant, while simultaneously recording facial reactions and neural activity in the VP. We replicated prior behavioral findings and extended them to investigate how the reward pathway tracks changes in hedonic value. One sweet taste started out 'liked' before being paired with LiCl injections and became 'disliked' after aversion learning. A control taste that was never paired with nausea remained 'liked' throughout the experiment, indicating that we induced a taste aversion to a specific sweet taste.

We hypothesized that the conversion of the hedonic value of a taste from 'liked' to 'disliked' would be reflected in the neural activity in the ventral pallidum (VP). We also hypothesized that the VP activity would not be altered if a taste remained 'safe' throughout the experiment. Prior to aversion learning the 'liked' tastes elicited significantly more increases in firing rate in VP cells than decreases in rate. After aversion learning, the sweet taste elicited significantly more decreases in firing rate than increases.

Project 2 (Chapter 3) – Ventral Pallidal Coding of TasteStimuli

This project was entirely novel from previous work in that for the first time I describe how dopamine and opioid modulation affects the hedonic value of a variety of tastes as well as how the VP responds to tastes under these drug conditions. We used

systemic administration of amphetamine and haloperidol to alter dopamine transmission and morphine and naltrexone to alter opioid activity. We also tested tastes that in control animals had different hedonic valences: sucrose which was 'liked', water which elicited a mixture of hedonic and aversive reactions, and quinine which was 'disliked'. We hypothesized that morphine would increase the hedonic value of tastes (quinine in particular, since it usually has the lowest hedonic value and therefore is less likely to encounter a ceiling effect), and that naltrexone would increase the aversive reactions to tastes (sucrose in particular, since it generally elicits the least aversive reactions. We hypothesized that dopamine modulation would have no effect on the hedonic values of tastes.

We found that morphine increased 'liking' reactions to quinine as hypothesized. Morphine was also the only drug condition that showed a VP response that mirrored the hedonic value of the three tastes. Naltrexone increased 'liking' of quinine but did not induce a population response that differentiated between the three tastes. As hypothesized, dopamine modulation did not significantly alter the hedonic values of tastes.

Project 3 (Chapter 4) – Ventral Pallidal Coding of Taste Cues and Rewards

This project used the same drug manipulations as project 2 above, but investigated whether these drugs affect how cues take on hedonic value via association with rewards. We used a sucrose solution as the reward, and a pair of water infusions as sequential cues to see if cues proximal to reward take on more hedonic value than cues distal to reward. We hypothesized that cues would acquire hedonic value though association with pleasant rewards. We hypothesized that morphine would boost this

attribution of hedonic value to cues (the second cue in particular, since it is most proximal to reward) and that naltrexone would decrease the attribution of hedonic value to cues. We hypothesized that amphetamine and haloperidol would have no effect on the hedonic value of the cues or rewards, and therefore there would be no effect on the VP response to those stimuli. As hypothesized, the cues did gain hedonic value by reward association in that they elicited as many hedonic reactions as the rewards themselves in most conditions (compared to the water infusions that were not associated with reward in experiment 2 which elicited fewer hedonic reactions than sucrose infusions).

Figures
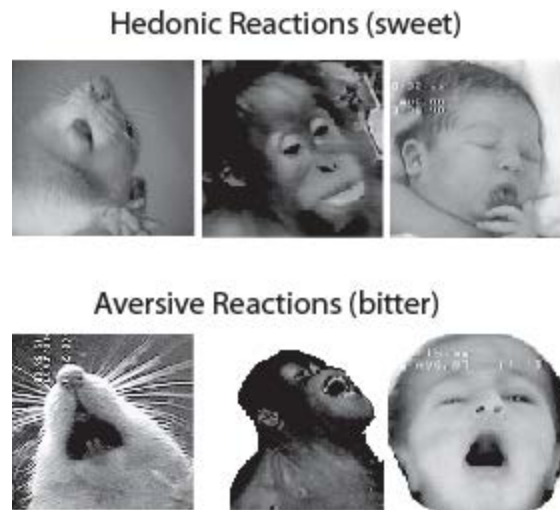
Figure 1.1 Examples of 'liking' taste reactions and 'disliking' in rodents, primates, and human infants. All species are doing homologous rhythmic tongue protrusions (an example of a hedonic reaction) to a sweet solution in the top row and homologous gapes (an example of an aversive reaction) to a bitter taste in the bottom row. Image modified from Berridge 2000 and Kyle Smith's Dissertation (Berridge, 2000; Smith, 2007).
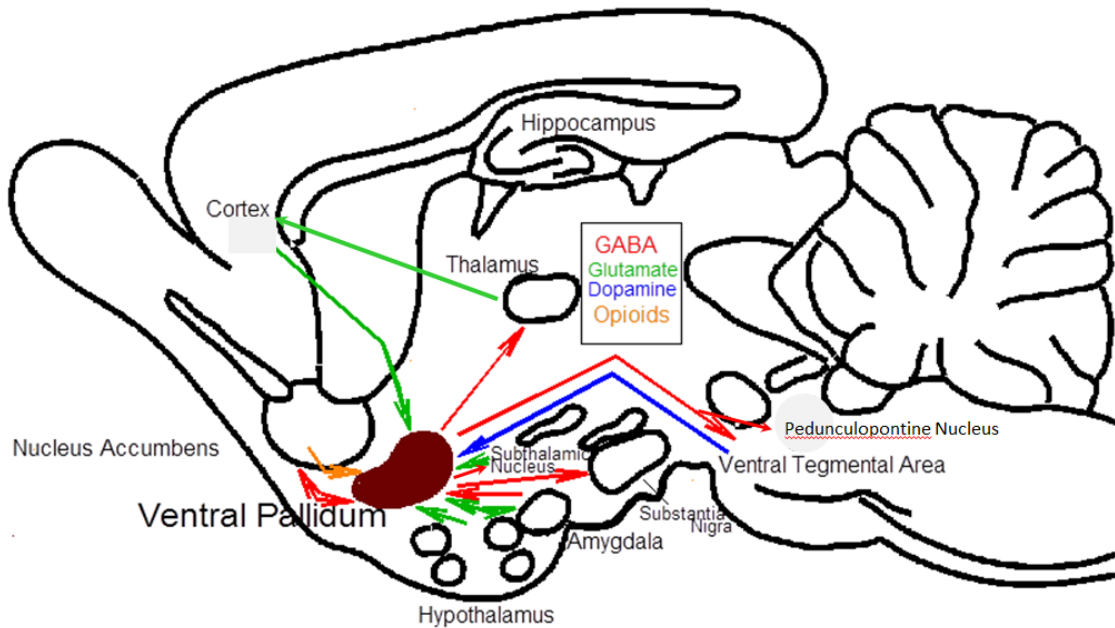
Figure 1.2 Sagittal view of a rat brain highlighting major reward pathway connections of the ventral pallidum. The ventral pallidum (VP) receives input from the amygdala (AMYG), nucleus accumbens (NAc), prefrontal cortex (PFC), and ventral tegmental area (VTA). The VP projects back to NAc, VTA, and AMYG. It also projects to thalamus (Thal), subthalamic nucleus (STN), substantia nigra (SN), and brainstem motor nuclei like pedunculopontine nucleus (PPN). Green lines represent glutamatergic projections, red lines represent GABAergic projections, blue lines represent dopaminergic projections, and the orange line represents opioid projections. Image modified from Amy Tindell's Dissertation (Tindell 2005).
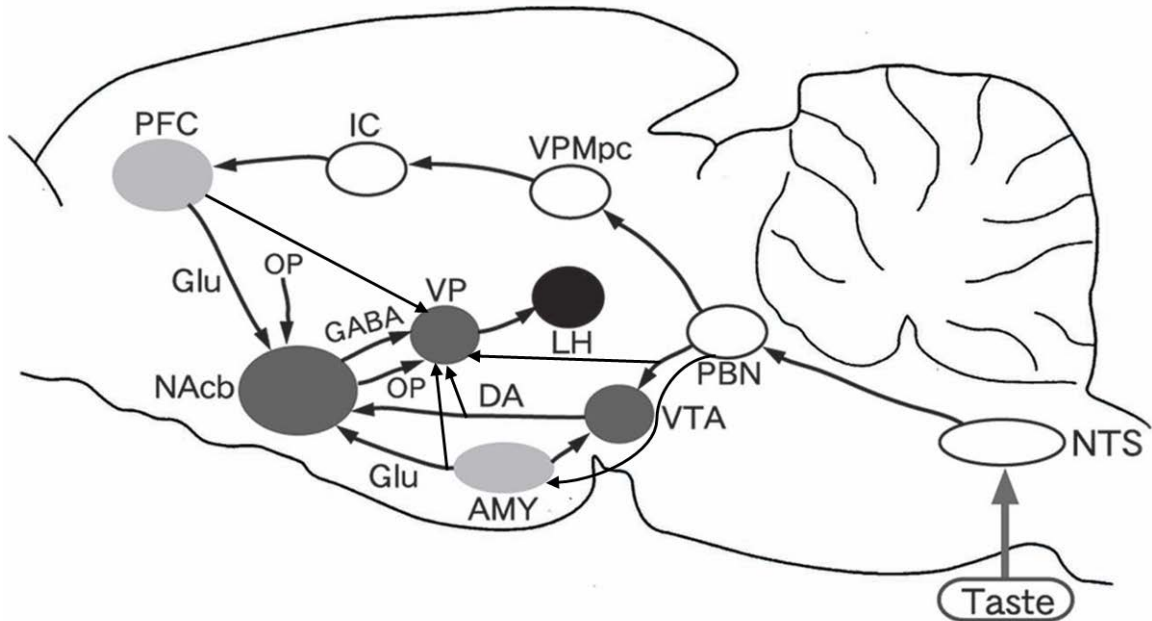
Figure 1.3 Sagittal view of a rat brain highlighting major taste pathway projections to the ventral pallidum (VP). Taste information travels from the cranial nerves (VII, IX, and X) to the nucleus of the tractus solitarus (NTS) and on to the parabrachial nucleus (PBN). From the PBN there are several routs to the VP: sparse projections from the PBN itself and indirect dopaminergic projections from the ventral tegmental area (VTA), GABAergic and opioid projections from the nucleus accumbens (NAcb), and glutamatergic projections from prefrontal cortex (PFC). Image modified from Yamamoto and Ueji 2011, Saper and Lowey 1980, and Kyle Smith's Dissertation (Smith 2007).

Reference List

 Berridge KC (1996) Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev 20:1-25.

Berridge KC (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. Neurosci Biobehav Rev 24:173-198.

Berridge KC, Flynn FW, Schulkin J, Grill HJ (1984) Sodium depletion enhances salt palatability in rats. Behav Neurosci 98:652-660.

Bourdelais A, Kalivas PW (1990) Amphetamine lowers extracellular GABA concentration in the ventral pallidum. Brain Res 516:132-136.

Carnes KM, Fuller TA, Price JL (1990) Sources of presumptive glutamatergic/aspartatergic afferents to the magnocellular basal forebrain in the rat. J Comp Neurol 302:824-852.

Chrobak JJ, Napier TC (1993) Opioid and GABA modulation of accumbens-evoked ventral pallidal activity. J Neural Transm Gen Sect 93:123-143.

Churchill L, Kalivas PW (1994) A topographically organized gamma-aminobutyric acid projection from the ventral pallidum to the nucleus accumbens in the rat. J Comp Neurol 345:579-595.

Churchill L, Zahm DS, Kalivas PW (1996) The mediodorsal nucleus of the thalamus in rats--I. forebrain gabaergic innervation. Neuroscience 70:93-102.

Cromwell HC, Berridge KC (1993) Where does damage lead to enhanced food aversion: the ventral pallidum/substantia innominata or lateral hypothalamus? Brain Res 624:1-10.

Fuller TA, Russchen FT, Price JL (1987) Sources of presumptive glutamergic/aspartergic afferents to the rat ventral striatopallidal region. J Comp Neurol 258:317-338.

Gong W, Neill D, Justice JB, Jr. (1996) Conditioned place preference and locomotor activation produced by injection of psychostimulants into ventral pallidum. Brain Res 707:64-74.

Grill HJ, Norgren R (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Research 143:263-279.

Grill HJ, Berridge KC (1985) Taste reactivity as a measure of the neural control of palatability. In: Progress in Psychobiology and Physiological Psychology (Sprague JM, Epstein AN, eds), pp 1-61. Orlando: Academic Press.

Groenewegen HJ, Berendse HW (1990) Connections of the subthalamic nucleus with ventral striatopallial parts of the basal ganglia in the rat. *The Journal of Comparative Neurology, 294*(4), 607-622. doi:10.1002/cne.902940408

Groenewegen HJ, Berendse HW, Haber SN (1993) Organization of the output of the ventral striatopallidal system in the rat: ventral pallidal efferents. Neuroscience 57:113-142.

Grove EA (1988a) Efferent connections of the substantia innominata in the rat. J Comp Neurol 277:347-364.

Grove EA (1988b) Neural associations of the substantia innominata in the rat: afferent connections. J Comp Neurol 277:315-346.

Haber SN, Groenewegen HJ, Grove EA, Nauta WJ (1985) Efferent connections of the ventral pallidum: evidence of a dual striato pallidofugal pathway. J Comp Neurol 235:322-335.

Hakan RL, Berg GI, Henriksen SJ (1992) Electrophysiological evidence for reciprocal connectivity between the nucleus accumbens septi and ventral pallidal region. Brain Res 581:344-350.

Hakan RL, Eyl C (1995) Neuropharmacology of the nucleus accumbens: iontophoretic applications of morphine and nicotine have contrasting effects on single-unit responses evoked by ventral pallidal and fimbria stimulation. Synapse 20:175-184.

Heimer L, Wilson RD (1975) The subcortical projections of allocortex: similarities in the neural associations of hte hippocampus, the periform cortex and the neocortex. in M Santini (Ed), Golgi centennial symposium proceedings (pp 173-193) New York: Raven Press.

Johnson PI, Napier TC (1997) GABA- and glutamate-evoked responses in the rat ventral pallidum are modulated by dopamine. Eur J Neurosci 9:1397-1406.

Kalivas PW, Nakamura M (1999) Neural systems for behavioral activation and reward. Curr Opin Neurobiol 9:223-227.

Kelley AE, Schiltz CA, Landry CF (2005) Neural systems recruited by drug- and food-related cues: studies of gene activation in corticolimbic regions. Physiol Behav 86:11-14.

Klitenick MA, Deutch AY, Churchill L, Kalivas PW (1992) Topography and functional role of dopaminergic projections from the ventral mesencephalic tegmentum to the ventral pallidum. Neuroscience 50:371-386.

Mitrovic I, Napier TC (1998) Substance P attenuates and DAMGO potentiates amygdala glutamatergic neurotransmission within the ventral pallidum. Brain Res 792:193-206.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69-97.

Morgane PJ (1961) Alterations in feeding and drinking behavior of rats with lesions in globi pallidi. Am J Physiol 201:420-428.

Murray B, Shizgal P (1996) Attenuation of medical forebrain bundle reward by anterior lateral hypothalamic lesions. Behav Brain Res 75:33-47.

Napier TC, Mickiewicz AL (2010) The role of the ventral pallidum in psychiatric disorders. *Neuropsychopharmacology, 35*(1), 337.

Napier TC, Mitrovic I (1999) Opioid modulation of ventral pallidal inputs. Annals of the New York Academy of Sciences 877:176-201.

Napier TC, Muench MB, Maslowski RJ, Battaglia G (1991) Is dopamine a neurotransmitter within the ventral pallidum/substantia innominata? Adv Exp Med Biol 295:183-195.

Olive MF, Maidment NT (1998a) Opioid regulation of pallidal enkephalin release: bimodal effects of locally administered mu and delta opioid agonists in freely moving rats. J Pharmacol Exp Ther 285:1310-1316.

Olive MF, Maidment NT (1998b) Repeated heroin administration increases extracellular opioid peptide-like immunoreactivity in the globus pallidus/ventral pallidum of freely moving rats. Psychopharmacology (Berl) 139:251-254.

Panagis G, Miliaressis E, Anagnostakis Y, Spyraki C (1995) Ventral pallidum self-stimulation: a moveable electrode mapping study. Behav Brain Res 68:165-172.

Peciña S, Smith KS, Berridge KC (2006) Hedonic hot spots in the brain. Neuroscientist 12:500-511.

Pfaffmann, C. and Norgren, R. (1977) Sensory Affect and Motivation. Annals of the New York Academy of Sciences, 290: 18–34.

Phillipson OT, Griffiths AC (1985) The topographic order of inputs to nucleus accumbens in the rat. Neuroscience 16:275-296.

Reep RL, Winans SS (1982) Efferent connections of dorsal and ventral agranular insular cortex in the hamster, Mesocricetus auratus. Neuroscience 7:2609-2635.

Richard JM, Castro DC, Difeliceantonio AG, Robinson MJ, Berridge KC (2012) Mapping brain circuits of reward and motivation: In the footsteps of Ann Kelley. *Neurosci Biobehav Rev,* doi: 10.1016/j.neubiorev.2012.12.008

Saper SB, Loewy AD (1980) Efferent connections of the parabrachial nucleus in the rat. Brain Res 197:291-317.

Schulkin J (1991) Sodium Hunger: the Search for a Salty Taste. New York: Cambridge University Press.

Simmons WK, Rapuano KM, Ingeholm JE, Avery J, Kallman S, Hall KD, Martin A (2013) The ventral pallidum and orbitofrontal cortex support food pleasantness inferences. Brain Struct Funct. 2013 Feb 9. [Epub ahead of print]

Smith, K. S., & Berridge, K. C. (2005). The ventral pallidum and hedonic reward: Neurochemical maps of sucrose "liking" and food intake. *The Journal of Neuroscience, 25*(38), 8637-8649.

Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: Interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *The Journal of Neuroscience, 27*(7), 1594-1605.

Smith, K. S., Berridge, K. C., & Aldridge, J. W. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proc Natl Acad Sci U S A, 108*(27), E255-E264.

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behav Brain Res, 196*(2), 155-167.

Steiner JE (1973) The gustofacial response: observation on normal and anencephalic newborn infants. Symposium on Oral Sensation and Perception 4:254-278.

Steiner JE, Glaser D (1995) Taste-induced facial expressions in apes and humans. Human Evolution 10:97-105.

Steiner JE, Glaser D, Hawilo ME, Berridge KC (2001) Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. Neurosci Biobehav Rev 25:53-74.

Tindell AJ, Berridge KC, Aldridge JW (2004) Ventral pallidal representation of pavlovian cues and reward: population and rate codes. J Neurosci 24:1058-1069.

Tindell AJ, Smith KS, Berridge KC, Aldridge JW (2005a) VP Neurons Integrate Learning and Physiological Signals to Code Incentive Salience of Conditioned Cues. Society for Neuroscience Abstracts.

Tindell AJ, Berridge KC, Zhang J, Pecina S, Aldridge JW (2005b) Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. Eur J Neurosci 22:2617-2634.

Tindell AJ, Smith KS, Peciña S, Berridge KC, Aldridge JW (2006) Ventral pallidum firing codes hedonic reward: when a bad taste turns good. J Neurophysiol 96:2399-2409.

Turner MS, Lavin A, Grace AA, Napier TC (2001) Regulation of limbic information outflow by the subthalamic nucleus: excitatory amino acid projections to the ventral pallidum. J Neurosci 21:2820-2832.

Usuda I, Tanaka K, Chiba T (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. Brain Research 797:73-93.

Yamamoto T, Ueji K (2011) Brain mechanisms of flavor learning. Frontiers in Systems Neuroscience 5:1-7.

Zaborszky L, Gaykema RP, Swanson DJ, Cullinan WE (1997) Cortical input to the basal forebrain. Neuroscience 79:1051-1078.

Zahm DS (1989) The ventral striatopallidal parts of the basal ganglia in the rat--II. Compartmentation of ventral pallidal efferents. Neuroscience 30:33-50.

Zahm DS (2000) An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neuroscience & Biobehavioral Reviews 24:85-105.

Zahm DS, Williams E, Wohltmann C (1996) Ventral striatopallidothalamic projection: IV. Relative involvements of neurochemically distinct subterritories in the ventral pallidum and adjacent parts of the rostroventral forebrain. J Comp Neurol 364:340-362.

Zahm DS, Zaborszky L, Alones VE, Heimer L (1985) Evidence for the coexistence of glutamate decarboxylase and Met-enkephalin immunoreactivities in axon terminals of rat ventral pallidum. Brain Res 325:317-321.

CHAPTER 2

VENTRAL PALLIDAL CODING OF TASTE AVERSION

LEARNING

Abstract

Pairing nausea-inducing LiCl injections with tastes that are normally pleasant suffices to induce a learned taste aversion that flips ingestion responses to 'disliking' reactions. We used two different sweet, caloric tastes in this experiment. One was never paired with aversion, and remained a 'safe' control taste (CS-) throughout the experiment. The other taste was 'liked' the first time it was experienced, but subsequent pairings with LiCl diminished the hedonic value of this taste over time (CS+). We recorded the neural activity in the ventral pallidum (VP) for each taste, before and after aversion training, and found that the population response to tastes that were hedonic was dominated by increases in firing rate, but after aversion learning reversed the hedonic value of the CS+, the predominate VP response was a decrease in firing rate.

Introduction

Learned Taste Aversions

The hazards of injecting a toxin are often not discovered until a relatively long period

of time has elapsed, potentially on the order of hours. An animal must decide whether or

not to ingest a substance based on whether or not the food previously resulted in

perceived toxicity. A biological solution to this problem is to develop a taste aversion for

any food that is toxic. A huge variety of species have shown experimentally induced taste aversion learning: many mammals, birds, fish, and reptiles; and a taste aversions have occurred to a wide range of foods and liquids across all taste categories (Garcia et al. 1974). Stronger tastes tend to result in stronger aversions. Likewise the severity of the illness is positively correlated with the strength of aversion. Lastly, while taste aversion learning can occur with a long delay, it is stronger if the illness onset is closer to the ingestion of the food.

Plechat et al. (1983) trained rats to avoid a sugar solution through pairing with LiCl toxicosis (upper gastrointestinal tract discomfort), shock (peripheral pain), or high levels of lactose (lower gastrointestinal tract discomfort). The LiCl pairing was the only condition that resulted in orofacial responses indicative of 'disliking', the rats in the other conditions continued to show positive orofacial responses to the sweet taste. They interpreted these results as representing a distinction between food rejection based on unpalatability (distaste) and rejection based on the anticipation of negative consequences of ingesting the food (hazards). This supports the hypothesis that upper gastrointestinal distress (nausea) is the key to negative palatability shifts (acquired distastes).

Conditioned flavor avoidance is an extremely robust form of learning. In addition to avoiding the consumption of a novel taste that was paired with illness, rats show conditioned gapes (aversive taste reactions) when they are unable to avoid the taste (Parker, 2013). This conditioned disgust is best measured by intraorally infused flavored solutions, as conditioned flavor avoidance makes it unlikely that an animal would approach and voluntarily consume a taste that had been paired with illness, making taste reactions difficult to gauge (Grill and Norgen 1978).

The purpose of this study was to investigate whether the VP response to tastes reflects hedonic value, independently from sensory input. We used two distinctly different sweet, caloric tastes in this experiment (counterbalanced between groups). We familiarized the animals with one of the tastes by allowing them several days of free access in their home cage. This taste remained "safe" throughout the experiment, as it was never paired with drug (the CS-). We tested the behavioral responses to this taste at the start of the experiment and found that it was 'liked'. The second taste was a novel taste when the animals first experienced it, but it was also 'liked'. Subsequently, that taste was paired with nausea inducing LiCl injections (the CS+) and the animals quickly developed a learned taste aversion and the hedonic value of this taste diminished. The 'safe' taste (CS-), on the other hand, remained 'liked' throughout the experiment. We recorded neural activity in the VP in response to each taste at the beginning and end of the experiment and found that the VP responded differently to the 'hedonically diminished' CS+ taste after aversion compared to before aversion training, whereas there was no significant effect of training on the VP response to the 'safe' CS-. These findings support our hypothesis that the conversion of the hedonic value of a taste from 'liked' to 'disliked' would be reflected in the neural activity in the ventral pallidum (VP) and that the VP activity would not be altered if a taste remained 'safe' throughout the experiment.

Materials and methods

We adapted our experimental paradigm from Experiment 5 of Berridge and Grill, 1981.

Subjects

Ten adult male Sprague-Dawley rats weighing 300 g – 400 g were used in this experiment. Animals were housed individually in tub cages on a 9:30 AM to 7:30 PM reversed light/dark schedule. Experiments were conducted during late morning to afternoon hours, coinciding with the rats' active (dark) period after acclimating to housing conditions for 1-2 days. Food and water were available ad libitum throughout testing, except when in the recording chamber.

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.1 for a schematic diagram of the chamber set-up.

Delivery of tastes and stimulation was controlled by a custom software program, MTASK. Neural activity was recorded during the testing sessions using a custom LabVIEW (National Instruments, Austin, TX) program, DataTask. Neural activity was amplified at a gain of 5000 and bandpass-filtered between 300 Hz and 6 kHz. Sessions

were recorded at 30 frames a second via a video camera placed underneath the glass floor. Timestamp clocks for the taste delivery program, video recording, and neural recording 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.

Pre-exposure to the 'safe' CS- solution

A pre-exposure paradigm was used to establish a specific sweet solution as a familiar and safe taste. This taste remained 'safe' throughout the experiment, as it was never paired with nausea inducing LiCl, and served as a control taste (CS-). For four days leading up to surgery, rats were given daily free access to a sipper tube containing 20 ml of the CS- solution: either 17.4% w/v sucrose solution or 16% polycose/0.2% saccharine w/v solution, in their home cage (the CS- assignment was counterbalanced). These two tastes were previously piloted found to be equal in hedonic and motivational value. The volume consumed was recorded every 24 hrs and tubes refilled for four consecutive days. Any rats that did not voluntarily consume 20 ml of a sweet solution within 24 hrs by the last day of pre-exposure were excluded from the experiment. In addition, for two days just before surgery, rats were also introduced to the test chamber. They were placed in the chamber for 5 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, pretreated with penicillin, 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. This implantation procedure allows for precise taste delivery via a computer controlled pump.

In the same surgery, rats were implanted with bilateral posterior VP-targeting electrodes (AP -0.8mm, ML ±2.8mm, DV 7-8.5mm). Each electrode consisted of two bundles of four 50 µm tungsten wires. Each bundle could be lowered or raised independently. Electrodes were implanted just dorsal to target and lowered to find cells as need on recording days. Both the electrode and the metal connecters to the oral cannulas were anchored to the skull with bone screws and acrylic cement. Animals were allowed to recover for at least 7 days while being monitored for complications due to surgery. Subcutaneous injections of penicillin were given daily for up to 4 days post-surgery to prevent infection. Rats received normal saline injections to keep the animal well hydrated and pain-management as needed during recovery. Rats had access to normal chow and water at all times, but were supplemented with infant cereal until swelling that resulted from the oral cannulation was gone, typically 2-4 days. Oral cannulae were kept clean and clear from blockage throughout the experiment and did not disrupt normal eating once the animal had recovered.

Habituation and re-exposure to 'safe' CS-

   After recovery, on two consecutive days the rats were placed in the experimental chamber for ten minutes each to allow the rats to become familiar with the test chamber. The experimental chamber is a transparent cylinder (25 cm diameter) placed within placed in a transparent Plexiglas chamber (28 X 35 cm). A glass floor allowed video recording from underneath for subsequent behavioral analysis of bodily and orofacial movements. In their home cages the rats again received 20 ml of the 'safe' CS- daily, to re-establish voluntary consumption post-recovery.

Training

   Training took place on each of 6 consecutive days. All training days were comprised of 1 bock of 10 trials, 1 taste per day. Trials consisted of single 0.1 ml taste infusions delivered by a computer-controlled pump over 1 sec, via intra-oral cannulae (variable 1 min ITI). Training began with the 'safe' CS- that the animals had access to during pre-exposure. Ten 'safe' taste (CS-) trials were delivered and synchronized with high speed digital video recordings of behavior and VP neural recordings. An injection of saline (drug control) was given after all ten 'safe' CS- taste trials were delivered, prior to the animal being returned to its home cage.

   The second day of training was the first time the rats ever encountered the 'novel' taste that would later be followed by a lithium chloride (LiCl) injection (the CS+ solution: either 17.4% w/v sucrose solution or 16% polycose/0.2% saccharine w/v solution, CS+ vs. CS-assignment was counterbalanced), which at this point was simply a novel sweet taste. 10 trials of the 'novel' taste were delivered, while neural and

behavioral data was collected. Lithium chloride (64 mg/kg, isotonic solution), which

induces moderate nausea, was injected i.p. after the CS+ training trials were completed,

prior to the rats being returned to their home cage. Therefore the 'hedonic devaluation'

did not occur until after the first CS+ training day, allowing us to use the data from this

day as another control condition. Tastes were alternated on the subsequent days so that

rats were exposed to each taste and injection pairing three times, but neural and

behavioral data was not collected on subsequent training days.

Testing

A single test day followed training, which was the only day the rats were exposed

to 2 blocks of trials: both the 'safe' CS- and the now 'hedonically diminished' CS+. The

first block of 10 trials was always the 'safe' CS-, so that any negative affect that resulted

from exposure to the 'hedonically diminished' CS+ (i.e. conditioned nausea) would not

taint the 'safe' CS- results. Time-stamped clocks were synchronized for taste infusions,

neural recordings, and videotape recordings.

Behavioral analysis: taste reactivity

Across a variety of species, there is a phylogenetically conserved behavior in

response to pleasant tastes ('liking' reactions). Rodent and primate species, including

human infants, do a variety of stereotyped mouth movements in response to a hedonic

taste, such as a sweet liquid; they do aversive taste reactions to liquids that are 'disliked',

such as bitter, sour, or extremely salty solutions (Grill and Norgren, 1978; Grill and

Berridge, 1985; Berridge, 2000; Steiner et al., 2001).

For all training and testing sessions, digital video of the stimulus duration and the subsequent 10 seconds of each trial was analyzed off-line in slow-motion (1/30 s frame-by-frame to 1/10th actual speed) using established procedures developed to assess hedonic, aversive and neutral taste reactivity patterns during liquid taste infusions (Grill and Berridge, 1985; Berridge, 2000) and the Datarat scoring program (developed by the Aldridge lab). Hedonic responses included rhythmic midline tongue protrusions, lateral tongue protrusions, and paw licks. Aversive responses included gapes, head-shakes, face washes, forelimb flails, and chin rubs. Neutral responses included passive dripping of solution out of the mouth, ordinary grooming, and rhythmic mouth movements (see Berridge and Grill, 1981, for technical definitions of these behavioral reactions). Individual reaction totals were calculated by adding all response scores within an affective category per rat (hedonic, aversive, or neutral).

A time-bin scoring procedure was used to ensure that taste reactivity components of different relative frequencies still contributed equally to the final affective hedonic/aversive totals (Berridge, 2000). For example, rhythmic mouth movements, passive dripping of solution, paw licking, rhythmic midline tongue protrusions, and grooming reactions occur in bouts. The onset and offset time of these bouts were recorded and the bout duration was converted into counts, with 1 second equaling one count of occurrence. The other behavioral components (lateral tongue protrusions, gapes, forelimb flails, head shakes, chin rubs) typically occur in discrete events and were thus scored as single occurrences each time they occurred (e.g., one lateral tongue protrusion equals one occurrence).

Individual rat totals were calculated for hedonic, neutral, and aversive categories by adding all response scores within an affective category for that rat. A hedonic 'liking' reaction total was the sum of scores for lateral tongue protrusions, rhythmic tongue protrusions, and paw licks. A neutral reaction total was the sum of passive drips and neutral mouth movement bouts. An aversive 'disliking' reaction total was the sum of gapes, head-shakes, face washes, forelimb flails, and chin rubs. These were statistically examined for CS- versus CS+ and Training versus Test Day effects using ANOVAs.

Ventral Pallidum Neuronal Activation Spike discrimination.

Single neurons (N=121) were identified using principle components or peak-width analysis of waveforms using Offline Sorter (Plexon Inc., Dallas TX). Neurons were verified by distinct spike waveforms (whose shapes remained consistent throughout the whole recording). Units with more than 2 % of spikes within a 1 ms refractory period window in an autocorrelation histogram were excluded. A cross-correlation analysis was also performed to ensure that neurons were counted only once. (NeuroExplorer, Nex Techologies, Littleton MA).

Firing rates.

Our epochs of interest was 0-250 ms after taste onset. Our baseline epoch was a 10 sec period one second prior to stimulus onset (-11 to -1 s). Normalized firing response to a stimulus event for each neuron was obtained by dividing the neuron's absolute firing response in epochs of interest by its baseline (1 would represent the baseline rate, > 1 hz an increase in rate, and <1 hz decrease in rate). The change in firing rate from baseline

was calculated by converting the normalized rate to a percentage change from baseline (0 would represent no change from baseline, a positive percentage would be an increase in rate, a negative percentage a decrease in rate).

Responsive Populations.

A neuron was considered 'responsive' if the epoch of interest contained a significant increase in rate, decrease in rate, or a mixed response. For each unit, raster plots and perievent time histograms (PETHs) with 50 ms bins were aligned to the onset of taste delivery. The criteria for an increase in rate were either one 50 ms bin being 3 SD greater than the baseline epoch, or two consecutive bins 2 SD above baseline. Decreases in rate were defined by one bin 2 SD below baseline or two bins 1 SD below baseline. Mixed responses were defined as any combination of an increase in rate and decrease in rate as defined above. Visual inspections of spike rasters were used to eliminate any false positives due to a single trial being the source of the rate change.

Histology

Anatomical localization of electrode sites was done after completion of testing. Rats were euthanized with FatalPlus at the end of the experiment. Brains were removed, flash-frozen in an isopentane and isopropyl alcohol solution, sectioned coronally into 40 μm sections on a CM 1850 cryostat (Leica Microsystems, Buffalo Grove, IL), and digitally photographed while wet using a bottom-lit microscope. The electrode placement was confirmed by observing the brain slices under a light microscope and a map illustrating electrode recording sites (figure 2.1) was constructed.
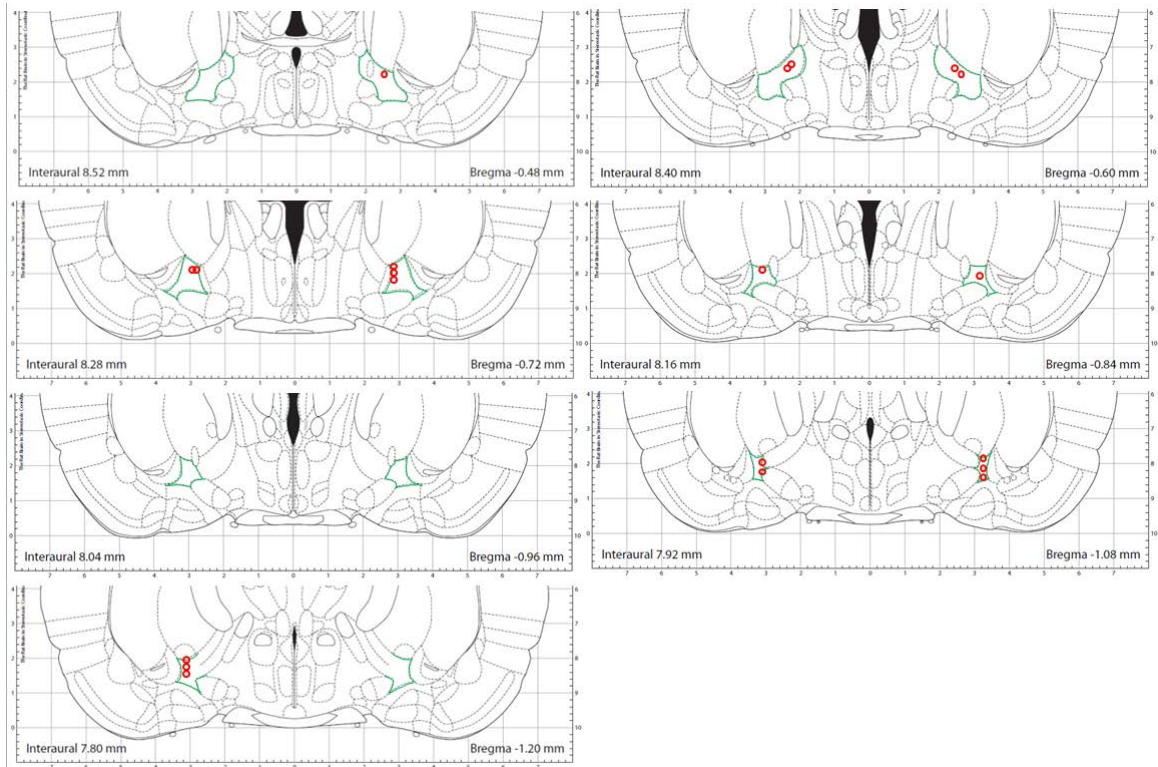
Figure 2.1 Coronal slices showing electrode placements of rats implanted bilaterally in the ventral pallidum (outlined in green). Red circles represent electrode bundle locations (n = 10 rats). Images modified from the Paxinos and Watson rat atlas, 6th edition (Paxinos & Watson, 2007).

Results

Behavioral Results

We induced a learned taste aversion of a specific sweet taste (the CS+) by repeatedly pairing it with nausea inducing injections of LiCl, while maintaining the hedonic value of a different sweet taste that was never paired with nausea (the CS-) (Figure 2.2). Before being paired with nausea, the novel sweet taste (CS+ on training day) elicited over 40 times more hedonic than aversive taste reactions (t-test, $p<0.001$). But after being repeatedly paired with nausea inducing LiCl injections (CS+ on test day),

there were more than 30 times the number of aversive than hedonic reactions (t-test, p<0.001).

The taste that was never paired with aversion, the 'safe' CS-, remained predominantly hedonic on the test day. While there was a minimal amount of generaliztion, resulting in a modest increase in aversive reactions to the safe CS-, this change was not statistically significant and the amount of hedonic or neutral reactions also didn't change (t-tests, $p > 0.05$ for all training vs. test comparisons of CS- taste reactions). Overall the CS- remained a "safe" taste throughout the experiment. As expected, the taste aversion was specific to a particular, distinctive sweet taste.

The taste that was paired with LiCl injections became 'disliked'. The 'hedonically diminished' CS+ taste on the test day resulted in more aversive taste reactions than hedonic (t-test, $p = 3.814^{-08}$) or neutral (t-test, $p = 0.006$) reactions (ANOVA; $F_{2,21}=23.443$, $p = 4.463^{-06}$). The CS- on the test day also had an overall difference in the valence of the taste reactions (ANOVA; $F_{2,21}=4.489$, $p = 0.02$) but while there were more hedonic reactions than aversive reactions, this difference did not quite reach statistical significance (t-test, $p = 0.057$).

For the 'safe' CS- taste on the first training day, there was an overall difference in the valence of the taste reactions (ANOVA; $F_{2,27}=11.238$, $p = 0.0003$). The quantity of hedonic and neutral taste reactions were significantly greater than that of aversive reactions (t-tests, $p = 0.001$ and $p = 0.0006$, respectively). The 'novel' CS+ on the training day, prior to LiCl pairings, had exactly the same pattern as the 'safe' CS- on the training day: (ANOVA; $F_{2,27}=19.452$, $p = 5.863^{-06}$; t-tests, $p = 0.00007$ and $p = 0.0019$,

respectively). For all test conditions, there was no statistical difference between the count of hedonic and neutral reactions (t-tests, all $p > 0.05$).
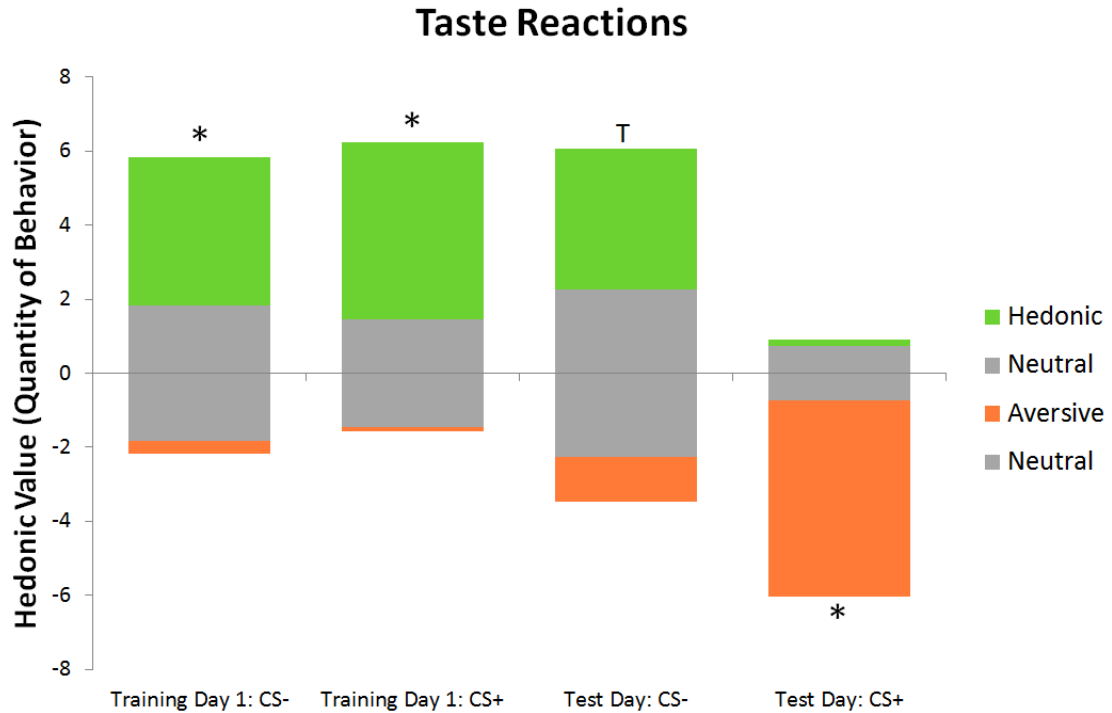
Figure 2.2 Overview of taste reactions for the taste aversions tudy: the 'safe' CS- on training day, the 'novel' CS+ before it was paired with nausea inducing LiCL injections, the 'safe'CS- taste on the test day, and the 'hedonically diminished' CS+ taste on the test day. As expected, the sweet tastes that were not paired with nausea were all 'liked', while the taste that underwent taste aversion learning became strongly aversive. * p < 0.001, ^{T} p = 0.066

To compare shifts in hedonic value across conditions, the behavioral data presented about was re-graphed to separate each category of taste reactions (hedonic, neutral, and aversive; figure 2.3 a-c). The 'hedonically diminished' CS+ on the test day resulted in far more aversive reactions than the three control conditions (the 'safe' CS- training day, the 'novel' CS+ training day, and the 'safe' CS- test day) (Figure 2.3 c, ANOVA; $F_{3,32}=69.040$, $p = 4.568^{-14}$). The amount of aversive reactions of each of the three control conditions were individually less than the count of aversive reactions in

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response to the taste that had been paired with aversion (t-tests, $p = 1.203^{-13}$, p= 7.723^{-15}, and $p = 0.00001$, respectively). The 'hedonically diminished' CS+ on the test day also resulted in significantly fewer hedonic reactions than the 'safe' CS- on training day, the 'novel' CS+ training day, and the 'safe' CS- test day (ANOVA; $F_{3,32}=7.667$, $p = 0.0005$, figure 2.3 a). The number of hedonic reactions of each of the three control conditions were individually greater than the count of hedonic reactions in response to the taste that had been paired with aversion (t-tests, $p = 0.0004$, p= 0.00001, and $p = 0.003$, respectively). There was a trend towards a difference in neutral taste reactions between the three control conditions and the 'hedonically diminished' CS+ test condition (figure 2.3 b), but it did not reach statistical significance (ANOVA; $F_{3,32}=2.641$, $p = 0.066$). Because there were few effects on the neutral reactions, our discussion will focus on the comparison of hedonic and aversive reactions.
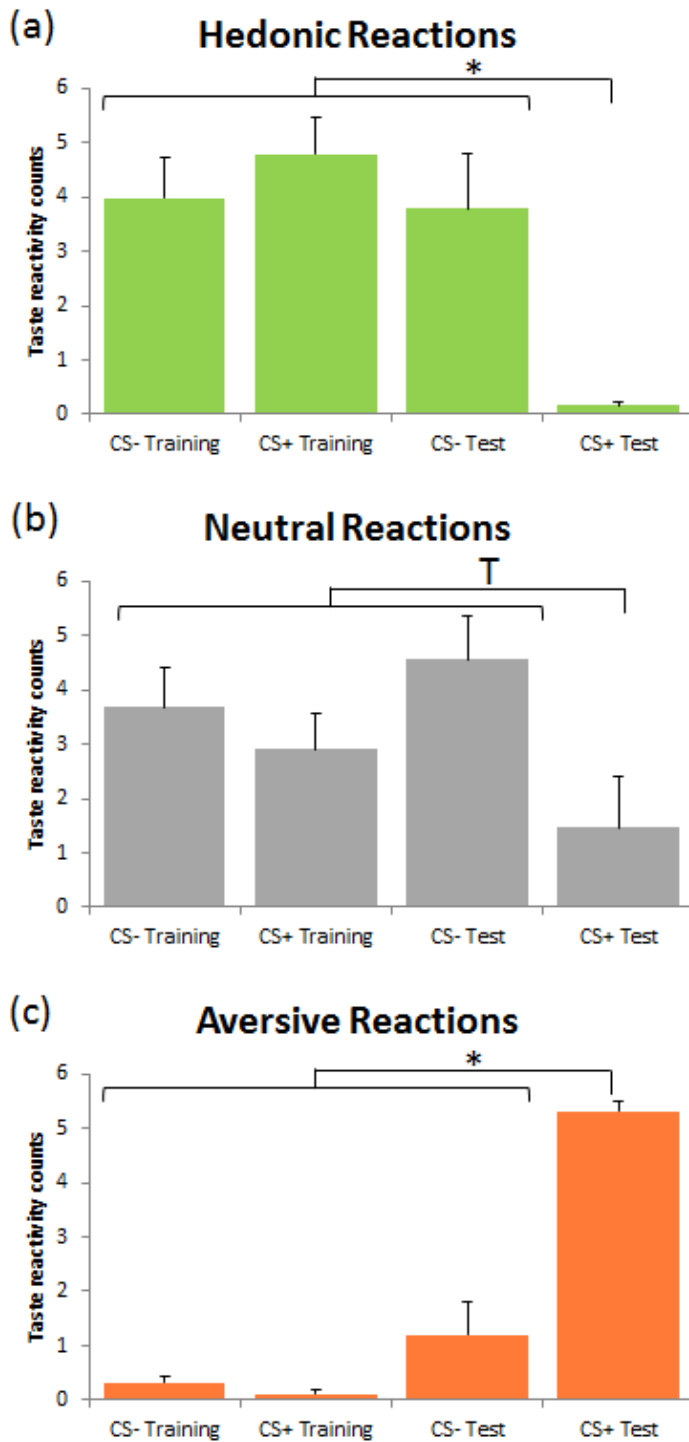
Figure 2.3 a-c Comparisons of 'liking' and 'disliking'. Prior to any aversion learning, both sweet tastes elicited 'liking' reactions and almost no aversive reactions. After the CS+ taste was repeatedly paired with LiCl injections, it became strongly aversive and produced almost no hedonic reactions. Error bars represent standard error. * p < 0.001, [T] p = 0.066

Population response types

VP neurons, as a population, tracked the hedonic valence of these sweet tastes with a greater proportion of increases in firing rate coding for 'liked' tastes and a greater proportion of decreases in firing rate indicating that a taste is aversive (figure 2.4 a). Before taste aversion learning, the 'safe' CS- only triggered increases in firing rate (27.5% showed an increase in firing rate, 0% decreased rate, X^2, p<0.001). The first time that the 'novel' CS+ sweet taste was experienced, before being paired with LiCL, it resulted in mostly excitation responses (32% increased rate, 5% decreased rate, X^2, p<0.05). This response shifted after taste aversion learning (test CS+) to mostly decreases in firing rate (3% increased rate, 11% decreased rate, X^2, p<0.05). In addition, the aversive taste resulted in fewer increases in rate than the three 'liked' tastes (all X^2 p<0.05).

When separated into anterior (-0.48 to -0.60 mm relative to Bregma) and posterior (-0.72 to -1.20 mm relative to Bregma) locations, the anterior VP data was very similar to the overall VP data, with the same statistically significant results. In the posterior VP, the aversive taste still resulted in more decreases in rate than increases in rate and the aversive taste resulted in fewer increases in rate than the three 'liked' tastes(all X^2 p<0.05). All three 'liked' tastes resulted in significantly more increases in rate than decreases (all X^2 p<0.05). Specific to the posterior VP, there were significantly more decreases in rate in response to the aversive taste than the three 'liked' tastes (all X^2

p<0.05). Therefore, while the pattern was similar across VP locations, the population

coding of hedonic value was most apparent in the posterior VP.
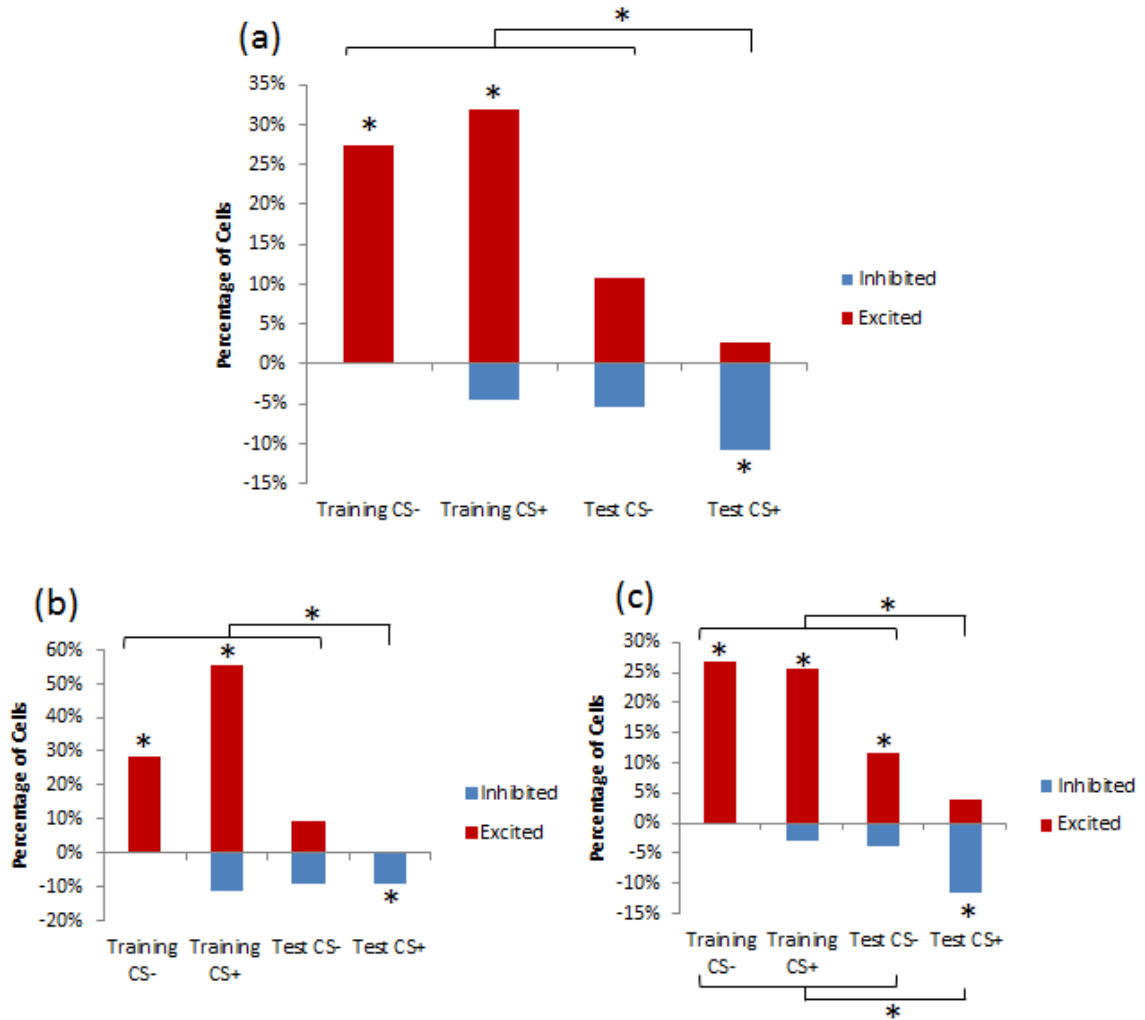
Figure 2.4 a-c Proportions per response category of all cells (not just the responsive cells), within the first 250 ms after taste onset. For each taste condition (the safe taste on the first day of training, the first exposure to the novel sweet taste prior to any aversion learning, the safe taste on the test day, and the now aversive taste on the test day) the distribution of responsive units that show deceases in firing rate (blue bars) or increases in firing rate (red bars) are plotted. Overall, there were fewer cells that responded to the aversive taste with an increase in firing rate compared to cells responding to 'liked' tastes. (a) All VP units: There were greater proportions of units that responded to the 'liked' tastes on the training days with an increase in firing rate than units that responded with a decrease in rate. There were more decreases in rate than increases in response to the aversive taste (Test CS+). (b) Anterior VP units: The significant findings were the same as those from all units. (c) Posterior VP Units: All three 'liked' tastes resulted in more increases in rate than decreases. The aversive taste still resulted in more decreases in rate than increases. In addition, there were more decreases in rate in response to the aversive taste than the three 'liked' tastes. While the pattern was similar across VP locations, the population coding of hedonic value was most apparent in the posterior VP. * p < 0.05

The population response data was converted to percentages of responsive units for figure 2.5 a. VP neurons, as a population, tracked the hedonic valence of these sweet tastes with a greater proportion of increases in firing rate coding for 'liked' tastes and a greater proportion of decreases in firing rate indicating that a taste is aversive (figure 2.5 a). All tastes conditions elicited neural responses, but the three control conditions ('safe' CS- and 'novel' CS+ before aversion training and 'safe' CS- on the test day) primarily resulted in increases in rate (100%, 88%, and 67% of the responsive cells, respectively). There were little to no decreased rate responses (0%, 12.5%, and 33% of the responses, respectively). The taste that was now aversive post-training (CS+ on test day) triggered far more decreases in rate (80% of responsive cells) than increases in rate (20%). The chi-squared test of the proportions of responses found there were fewer increases in rate ($p < 0.001$) and more decreases in rate ($p < 0.01$) for the 'hedonically diminished' CS+ on test day compared to the three control conditions. Furthermore, the 'safe' CS- and 'novel' CS+ tastes on the training days had significantly more increases in rate than decreases in rate ($p < 0.001$ and $p < 0.05$, respectively), whereas the 'hedonically diminished' CS+ on test day had more decreases in rate than increases in rate ($p < 0.05$).

When separated into anterior (-0.48 to -0.60 mm relative to Bregma) and posterior (-0.72 to -1.20 mm relative to Bregma) locations, the anterior VP data was similar to the overall VP data but the 'safe' CS- taste on the test day did not have a significant difference between the proportions of increases or decreases in rates aversive (figure 2.5 b). This might reflect a slight generalization of the taste aversion to the sweet taste that

had not been paired with LiCl injections in the anterior VP population. The posterior VP

data had the same significant results as the overall VP data (figure 2.5 c).
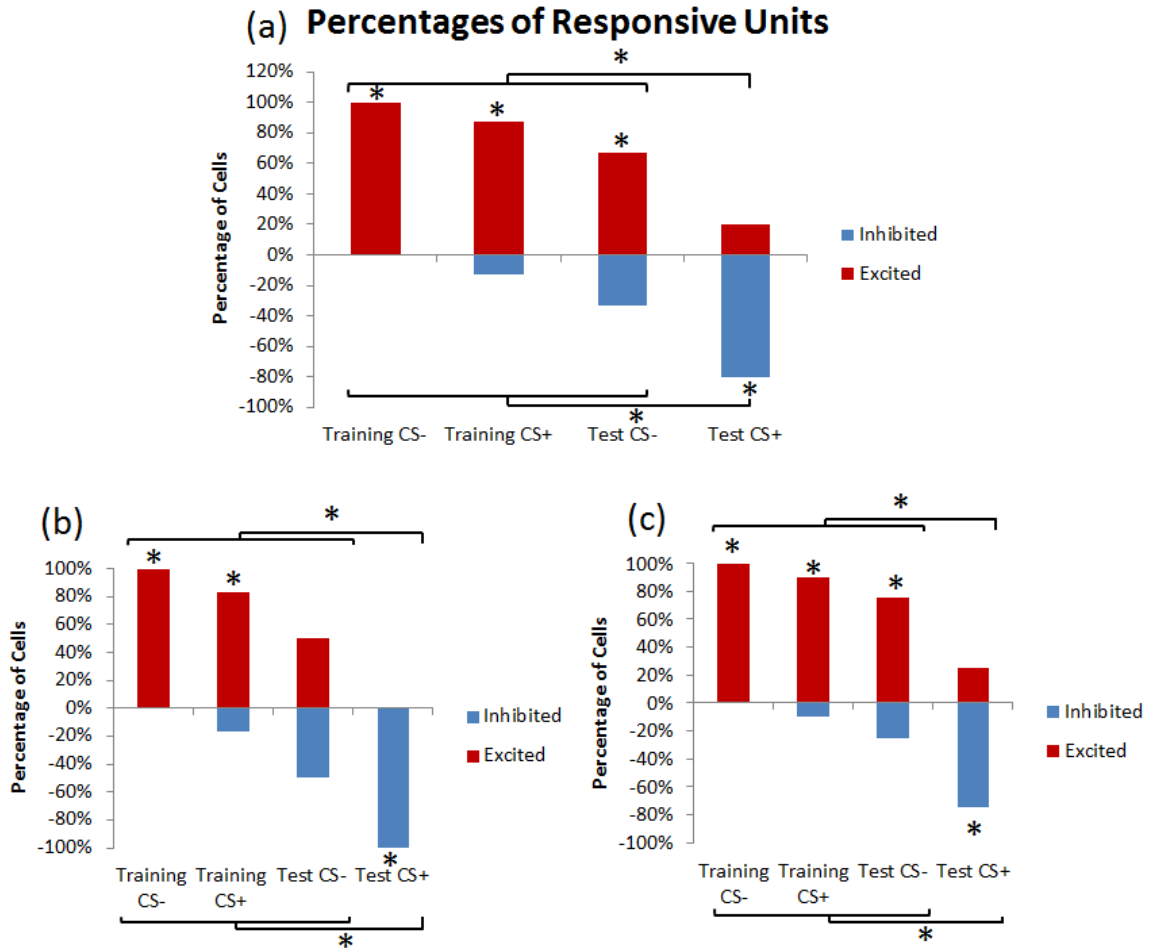
Figure 2.5 a-c Proportions per response category of the cells that responded within the first 250 ms after taste onset. For each taste condition (the safe taste on the first day of training, the first exposure to the novel sweet taste prior to any aversion learning, the safe taste on the test day, and the now aversive taste on the test day) the distribution of responsive units that show deceases in firing rate (blue bars) or increases in firing rate (red bars) are plotted. Overall, the aversive taste (Test CS-) resulted in a smaller proportion of increases in firing rate and larger proportion of decreases in firing rate compared to cells responding to 'liked' tastes. (a) All VP units: There were greater proportions of units that responded to the 'liked' tastes with an increase in firing rate than units that responded with a decrease in rate. There were more decreases in rate than increases in response to the aversive taste (Test CS+). (b) Anterior VP units: There was no difference in the proportion of increases vs. decreases in firing rate in response to the safe taste on the test day, which might indicate that there was more generalization of the taste aversion in the anterior VP population. (c) Posterior VP Units: The significant findings were the same as those from all units. While the pattern was similar across VP locations, the population coding of hedonic value was most apparent in the posterior VP. * $p < 0.05$

While the most reliable coding of the hedonic value of the sweet tastes lies in the population responses, because both tastes were evaluated in the same session on the test days, we were able to compare the effects of the two tastes directly on individual neurons. We found that individual VP units were able to code the opposing hedonic values of the sweet tastes by having one type of response to the 'liked' taste and the opposite response to the aversive taste. For example, a single unit that showed a strong increase in rate in response to the safe sweet taste on the test day, but the same unit showed a strong decrease in rate in response to the sweet taste that was now aversive (figure 2.6). Some cells also responded differently to the opposing hedonic values, but did so by having a response to one taste but had little or no response to the other taste (figure 2.7). However, not all units had different responses to the two tastes, some responded to both tastes in a similar way (figure 2.7).

(a)

(b)
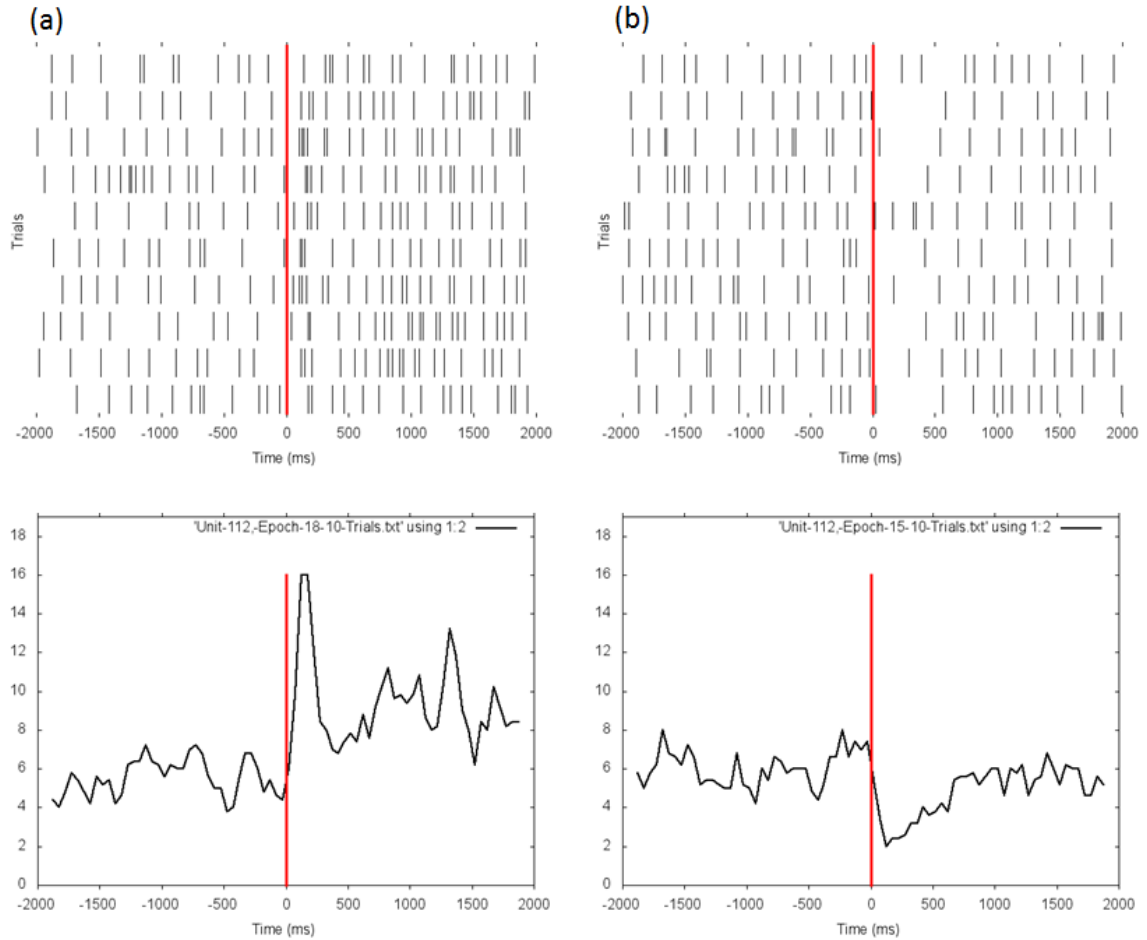
Figure 2.6 Example 1 PETHs and raster plots for (a) a unit responding to the "safe" sweet CS- taste and (b) the same unit responding to the now aversive CS+ sweet taste which had previously been paired with LiCl injections. Plots show unit activity up to 2 s before and after taste onset, which is centered at time 0 and indicated by the red lines. Each mark in a raster indicates a spike and each horizontal row is a trial with consecutive trials going from top to bottom. Gaussian Smoothed Histograms (bin width = 50 ms) show the average firing rate across all trials. This is an example of a single unit that showed opposing responses to the 'liked' and 'disliked' tastes.
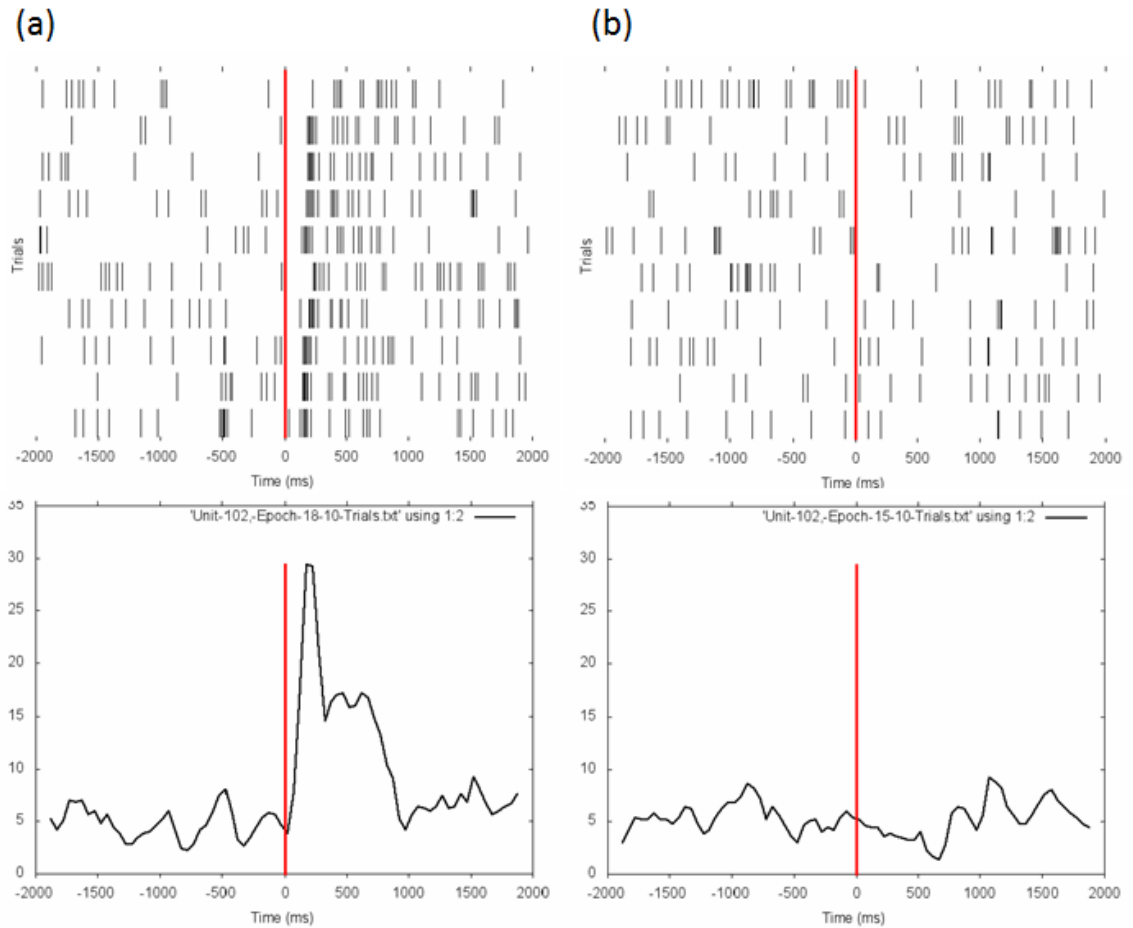
Figure 2.7 Example 2 PETHs and raster plots for (a) a unit responding to the "safe" sweet CS- taste and (b) this same unit showing very little response to the now aversive CS+ sweet taste which had previously been paired with LiCl injections. Plots show unit activity up to 2 s before and after taste onset, which is centered at time 0 and indicated by the red lines. Each mark in a raster indicates a spike and each horizontal row is a trial with consecutive trials going from top to bottom. Gaussian Smoothed Histograms (bin width = 50 ms) show the average firing rate across all trials. This is an example of a single unit that showed a strong increase in firing rate in response to the safe sweet taste on the test day, but very little change in firing rate in response to the now aversive sweet taste.
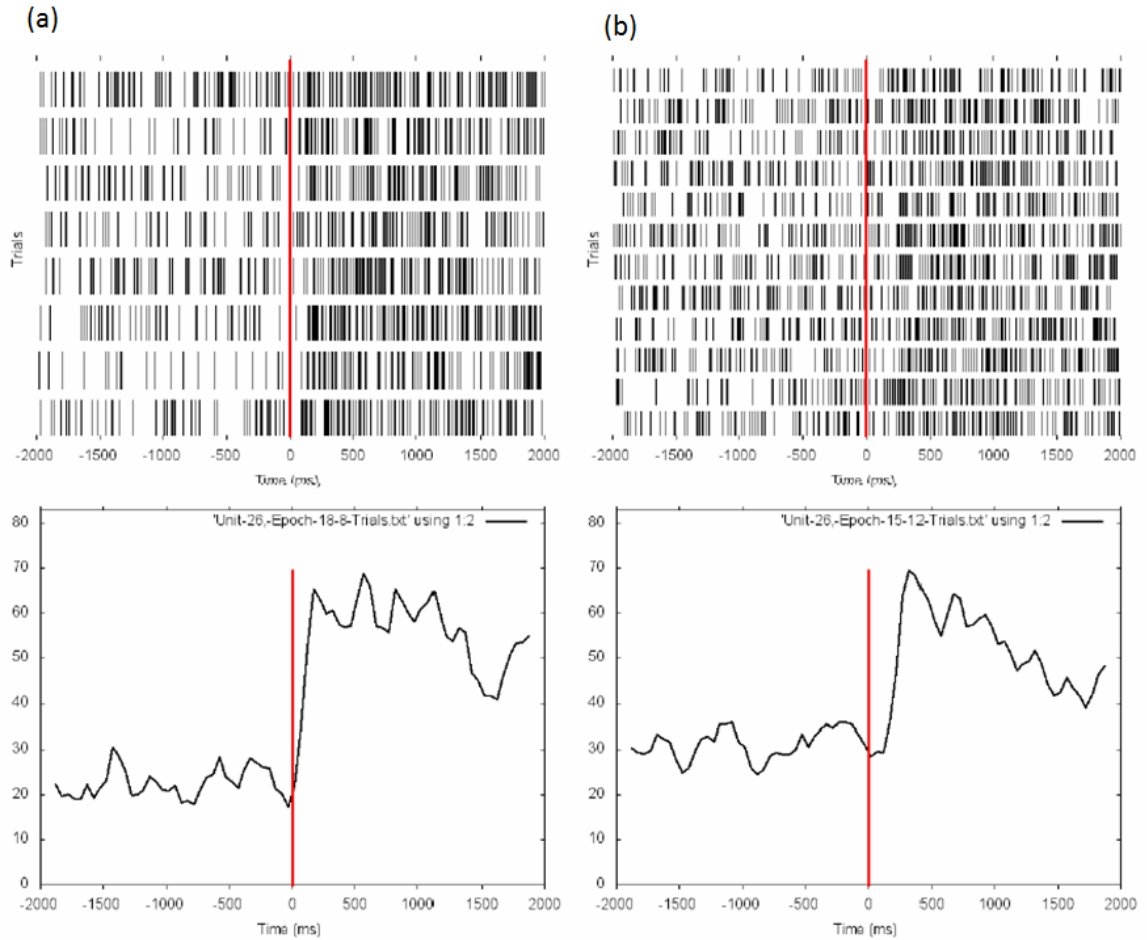
Figure 2.8 Example 3 PETHs and raster plots for (a) a unit responding to the "safe" sweet CS- taste and (b) this same unit's response to the now aversive CS+ sweet taste. Plots show unit activity up to 2 s before and after taste onset, which is centered at time 0 and indicated by the red lines. Each mark in a raster indicates a spike and each horizontal row is a trial with consecutive trials going from top to bottom. Gaussian Smoothed Histograms (bin width = 50 ms) show the average firing rate across all trials. This is an example of a single unit that showed strong increases in firing rate to both tastes on the test day.

Rate coding of tastes

The aversive sweet taste ('hedonically diminished' CS+ on the test day) evoked an overall slower increase in the average firing rate of the VP cells when compared to the same taste before it was paired with LiCl. The averaged population rate changes for the 'safe' taste (CS-) did not change on the test day. It's pattern of activation was the same as that observed on the training day for the 'safe' taste. The undiminished 'safe' taste on both training and test days and the sweet taste that was later paired with LiCl all evoked similar patterns of neural activation evident on the averaged population signals. The pattern consisted of a sharp rise in average firing rate that was sustained or diminished gradually for at 2 seconds (black line, figure 2.9 a-c). The 'hedonically diminished' taste evoked a different pattern. It produced an overall gradual and transient rise in the average firing rate (black line, figure 2.9 d).   The altered rise in neural activation was evident in measures of the time to peak: Training 'safe' CS-: 275 ms, Training 'novel' CS+: 375 ms, Test 'safe' CS-: 175 ms, Test 'hedonically diminished' CS+: 925 ms.

This change in temporal profile of the averaged population response to the 'hedonically altered' taste was due a dramatic alteration in the mix of neural response to the altered taste. After the hedonic conversion the mix of increases and decreases in neural activation to the 'hedonically diminished' taste was changed dramatically. There were now many more decreases in activity in response to the taste and fewer increases. The average normalized rate of firing in initial 250 msec also rose in response to 'novel' CS+ before training, but dropped after aversion training (before 1.47, after 0.85, p<0.01). By contrast, the average 'safe' CS- triggered firing rate increased from baseline both before and after training in first 250 msec (before 1.51, after 1.85, NS). When examined

separately, the increases and decreases to the altered taste produced response profiles that

did not differ dramatically from the separate profiles in the 'safe' tastes (CS-) or the

sweet taste before it was altered by pairing it with LiCl. The red lines (increases in

activation) and blue lines (decreases) illustrate this in Figure 2.9. Overall, it appears that

the population and rate response of the VP tracked the selectively decreased hedonic

value of a CS+ sweet taste, even when the sensory value of the stimulus is unchanged.
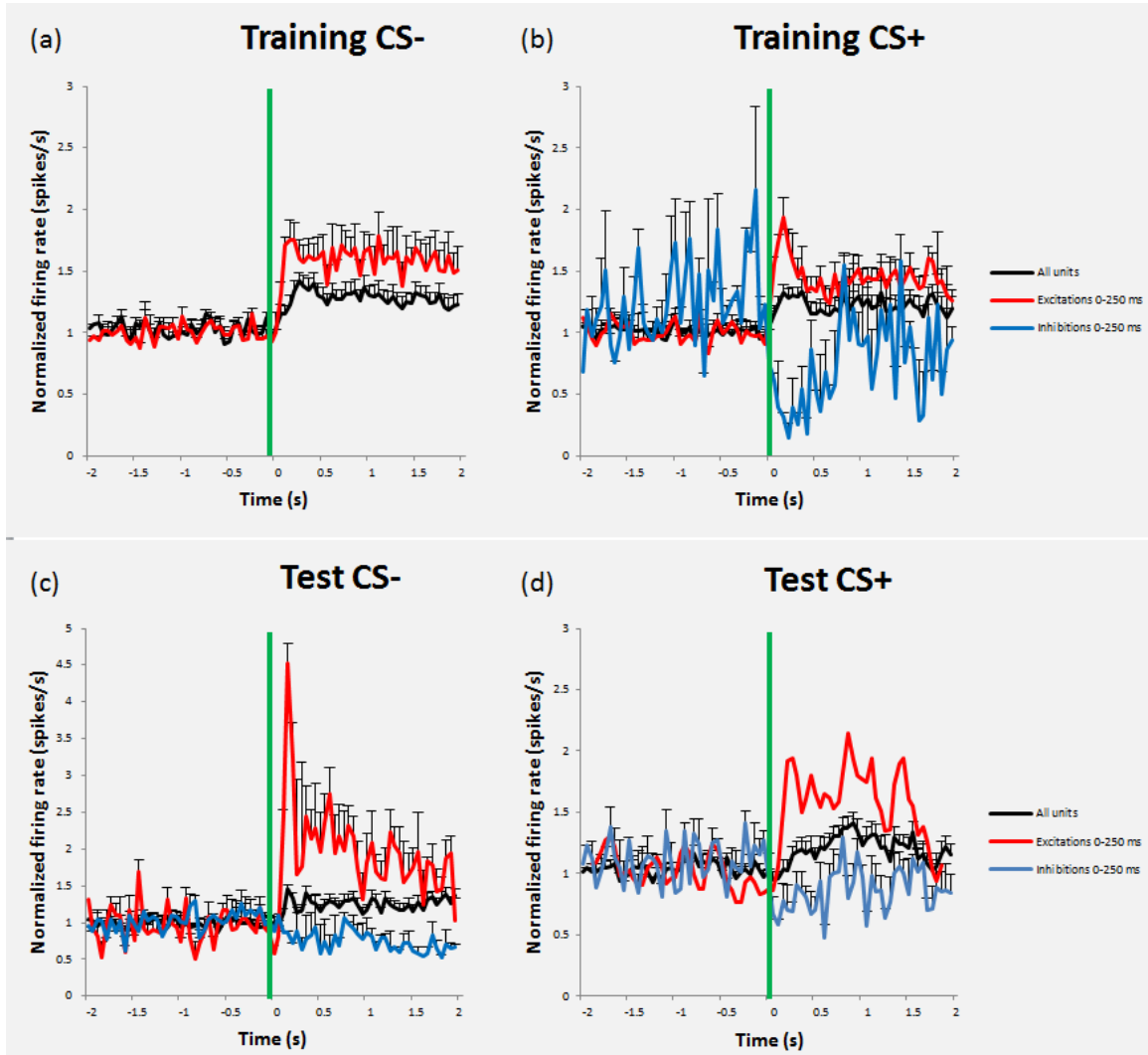
Figure 2.9 a-d Average taste-evoked firing rates of units in the VP: composite histograms for all units (black), units that had an increase in rate within 250 ms of taste onset (red), and units that had an decrease in rate within 250 ms of taste onset (blue). Error bars represent standard error. Histograms are lined to taste onset showing 2 s before and after the taste infusion: (a) the 'safe' CS- on the first training day (there were no decreases in rate within the first 250 ms of taste onset), (b) the 'novel' CS+ before being paired with LiCl, (c) the 'safe' CS- on test day, and (d) the 'hedonically diminished' CS+ after taste aversion learning, on the test day (there was only one unit that showed an increase in rate within 250 ms of taste onset). All units are normalized to their respective mean firing rates during the 10 seconds preceding taste onset. Y-axis is the normalized firing rate, the green line represents taste onset.

Across the VP (figure 2.10 a), after aversion learning, the average firing rate of taste responsive units decreased in response to the taste paired with aversion (the CS+), which was significantly different from the response on the training day ($p = 0.01$) and the 'safe' CS- on the training day ($p = 0.006$). There was a trend for a difference between the 'safe' CS- and 'hedonically diminished' CS+ on the test day, but this difference was not significant ($p = 0.09$). The average firing rate was not changed in the 'safe' taste that was never paired with LiCl (NS).

In the anterior VP (-0.48 to -0.60 mm relative to Bregma) there was a lot of variability in the change in firing rates in response to the 'safe' taste on the test day. In addition, there was only one responsive unit to the aversive taste on the test day, so statistical comparisons of rate between the test condition and the three controls were not possible (figure 2.10 b). The posterior VP (-0.72 to -1.20 mm relative to Bregma) changes in firing rates were similar to the overall VP data with slightly less variability in the 'safe' test day data (test CS-, figure 2.10 c).
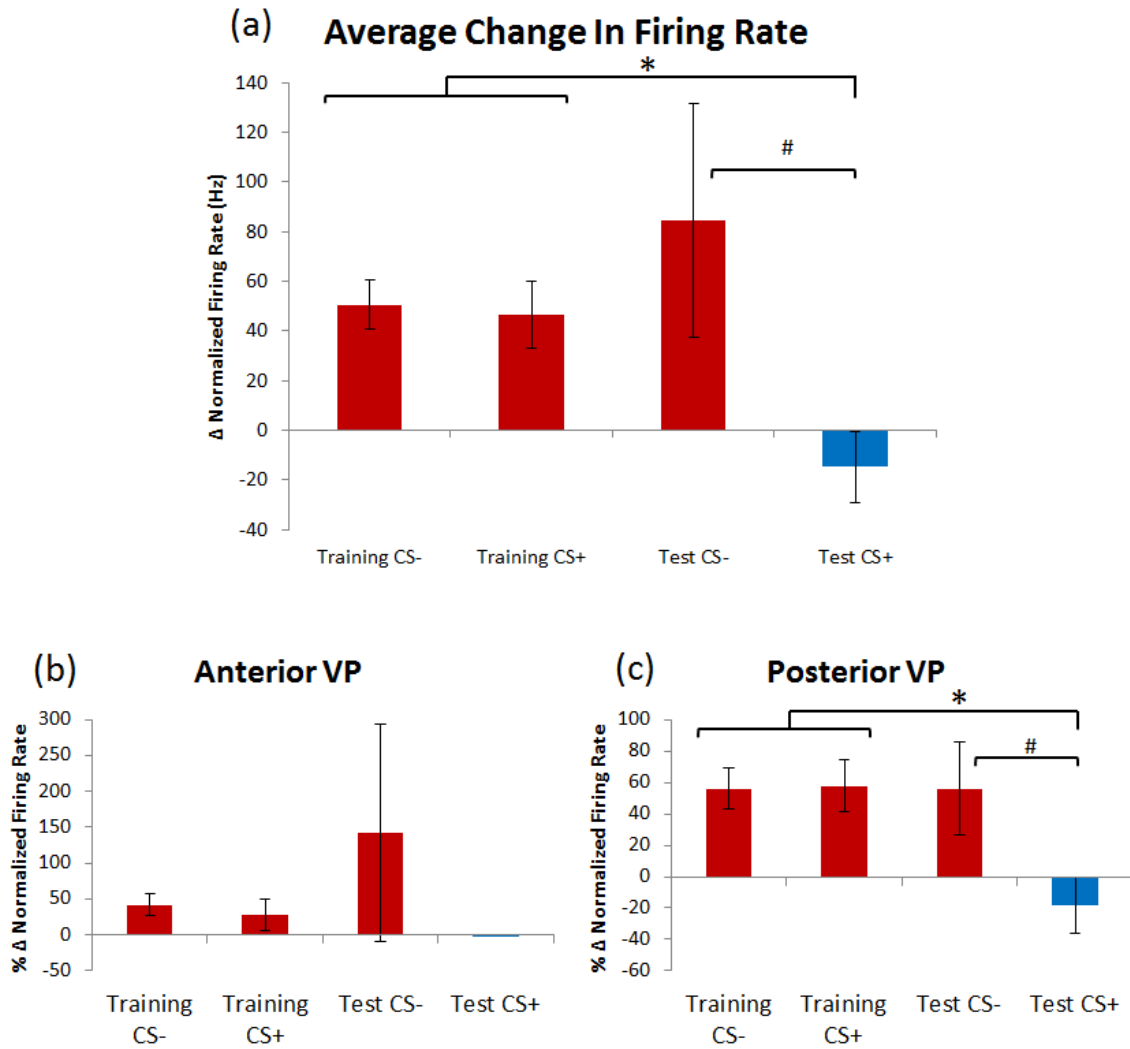
Figure 2.10a-c The average percentage change in normalized firing rate from the 10 s baseline epoch preceding each taste to the 250 ms epoch after each taste is depicted. 0 represents no change in firing rate, a positive value represents an increase in firing rate (red bars), and a negative value represents a decrease in firing rate (blue bar). Error bars represent standard error. * p < 0.05

Table 2.1 VP unit responses for 'safe' CS- and 'novel' CS+ on training days and the 'safe' CS- and 'hedonically diminished' CS+ tastes on the test day.

Epoch Window	Rate Response	CS- Training (n = 40)	CS+ Training (n = 44)	CS- Test (n = 37)	CS+ Test (n = 37)
		n (% total)	n (% total)	n (% total)	n (% total)
0-250 ms post taste	increase	11 (27.5%)	14 (31.8%)	4 (10.8%)	1 (2.7%)
0-250 ms post taste	decrease	0 (0%)	2 (4.5%)	2 (5.4%)	4 (10.8%)

Discussion

Taste aversion learning

By pairing a particular sweet taste with LiCl, we were able to induce an aversive reaction to that sweet taste alone without altering taste reactions to other sweet tastes. Taste reactivity shifted from hedonic to aversive when the novel sweet taste (CS+ before LiCl pairing) became 'hedonically diminished' (the CS+ on the test day), but the 'safe' sweet taste that was never paired with LiCl injections (CS-) remained 'liked' throughout the experiment. Although there was a modest amount of generalization of aversion to sweet tastes, as indicated by the slight shift in the hedonic value of the 'safe' CS- taste on test day, the change from baseline was not significant. In other words, we did not induce aversion learning to all sweet tastes, but rather to a specific and distinct taste. Our behavioral results matched those of the study that we adapted our experimental paradigm

from (Experiment 5 of Berridge et al., 1981) and many other taste aversion studies

(Plechat, 1983; Parker and Jenson 1992; Parker, 2013; Carelli and West 2014).

VP coding of the hedonic value of tastes

Within the pool of VP neurons that responded rapidly (<250 ms) to the tastes, the

proportions of "excitations" to "inhibitions" flipped when the hedonic value of the taste

flipped. The neural coding of this taste aversion in the ventral pallidum (VP) was most

apparent in the proportion of increases in firing rate vs. decreases in firing rate. In the

three conditions where the sweet tastes were liked (the 'safe' taste before and after

training, and the 'novel' CS+ before being paired with aversion), the majority of the VP

cells that responded to the tastes showed an increase in firing rate (67-100% of

responsive cells). There were no or few decreases in firing rate (0-33% of responsive

cells). The sweet taste that became aversive, on the other hand, resulted primarily in

decreases in firing rate (80% of responses), with a few increases in rate (20% of

responses). As a population, the hedonic value of the tastes was also tracked by the

change in average firing rate in response to the tastes. The three control conditions

resulted in an average increase in firing rate 0-250 ms post-taste onset (47-85% increase

in rate from baseline). The aversive taste yielded a decrease in average firing rate in this

epoch (15% decrease in rate from baseline).

Conclusion

We confirmed that the VP is able to encode the decrease in hedonic value of a sweet, caloric taste. This finding, in conjunction with a previous study that found that the VP can track the change in hedonic value of a salt solution from aversive to hedonic via salt depletion (Tindell et al. 2006), provides evidence that the VP can encode for shifting hedonic values of a given taste. This is a fairly complex psychological phenomenon, which indicates that the Basal Ganglia provide rich reward related information, far beyond simple prediction learning.

Reference List

Berridge KC (1996) Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev 20:1–25

Berridge KC (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. Neurosci Biobehav Rev 24:173-198.

Berridge KC (2003) Pleasures of the brain. Brain Cogn 52:106-128.

Berridge KC, Grill HJ (1981) Relation of Consummatory Responses and Preabsorptive Insulin Release to Palatability and Learned Taste Aversions. Journal of Comparative and Physiological Psychology, 95(3): 363-382.

Carelli RM, West EA (2014) When a good taste turns bad: Neural mechanisms underlying the emergence of negative affect and associated natural reward devaluation by cocaine. Neuropharmacology 76:360-369.

Feigin MB, Sclafani A, Sunday SR (1987) Species differences in polysaccharide and sugar taste preferences, Neurosci. Biobehav. Rev. 11: 231–240.

Garcia J, Hankins WG, Rusiniak KW (1974) Behavioral regulation of the milieu interne in man and rat. Science 185, 824–883.

Garcia J, Koelling RA (1966) Relation of cue to consequence in avoidance learning. Psychonomic Science, 4: 123-124.

Giza BK, Scott TR, Sclafani A, Antonucci RF (1991) Polysaccharides as taste stimuli: their effect in the nucleus tractus solitarius of the rat, Brain Res. 555:1–9.

Grill HJ, Norgren R (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 143:263-279.

Grill HJ, Berridge KC (1985) Taste reactivity as a measure of the neural control of palatability. Progress in Psychobiology and Physiological Psychology 2.

Jaeger D, Gilman S, Aldridge JW (1990) A multiwire microelectrode for single unit recording in deep brain structures. J Neurosci Methods 32:143-148.

Parker LA (2013) Conditioned flavor avoidance and conditioned gaping: Rat models of conditioned nausea. Eur J Pharmacol. In Press.

Parker L, Jensen K (1992) Food Aversions: Taste Reactivity Responses Elicited by Lithium-Paired Food. Pharm Biochem & Behavior 41: 239-240.

Parker LA, Limebeer CL, Rana SA (2009) Conditioned disgust, but not conditioned taste avoidance, may reflect conditioned nausea in rats. In: Reilly S, Schachtman TR (Eds.), Conditioned Taste Aversions: Behavioral and Neural Processes. Oxford Univ Press, NY, pp. 92–113.

Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates* (6th ed.). Amsterdam, the Netherlands: Elsevier Inc.

Peciña S, Smith KS, Berridge KC (2006) Hedonic hot spots in the brain. Neuroscientist 12:500-511.

Pelchat ML, Grill HJ, Rozin P, Jacobs J. (1983) Quality of acquired responses to tastes by Rattus norvegicus depends on type of associated discomfort. J Comp Psychol 97:140-53.

Sclafani A, Einberg LT, Nissenbaum JW (1987) Influence of saccharin on Polycose, sucrose, and glucose intake and preference in rats, Neurosci. Biobehav. Rev. 11:223–229.

Sclafani A, Nissenbaum JW, Vigorito M (1987) Starch preference in rats, Neurosci. Biobehav. Rev. 11:253–262.

Smith, K. S., & Berridge, K. C. (2005). The ventral pallidum and hedonic reward: Neurochemical maps of sucrose "liking" and food intake. *The Journal of Neuroscience, 25*(38), 8637-8649.

Steiner JE, Glaser D, Hawilo ME, Berridge KC (2001) Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. Neurosci Biobehav Rev 25:53-74.

Tindell AJ, Smith KS, Pecina S, Berridge KC, Aldridge JW (2006) Ventral pallidum firing codes hedonic reward: when a bad taste turns good. J Neurophysiol 96:2399-2409.

Wheeler RA, Carelli RM (2006) The neuroscience of pleasure. Focus on "Ventral pallidum firing codes hedonic reward: when a bad taste turns good". J Neurophysiol 96:2175-2176.

CHAPTER 3

VENTRAL PALLIDAL CODING OF TASTE STIMULI

Abstract

We investigated whether increased dopamine levels (amphetamine), D2 antagonism (haloperidol), opioid stimulation (morphine), or opioid antagonism (naltrexone) affects the hedonic value of a 'liked' sweet taste (sucrose), neutral taste (water), or a 'disliked' bitter taste (quinine). We recorded from the ventral pallidum (VP) while these tastes were presented to examine how the VP encodes these drug effects on taste hedonia. Opioid modulation (morphine and naltrexone) increased the 'liking' reactions to quinine. The only drug condition that showed a VP response that mirrored the hedonic value of the three tastes was morphine.

Introduction

Several studies have looked at the effects of opioid agonists within discreet nuclei, even sub-regions within a structure. Microinjections of an opioid agonist (DAMGO) in the NAcc and VP 'hedonic hot spots' (the rostral half of the medial shell of the NAcc and the caudal half of the VP) increased 'liking' reactions and decreased 'disliking' reactions to orally infused sucrose (Peciña and Berridge 2005, Smith and Berridge 2007). However, the NAcc and VP also have small sub-regions in which DAMGO can decrease 'liking'. DAMGO administered into the central nucleus of the amygdala did not enhance liking responses for sugar (Mahler and Berridge 2012). This indicates that opioid elevation can have different and potentially opposing effects in

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different brain areas. However, human abuse of opioids occurs via systemic administration.

Systemically administered morphine increases the number of hedonic reactions to a sucrose-quinine mixture (Doyle et al. 1993) and other tastes (Berridge 1996). Systemic morphine also enhances saline intake in sodium-depleted rats and respectively reduces negative responses (Na et al. 2012). These studies give us a better picture of how a systemic opioid agonist might affect behavior, by enhancing hedonic reactions and decreasing aversive reactions, in particular to tastes that would normally be disliked. However, how the VP codes for morphine induced changes in the hedonic value of tastes was not yet known.

Human studies have found that naloxone and naltrexone decrease ratings of how pleasant food is, particularly for foods that are normally rated as highly palatable (Drewnowski et al. 1992, Yeomans and Grey 1997, Kelley et al. 2002). In addition, an anorectic effect is seen in animal studies of naloxone (Giraudo et al. 1993, O'Hare et al. 1997). Naltrexone attenuated saline intake in sodium-depleted rats and reduced hedonic taste responses (Na et al. 2012). The value of sweet foods seem to be especially vulnerable to naloxone or naltrexone decreasing their hedonic value (Giraudo et al. 1993, Agmo et al. 1995, Yeomans and Gray 1997 and 2002, Glass et al. 1999, Kelley et al. 2002, Barbano and Cador 2006). This led us to hypothesize that naltrexone would decrease hedonic reactions to palatable tastes, and our goal was to see how the VP encodes this shift in hedonic value.

Mesolimbic dopamine levels sometime modulate hedonic values of tastes, but the effects have not been consistent across all studies. Some have found that amphetamine

increases subjective rating of hedonic impact (Wise 1980). However, the majority of studies that have found that boosting dopaminergic activity doesn't alter the hedonic value of tastes (Berridge et al. 2010 and 1989, Leyton et al. 2002, Peciña et al. 1997 and 2003, Robinson et al. 2005, Smith et al. 2011, Tindell et al. 2005 a and b, Treit and Berridge 1990, Volkow et al. 2002, Wyvell and Berridge 2000 and 2001). Based on previous work in our lab, we hypothesized that any amphetamine induced change in VP coding of tastes would not be due to a change in the hedonic value of taste stimuli.

Rats given the dopamine antagonist pimozide still exhibited 'liking' reactions in response to sucrose solutions (Peciña et al. 1997). Likewise, 6-OHDA lesions, which destroy 99% of dopamine neurons in rats, did not alter 'liking' reactions to sweet tastes (Berridge and Robinson 1998). Furthermore, systemic haloperidol and apomorphine had no effect on hedonic or aversive taste reactions to a strongly hedonic sucrose solution, weak sucrose solution, or bitter quinine solution (Treit and Berridge 1990). Therefore we hypothesized that there would be no changed in the hedonic value of taste stimuli under systemic haloperidol and likely no change in VP coding of tastes.

The purpose of this study was to investigate whether the ventral pallidum tracked any drug induced changes in the hedonic value of taste stimuli. We compared opioid modulation via systemic morphine or naltrexone, dopamine modulation via amphetamine or haloperidol, and vehicle controls. We hypothesized that opioid modulation would alter the hedonic values of tastes in an opposing manner (morphine would boost hedonia, naltrexone would decrease it) but that dopamine modulation would not alter behavior from vehicle control. We also analyzed the effects of these drug conditions on neural activity to determine whether the VP tracks drug induced changes in hedonic value. We

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found that morphine did increase the hedonic value of bitter quinine, but naltrexone did as well. But morphine was the only drug that produced a population response that correlated with the hedonic value of the three tastes. Under morphine the proportion of excitations to inhibitions was highest for sweet sucrose and lowest for bitter quinine. The other drug conditions did not have significantly different neural responses to the different tastes. As hypothesized, there was no effect of dopamine modulation on taste reactions or VP coding that differed from control.

Materials and methods

Subjects

Six adult male Sprague-Dawley rats weighing 270g – 305g were used in this experiment. Animals were housed individually in tub cages containing wood corn cob bedding before implantation and shredded paper bedding post-implantation to avoid bedding being trapped in the electrode covers on a 9:30 AM to 7:30 PM reversed light/dark schedule at ~21°C. Experiments were conducted during late morning to afternoon hours, coinciding with the rats' active (dark) period after acclimating to housing conditions for 1-2 days. Food (Purina Rat Chow) and water were available *ad libitum* in their home cages.

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.1 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). 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 5000 and bandpass-filtered between 300 Hz and 6 kHz. Sessions were recorded at 30 frames a second via a video camera placed underneath the glass floor. Timestamp clocks for the taste delivery program, video recording, and neural recording 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.

Surgical procedures

Rats were pretreated penicillan and anesthetized with 100 mg/kg ketamine HCl and 10 mg/kg xylazine. Rats were positioned in a stereotaxic apparatus (Kopf; Tujunga, CA).   Bilateral electrodes were implanted in the caudal VP (ML ±2.7 to 2.8 mm; AP -0.8 to -1.1 mm; DV 5.96 to 7.3265 mm; incisor bar set for flat skull), with the tips of the electrode bundles positioned approximately 1 mm above target sites in the VP (DV of

recording sites were 7.004 to 8.4535 mm) (Paxinos and Watson 2007). Each electrode

consisted of 2 bundles with 4 wires each (50 µm tungsten). Each 4 wire bundle was a

screw-driven brass microdrive, which allowed each electrode to be moved individually to

optimize recording placement prior to testing.

In the same surgery, each animal was implanted with bilateral intraoral cannulae

(PE-100 tubing) to permit oral taste infusion. Oral cannulae enter the mouth in the upper

cheek lateral to the first maxillary molar, travel beneath the zygomatic arch, and exit the

dorsal head lateral to the skull (Grill and Norgren, 1978; Grill and Berridge, 1985).

Electrodes and oral cannulae were anchored to the skull with 8 bone screws and acrylic

dental cement. A stainless steel obturator was inserted in the intra-NAc cannulae to

prevent occlusion. Rats were allowed to recover for at least 7 days before behavioral

testing. They were maintained on daily injections of penicillin and normal saline as

needed to prevent infection and keep the animal well hydrated during recovery. They

had access to normal chow and water at all times, but were supplemented with infant

cereal until swelling that resulted from the oral cannulation was gone, typically 2-4 days.

Oral cannulae did not disrupt normal eating.

Habituation

After recovery, on two consecutive days the rats were placed in the experimental

chamber for ten minutes each to allow the rats to become familiar with the test chamber.

The experimental chamber is a transparent cylinder (25 cm diameter) placed within

placed in a transparent Plexiglas chamber (28 X 35 cm). A glass floor allowed video

recording from underneath for subsequent behavioral analysis of bodily and orofacial movements.

Testing

We tested affective orofacial reactions of rats to a series of solution infusions into the mouth via oral cannula 15 minutes after amphetamine, haloperidol, naltrexone, or saline control injections or 90 minutes after morphine injections. The 90-min morphine time point was chosen because a previous study found maximal drug induced increases in hedonic reactions to sucrose at approximately 90-min after systemic (subcutaneous) morphine injections (Doyle et al, 1993). Naltrexone has a faster time course, with no difference in hedonic reactions at 25 and 40 minutes post-injection (Richardson et al, 2005). Early time course effects of morphine include a sedative effect which potentially masks the hedonic response.

Animals were injected with drug or vehicle (order of testing was randomized), connected to the taste delivery tubes and headstage cable, then placed in the testing chamber where they were allowed to habituate to the chamber while waiting for the drug to take effect (15-90 min). All test days were comprised of 3 blocks of 10 trials, 1 block per taste. To infuse solutions into the mouth, a 3-ml syringe containing either sucrose (17%; 0.5 M), tap water, or quinine (0.01%; 2.6×10^{-4} M) was attached via hollow tubing (PE-50 connected to a PE-10 delivery nozzle) to a single oral cannula. In each taste reactivity trial, there was a 45-75 second variable wait, then a 0.1 ml volume of solution was infused via syringe pump in 1 second. The tastes were always presented in that order. Quinine was always presented last so that any negative affect or lingering bitter

taste would not alter the response to the other tastes. Between blocks of taste, an extra

infusion of distilled water was given in order to rinse the mouth between tastes. Trials

consisted of single 0.1 ml taste infusions delivered by a computer-controlled pump over 1

sec, via intra-oral cannulae with a variable interval of 40 s to 60 s separating infusions.

Time-stamped clocks were synchronized for taste infusions, neural recordings, and

videotape recordings.

Behavioral analysis: taste reactivity

Hedonic, aversive and neutral taste reactivity patterns were later scored off-line

for the duration of the infusion and 5 seconds after that, in slow-motion (1/30 s frame-by-

frame to $1/10^{th}$ actual speed) using established procedures developed to assess hedonic

versus aversive taste valuation (Grill and Berridge, 1985; Berridge, 2000) and the Datarat

scoring program (developed by the Aldridge lab). Hedonic responses included rhythmic

midline tongue protrusions, lateral tongue protrusions, and paw licks. Aversive responses

included gapes, head-shakes, face washes, forelimb flails, and chin rubs. Neutral

responses included passive dripping of solution out of the mouth and rhythmic mouth

movements.

A time-bin scoring procedure was used to ensure that taste reactivity components

of different relative frequencies still contributed equally to the final affective

hedonic/aversive totals (Berridge, 2000). Specifically, this ensured that shifts in frequent

components (e.g., rhythmic tongue protrusions) did not swamp shifts in rarer but equally

informative components (e.g., lateral tongue protrusions). The other behavioral

components (lateral tongue protrusions, gapes, forelimb flails, head shakes) typically

occur in discrete events and were thus scored as single occurrences each time they occurred (e.g., one gape equals one occurrence). Individual rat totals were calculated for hedonic, neutral, and aversive categories by adding all response scores within an affective category for that rat.

We tested affective orofacial reactions of rats to a series of solution infusions into the mouth via oral cannula 15 minutes after amphetamine, haloperidol, naltrexone, or saline control injections or 90 minutes after morphine injections. The 90-min morphine time point was chosen because a previous study found maximal drug induced increases in hedonic reactions to sucrose at approximately 90-min after systemic (subcutaneous) morphine injections (Doyle et al, 1993). Naltrexone has a faster time course, with no difference in hedonic reactions at 25 and 40 minutes post-injection (Richardson et al, 2005). Early time course effects of morphine include a sedative effect which potentially masks the hedonic response.

Neural analysis.

Spike discrimination.

Single neurons (N=154) were identified using principle components or peak-width analysis of waveforms using Offline Sorter (Plexon Inc., Dallas TX). Neurons were verified by distinct spike waveforms (whose shapes remained consistent throughout the whole recording). Units with more than 2 % of spikes within a 1 ms refractory period window in an autocorrelation histogram were excluded. A cross-correlation analysis was also performed to ensure that neurons were counted only once. (NeuroExplorer, Nex Techologies, Littleton MA).

Firing rates.

Rates were calculated for the first 600 ms after taste onset. Our baseline epoch was a 10 sec period one second prior to stimulus onset (-11 to -1 s). Normalized firing response to a stimulus event for each neuron was obtained by dividing the neuron's absolute firing response in epochs of interest by its baseline (1 would represent the baseline rate, > 1 hz an increase in rate, and <1 hz a decrease). The change in firing rate from baseline was calculated by converting the normalized rate to a percentage change from baseline (0 would represent no change from baseline, a positive percentage would be an increase in rate, a negative percentage an decrease in rate).

Responsive Populations.

A neuron was considered 'responsive' if the epoch of interest contained a significant excitation, inhibition, or mixed response. For each unit, raster plots and perievent time histograms (PETHs) with 50 ms bins were aligned to the onset of taste delivery. The criteria for increases in firing rate (labeled "excitations" in figures for brevity, although this could include disinhibition responses) were either one 50 ms bin being 3 SD greater than the baseline epoch, or two consecutive bins 2 SD above baseline. Decreases in firing rate (labeled "inhibitions" in figures for brevity, although this could include disfacilitation responses) were defined by one bin 2 SD below baseline or two bins 1 SD below baseline. Mixed responses were defined as any combination of both an increase and a decrease in firing rate, as defined above. Visual inspections of spike

rasters were used to eliminate any false positives due to a single trial being the source of the rate change.

Histology

Anatomical localization of electrode sites was done after completion of testing. Rats were euthanized with pentobarbital (FatalPlus) at the end of the experiment. The electrodes were then retracted the full extent to minimize further damage. Once the animal had expired, the brains were removed, frozen with dry ice, and sliced into 40 µm coronal sections using a CM 1850 cryostat (Leica Microsystems, Buffalo Grove, IL), stained with cresyl violet, covered in Permount, and coverslipped. Electrode placement was later confirmed by observing the brain slices under a light microscope.

Results

Behavioral Results

When the animal received systemic morphine or naltrexone, bitter quinine (2.6 x 10^{-4} M) elicited more hedonic reactions compared to a vehicle control, amphetamine, or haloperidol (figures 3.1 and 3.2). Overall, there were significant drug and taste effects (two-way ANOVA; $F_{4,42}=4.758$, $p = 8.639^{-06}$; $F_{2,42}=30.483$, $p = 6.632^{-09}$; respectively) for hedonic taste reactions. When we collapsed opioid modulation (morphine and naltrexone) and dopamine modulation (amphetamine and haloperidol), there were significantly more hedonic reactions under opioid modulation than control or dopamine modulation in response to quinine (figure 3.1, ANOVA; $F_{2,19}=6.184$, $p = 0.009$), but the only individual drug comparison that was significant in the post hock analysis was that

naltrexone yielded more hedonic reactions than haloperidol in response to quinine ($p = 0.04$).

As hypothesized, dopamine modulation (amphetamine and haloperidol) did not alter the hedonic value of tastes (0.5 M sucrose , tap water, or 2.6 x 10^{-4} M quinine) compared to control conditions. There were no significant drug effects for the number of hedonic reactions to 0.5 M sucrose or hedonic reactions to tap water. As expected, sucrose consistently evoked more hedonic than aversive taste reactions. In contrast, under control, amphetamine and haloperidol conditions, quinine elicited strongly aversive "disliking" reactions. Tap water elicited a balance of hedonic and aversive reactions (Figure 3.1). Post hock analysis confirmed that the three tastes each had significantly different amounts of hedonic taste reactions: sucrose elicited more hedonic reactions than water ($p = 0.01$) and quinine ($p = 1.083^{-06}$), and water was more 'liked' than quinine ($p = 0.03$).

Like the hedonic reactions, the aversive reactions also had significant drug and taste effects (two-way ANOVA; $F_{4,42}=2.116$, $p = 0.019$; $F_{2,42}=48.568$, $p = 1.191^{-11}$; respectively). Post hock analysis confirmed that the three tastes each had significantly different amounts of aversive taste reactions: sucrose elicited fewer aversive reactions than water ($p = 0.0187$) and quinine ($p = 2.731^{-11}$), and water was less aversive than quinine ($p = 7.302^{-06}$). However there were no significant drug effects for the aversive reactions within a specific taste category. The neutral reactions had an overall drug effect (but no drug effects within a taste category), and no taste effects (two-way ANOVA; $F_{4,42}=1.846$, $p = 0.045$; $F_{2,42}=0.419$, $p > 0.05$; respectively).
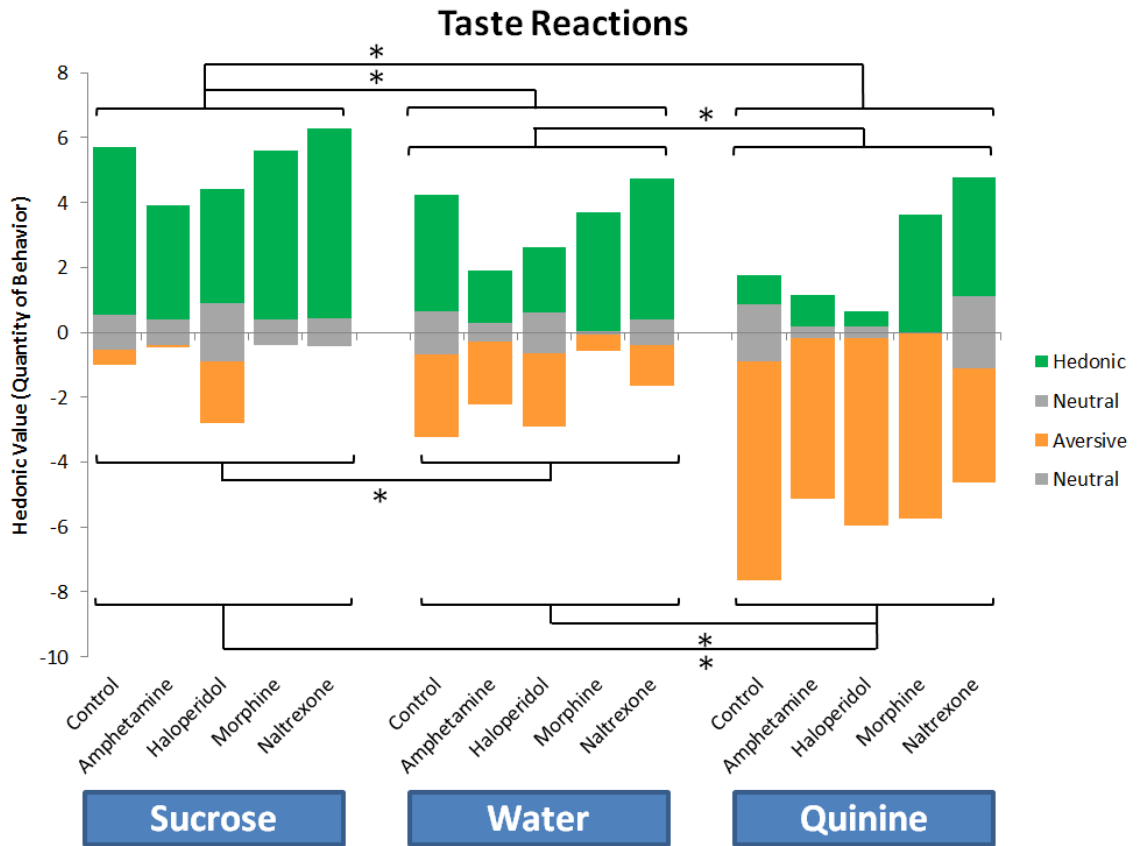
Figure 3.1 Overview of taste reactions for all tastes and drug conditions. Sucrose was 'liked' the most, water was less hedonic, and quinine was the most aversive taste. * p < 0.05

Morphine and naltrexone boosted the hedonic value of bitter quinine (figure 3.2), but did not lessen the aversive reactions to quinine, nor alter overall taste reactions to sucrose or water.

Hedonic Reactions to Quinine
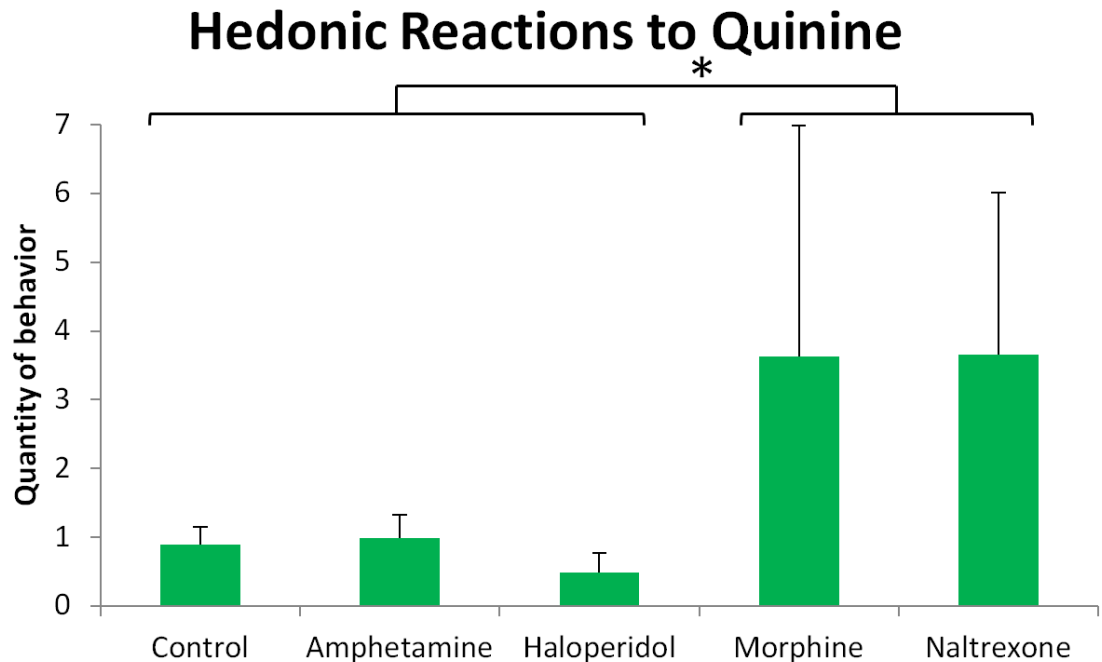
Figure 3.2 Opioid modulation increased the amount of hedonic taste reactions to bitter quinine. Error bars represent standard error. * p < 0.05

Ventral Pallidum Neuronal Activation

The predominant VP response to the taste stimuli was an increase in firing rate (245 out of 341 neural responses to a taste, or 72%), regardless of the hedonic valence of the tastes. There were also a smaller population that responded to the tastes with a decrease in firing rate (74/341, or 22%), and even fewer units (22/341, or 6%) that had both an increase and a decrease in rate within 600 ms of taste onset (Figure 3.3). Units that responded to the tastes with a decrease in firing rate ("inhibitions") and units that had both increases and decreases in firing rate within the epoch of interest ("complex responses") did not encode hedonic value in any set pattern (X^2, each NS). Haloperidol resulted in roughly equal proportions of excitations (37-43%) and inhibitions (27-30%),

65

across all tastes, compared to other drug conditions, which are mostly excitations (21-73%) and very few inhibitions (0-21%) (Table 3.1).

Population Coding
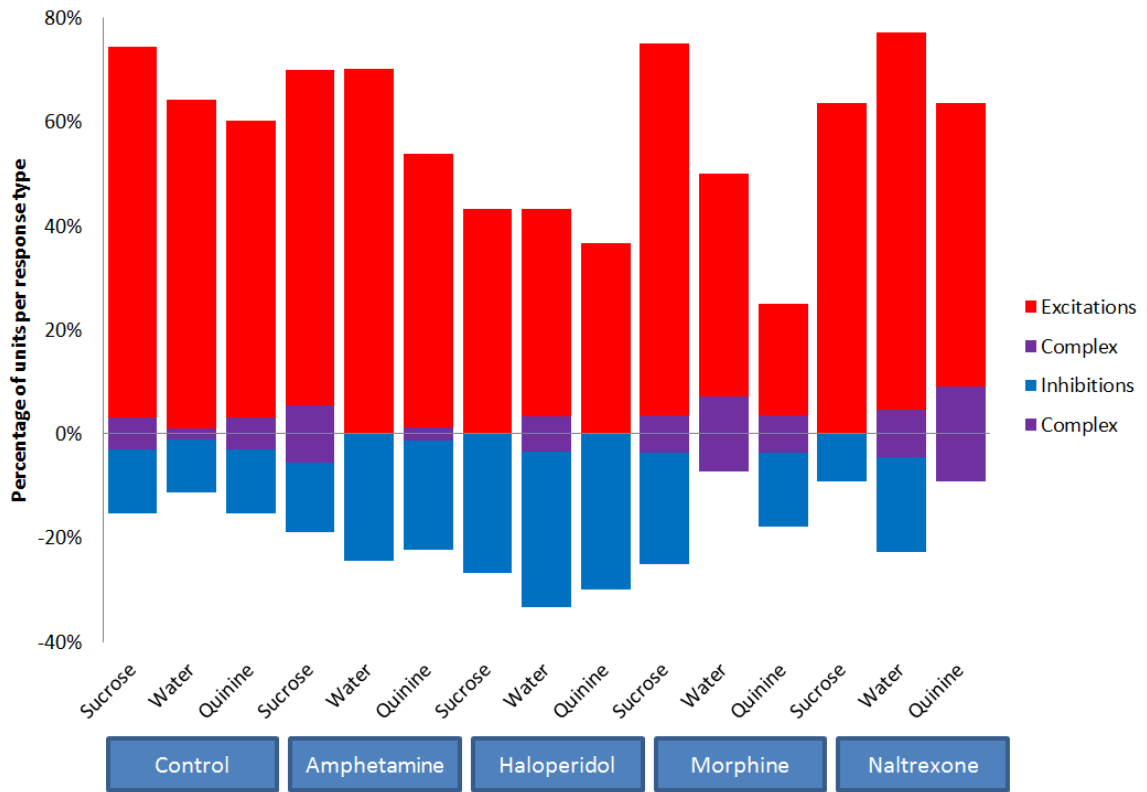
Figure 3.3 Overview of neural responses for all tastes and drug conditions. Sucrose and water generally triggered more excitation responses than quinine.

Morphine was the only drug that resulted in a significant difference in neural coding between the three tastes (figure 3.4; X^2 (2, N = 14) = 27.82, p <0.001). The proportion of units that had an increase in firing rate in response the tastes was highest to sucrose (71%), which was 'liked', there were fewer excitations in response to tap water (43%), and even fewer in response to bitter quinine (21%). The individual tastant comparisons of excitation responses were also significant: sucrose yielded more

excitations than water (X^2 (1, N = 14) = 7.14, p <0.01), water more than quinine (X^2 (1, N = 14) = 7.14, p <0.01), and therefore sucrose more quinine (X^2 (1, N = 14) = 26.92, p <0.001). No other drug conditions (vehicle control, amphetamine, haloperidol, and naltrexone) were statistically significant for encoding hedonic value in this manner, although there were fewer excitations in response to quinine than sucrose in all six drug conditions.
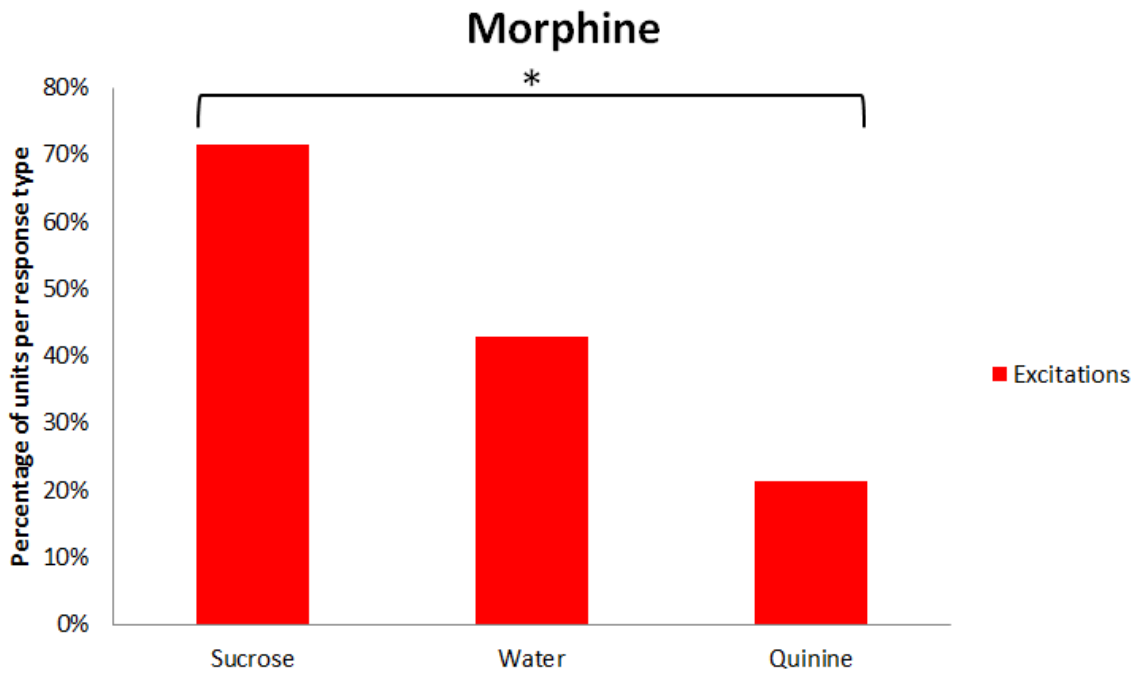
Morphine

Figure 3.4 With systemic morphine, VP neurons code the hedonic value of tastes via the proportion of units that showed an excitation response. * p < 0.05

Table 3.1 VP unit responses as a percentage of all units, for all drug conditions and tastes.

		Control			Amphetamine			Haloperidol			Morphine			Naltrexone		
		Sucrose	Water	Quinine	Sucrose	Water	Quinine	Sucrose	Water	Quinine	Sucrose	Water	Quinine	Sucrose	Water	Quinine
Excitation		71%	63%	57%	64%	70%	53%	43%	40%	37%	71%	43%	21%	64%	73%	55%
Complex		6%	2%	6%	11%	0%	3%	0%	7%	0%	7%	14%	7%	0%	9%	18%
Inhibition		12%	10%	12%	13%	24%	21%	27%	30%	30%	21%	0%	14%	9%	18%	0%

Rate coding of tastes

The raw firing rates during the 600 ms epoch after taste onset were lower under amphetamine than control for all three tastes. The firing rate was lower under amphetamine than vehicle control in response to sucrose (37 spikes/sec and 48 spikes/sec respectively; $t = 2.657$, $p = 0.009$, $df = 97$); in response to tap water (35 spikes/sec and 45 spikes/sec respectively; $t = 2.340$, $p = 0.02$, $df = 90$); and to quinine (36 spikes/sec and 48 spikes/sec respectively; $t = 2.710$, $p = 0.008$, $df = 84$). There were no significant drug effects on the change in normalized rate in response to any of the three tastes (Table 3.2; ANOVAs, all NS).

Table 3.2 Average firing rates 0-600 ms after taste onset for all drug and taste conditions.

	Control	Amphetamine	Haloperidol	Morphine	Naltrexone
Sucrose	48 Hz	37 Hz	47 Hz	42 Hz	43 Hz
Water	45 Hz	35 Hz	45 Hz	43 Hz	42 Hz
Quinine	48 Hz	36 Hz	41 Hz	39 Hz	40 Hz

Discussion

As predicted, boosting dopamine levels (amphetamine) or blocking D2 receptors (haloperidol) did not alter taste reactions compared to vehicle control. Under vehicle control conditions and dopamine modulation, sucrose was 'liked', water elicited a mixture of hedonic and aversive reactions, and quinine was primarily aversive. This matches previous studies that have also found that boosting dopaminergic activity doesn't alter the hedonic value of tastes (Smith et al. 2011, Berridge et al. 2010, Berridge et al. 1989, Peciña et al. 1997, Wyvell and Berridge 2000 and 2001, Leyton et al. 2002, Volkow et al. 2002, Peciña et al. 2003, Robinson et al. 2005, Tindell et al. 2005, Evans et al. 2006). Likewise, rats given the dopamine antagonist pimozide does not block 'liking' reactions in response to sucrose solutions (Peciña et al, 1997), and rats with 6-OHDA lesions which destroyed 99% of the animal's dopamine neurons exhibit the same amount of 'liking' reactions to sweet tastes as control animals (Berridge and Robinson, 1998).

Opioid modulation shifted behavior towards the hedonic end of the taste reaction spectrum. Under opioid modulation, water primarily resulted in hedonic reactions and quinine was a fairly even mix of hedonic and aversive. In other words, tastes were more hedonic under the influence of systemic morphine and naltrexone. These results are consistent with previous studies which found that systemic morphine increased the increased the number of hedonic reactions to a sucrose-quinine mixture (Doyle et al., 1993) and tastes in general (Berridge, 1996). But the previous studies on the effect of naltrexone have found that in humans it decreases the hedonic value of tastes, particularly the value of sweet foods (Yeomans and Gray 1997 and 2002). We did not see a decrease in the hedonic value of 17% sucrose solution under systemic Naltrexone compared to control. Our dose of naltrexone (1 mg/kg) matched those of a previous study in rats that found a decrease in hedonic reactions to salt in salt deprived rats (Na et al. 2012) and decreased the palatability and consumption of 10% ethanol in rats (Coonfield et al. 2002). However, higher doses (3 to 10 mg/kg) have also been found to be effective (Na et al. 2012, Ferraro et al. 2002), so it's possible that our dose was lower than ideal.

The only drug condition that reflected the hedonic value of the tastes via neural coding was morphine. Under systemic morphine, the proportion of cells that showed an excitation response was inversely related to the hedonic value of the taste. So systemic morphine boosted the hedonic value of quinine compared to vehicle control while enhancing the difference in VP response to tastes with different hedonic values. The population responses to the three tastes were not strikingly different in the vehicle control, amphetamine, haloperidol, or naltrexone conditions. Overall, the predominant neural response to a taste was an increase in firing rate. There were few inhibitions and

and even fewer cells that responded with both an increase and a decrease in firing rate within the 600 ms epoch following taste delivery.

Further investigation would be needed to determine if our finding for naltrexone's effect on the hedonic value of tastes truly counters previous studies, or whether this result is a product of a small sample size or too low of a dose. It would also be interesting to further elucidate the contributions of specific dopamine and opioid receptor types on VP coding of hedonic taste value.

Conclusion

Overall, there is an interesting disconnect between the behavioral and neural results. While the control, amphetamine, and haloperidol showed the strongest behavioral difference between tastes, those same drug conditions showed the weakest neural differences between tastes. Morphine and naltrexone both increased the quantity of hedonic taste reactions to bitter quinine, making the behavioral responses closer to those in response to sucrose and water. While we expected morphine to boost the hedonic value of quinine, we did not expect naltrexone to do the same. Rather, we hypothesized that naltrexone would decrease the hedonic value of sucrose. We did not see this drug effect. Morphine was the only drug condition that resulted in a neural difference between tastes. The proportion of "excitations" to "inhibitions" was highest for the taste that was 'liked' the most (sucrose) and lowest for the taste that was 'disliked' the most (quinine).

Reference List

Agmo A, Galvan A, Talamantes B (1995) Reward and reinforcement produced by drinking sucrose: two processes that may depend on different neurotransmitters. Pharmacol Biochem Behav 52:403–414

Barbano MF, Cador M (2006) Opioids for hedonic experience and dopamine to get ready for it. Psychopharmacology (Berl). (3):497-506.

Berridge KC (1996) Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev 20:1–25

Berridge KC (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. Neurosci Biobehav Rev 24:173-198.

Berridge KC (2003) Pleasures of the brain. Brain Cogn 52:106-128.

Berridge, K. C., Ho, C., Richard, J. M., & DiFeliceantonio, A. G. (2010). The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders. *Brain Res, 1350*, 43-64.

Berridge KC, Treit D (1990) A comparison of benzodiazepine, serotonin, and dopamine agents in the taste-reactivity paradigm. Pharmacology Biochemistry & Behavior, 37:451-456.

Berridge KC, Valenstein ES (1991) What psychological process mediates feeding evoked by electrical stimulation of the lateral hypothalamus? Behav Neurosci 105:3-14.

Berridge KC, Venier IL, Robinson TE (1989) Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: implications for arousal and anhedonia hypotheses of dopamine function. Behav Neurosci 103:36-45.

Doyle TG, Berridge KC, Gosnell BA (1993) Morphine enhances hedonic taste palatability in rats. Pharmacol Biochem Behav 46:745–749Evans AH, Pavese N, Lawrence AD, Tai YF, Appel S, Doder M, Brooks DJ, Lees AJ, Piccini P (2006) Compulsive drug use linked to sensitized ventral striatal dopamine transmission. Ann Neurol 59:852-858.

Drewnowski A, Krahn DD, Demitrack MA, Nairn K, Gosnell BA (1992) Taste responses and preferences for sweet high-fat foods: evidence for opioid involvement. Physiol Behav 1992;51:371–9.

Giraudo SQ, Grace MK, Welch CC, Billington CJ, Levine AS (1993) Naloxone's anorectic effect is dependent upon the relative palatability of food. Pharmacol, Biochem Behav 46:917– 21.

Glass MJ, Billington CJ, Levine AS (1999) Opioids and food intake:distributed functional neural pathways? Neuropeptides 33:360–368

Grill HJ, Norgren R (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 143:263-279.

Grill HJ, Berridge KC (1985) Taste reactivity as a measure of the neural control of palatability. Progress in Psychobiology and Physiological Psychology 2.

Jaeger D, Gilman S, Aldridge JW (1990) A multiwire microelectrode for single unit recording in deep brain structures. J Neurosci Methods 32:143-148.

Kelley AE, Bakshi VP, Haber SN, Steininger TL, Will MJ, Zhang M (2002) Opioid modulation of taste hedonics within the ventral striatum. Physiol Behav 76:365-377.

Kelley AE, Bless EP, Swanson CJ (1996) Investigation of the effects of opiate antagonists infused into the nucleus accumbens on feeding and sucrose drinking in rats. J Pharmacol Exp Ther 278:1499-1507.

Leyton M, Boileau I, Benkelfat C, Diksic M, Baker G, Dagher A (2002) Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[11C]raclopride study in healthy men. Neuropsychopharmacology 27:1027-1035.

Mahler SJ, Berridge KC (2012) What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. Psychopharmacology (Berl) 221:407–28.

Na ES, Morris MJ, Johnson AK (2012) Opioid mechanisms that mediate the palatability of and appetite for salt in sodium replete and deficient states. Physiol Behav 106:164–70.

O'Hare EO, Cleary J, Bartz PJ, Weldon DT, Billington CJ, Levine AS (1997) Naloxone administration following operant training of sucrose/water discrimination in the rat. Psychopharmacology (Berlin) 129:289–94.

Olds ME (1982) Reinforcing effects of morphine in the nucleus accumbens. Brain Res 237:429-440.

Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates* (6th ed.). Amsterdam, the Netherlands: Elsevier Inc.

Peciña S, Berridge KC (2000) Opioid site in nucleus accumbens shell mediates eating and hedonic 'liking' for food: map based on microinjection Fos plumes. Brain Res 863:71-86.

Peciña S, Berridge KC (2005) Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci 25:11777-11786.

Peciña S, Berridge KC, Parker LA (1997) Pimozide does not shift palatability: separation of anhedonia from sensorimotor suppression by taste reactivity. Pharmacol Biochem Behav 58:801-811.

Peciña S, Smith KS, Berridge KC (2006) Hedonic hot spots in the brain. Neuroscientist 12:500-511.

Peciña S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. J Neurosci 23:9395-9402.

Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD (2005) Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. Behav Neurosci 119:5-15.

Sills TL, Vaccarino FJ (1996) Individual differences in sugar consumption following systemic or intraaccumbens administration of low doses of amphetamine in nondeprived rats. Pharmacol Biochem Behav 54:665-670.

Smith, K. S., & Berridge, K. C. (2005). The ventral pallidum and hedonic reward: Neurochemical maps of sucrose "liking" and food intake. *The Journal of Neuroscience, 25*(38), 8637-8649.

Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: Interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *The Journal of Neuroscience, 27*(7), 1594-1605.

Smith, K. S., Berridge, K. C., & Aldridge, J. W. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proc Natl Acad Sci U S A, 108*(27), E255-E264.

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behav Brain Res, 196*(2), 155-167.

Tindell AJ, Berridge KC, Aldridge JW (2004) Ventral pallidal representation of pavlovian cues and reward: population and rate codes. J Neurosci 24:1058-1069.

Tindell AJ, Smith KS, Berridge KC, Aldridge JW (2005 a) VP Neurons Integrate Learning and Physiological Signals to Code Incentive Salience of Conditioned Cues. Society for Neuroscience Abstracts.

Tindell AJ, Berridge KC, Zhang J, Pecina S, Aldridge JW (2005 b) Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. Eur J Neurosci 22:2617-2634.

Tindell AJ, Smith KS, Pecina S, Berridge KC, Aldridge JW (2006) Ventral pallidum firing codes hedonic reward: when a bad taste turns good. J Neurophysiol 96:2399-2409.

Toates FM (1986) Motivational Systems. Cambridge: Cambridge University Press.

van der Kooy D, Mucha RF, O'Shaughnessy M, Bucenieks P (1982) Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Res 243:107-117.

Volkow ND, Wang GJ, Fowler JS, Logan J, Jayne M, Franceschi D, Wong C, Gatley SJ, Gifford AN, Ding YS, Pappas N (2002) "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. Synapse 44:175-180.

Wheeler RA, Carelli RM (2006) The neuroscience of pleasure. Focus on "Ventral pallidum firing codes hedonic reward: when a bad taste turns good". J Neurophysiol 96:2175-2176.

Wise RA (1980) The dopamine synapse and the notion of 'pleasure centers' in the brain. Trends in Neurosciences 3:91-95.

Woolley JD, Lee BS, Fields HL (2006) Nucleus accumbens opioids regulate flavor-based preferences in food consumption. Neuroscience 143:309-317.

Wyvell CL, Berridge KC (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. J Neurosci 20:8122-8130.

Wyvell CL, Berridge KC (2001) Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. J Neurosci 21:7831-7840.

Yang CR, Mogenson GJ (1989) Ventral pallidal neuronal responses to dopamine receptor stimulation in the nucleus accumbens. Brain Res 489:237-246.

Yeomans MR, Gray RW (1997) Effects of naltrexone on food intake and changes in subjective appetite during eating: evidence for opioid involvement in the appetizer effect. Physiol Behav 62:15 –21

Yeomans MR, Gray RW (2002) Opioid peptides and the control of human ingestive behaviour. Neurosci Biobehav Rev. 26(6):713-28.

CHAPTER 4

VENTRAL PALLIDAL CODING OF TASTE CUES AND REWARDS

Abstract

We investigated whether cues that signal the delivery of unconditioned taste stimuli (sucrose, which is generally intrinsically 'liked') can take on the hedonic properties of the rewards through associative learning. We also examined the effect of increased dopamine levels (amphetamine), D2 antagonism (haloperidol), opioid stimulation (morphine), or opioid antagonism (naltrexone) on the hedonic value of cues and rewards and how the ventral pallidum (VP) encodes these changes. A pair of infusions of tap water (CS+1 and CS+2) preceded sucrose delivery (a natural reward) in classical conditioning trials. We also tested blocks of extinction trials, which consisted of the cues without the reinforcement of rewards, and blocks of uncued rewards. Overall, the cues did take on hedonic value via reward association.

Introduction

Incentive salience of cues

A study by Tindell et al. (2005b) demonstrated that VP neurons integrate

dopamine signals to track the incentive salience of cues for rewards. Rats were trained

to associate a series of cues (CS+1, which was temporally distal from the reward,

followed by CS+2, which was proximal to reward) with a sucrose reward. Acute

amphetamine effects, amphetamine sensitization effects, and sensitized rats with

amphetamine on board were tested for VP neuronal coding of reward cues. All three

manipulations of mesolimbic dopamine levels preferentially elevated CS+2 firing more

than CS+1 firing. Because motivation for reward is thought to build with temporal proximity, this dopamine induced boost in CS+2 responses was attributed to an increase in incentive salience. If there had been a general increase in reward prediction learning, there would not have been a preferential increase in CS+2 responses compared to CS+1.

Opioid elevation of the incentive salience and hedonic value of cues

Opioids increase eating behavior and food intake (Cooper and Kirkham 1993, Berthoud 2002, Kelley et al. 2002, Berridge 2003, Levine and Billington 2004, Peciña et al. 2006). Elevated eating behavior also follows stimulation of opioid receptors in the hotspot (Bakshi and Kelley 1993, Zhang and Kelley 2000,Peciña and Berridge 2005), perhaps reflecting a role in 'wanting' as well as 'liking'. Microinjections of an opioid agonist (DAMGO) in the NAcc and VP increased eating of normal chow (Smith and Berridge 2007). This was true even in the 'hedonic cold spot' (the caudal half of the medial shell of the NAcc) where 'liking' reactions were actually suppressed by DAMGO below vehicle control levels (Peciña and Berridge, 2005). Opioids may cause 'liking' only in a specialized hotspot of the NAcc, but cause eating and food 'wanting' throughout the structure. The effect of opioids on cue-triggered incentive motivation has not been studied to as much as the effects of dopamine. Rats will show a place preference for morphine and will work by pressing a lever to receive a morphine infusion (Bodnar 2013), indicating that incentive salience likely contributes to morphine abuse.

The effect of opioid elevation on the hedonic value of tastes has been studied more extensively. Opioid agonist (DAMGO) microinjections of an in the NAcc and VP 'hedonic hot spots' (the rostral half of the medial shell of the NAcc and the caudal half of

the VP) increased 'liking' reactions and decreased 'disliking' reactions to orally infused sucrose (Peciña and Berridge 2005, Smith and Berridge 2007). However, DAMGO administered into the central nucleus of the amygdala did not enhance liking responses for sugar (Mahler and Berridge 2012). Morphine enhanced saline intake in sodium-depleted rats and respectively reduced negative responses (Na et al. 2012). Systemic morphine also increased the increased the number of hedonic reactions to a sucrose-quinine mixture (Doyle et al., 1993) and other tastes (Berridge, 1996).

Previous studies have shown that Pavlovian cues can take on some level of hedonic value though association with reward (Berridge and Schulkin 1989, Bindra 1974, Bolles 1972, Delamater et al. 1986, Kerfoot et al. 2007, Toates 1986). Intra-accumbal microinjections of DAMGO (a μ-opioid receptor agonist), increased the hedonic reactions to a pair of CS+ cues that were tones (although the absolute quantity of these reactions were fairly modest, as there was no taste present for the animal to process) and sucrose reward compared to control (Smith et al. 2011). Furthermore, there was a greater increase in hedonic value of the second cue than the first cue.

We hypothesized that morphine would boost the hedonic value of cues and rewards and that the VP would reflect this change in hedonic value compared to vehicle controls. What's more, we proposed that hedonic value might increase with temporal proximity to reward, such that the hedonic value of the second cue might be boosted more than the first cue. We hypothesized that naltrexone would decrease the hedonic value (via an increase in aversive taste reactions) of cues and rewards and that this effect would like wise be reflected in the neural response to the stimuli.

Dopamine elevates the incentive salience but not the hedonic value of cues

There is no clear evidence that increased dopamine levels enhances the hedonic value of cues that predict reward (Smith et al. 2011). Instead, dopamine seems to signal the incentive salience of Pavlovian CS+ cues for reward and trigger motivation for rewards. Studies using manipulations that increased accumbal dopamine have shown increases in conditioned reinforcement, Pavlovian-instrumental transfer, instrumental breakpoint, runway performance, second-order conditioning, and conditioned instrumental reinforcement (Wise and Bozarth 1985, Berridge 1996, Robbins and Everitt 1996, Berridge and Robinson 1998, Wise 1998, Everitt et al. 1999, Berke and Hyman 2000, Wyvell and Berridge 2000, Salamone and Correa 2002, Peciña et al. 2003, Di Ciano and Everitt 2005, Everitt and Robbins 2005, Wise 2005, Uslaner et al. 2006, Baldo and Kelley 2007, Berridge 2007). However, motivation is not the focus of this study, as we don't have a clean way to measure motivation directly (tastes were delivered intra-orally and therefore there was no instrumental or approach response required of the rats to receive the stimuli). Rather, we hypothesized that amphetamine and haloperidol would have no effect on the hedonic value of the cues or rewards, and therefore there would be no effect on the VP response to those stimuli.

Materials and methods

Subjects

Twelve adult male Sprague-Dawley rats weighing 250g – 400g were used in this experiment. Animals were housed individually in tub cages containing wood corn cob

bedding before implantation and shredded paper bedding post-implantation to avoid

bedding being trapped in the electrode covers on a 9:30 AM to 7:30 PM reversed

light/dark schedule at ~21°C. Experiments were conducted during late morning to

afternoon hours, coinciding with the rats' active (dark) period after acclimating to

housing conditions for 1-2 days. Food (Purina Rat Chow) and water were available *ad*

libitum in their home cages.

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.1 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). 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 5000 and bandpass-filtered

between 300 Hz and 6 kHz. Sessions were recorded at 30 frames a second via a video

camera placed underneath the glass floor. Timestamp clocks for the taste delivery

program, video recording, and neural recording 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.

Surgical procedures

Rats were pretreated penicillan and anesthetized with 100 mg/kg ketamine HCl

and 10 mg/kg xylazine. Rats were positioned in a stereotaxic apparatus (Kopf; Tujunga,

CA).   Bilateral electrodes were implanted in the caudal VP (ML ±2.7 to 2.8 mm; AP -0.8

to -1.1 mm; DV 5.96 to 7.3265 mm; incisor bar set for flat skull), with the tips of the

electrode bundles positioned approximately 1 mm above target sites in the VP (DV of

recording sites were 7.004 to 8.4535 mm) (Paxinos and Watson, 2007). Each electrode

consisted of 2 bundles with 4 wires each (50 µm tungsten). Each 4 wire bundle was a

screw-driven brass microdrive, which allowed each electrode to be moved individually to

optimize recording placement prior to testing.

In the same surgery, each animal was implanted with bilateral intraoral cannulae

(PE-100 tubing) to permit oral taste infusion.   Oral cannulae enter the mouth in the upper

cheek lateral to the first maxillary molar, travel beneath the zygomatic arch, and exit the

dorsal head lateral to the skull (Grill and Norgren, 1978; Grill and Berridge, 1985).

Electrodes and oralcnnulae were anchored to the skull with 8 bone screws and acrylic

cement. A stainless steel obturator was inserted in the intra-NAc cannulae to prevent

occlusion. Rats were allowed to recover for at least 7 days before behavioral testing.

They were maintained on daily injections of pennicillan and normal saline as needed to

prevent infection and keep the animal well hydrated during recovery. They had access to

normal chow and water at all times, but were supplemented with infant cereal until swelling that resulted from the oral cannulation was gone, typically 2-4 days. Oral cannulae did not disrupt normal eating.

Training

After surgical recovery, rats underwent five days of classical conditioning (table 4.1). Animals were placed in the testing chamber which was illuminated from underneath with white light. Sessions consisted of a five-minute habituation period, followed by 20 trials of intraoral infusions. Each trial consisted of a variable wait interval of 45 s to 75 s, followed by a 0.1 ml infusion of tap water, a five to seven second variable wait, a second water infusion, a fixed five second wait, a 0.1 ml delivery of sucrose (17%, 0.5M) (figure 4.1a). Tastants were 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 one of the oral cannula.

Testing

We tested affective orofacial reactions of rats to a series of solution infusions into the mouth via oral cannula 15 minutes after amphetamine, haloperidol, naltrexone, or saline control injections or 90 minutes after morphine injections. The 90-min morphine time point was chosen because a previous study found maximal drug induced increases in hedonic reactions to sucrose at approximately 90-min after systemic (subcutaneous) morphine injections (Doyle et al, 1993). Naltrexone has a faster time course, with no

difference in hedonic reactions at 25 and 40 minutes post-injection (Richardson et al, 2005). Early time course effects of morphine include a sedative effect which potentially masks the hedonic response.

Animals were injected with drug or vehicle (order of testing was randomized), connected to the taste delivery tubes and headstage cable, then placed in the testing chamber where they were allowed to habituate to the chamber while waiting for the drug to take effect (15-90 min). All test days were comprised of 3 blocks of 10 trials, the order of the blocks was randomized: classical conditioning trials that were identical to the training trials (figure 4.1a), extinction trials that were similar to the classical conditioning trials but were not reinforced with the sucrose reward (figure 4.1b), and uncued reward trials that had the sucrose rewards but no water cues (figure 4.1c). To infuse solutions into the mouth, a 3-ml syringe containing either sucrose (17%; 0.5 M) was attached via hollow tubing (PE-50 connected to a PE-10 delivery nozzle) to one of the oral cannula. Another syringe containing tap water was connected to the other oral cannula. Time-stamped clocks were synchronized for taste infusions, neural recordings, and videotape recordings.

Behavioral analysis: taste reactivity

Hedonic, aversive and neutral taste reactivity patterns were later scored off-line for the duration of the infusion and 5 seconds after that, in slow-motion (1/30 s frame-by-frame to $1/10^{th}$ actual speed) using established procedures developed to assess hedonic versus aversive taste valuation (Grill and Berridge, 1985; Berridge, 2000) and the Datarat scoring program (developed by the Aldridge lab). Hedonic responses included rhythmic

midline tongue protrusions, lateral tongue protrusions, and paw licks. Aversive responses included gapes, head-shakes, face washes, forelimb flails, and chin rubs. Neutral responses included passive dripping of solution out of the mouth and rhythmic mouth movements.

A time-bin scoring procedure was used to ensure that taste reactivity components of different relative frequencies still contributed equally to the final affective hedonic/aversive totals (Berridge, 2000). Specifically, this ensured that shifts in frequent components (e.g., rhythmic tongue protrusions) did not swamp shifts in rarer but equally informative components (e.g., lateral tongue protrusions). The other behavioral components (lateral tongue protrusions, gapes, forelimb flails, head shakes) typically occur in discrete events and were thus scored as single occurrences each time they occurred (e.g., one gape equals one occurrence). Individual rat totals were calculated for hedonic, neutral, and aversive categories by adding all response scores within an affective category for that rat.

Neural analysis.

Spike discrimination.

Single neurons (N=154) were identified using principle components or peak-width analysis of waveforms using Offline Sorter (Plexon Inc., Dallas TX). Neurons were verified by distinct spike waveforms (whose shapes remained consistent throughout the whole recording). Units with more than 2 % of spikes within a 1 ms refractory period window in an autocorrelation histogram were excluded. A cross-correlation analysis

was also performed to ensure that neurons were counted only once. (NeuroExplorer, Nex Techologies, Littleton MA).

Firing rates.

Rates were calculated for the first 600 ms after taste onset. Our baseline epoch was a 10 sec period one second prior to stimulus onset (-11 to -1 s). Normalized firing response to a stimulus event for each neuron was obtained by dividing the neuron's absolute firing response in epochs of interest by its baseline (1 would represent the baseline rate, > 1 an increase in rate, and <1 a decrease). The change in firing rate from baseline was as a percentage change from the normalized rate (0 would represent no change from baseline, a positive percentage would be an increase in rate, a negative percentage an decrease in rate).

Responsive Populations.

A neuron was considered 'responsive' if the epoch of interest contained a significant excitation, inhibition, or mixed response. For each unit, raster plots and perievent time histograms (PETHs) with 50 ms bins were aligned to the onset of each taste delivery. The criteria for increases in firing rate (labeled "excitations" in figures and occasionally referred to as "excitations" as a shorthand in the text, although this could include disinhibition responses) were either one 50 ms bin being 3 SD greater than the baseline epoch, or two consecutive bins 2 SD above baseline. Decreases in firing rate (labeled "inhibitions" in figures in and occasionally referred to as "inhibitions" as a shorthand in the text, although this could include disfacilitation responses) were defined

by one bin 2 SD below baseline or two bins 1 SD below baseline. Mixed responses were defined as any combination of both an increase and a decrease in firing rate, as defined above. Visual inspections of spike rasters were used to eliminate any false positives due to a single trial being the source of the rate change.

Histology

Anatomical localization of electrode sites was done after completion of testing. Rats were euthanized with pentobarbital (FatalPlus) at the end of the experiment. The electrodes were then retracted the full extent to minimize further damage. Once the animal had expired, the brains were removed, frozen with dry ice, and sliced into 40 µm coronal sections using a CM 1850 cryostat (Leica Microsystems, Buffalo Grove, IL), stained with cresyl violet, covered in Permount, and coverslipped. Electrode placement was later confirmed by observing the brain slices under a light microscope.

Table 4.1 Experimental timeline for classical conditioning study.

| Day 1	Days 2-7	Days 8-12	Days 13-23	Days 24-27
Bilateral oral canullation & bilateral VP targeting electrode implantation	Recovery	5 training days: 20 cued rewarded trials per day	Test days: 10 cued rewarded trials, 10 uncued reward trials, 10 extinction trials 6 drug/vehicle days, 48 hr washout between tests	Histology: Electrolytic lesion, glial growth, perfuse, slice, CV stain, mark figures
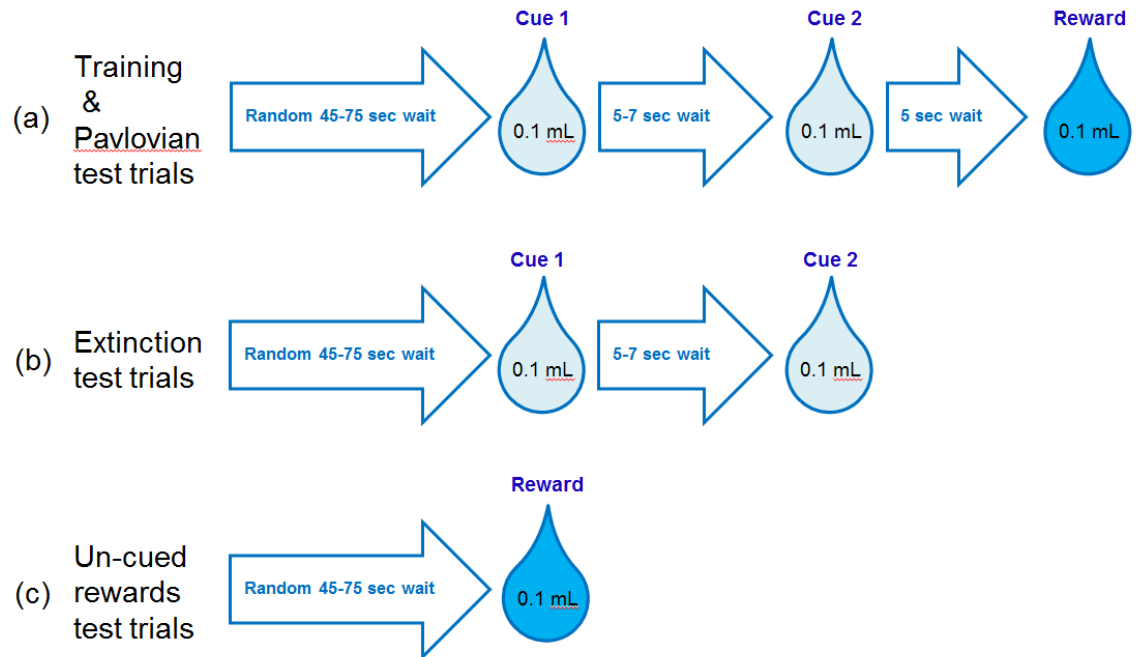
Figure 4.1 Schematic of a taste infusion trials showing timing of the different events. (a) The classical conditioning trials were identical on training and text days. (b) The extinction trials were only presented on test days and included the cues but not the rewards. (c) The uncued reward trials were also only presented on test days and consisted of just the rewards, with no cues.

Results

Behavioral Results

As hypothesized, all taste stimuli (water infusions that acted as cues and sucrose solutions that served as rewards) in this experiment were predominantly hedonic, regardless of the drug condition or trial type (figures 4.2-4.9). The neutral tasting cues often elicited as many hedonic reactions as the sweet rewards themselves. There were some neutral reactions, but very few to no aversive reactions. The test trials (titled Classical Conditioning Trials in figures 4.2-4.4) were identical to the training trials: a pair of water cues followed by a sucrose reward.

Classical Conditioning Trials
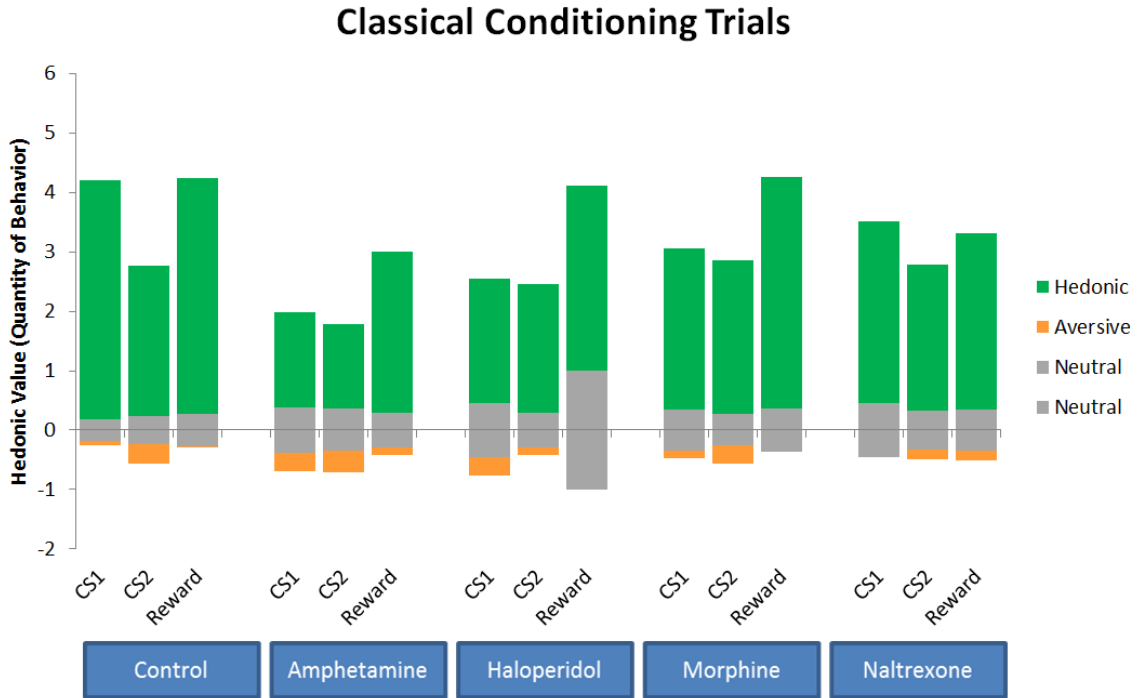
Figure 4.2 Overview of taste reactions from classical conditioning trials for all drugs.

Given how few neutral and aversive taste reactions there were, we focused analysis of behavior on the hedonic taste reactions. The only significant stimulus difference in the classical conditioning trials occurred in the vehicle control condition. The second cue elicited fewer hedonic reactions than the first cue and the reward (figure 4.3; ANOVA, $F_{2,45}=7.905$, $p = 0.001$; post hock t-test, $p = 0.013$, $p = 0.029$, respectively). For the four drug conditions, there were no stimulus differences on the number of hedonic reactions (ANOVAs, all NS).

Classical Conditioning Trials

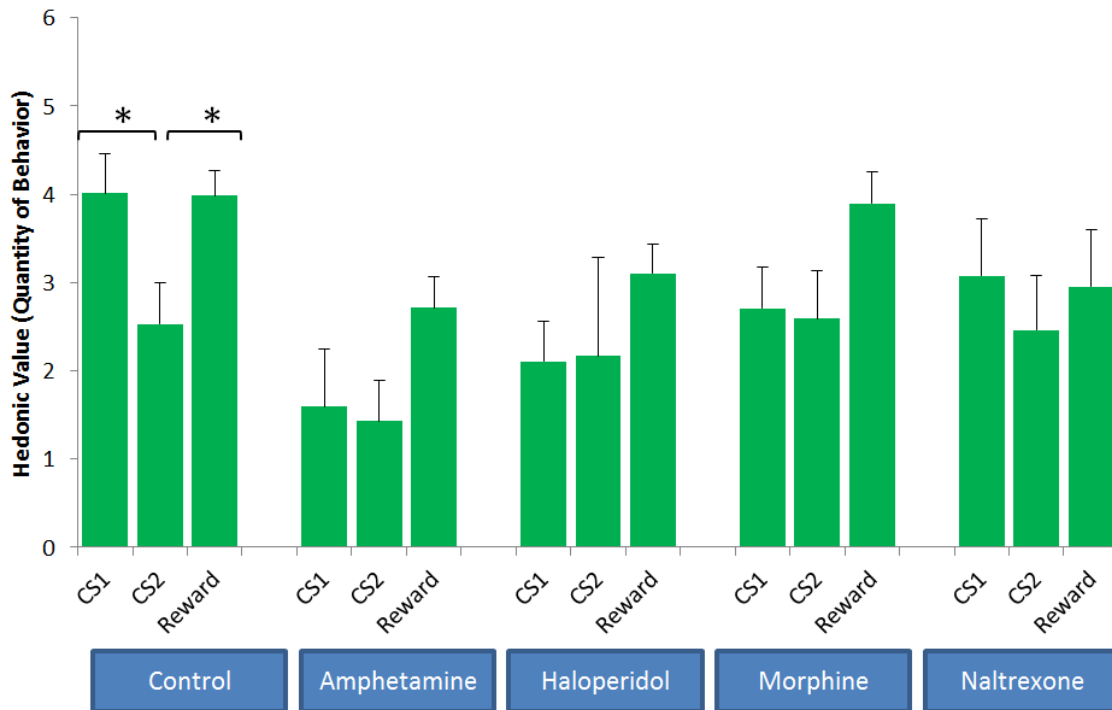Figure 4.3 Hedoinc taste reactions in response to classical conditioning trials arranged for stimulus comparisons per drug condition. Error bars represent standard error. * p < 0.05

We confirmed that dopamine modulation did not increase the hedonic value of taste cues or taste rewards. However, compared to vehicle control, there were fewer hedonic reactions to the first cue under the effect of amphetamine and haloperidol (figure 4.4; ANOVA, $F_{4,43}=3.510$, $p = 0.014$; post hock t-test, $p = 0.0006$, $p = 0.014$, respectively).   These drugs did not differ from control for the other two stimuli: cue 2 and reward (ANOVAs, all NS). In chapter 3, we saw that amphetamine and haloperidol had no effect on the hedonic value of water stimuli compared to vehicle control.

An alternative explanation for the effect of dopaminergic modulation of the hedonic value of CS+1 lies in motor effects. With amphetamine, there were generally far more non-taste related behaviors (such as ambulatory, sniffing, grooming, and rearing

behaviors) during the inter-trial interval (ITI), which makes more likely that the animal was engaged in another behavior at the onset of the first cue. This distraction might slightly delay their reaction to CS+1, making the count of hedonic reactions lower. This might be particularly apparent in this experiment because we analyzed such short time intervals (6 seconds) compared to previous experiments which measured hedonic reactions over 10-60 seconds. These general sensorimotor behaviors also may have made it more difficult to accurately score taste reactions. With haloperidol there was little to no exploratory behavior during the ITI, but while they were able to ultimately express taste reactions, perhaps they were a little slower to initiate the reactions. Hypodopaminergic states can make initiating a behavior harder, so this could explain both the decrease in exploratory behavior and taste reactions to the first cue. Further analysis is needed to determine whether general sensorimotor behaviors affected the taste reaction scoring.

The other drugs, morphine and naltrexone, did not differ from vehicle control in the number of hedonic reactions to CS+1, CS+2, or the reward (ANOVAs, all NS). Therefore, contrary to our hypothesis, we did not see a morphine enhancement of hedonic value of any of the stimuli. Nor did we see a naltrexone induced decrease in hedonic value of the cues or rewards.
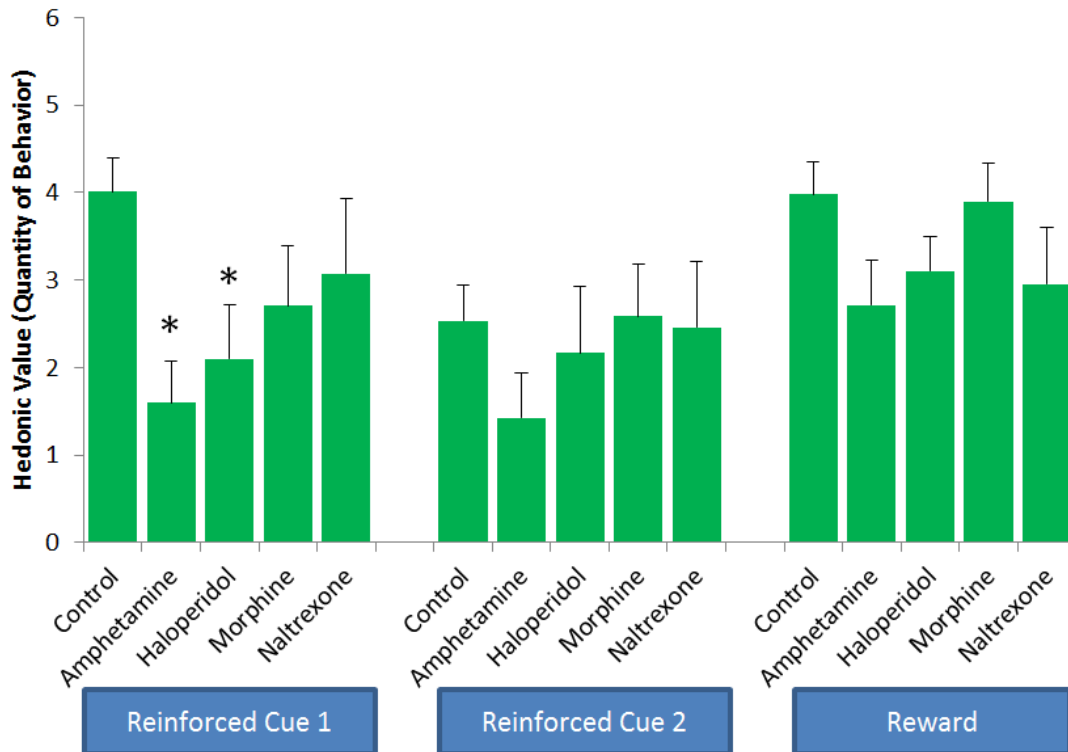
Classical Conditioning Trials

Figure 4.4 Hedoinc taste reactions in response to classical conditioning trials arranged for drug comparisons per stimulus. Error bars represent standard error. * p < 0.05

The extinction trials had slightly fewer hedonic taste reactions than the reinforced trials, but this difference was not significant. All stimuli were still predominantly hedonic (figure 4.5). Since there was no reward presented, it's not surprising that there were not many taste reactions during the six seconds following the taste onset (the "No Reward" conditions in figure 4.5), but there were a few taste reactions, mostly hedonic, during this epoch. These taste reactions could be the tail end of the reactions to the second cue, a conditioned behavior that occurs in anticipation of reward during the time it would have been delivered if it was a classical conditioning trial, or a combination of these reasons.
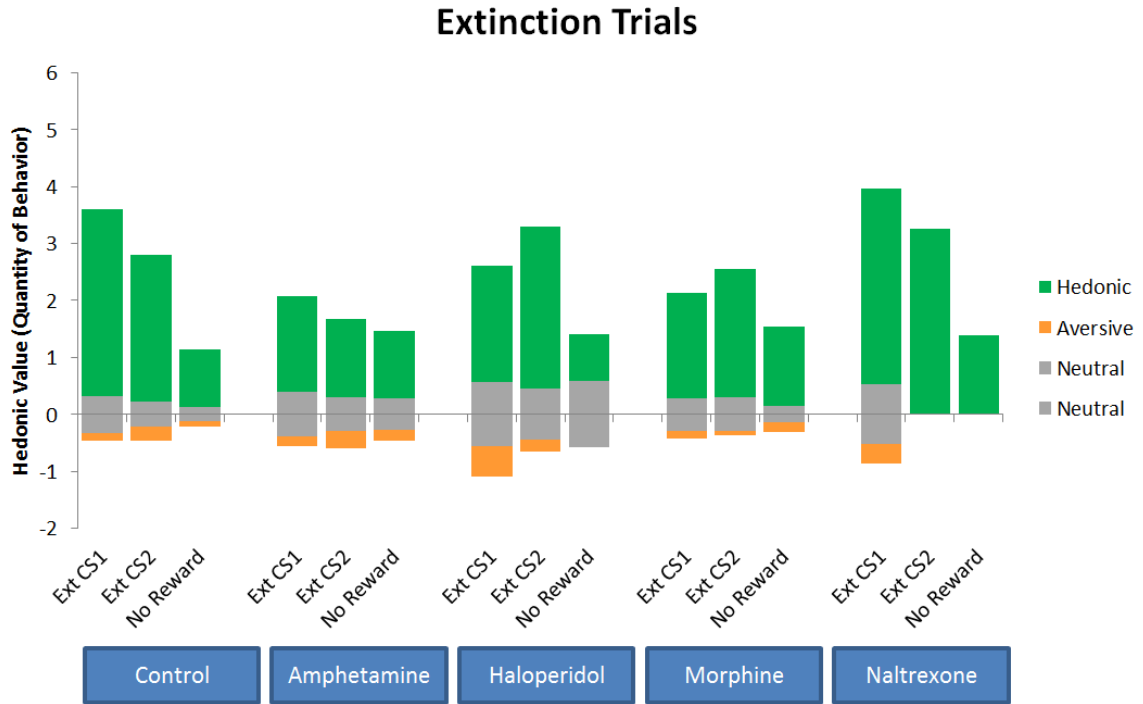
Extinction Trials

Figure 4.5 Overview of taste reactions from extinction trials, in which cues were delivered but they were not followed by the sucrose reward.

There was no difference in the number of hedonic reactions to the first and second cue for any drug condition in the extinction trials (figure 4.6). There were more hedonic reactions to cue 1 and cue 2 than to the missing reward in the vehicle control condition (ANOVA, $F_{2,45}$=7.905, $p = 0.001$; post hock t-test, $p = 0.0002$, $p = 0.008$, respectively). For the other drug conditions, there were no differences in the number of hedonic reactions between the first cue, second cue, or the missing reward.

Figure 4.6 Hedoinc taste reactions in response to classical conditioning trials arranged for stimulus comparisons per drug condition. Error bars represent standard error. * p < 0.05

There were no significant drug effects on the hedonic taste reactions for any stimulus epoch in the extinction trials (ANOVAs, all NS). However, there was a trend towards slightly fewer hedonic reactions to the first extinction cue (ANOVA, $F_{4,43}=2.310$, $p = 0.07$) in amphetamine and morphine conditions compared to control (post-hock t-test; $p = 0.050$, $p = 0.054$, respectively).

Extinction Trials
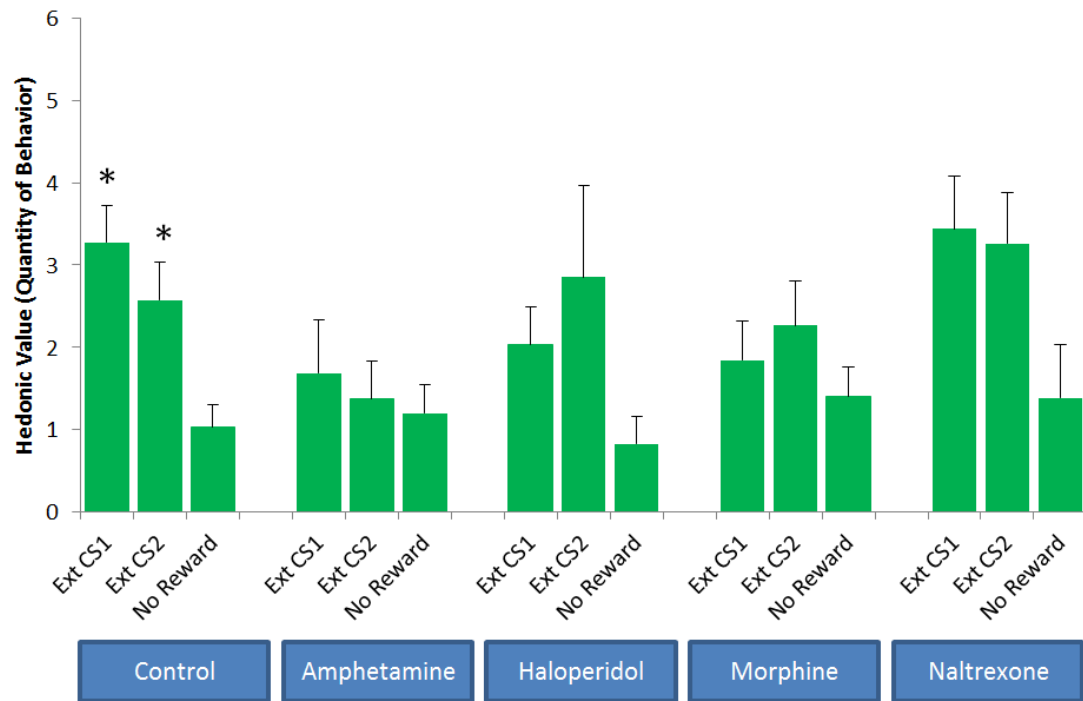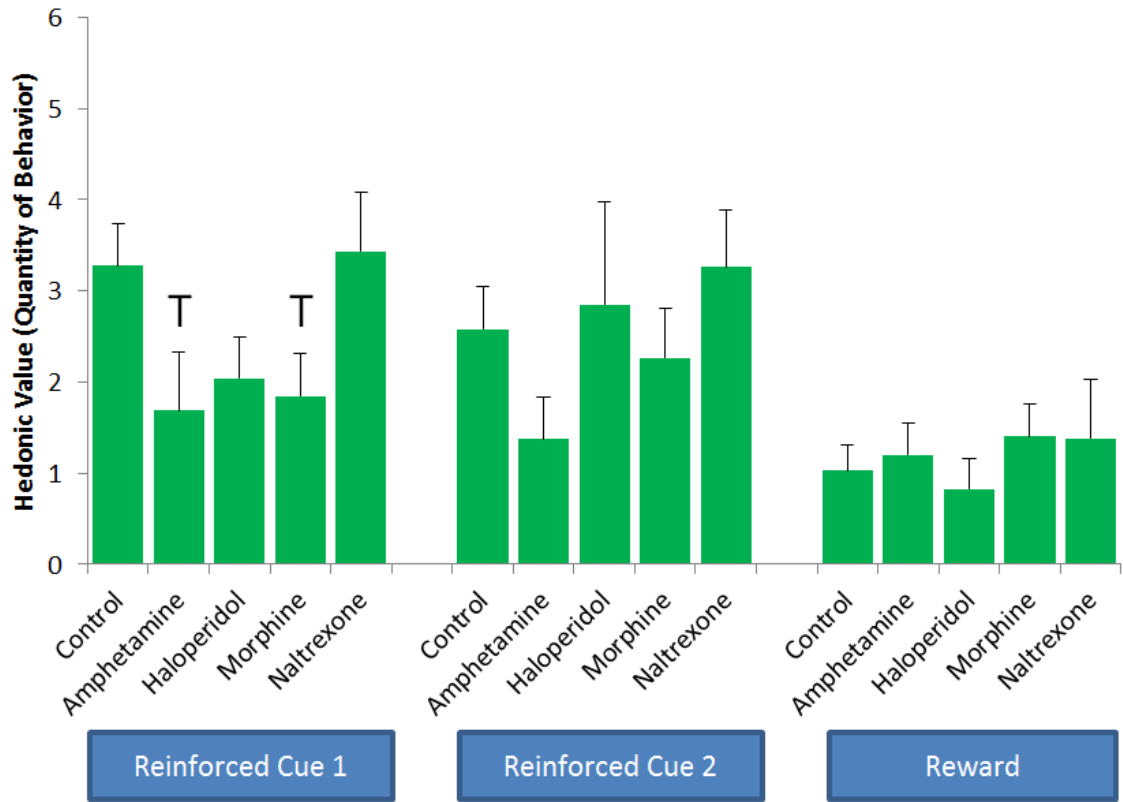

Figure 4.7 Hedoinc taste reactions in response to classical conditioning trials arranged for drug comparisons per stimulus. Error bars represent standard error. [T] $p < 0.10$

Like the cued rewards, the uncued sweet rewards strongly elicited hedonic

reactions, fewer neutral reactions, but very few to no aversive reactions (figure 4.8).

Uncued Reward Trials
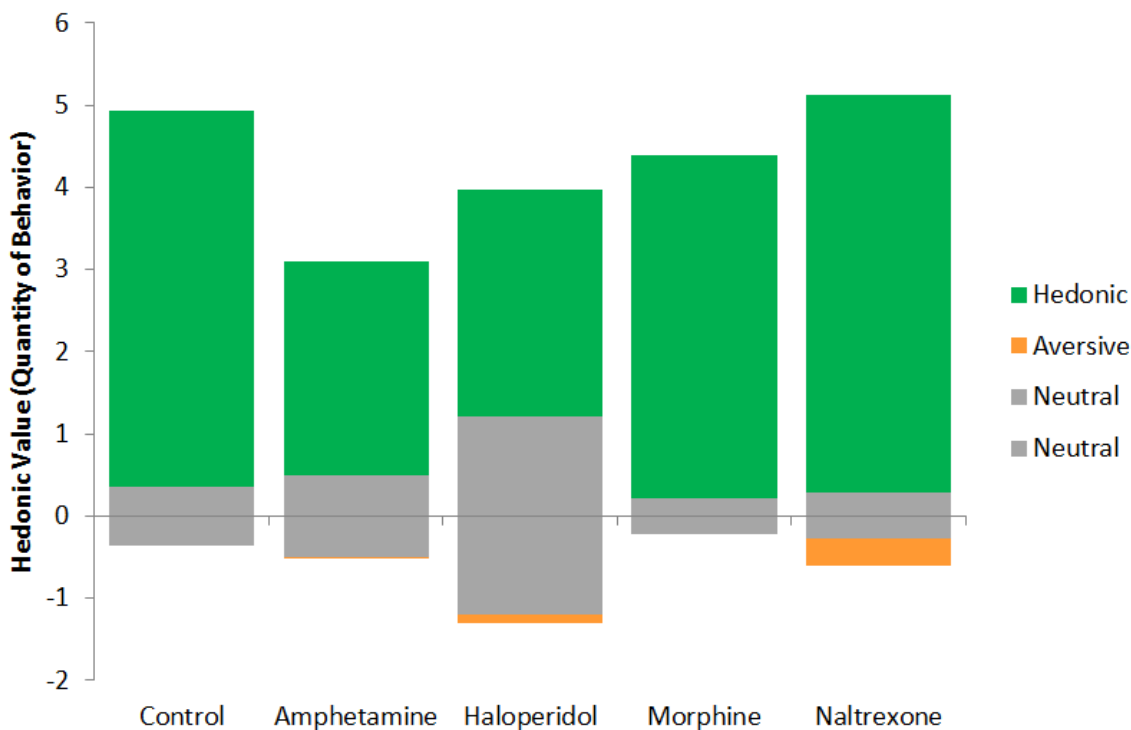
Figure 4.8 Overview of taste reactions in uncued reward trials, in which the sucrose reward was not preceded by the cues.

There were fewer hedonic reactions to uncued rewards under amphetamine and haloperidol compared to control (figure 4.9; ANOVA, $F_{4,43}$=5.404, $p = 0.001$; t-tests, $p = 0.0007$, $p = 0.008$, respectively). Amphetamine also made the uncued rewards less hedonic compared to morphine and naltrexone (t-tests, $p = 0.017$, $p = 0.004$, respectively). Haloperidol made uncued rewards less hedonic compared to naltrexone (t-tests, $p = 0.027$). Morphine and naltrexone had no effect on the number of hedonic reactions compared to vehicle control or each other (t-tests, all NS).
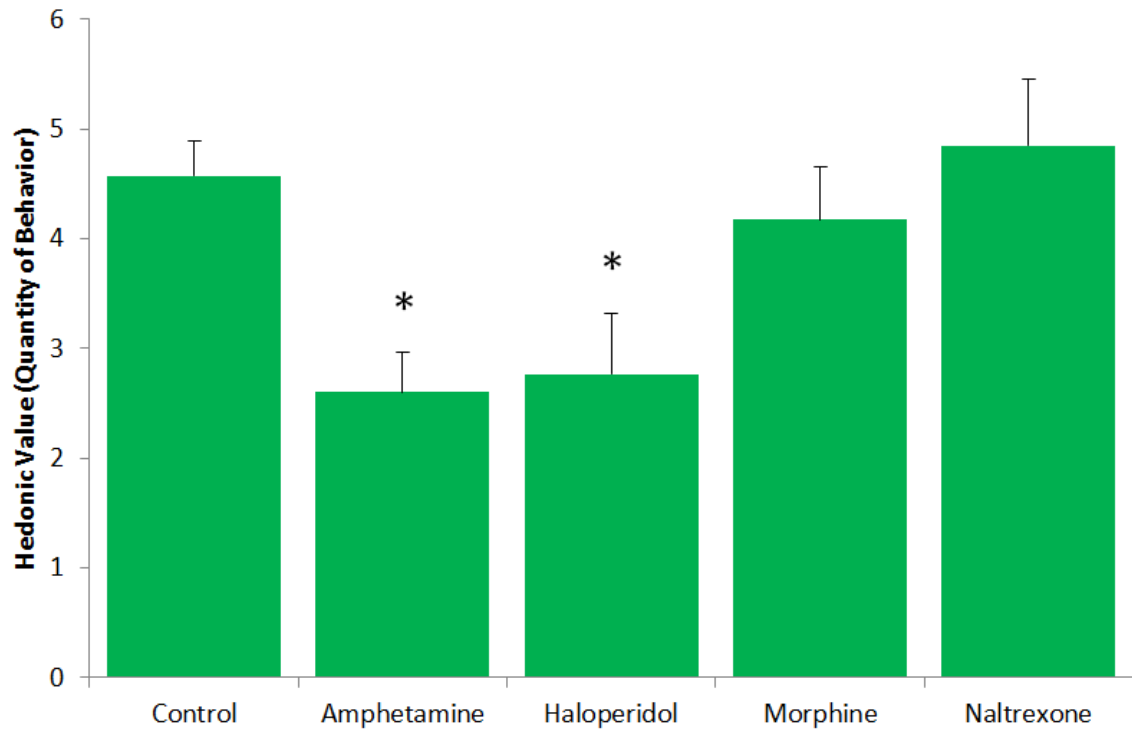
Figure 4.9 Hedoinc taste reactions in response to uncued sucrose rewards. Error bars represent standard error. * p < 0.05

Overall there was little difference in the count of hedonic reactions to cued vs. uncued rewards. There was a trend for a difference under naltrexone (t-test, $p = 0.058$), but there were no significant differences (figure 4.10; t-tests, all NS).
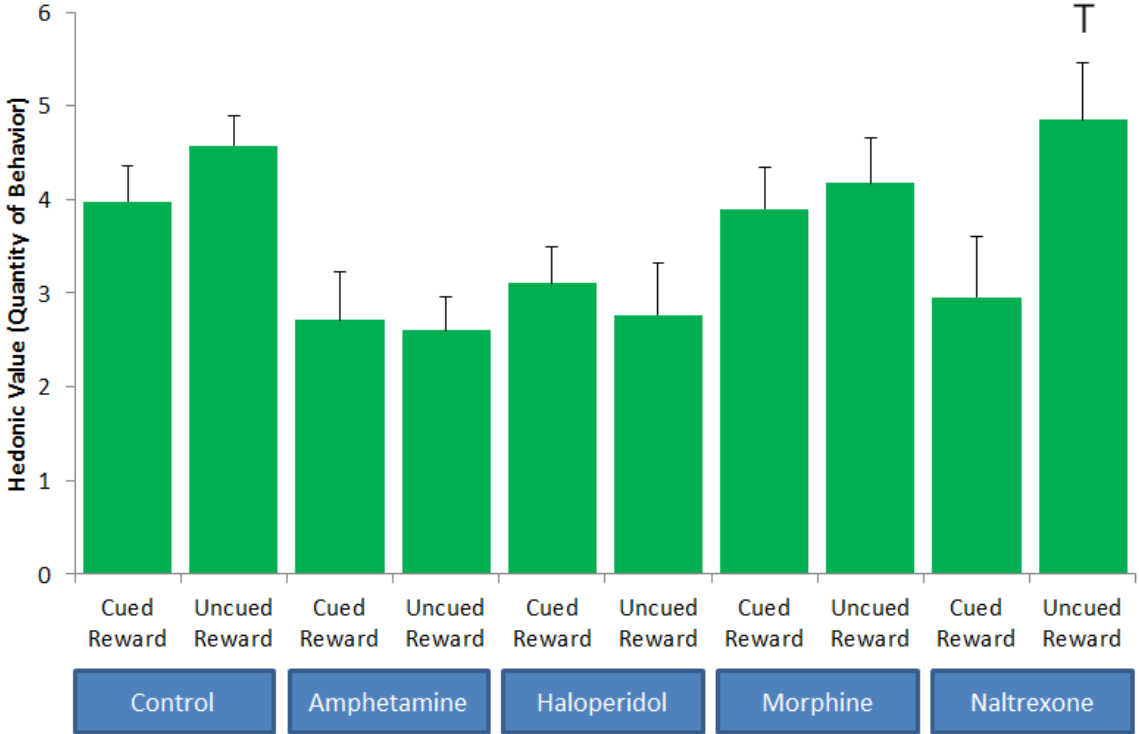
Figure 4.10 Comparison of rewards that were cued or not cued, per drug condition. Error bars represent standard error. ^{T} $p < 0.10$

There were no significant differences in the number of hedonic reactions to cue 1 when it was reinforced by reward compared to when it was presented under extinction conditions (figure 4.11; t-tests, all NS).

Reinforced vs. Extinction: Cue 1
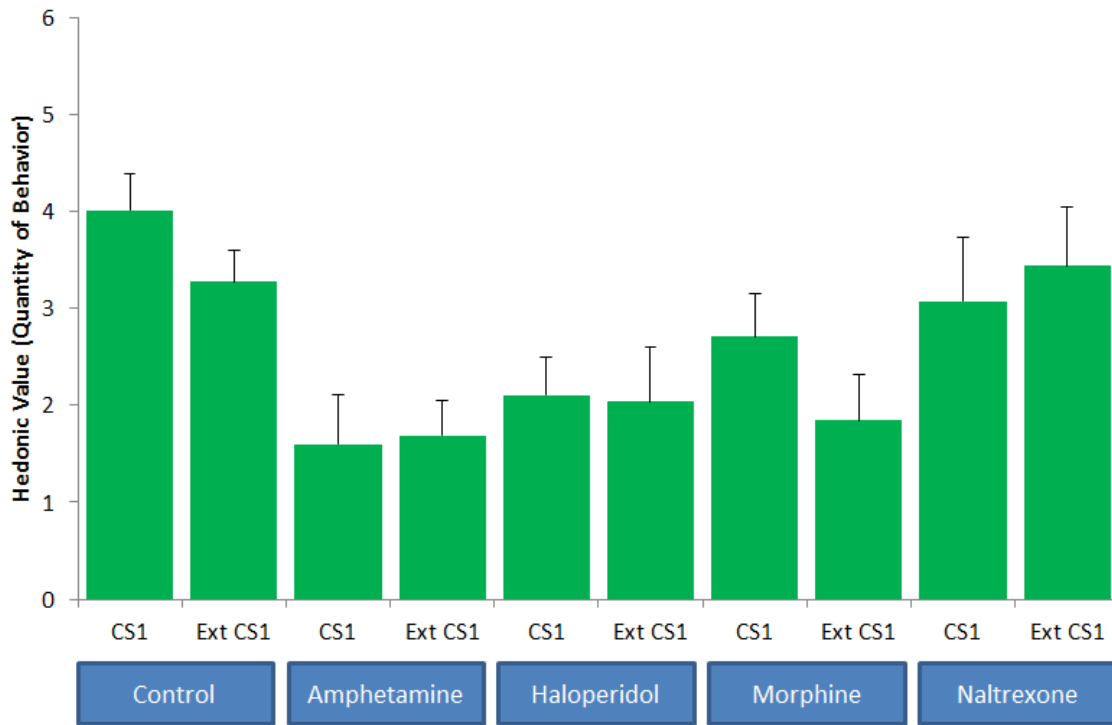
Figure 4.11 Comparison of the first cues that were reinforced (CS1) or not (Ext CS1), per drug condition. Error bars represent standard error.

Likewise, there were no differences between reinforced or extinction trials for the second cues (figure 4.12; t-tests, all NS).

Reinforced vs. Extinction: Cue 2
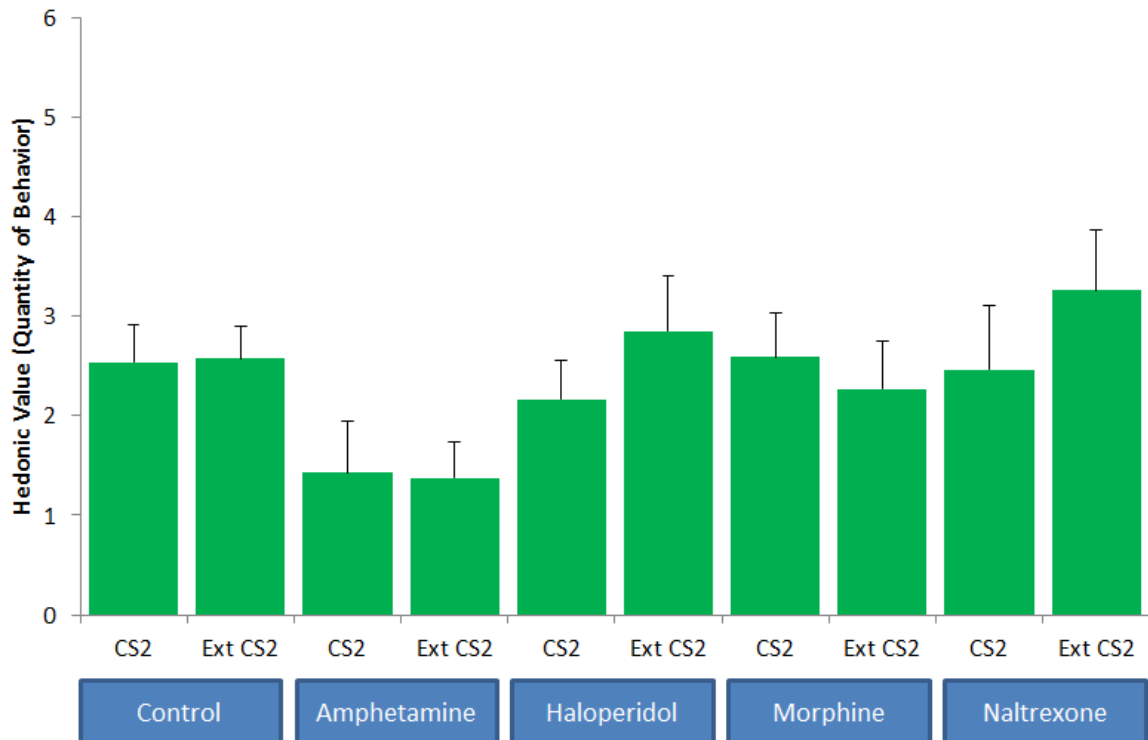
Figure 4.12 Comparison of the second cues that were reinforced (CS2) or not (Ext CS2), per drug condition. Error bars represent standard error.

Ventral Pallidum Neuronal Activation

Single unit examples

While the most common response to the cues and rewards was an increase in firing rate, there was a variety of responses overall. Some cells had the same response to all tastes (figures 4.13-4.14). Others had different responses to the different stimuli or conditions (figure 4.15). The trials used for the behavioral and population neural data had a variable 5-7 second wait between cue 1 and cue 2, making it not possible to align taste onset for the three stimuli in the classical conditioning trials within a single

continuous perievent time histogram (PETH). Instead, PETHs were created per stimulus

and analyzed individually. But a few animals from a pilot study were tested with a fixed

5 second interval between cue 1 and cue 2. These sessions were used to generate the

following PETHs (figures 4.13-4.15) for examples of VP responses.
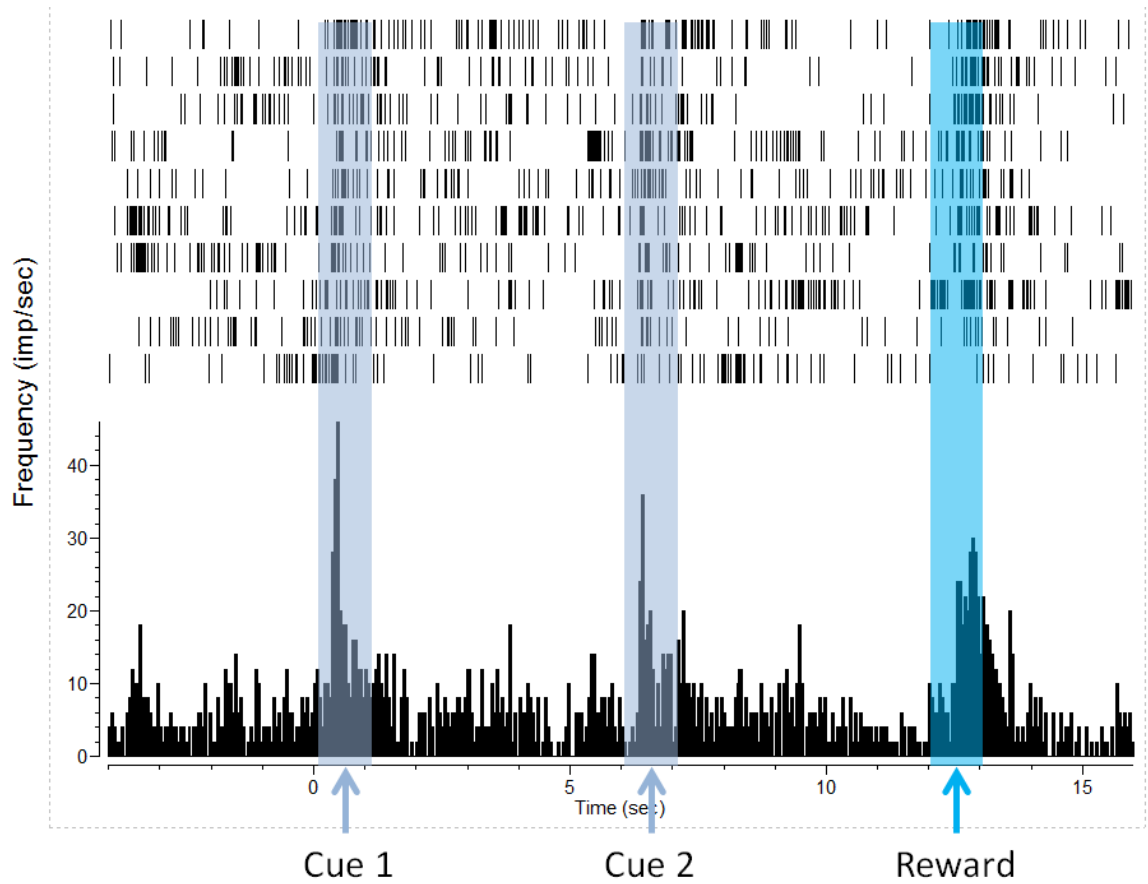
Figure 4.13 Example of a VP cell that responded to both cues and the reward with increases in firing rate. The pale blue rectangles indicate the one second intervals during which 0.1 ml of tap water was delivered intra-orally. The aqua rectangle indicates the one second interval during which 0.1 ml of 0.5 M sucrose was delivered intra-orally.
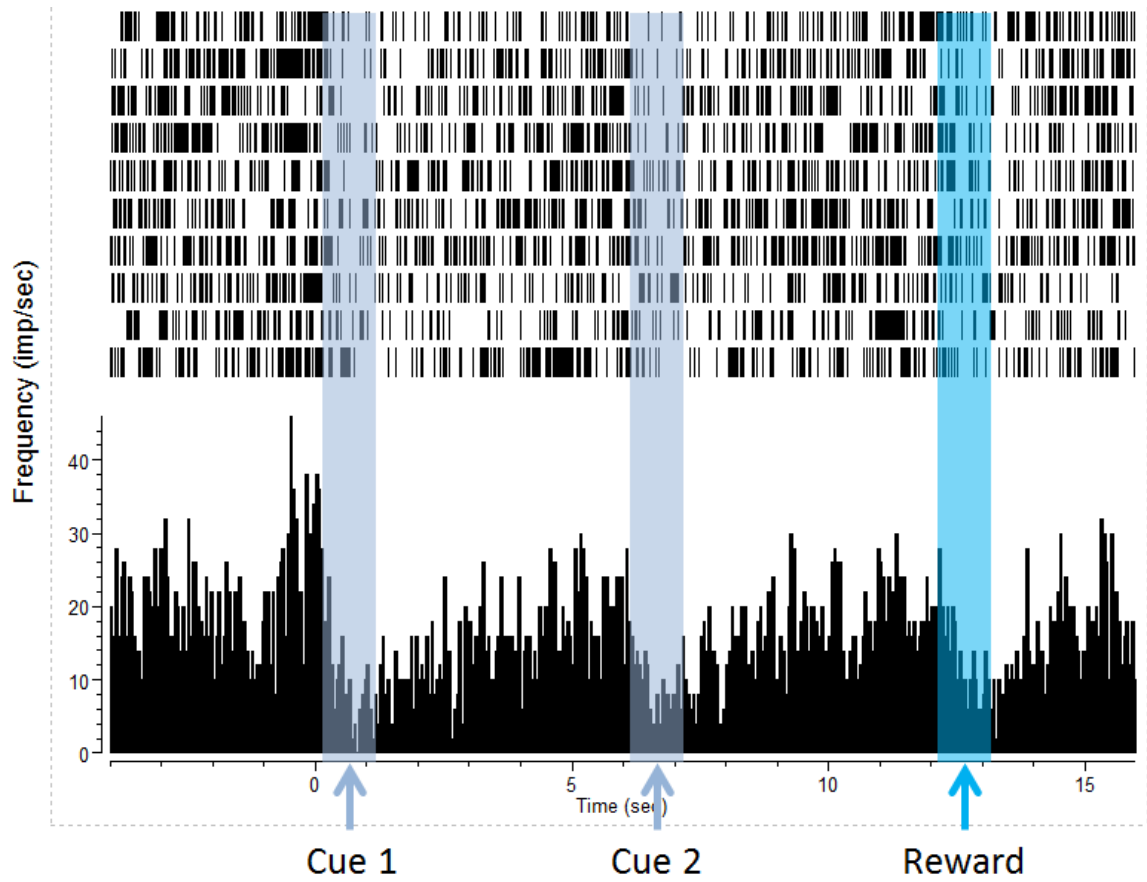
Figure 4.14 Example of a VP cell that responded to both cues and the reward with decreases in firing rate. The pale blue rectangles indicate the one second intervals during which 0.1 ml of tap water was delivered intra-orally. The aqua rectangle indicates the one second interval during which 0.1 ml of 0.5 M sucrose was delivered intra-orally.
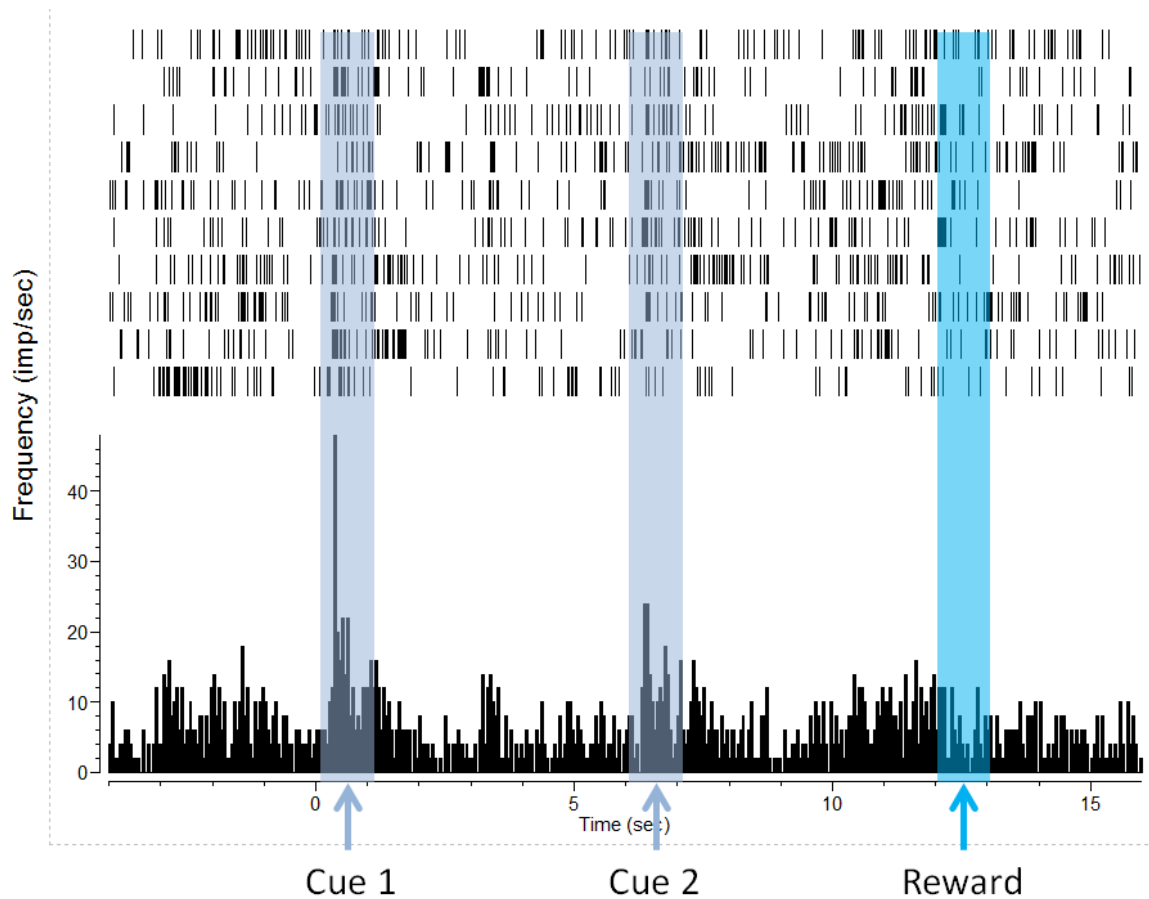
Figure 4.15 Example of a VP cell that had a stronger increase in firing rate to cue 1 than cue 2 and a decrease in firing rate in response to the reward. The pale blue rectangles indicate the one second intervals during which 0.1 ml of tap water was delivered intra-orally. The aqua rectangle indicates the one second interval during which 0.1 ml of 0.5 M sucrose was delivered intra-orally.

Population coding

The predominant VP response to the taste stimuli (CS+1, CS+2, Reward) in the classical conditioning trials was an increase in firing rate (345 out of 469 neural responses to a taste, or 74%, figure 4.16). There were also a smaller population that responded to the stimuli with a decrease in firing rate (94/469, or 20%), and even fewer units (30/469, or 6%) that had both an increase and a decrease in rate within 600 ms of taste onset (figure 4.16). When the cues were reinforced by rewards, there was almost no

difference in proportion of "excitations" to "inhibitions" between the cues and the

rewards. This mirrors the behavioral findings that there was hardly any difference in the

hedonic value of the cues and rewards. There were no stimulus differences in the

percentage of "inhibitions" or "complex responses" for any drug condition (X^2s, all NS).

The only stimulus difference that was statistically significant was that there were fewer

excitations in response to the second cue under naltrexone (X^2 (2, N = 51) = 6.03, p

<0.05).

For drug effects, there were fewer excitations to cue 1 for amphetamine than

control and fewer inhibitions to reward under haloperidol and cue 1 under naltrexone

compared to control (X^2s, p <0.05 for all differences listed above).
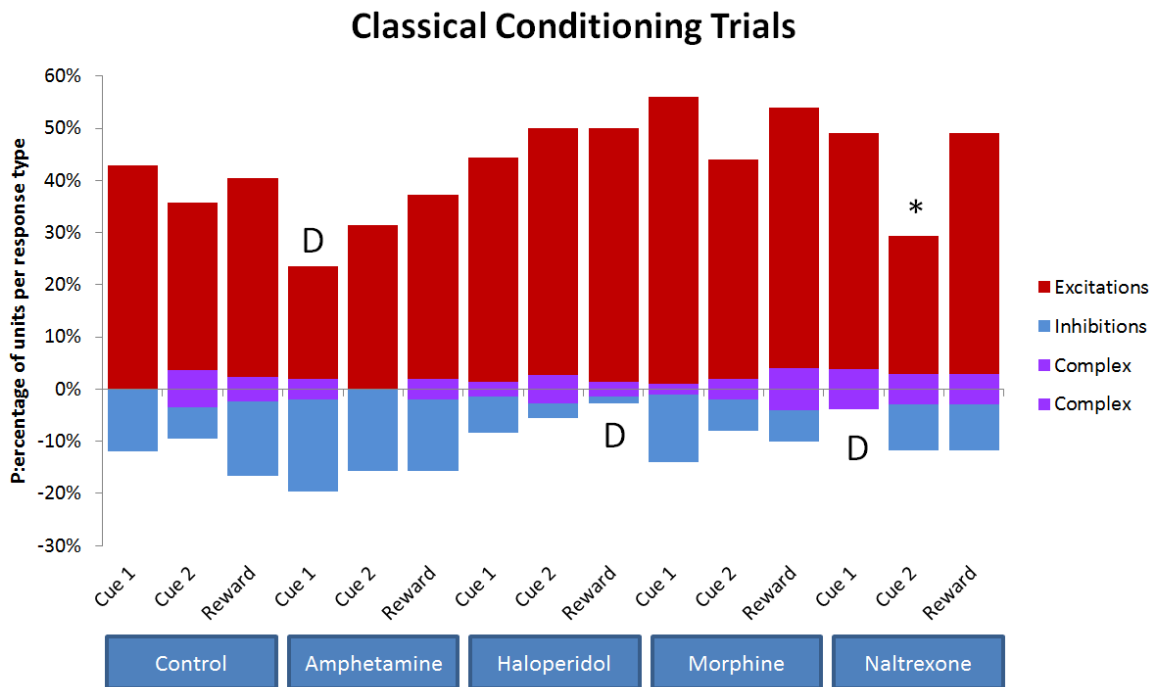
Figure 4.16 Overview of neural responses for all classical conditioning taste stimuli and drug conditions. *
p < 0.05 for stimulus effect, [D] p < 0.05 for drug effect compared to control.

Unlike the classical conditioning trials, there was more of a balance between the proportion of excitations (251 out of 438 neural responses to a taste, 57%) and inhibitions (188/438, 43%) in the extinction trials, although the proportion of "complex" responses was still low (13/438, 3%) (figure 4.17). Under vehicle control, haloperidol, and naltrexone conditions, there were more increases in firing rate in response to either cues than the missing reward (X^2s, p <0.05 for all differences listed above). Morphine resulted in more increases in firing rate in response to the first extinction cue than the second cue or the missing reward (X^2 (2, N = 50) = 4.15, p <0.05). This was the only significant difference between the first extinction cue and the second cue, for all other drug conditions there were no differences between cue 1 and cue 2 under extinction (X^2s, all NS).

Under haloperidol, there was also an increase in the percentage of "inhibitions" in response to the withheld reward than to either cue under extinction (X^2s, p <0.001 for both). Under amphetamine, there were more "inhibitions" to the missing reward than the first extinction cue (X^2 (1, N = 51) = 8.35, p <0.01). Amphetamine also caused the only significant drug effect on the response to stimuli: there were more "inhibitions" in response to the withheld reward compared to control (X^2 (1, N = 135) = 8.69, p <0.01).
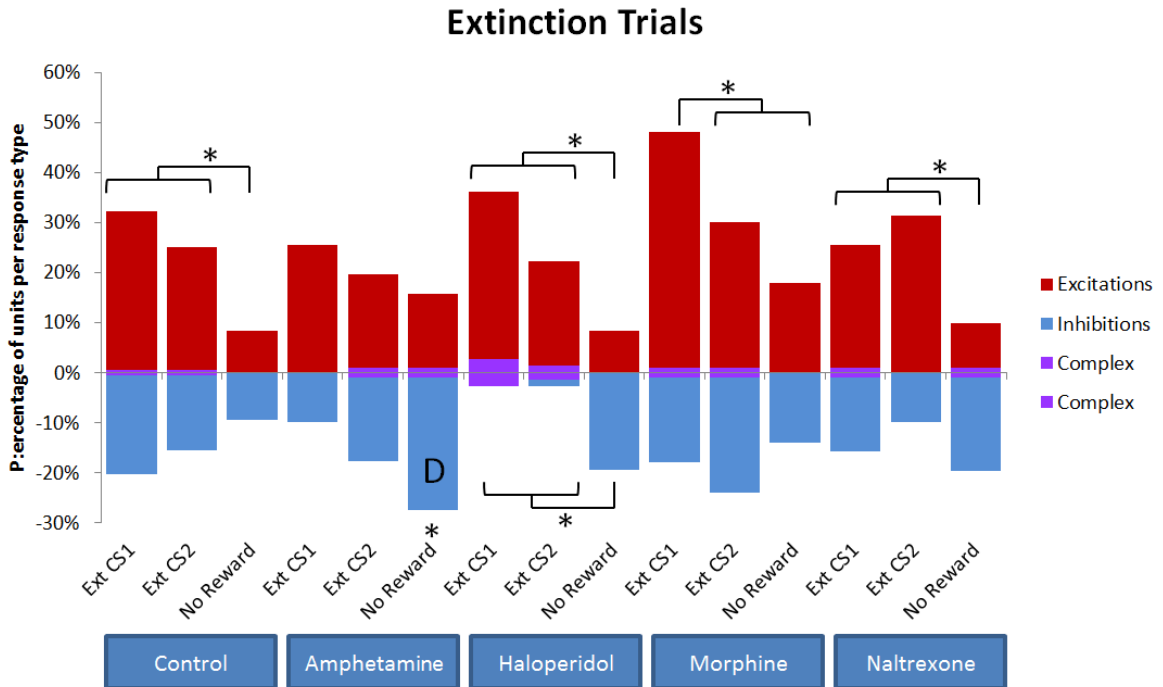
Figure 4.17 Overview of neural responses from the extinction trials, per drug conditions. * $p < 0.05$ for stimulus effect, [D] $p < 0.05$ for drug effect.

Overall, the uncued reward trials triggered many excitation responses but few inhibitions or mixed responses (figure 4.18). Haloperidol only elicited excitation responses. In addition, it elicited more excitations than vehicle control or amphetamine, and fewer inhibitions than any other drug condition (X^2s, $p < 0.05$ for all differences listed above).
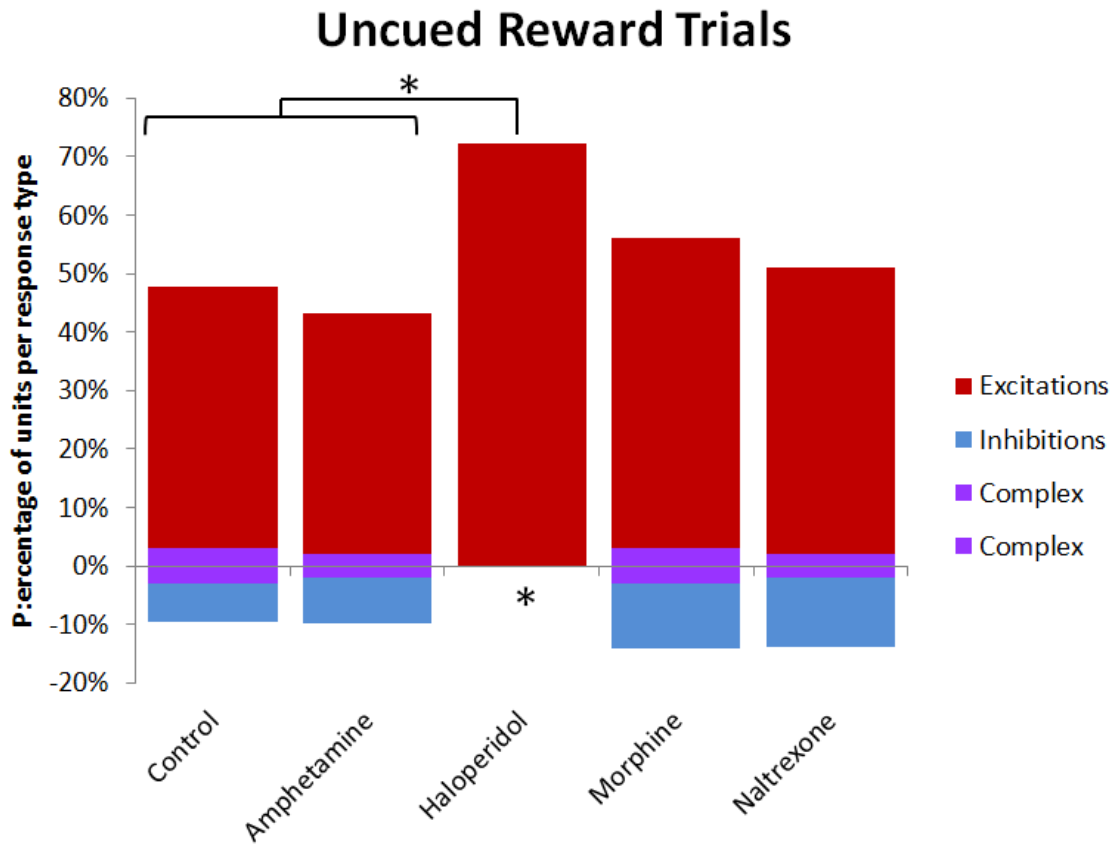
Figure 4.18 Overview of neural responses to uncued rewards per drug condition. * p < 0.05

Rate coding

There was a significant overall drug effect on average firing rate for most stimuli: Pavlovian Cue 1, Reward, Extinction Cue 1, No reward, and Uncued Reward (ANOVAs, all p<0.05). However, there was no consistent drug effect across all stimuli. Amphetamine resulted in a lower average firing rate than control in response to CS1; naltrexone resulted in a higher average firing rate compared to control in response to cued rewards; haloperidol resulted in a higher firing rate in response to the first cue under extinction compared to control; naltrexone resulted in a faster firing rate when reward was withheld compared to amphetamine, morphine and naltrexone; and amphetamine

resulted in a lower firing rate in response to uncued rewards than haloperidol or morphine (post hock results, Table 4.2). There was no drug effect for the second cue, in the classical conditioning trials or the extinction trials (ANOVAs, both NS).

Table 4.2 Normalized rates (1=baseline rate) for the 600 ms epoch after taste onset, or for "No Reward", the time that reward would have been delivered in a Pavlovian trial. Significant drug effects on rate are noted.

	Stimulus	Control	Amphetamine	Haloperidol	Morphine	Naltrexone
Pavlovian Trials	Pav CS1	1.273512	1.119968 < control	1.346169	1.392742	1.309056
	Pav CS2	1.369767	1.274953	1.513622	1.369551	1.456748
	Reward	1.293394	1.243461	1.338759	1.306939	1.487557 > control
Extinction Trials	Ext CS1	1.248968	1.176851	1.403937 > control	1.314081	1.28677
	Ext CS2	1.315275	1.209252	1.297464	1.27232	1.397793
	No Reward	1.287724	1.199691 < naltrexone	1.183001 < naltrexone	1.215288 < naltrexone	1.36549
UCS	Uncued Reward	1.308505	1.216875	1.46105 > amphetamine	1.41573 > amphetamine	1.383043

Discussion

Evidence that we can measure the hedonic value of cues

Usually, there is a clear difference in the hedonic value of a sweet sucrose solution and tap water (as discussed in chapter 3, Berridge 2000). But in this experiment, using tap water as cues for sucrose delivery, the hedonic value of the two tastes became very similar. In fact, there was no significant difference in the quantity of hedonic reactions between the first cue and the sucrose reward in any drug condition. This provides evidence that we were able to boost the hedonic value of water via an association with the delivery of a natural reward, supporting previous studies on

Pavlovian cues taking on hedonic value though association with reward (Berridge and Schulkin, 1989; Bindra, 1974; Bolles, 1972; Delamater et al., 1986; Kerfoot et al., 2007; Toates, 1986;).

A previous study found that intra-accumbal microinjections of DAMGO (a μ-opioid receptor agonist), increased the hedonic reactions to a pair of CS+ cues that were tones and sucrose reward compared to control (Smith et al. 2011). Furthermore, they saw a greater increase in hedonic value of the second cue than the first cue. We did not see an opioid enhancement of cues or reward via systemic morphine, but this might be due to the cues and rewards already having such a high hedonic value in the vehicle control condition. The cues were already about as hedonic as the sucrose rewards. The hedonic enhancement of the tone cues found by Smith et al. was, in absolute terms, quite modest: a count of about 1-2 over 10 seconds (Smith et al. 2011). Our behavior was measured over 6 seconds, but would approximate to roughly 2.5-6.7 counts per 10 seconds if we were to extrapolate. Perhaps the cues were already so hedonic, that boosting the hedonic values via morphine was unlikely. Likewise, perhaps our sucrose solution was already maximally hedonic. The UCS used in the Smith et al. study was 9% sucrose, delivered over 10 seconds. We used 17% sucrose delivered over 1 sec. The concentration was increased because we've found that shorter infusions tend to require a more concentrated solution to yield consistent behavior. Perhaps we would have seen an enhancement in the hedonic value of sucrose if we had used a weaker solution, to eliminate a ceiling effect. The systemic effect may be complicated by conflicting effects in different brain areas (for example both the NAcc and VP have both hedonic "hotspots" and "coldspots" which can

have contrasting effects (Peciña and Berridge, 2000; Peciña and Berridge, 2005; Smith and Berridge, 2007) and/or stimulation of a mixture of receptors.

The same Smith et al. study found that intra-accumbal microinjections of amphetamine did not enhance hedonic reactions to tone CSs or the UCS. Our results found that systemic amphetamine and systemic haloperidol likewise did not enhance the hedonic value of taste CSs or the UCS. Rather, both drugs decreased the hedonic value of the CS+1 taste cue compared to control. Given how low the quantity of hedonic reactions to the cues were in the Smith et al. study, it would have been difficult to find a statistically significant decrease in reactions via intra-accumbal injections of amphetamine due to a floor effect. Therefore our findings do not necessarily conflict. Furthermore, systemic amphetamine effects may well be different.

There are several alternative explanations for the effect of dopaminergic modulation of the hedonic reaction to the CS+1. With amphetamine, there were generally far more non-taste related behaviors (such as ambulatory, sniffing, grooming, and rearing behaviors), which makes more likely that the animal was engaged in another behavior at the onset of the first cue. Being engaged in another behavior might slightly delay their reaction to CS+1, making the count of hedonic reactions lower. It's important to note that there was no significant increase in aversive reactions, so it wasn't a shift in hedonic value per se. All tastes were still predominantly hedonic, like they were in the vehicle control condition. This might be particularly apparent in this experiment because we analyzed such short time intervals (6 seconds) compared to previous experiments which measured hedonic reactions over 10-60 seconds. These general sensorimotor behaviors also may have made it more difficult to accurately score taste reactions. With

haloperidol there was little to no exploratory behavior during the ITI, but while they were able to ultimately express taste reactions, perhaps they were a little slower to initiate the reactions. Hypodopaminergic states can make initiating a behavior harder, so this could explain both the decrease in exploratory behavior and taste reactions to the first cue. Further analysis is needed to determine whether general sensorimotor behaviors affected the taste reaction scoring, as has been the case in a previous study (Pecina et al. 1997).

Conclusion

There is evidence that we were able to enhance the hedonic value of cues via association with reward. Compared to a water stimulus that was never associated with another taste, cues that consisted of infusions of tap water which preceded delivery of the natural reward of a sucrose solution were more hedonic. Indeed, the water infusion cues were nearly indistinguishable from the hedonic value of the sweet tastes. However, we did not see the boost in hedonic value of cues or reward via systemic morphine that we had hypothesized. We did get unexpected amphetamine and haloperidol effects on decreasing the hedonic value of the first water cue relative to vehicle control. Amphetamine also resulted in fewer increases in firing rate in response to the first cue compared to vehicle control. Further investigation is needed to determine how stable these drug effects are.

Reference List

Bakshi VP, Kelley AE (1993) Striatal regulation of morphine-induced hyperphagia: an anatomical mapping study. Psychopharmacology (Berl) 111:207-214.

Baldo BA, Kelley AE (2007) Discrete neurochemical coding of distinguishable motivational processes: insights from nucleus accumbens control of feeding. Psychopharmacology (Berl) 191:439-459.

Berke JD, Hyman SE (2000) Addiction, dopamine, and the molecular mechanisms of memory. Neuron 25:515-532.

Berridge KC (1996) Food reward: brain substrates of wanting and liking. Neurosci Biobehav Rev 20:1–25

Berridge KC (2000) Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. Neurosci Biobehav Rev 24:173-198.

Berridge KC (2003) Pleasures of the brain. Brain Cogn 52:106-128.

Berridge KC (2004) Motivation concepts in behavioral neuroscience. Physiol Behav 81:179-209.

Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology (Berl) 191:391-431.

Berridge, K. C., Ho, C., Richard, J. M., & DiFeliceantonio, A. G. (2010). The tempted brain eats: Pleasure and desire circuits in obesity and eating disorders. *Brain Res, 1350*, 43-64.

Berridge KC, Schulkin J (1989) Palatability shift of a salt-associated incentive during sodium depletion. Q J Exp Psychol B 41:121-138.

Berridge, Kent C. and Robinson, Terry E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Reviews, 28, 309–369.

Berridge KC, Robinson TE (2003) Parsing reward. Trends Neurosci 26:507-513.

Berridge KC, Venier IL, Robinson TE (1989) Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: implications for arousal and anhedonia hypotheses of dopamine function. Behav Neurosci 103:36-45.

Berridge, Kent C.; Robinson, Terry E.; and Aldridge, J. Wayne (2009). Dissecting components of reward: 'liking', 'wanting', and learning. Current Opinion in Pharmacology, 9, 65-73.

Berthoud HR (2002) Multiple neural systems controlling food intake and body weight. Neurosci Biobehav Rev 26:393-428.

Bindra D (1974) A motivational view of learning, performance, and behavior modification. Psychological Review 81:199-213.

Bodnar RJ (2013) Endogenous opiates and behavior: 2012. Peptides 50:55-95.

Bolles RC (1972) Reinforcement, expectancy, & learning. Psychological Review 79:394-409.

Cooper SJ (1983) Effects of opiate agonists and antagonists on fluid intake and saccharine choice in the rat. Neuropharmacology 22:323-328.

Cooper SJ, Kirkham TC (1993) Opioid mechanisms in the control of food consumption and taste preferences. in: AA Herz, EJ Simon (Eds), Opioids, part I and II, Springer-Verlag, Berlin, 1993, 239-262.

Cooper SJ, Higgs S (1994) Neuropharmacology of appetite and taste preferences. In: Appetite: Neural and Behavioural Bases (Legg CR, Booth DA, eds), pp 212-242. New York: Oxford University Press.

Delamater AR, LoLordo VM, Berridge KC (1986) Control of fluid palatability by exteroceptive Pavlovian signals. J Exp Psychol Anim Behav Process 12:143-152.

Di Ciano P, Everitt BJ (2005) Neuropsychopharmacology of drug seeking: Insights from studies with second-order schedules of drug reinforcement. Eur J Pharmacol 526:186-198.

Doyle TG, Berridge KC, Gosnell BA (1993) Morphine enhances hedonic taste palatability in rats. Pharmacol Biochem Behav 46:745–749Evans AH, Pavese N, Lawrence AD, Tai YF, Appel S, Doder M, Brooks DJ, Lees AJ, Piccini P (2006) Compulsive drug use linked to sensitized ventral striatal dopamine transmission. Ann Neurol 59:852-858.

Everitt BJ, Robbins TW (2005) Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. Nat Neurosci 8:1481-1489.

Everitt BJ, Parkinson JA, Olmstead MC, Arroyo M, Robledo P, Robbins TW (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. Annals of the New York Academy of Sciences 877:412-438.

Grill HJ, Norgren R (1978) The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 143:263-279.

Grill HJ, Berridge KC (1985) Taste reactivity as a measure of the neural control of palatability. Progress in Psychobiology and Physiological Psychology 2.

Jaeger D, Gilman S, Aldridge JW (1990) A multiwire microelectrode for single unit recording in deep brain structures. J Neurosci Methods 32:143-148.

Kalivas PW, Nakamura M (1999) Neural systems for behavioral activation and reward. Curr Opin Neurobiol 9:223-227.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. Am J Psychiatry 162:1403-1413.

Kelley AE, Bakshi VP, Haber SN, Steininger TL, Will MJ, Zhang M (2002) Opioid modulation of taste hedonics within the ventral striatum. Physiol Behav 76:365-377.

Kerfoot EC, Agarwal I, Lee HJ, Holland PC (2007) Control of appetitive and aversive taste-reactivity responses by an auditory conditioned stimulus in a devaluation task: A FOS and behavioral analysis. Learn Mem 14:581–589.

Levine AS, Billington CJ (2004) Opioids as agents of reward-related feeding: a consideration of the evidence. Physiol Behav 82:57-61.

Mahler SJ, Berridge KC (2012) What and when to "want"? Amygdala-based focusing of incentive salience upon sugar and sex. Psychopharmacology (Berl) 221:407–28.

Paxinos, G., & Watson, C. (2007). *The rat brain in stereotaxic coordinates* (6th ed.). Amsterdam, the Netherlands: Elsevier Inc.

Peciña S, Berridge KC (2000) Opioid site in nucleus accumbens shell mediates eating and hedonic 'liking' for food: map based on microinjection Fos plumes. Brain Res 863:71-86.

Peciña S, Berridge KC (2005) Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci 25:11777-11786.

Peciña S, Berridge KC (1996) Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? J Neurosci 25:11777-11786.

Peciña S, Berridge KC, Parker LA (1997) Pimozide does not shift palatability: separation of anhedonia from sensorimotor suppression by taste reactivity. Pharmacol Biochem Behav 58:801-811.

Peciña S, Smith KS, Berridge KC (2006) Hedonic hot spots in the brain. Neuroscientist 12:500-511.

Peciña S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. J Neurosci 23:9395-9402.

Rescorla RA (1988) Behavioral studies of Pavlovian conditioning. Annu Rev Neurosci 11:329-352.

Rescorla RA, Wagner AR, eds (1972) A theory of Pavlovian conditioning: variations in the effectiveness of reinforcement and nonreinforcement. NEw York: Appleton Century Crofts.

Robbins TW, Everitt BJ (1996) Neurobehavioural mechanisms of reward and motivation. Current Opinion in Neurobiology 6:228-236.

Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD (2005) Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. Behav Neurosci 119:5-15.

Salamone JD, Correa M (2002) Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. Behavioural Brain Research 137:3-25.

Schultz W (1998) Predictive reward signal of dopamine neurons. J Neurophysiol 80:1-27.

Schultz W (2000) Multiple reward signals in the brain. Nature Reviews Neuroscience 1:199-207.

Schultz W (2002) Getting formal with dopamine and reward. Neuron 36:241-263.

Schultz W, Tremblay L, Hollerman JR (1998) Reward prediction in primate basal ganglia and frontal cortex. Neuropharmacology 37:421-429.

Sills TL, Vaccarino FJ (1996) Individual differences in sugar consumption following systemic or intraaccumbens administration of low doses of amphetamine in nondeprived rats. Pharmacol Biochem Behav 54:665-670.

Smith, K. S., & Berridge, K. C. (2005). The ventral pallidum and hedonic reward: Neurochemical maps of sucrose "liking" and food intake. *The Journal of Neuroscience, 25*(38), 8637-8649.

Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: Interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. *The Journal of Neuroscience, 27*(7), 1594-1605.

Smith, K. S., Berridge, K. C., & Aldridge, J. W. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proc Natl Acad Sci U S A, 108*(27), E255-E264.

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behav Brain Res, 196*(2), 155-167.

Tindell AJ, Berridge KC, Aldridge JW (2004) Ventral pallidal representation of pavlovian cues and reward: population and rate codes. J Neurosci 24:1058-1069.

Tindell AJ, Smith KS, Berridge KC, Aldridge JW (2005 a) VP Neurons Integrate Learning and Physiological Signals to Code Incentive Salience of Conditioned Cues. Society for Neuroscience Abstracts.

Tindell AJ, Berridge KC, Zhang J, Pecina S, Aldridge JW (2005 b) Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. Eur J Neurosci 22:2617-2634.

Tindell AJ, Smith KS, Pecina S, Berridge KC, Aldridge JW (2006) Ventral pallidum firing codes hedonic reward: when a bad taste turns good. J Neurophysiol 96:2399-2409.

Toates FM (1986) Motivational Systems. Cambridge: Cambridge University Press.

Uslaner JM, Acerbo MJ, Jones SA, Robinson TE (2006) The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. Behav Brain Res 169:320-324.

van der Kooy D, Mucha RF, O'Shaughnessy M, Bucenieks P (1982) Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Res 243:107-117.

Volkow ND, Wang GJ, Fowler JS, Logan J, Jayne M, Franceschi D, Wong C, Gatley SJ, Gifford AN, Ding YS, Pappas N (2002) "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. Synapse 44:175-180.

Wheeler RA, Carelli RM (2006) The neuroscience of pleasure. Focus on "Ventral pallidum firing codes hedonic reward: when a bad taste turns good". J Neurophysiol 96:2175-2176.

Wise RA (1985) The anhedonia hypothesis: Mark III. Behavioral and Brain Sciences 8:178-186.

Wise RA (1989) Opiate reward: sites and substrates. Neuroscience and Biobehavioral Review 13:129-133.

Wise RA (1998) Drug-activation of brain reward pathways. Drug & Alcohol Dependence 51:13-22.

Wise RA (2005) Forebrain substrates of reward and motivation. J Comp Neurol 493:115-121.

Wise RA, Bozarth MA (1985) Brain mechanisms of drug reward and euphoria. Psychiatric Medicine 3:445-460.

Woolley JD, Lee BS, Fields HL (2006) Nucleus accumbens opioids regulate flavor-based preferences in food consumption. Neuroscience 143:309-317.

Wyvell CL, Berridge KC (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. J Neurosci 20:8122-8130.

Wyvell CL, Berridge KC (2001) Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. J Neurosci 21:7831-7840.

Yang CR, Mogenson GJ (1989) Ventral pallidal neuronal responses to dopamine receptor stimulation in the nucleus accumbens. Brain Res 489:237-246.

Zahm DS (2000) An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neurosci Biobehav Rev 24:85-105.

Zhang M, Kelley AE (2000) Enhanced intake of high-fat food following striatal mu-opioid stimulation: microinjection mapping and fos expression. Neuroscience 99:267-277.

Zhang M, Gosnell BA, Kelley AE (1998) Intake of high-fat food is selectively enhanced by mu opioid receptor stimulation within the nucleus accumbens. J Pharmacol Exp Ther 285:908-914.

CHAPTER 5

GENERAL DISCUSSION

A direct comparison of water cues and water stimuli

In chapter 4 we saw that the cues had become very similar to sucrose rewards in terms of hedonic value, whereas we had seen a contrast between sucrose and water stimuli in chapter 3. Here we will compare data from chapters 3 and 4 directly. The water stimuli from chapter 3 elicited a balance of hedonic and aversive taste reactions, whereas the water oral infusions that predicted reward elicited far fewer aversive reactions. There was no statistical difference between the quantities of hedonic reactions to water cues associated with a reward compared to water stimuli that had no association with rewards (figure 5.1). However, there are fewer aversive reactions to water cues associated with a reward compared to water stimuli that had no association with rewards (figure 5.2). Collapsed across drug conditions, the water stimuli elicited more aversive taste reactions than cues that were associated with reward (t-test, $p = 9.992^{-07}$). Within drug conditions, there were more aversive reactions to stimuli than cues under vehicle control (t-test, $p = 0.0003$) and naltrexone (t-test, $p = 0.012$). In summary, association with rewards can shift the net hedonic value of oral infusions of water from neutral to 'liked'.

Hedonic Reactions
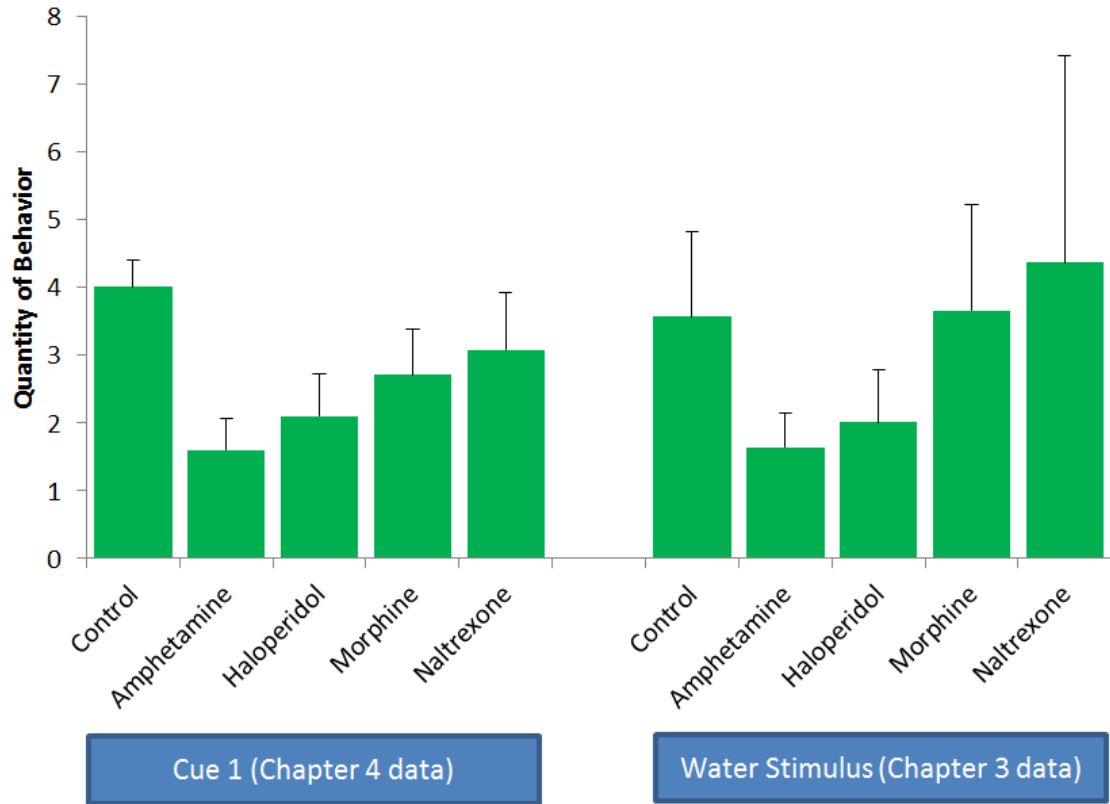
Figure 5.1 A comparison of the hedonic taste reactions to water oral infusions that predicted reward (cue 1 from Chapter 4) and water oral infusions that had no association with reward (water stimuli from Chapter 3). There were no statistical differences in hedonic value between the cues and stimuli. Error bars represent standard error.
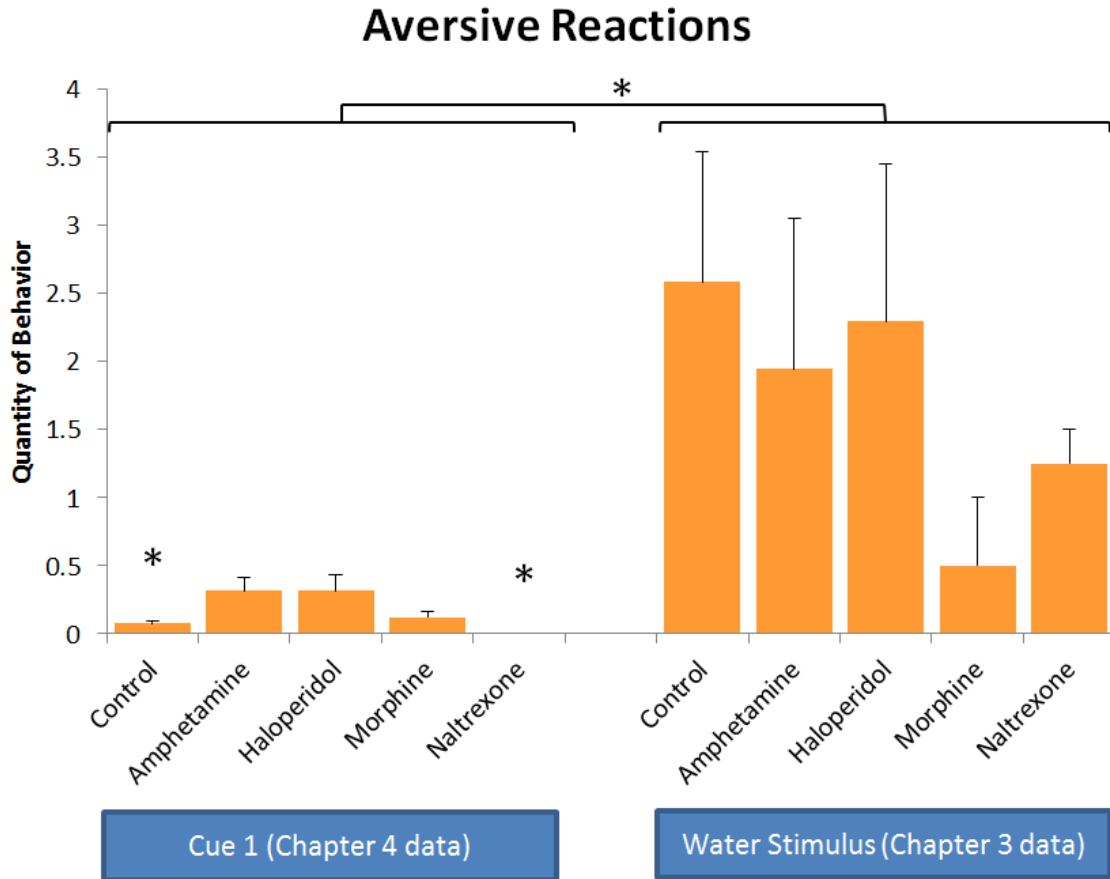
Figure 5.2 A comparison of the aversive taste reactions to water oral infusions that predicted reward (cue 1 from Chapter 4) and water oral infusions that had no association with reward (water stimuli from Chapter 3). Collapsed across drug conditions, the water stimuli elicited more aversive taste reactions than cues that were associated with reward. Within drug conditions, there were more aversive reactions to stimuli than cues under vehicle control and naltrexone. Error bars represent standard error. *p < 0.05

Is there a more powerful way to measure the hedonic value of cues?

There are many ways to measure motivation behaviorally: Conditioned Place Preference, Pavlovian Instrumental Transfer, break points, reaction times, pursuit despite aversive consequences, etc. However, these methods do not allow one to specifically isolate how much an animal *enjoys* the pursuit or anticipation of rewards. Yet for humans, at least, the pleasure inherent in pursuit is often a substantial aspect of motivation. The "thrill of the chase", as they say. Recall the joy and excitement of a child counting down the days until their birthday or any holiday involving gifts. We were able to increase the hedonic value of cues that consisted of water infusions compared to water stimuli that had no associative value, but perhaps there are alternative ways to increase the transfer of hedonia to cues from natural rewards.

Perhaps tastes are so intrinsically salient that they are not the best candidates for being valueless stimuli that later take on meaning through repeated paring with an unconditioned stimulus, compared to tones or lights. We chose water to be the most neutral, most innocuous liquid possible, as the cues, but since they had free access to it in their home cages, the generalization and familiarity may have worked against us. Compared to a distinct tone or a localized light (far more stereotypical examples of CSs) that is only experienced in a test chamber, water cues may not take on as strong of a reward predictive value because the majority of the time it is experienced in a non-experimental environment, where it predicts nothing.

Some studies have found that there are more spontaneous mouth movements to CS+ tones even in the absence of a liquid taste to react to (Smith et al. 2011, Kerfoot et

al. 2007). I did a pilot study where I had layered tones with the water cues (similar to Delamater et al. 1986 and Kerfoot et al. 2007), to try to make the context more distinct. This was dropped from the design because it made it difficult to assess whether the VP neural coding changes were in response to the tone, the taste, or both. Furthermore, the number of hedonic reactions to the tone alone was far lower than when tones were layered with water infusions, and highest to water alone after the tone was shut off (Kerfoot et al. 2007), so it's not clear that tone cues are better at taking on hedonic value via association with sweet rewards than cues comprised of water infusions.

A previous graduate student from the Berridge Lab, Dr. Ho, and I piloted a taste association experiment. It involved mixing mildly aversive, weak bitter or sour tastes with hedonic tastes (sucrose or mildly salty solutions). Taste associations maybe a far more natural taste learning paradigm than sequential classical conditioning. Unfortunately, we did not get consistent behavioral responses with the taste association paradigm.

Another possibility is that the pleasure that comes from anticipation of rewards is keenest when the rewards are rare and illusive. Predators have to wait patiently for extended periods of time, often nearing the brink of starvation, waiting for the perfect opportunity to take down prey. They need to watch, calculate risk, and in the case of pack hunters, coordinate their efforts. The majority of hunting attempts will be unsuccessful. Perhaps the "thrill of the hunt" is part of an evolutionary strategy to reinforce hunting instincts even in the absence of a successful kill. Wolves working as a pack to bring down an elk may be having more fun than a herd of elk migrating to a new meadow. Both are pursuing their food source, but the risk and uncertainty inherent in

hunting may be a source of enjoyment. Likewise in humans, I posit that the pleasure derived from the anticipation for rare rewards are far keener than those for common or guaranteed rewards.

What is driving the VP coding of hedonia and reward learning?

Both the NAc and VP receive dopaminergic projections from the VTA, bringing in motivation and reward learning signals. The dopamine pathway has been studied extensively in relation to reward learning and dopamine projections were at one time proposed as a pleasure pathway (Wise 1985, Hoebel et al. 1999, Shizgal 1999). While dopamine neurons in the ventral tegmental area and substantia nigra respond to many pleasurable stimuli (Fiorino et al. 1997, Schultz 1998, Wise 1998, Ahn and Phillips 1999, Becker et al. 2001, Robinson et al. 2005, Aragona et al. 2006), newer evidence suggests that dopamine is coding the motivational or rewarding aspects of these stimuli rather than the hedonic values. Previous findings of dopamine modulation effects on the hedonic value of tastes were later shown to be sensorimotor effects rather than true shifts in hedonic value (Pecina et al. 1997). Destruction of mesolimbic dopamine projections via 6-OHDA lesions does not impair affective 'liking' reactions elicited by tastes (Berridge et al. 1989, Berridge and Robinson 1998). In human patients with Parkinson's disease, the loss of dopaminergic neurons in does not suppress the pleasantness of sweet foods (Sienkiewicz-Jarosz et al. 2005). Acute and long-term amphetamine administration, which boosts dopamine levels, failed to increase hedonic reactions to tastes (Wyvell and Berridge 2000, Tindell et al. 2005). Even in humans, dopamine elevation does not seem

to elevate pleasure. Drug-induced increases in dopamine activity correlates better with subjective ratings for how much people 'want' the drug than ratings for how much they 'like' the drug and can increase desire for visually presented food in a motivational but non-hedonic manner (Leyton et al. 2002 and 2005, Volkow et al. 2002). In summary, recent evidence strongly supports the assertion that dopamine is neither necessary for normal pleasure generation, nor sufficient for enhancing pleasure above normal levels. Rather, dopamine drives the motivational aspects of reward learning.

So what drives the hedonic aspects of reward learning? There are opioid projections from the NAc to the VP that modulate hedonia. Prior studies have shown that cells in the NAc hotspot region project directly to the VP hotspot region (Usuda et al. 1998, Mogenson et al. 1980). Opioid hedonic hotspots in the NAc and VP synergistically enhance 'liking' of oral infusion of sucrose. Mu opioid blockade via naloxone microinjections in one hotspot suppressed the increase in hedonic 'liking' reactions to sucrose taste normally caused by DAMGO microinjection in the other hotspot (Smith and Berridge 2007). In summary, the two limbic sites acted together in a single hedonic circuit to enhance the hedonic impact of sucrose taste.

Other sites that project hedonic information to the VP include the orbitofrontal cortex and insular cortex (O'Doherty et al. 2000, Small et al. 2001, Kringelbach et al. 2003). Neural correlates of palatability devaluation have also been found in the lateral hypothalamus and brainstem parabrachial nucleus (Burton et al. 1975, Rolls et al. 1989, Nakamura and Norgren 1995, Critchley and Rolls 1996, Giza et al. 1997, Schoenbaum et al. 1998, Reilly 1999, Rolls 2000, Schultz 2000, de Araujo et al. 2006, Peciña et al. 2006, Simon et al. 2006). Small increments in taste palatability encoding (e.g. caused by a

weak salt-appetite induction) have been observed in the parabrachial nucleus and in the nucleus of the solitary tract (Jacobs et al. 1988, McCaughey and Scott 2000).

There are also heavy reciprocal GABAergic projections between the NAc and VP. Micro injections of bicuculline (a GABA$_A$ receptor antagonist) into the VP increased voluntary consumption of a sweet taste that had previously acquired a conditioned taste aversion and increased 'liking' of this taste (Inui et al. 2007). In other words, disinhibition of the VP increased the pleasantness of a conditioned aversive taste. VP microinjections of bicuculline increased consumption of sucrose but not water or quinine and in naïve rats, while micro injections of muscimol (a GABA$_A$ receptor agonist) suppressed intake of all three solutions and resulted in strongly aversive taste reactions to all three tastes (Shimura at al. 2006). Therefore it's possible that GABA has different effects on the hedonic value of taste stimuli compared to conditioned tastes.

The activation of NAc projection neurons to the VP has been shown to be functionally active in response to a learned aversive taste in a rat manganese-enhanced MRI study (Inui et al. 2011). Rats with a conditioned taste aversion (CTA) to a sweet taste and naïve rats received NAc microinjections of manganese chloride. Both groups were given intra-oral infusions of a saccharin solution and infusions of water. The CTA rats receiving the aversive taste showed significantly more movement of manganese from the NAc to the VP than when they were given water infusions and compared to the naïve rats in response to either taste. Therefore NAc to VP projections are directly involved in the neural coding of learned taste aversions.

Our results from Experiment 1, which indicate that the VP typically responded to 'liked' tastes with increases in firing rate and the 'disliked' taste with decreases in firing

rate, is consistent with studies that have shown that NAc neurons were generally inhibited by sucrose or excited by quinine (Roitman et al 2005, Nicola et al. 2004, Taha and Fields 2006). Bi-directional GABAergic inhibition between the NAc and VP may serve to amplify the inverted responses to hedonic vs. aversive tastes, although we only saw a VP population coding of hedonic valence under the effect of systemic morphine in the naïve rats from Experiment 2. NAc opioids may further enhance coding of hedonia by predominantly inhibiting NAc firing rate (Hakan et al. 1992, Hakan et al. 1994, Hakan and Eyl 1995). In Experiment 3, we found that the predominant response to taste cues for sucrose were increases in firing rate. This is consistent with previous studies of VP responses to auditory cues (tones) that predict sucrose delivery (Tindell et al. 2004, 2005a, and 2005b).

Future Directions

If someone would like to pursue layering more typical cues with the taste cues, the issue of disentangling the tones/lights from the tastes could be approached by over-training the animals and then testing the cues separately, to see the response to the taste cues in isolation and the tone/light cues in isolation. This has the complication of partial extinction learning, however. Another option would be to have a partial overlap between the tone and water cues (Kerfoot et al. 2007) rather than a complete overlap, to allow for comparisons of neural responses to the tones alone, tones plus water, and just water after tones are presented.

The taste association paradigm should work, in theory. There were perhaps some unknown issues, or conditions were not optimal. Or perhaps there is simply more individual variability in taste association learning than our typical studies on classical conditioning and the study simply needed more power. This is a reasonable possibility, as acquired tastes tend to be fairly specific and individual. It's not something that can be forced on everyone. While adults tend to like a greater variety of bitter substances than children do, not all adults prefer dark chocolate to milk chocolate. Likewise, maybe some animals learn taste associations better than others.

It would be interesting to explore the effect of anticipation on the hedonic value of cues. One could combine and instrumental aspect with classical conditioning, by making the animals "work" for a reward by pushing a lever on a variable or progressive ratio and then have a taste cue precede a taste reward. Or one could try a Pavlovian Insturmental Transfer paradigm. However, the problem of later separating out the neural coding of the instrumental and classical conditioning components of the experiment would need to be addressed.

Other techniques could be used to map out and better understand how various structures contribute to VP coding of hedonic value. There are ongoing optogenetic studies using intracranial virus delivery to selectively activate lateral hypothalamus (LH) projections to the VP and comparing that to general activation of the LH or VP (Castro and Berridge 2013). At the time of publication of this thesis, the results of this study so far indicate that general activation of the LH increased food consumption but did not change the hedonic value of tastes, general activation of the VP hedonic hotspot increased the hedonic value of tastes but did not affect eating, whereas activating LH

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projections to the hedonic hotspot of the VP increased both 'liking' and 'wanting'/eating. The contribution of other VP inputs on hedonia and motivation could be explored in the future. Optogenteics can also be combined with neuropharmacology to further delve into the relative contributions of various reward pathway structures. Opioid and orexin antagonists blocked the optogeneticly triggered enhancement of hedonia in the VP. Other pharmacological manipulations such as D1 or D2 antagonists could be used to tease apart the changes in motivation. In addition, transgenic mice that express Cre recombinase in either D1 or D2 expressing medium spiny neurons (MSN) combined with Cre-targeting channel rhodopsin viral vectors are currently being used to separate the effects of stimulating D1 or D2 expressing neurons in the nucleus accumbens shell (Cole et al. 2013). Similar methods could be applied to the VP in future studies.

Conclusion

In Experiment 1, we showed that the VP was able to track the shift in hedonic value when a sweet taste that was initially liked became aversive through LiCl pairings. VP neurons typically responded to 'liked' tastes with an increase in firing rate and were more likely to respond to the 'disliked' taste with a decrease in firing rate. Given previous findings that NAc neurons were generally inhibited by sucrose (a sweet 'liked' taste) or excited by quinine (a bitter 'disliked' taste) (Roitman et al 2005, Nicola et al. 2004, Taha and Fields 2006) and the presence of reciprocal GABAergic inhibition between the NAc and VP (Churchill and Kalivas 1994, Hakan et al. 1992, Phillipson and Griffiths 1985,

Usuda et al. 1998, Zahm 2000), the inversion of neural coding of 'liked' vs. 'disliked'

tastes between the NAc and VP may create a key circuit in the neural coding of hedonia.

Experiment 2 showed that there was a difference in the hedonic value of water

stimuli and sucrose stimuli in that water elicited fewer hedonic reactions and more

aversive reactions than sucrose. However, in Experiment 3, we saw that in classical

conditioning trials, water infusions that are acting as cues for a natural reward took on

nearly identical hedonic value as the reward itself. Furthermore, the VP responded

similarly to the water cues and sucrose rewards, mirroring the similarity in hedonic value:

the predominant response to both taste cues for sucrose and for the sucrose rewards

themselves were increases in firing rate. Together, the findings of these three

experiments provide strong evidence that the VP codes for the hedonic values of tastes,

independently from the stimulus identity.

Reference List

Ahn S, Phillips AG (1999) Dopaminergic correlates of sensory-specific satiety in the medial prefrontal cortex and nucleus accumbens of the rat. Journal of Neuroscience 19:B1-B6.

Aragona BJ, Liu Y, Yu YJ, Curtis JT, Detwiler JM, Insel TR, Wang Z (2006) Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat Neurosci 9:133-139.

Becker JB, Rudick CN, Jenkins WJ (2001) The role of dopamine in the nucleus accumbens and striatum during sexual behavior in the female rat. Journal of Neuroscience 21:3236-3241.

Berridge KC, Robinson TE (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Reviews 28:309-369.

Berridge KC, Venier IL, Robinson TE (1989) Taste reactivity analysis of 6-hydroxydopamine-induced aphagia: Implications for arousal and anhedonia hypotheses of dopamine function. Behavioral Neuroscience 103:36-45.

Burton MJ, Mora F, Rolls ET (1975) Proceedings: Visual and taste neurones in the lateral hypothalamus and substantia innominata: modulation of responsiveness by hunger. J Physiol 252:50P-51P.

Castro DC, Berridge KC (2013) Optogenetic enhancement of food 'liking' versus 'wanting' in the ventral pallidum hotspot and lateral hypothalamus. Abstract from Society For Neuroscience 2013.

Churchill L, Kalivas PW (1994) A topographically organized gamma-aminobutyric acid projection from the ventral pallidum to the nucleus accumbens in the rat. J Comp Neurol 345:579-595.

Cole SL, Robinson MJF, Berridge KC (2013) Reinforcing effects of optogenetic excitation of d1 and/or d2 cells in the nucleus accumbens and lateral septum. Abstract from Society For Neuroscience 2013.

Critchley HD, Rolls ET (1996) Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. J Neurophysiol 75:1673-1686.

de Araujo IE, Gutierrez R, Oliveira-Maia AJ, Pereira A, Jr., Nicolelis MA, Simon SA (2006) Neural ensemble coding of satiety states. Neuron 51:483-494.

Delamater AR, LoLordo VM, Berridge KC (1986) Control of fluid palatability by exteroceptive Pavlovian signals. J Exp Psychol Anim Behav Process 12:143-152.

Fiorino DF, Coury A, Phillips AG (1997) Dynamic changes in nucleus accumbens dopamine efflux during the Coolidge effect in male rats. Journal of Neuroscience 17:4849-4855.

Giza BK, Ackroff K, McCaughey SA, Sclafani A, Scott TR (1997) Preference conditioning alters taste responses in the nucleus of the solitary tract of the rat. Am J Physiol 273:R1230-1240.

Hakan RL, Eyl C (1995) Neuropharmacology of the nucleus accumbens: iontophoretic applications of morphine and nicotine have contrasting effects on single-unit responses evoked by ventral pallidal and fimbria stimulation. Synapse 20:175-184.

Hakan RL, Berg GI, Henriksen SJ (1992) Electrophysiological evidence for reciprocal connectivity between the nucleus accumbens septi and ventral pallidal region. Brain Res 581:344-350.

Hakan RL, Eyl C, Henriksen SJ (1994) Neuropharmacology of the nucleus accumbens: systemic morphine effects on single-unit responses evoked by ventral pallidum stimulation. Neuroscience 63:85-93.

Hoebel BG, Rada PV, Mark GP, Pothos EN (1999) Neural systems for reinforcement and inhibition of behavior: Relevance to eating, addiction, and depression. In: Well-being: The foundations of hedonic psychology, pp 558-572. New York, NY: Russell Sage Foundation.

Inui T, Inui-Yamamoto C, Yoshioka Y, Ohzawa I, Shimura T (2011) Activation of projective neurons from the nucleus accumbens to ventral pallidum by a learned aversive taste stimulus in rats: a manganese-enhanced magnetic resonance imaging study. Neuroscience 177:66-73.

Inui T, Shimura T, Yamamoto T (2007) The role of the ventral pallidum GABAergic system in conditioned taste aversion: Effects of microinjections of a GABAA receptor antagonist on taste palatability of a conditioned stimulus. Brain Research 1164:117-124.

Jacobs KM, Mark GP, Scott TR (1988) Taste responses in the nucleus tractus solitarius of sodium-deprived rats. J Physiol 406:393-410.

Kerfoot EC, Agarwal I, Lee HJ, Holland PC (2007) Control of appetitive and aversive taste-reactivity responses by an auditory conditioned stimulus in a devaluation task: A FOS and behavioral analysis. Learn Mem 14:581–589.

Kringelbach ML, O'Doherty J, Rolls ET, Andrews C (2003) Activation of the human orbitofrontal cortex to a liquid food stimulus is correlated with its subjective pleasantness. Cereb Cortex 13:1064-1071.

Leyton M, Casey KF, Delaney JS, Kolivakis T, Benkelfat C (2005) Cocaine craving, euphoria, and self-administration: a preliminary study of the effect of catecholamine precursor depletion. Behav Neurosci 119:1619-1627.

Leyton M, Boileau I, Benkelfat C, Diksic M, Baker G, Dagher A (2002) Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[11C]raclopride study in healthy men. Neuropsychopharmacology 27:1027-1035.

McCaughey SA, Scott TR (2000) Rapid induction of sodium appetite modifies taste-evoked activity in the rat nucleus of the solitary tract. Am J Physiol Regul Integr Comp Physiol 279:R1121-1131.

Mogenson GJ, Jones DL, Yim CY (1980) From motivation to action: functional interface between the limbic system and the motor system. Prog Neurobiol 14:69-97.

Nakamura K, Norgren R (1995) Sodium-deficient diet reduces gustatory activity in the nucleus of the solitary tract of behaving rats. Am J Physiol 269:R647-661.

Nicola SM, Yun IA, Wakabayashi KT, Fields HL (2004) Firing of nucleus accumbens neurons during the consummatory phase of a discriminative stimulus task depends on previous reward predictive cues. J Neurophysiol 91:1866-1882.

O'Doherty J, Rolls ET, Francis S, Bowtell R, McGlone F, Kobal G, Renner B, Ahne G (2000) Sensory-specific satiety-related olfactory activation of the human orbitofrontal cortex [corrected and republished article originally printed in Neuroreport 2000 Feb 7;11(2):399-403]. Neuroreport 11:893-897.

Peciña S, Berridge KC, Parker LA (1997) Pimozide does not shift palatability: Separation of anhedonia from sensorimotor suppression by taste reactivity. Pharmacology Biochemistry and Behavior 58:801-811.

Peciña S, Smith KS, Berridge KC (2006) Hedonic hot spots in the brain. Neuroscientist 12:500-511.

Phillipson OT, Griffiths AC (1985) The topographic order of inputs to nucleus accumbens in the rat. Neuroscience 16:275-296.

Reilly S (1999) The parabrachial nucleus and conditioned taste aversion. Brain Res Bull 48:239-254.

Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD (2005) Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. Behav Neurosci 119:5-15.

Roitman MF, Wheeler RA, Carelli RM (2005) Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron 45:587-597.

Rolls ET (2000) The orbitofrontal cortex and reward. Cereb Cortex 10:284-294.

Rolls ET, Sienkiewicz ZJ, Yaxley S (1989) Hunger Modulates the Responses to Gustatory Stimuli of Single Neurons in the Caudolateral Orbitofrontal Cortex of the Macaque Monkey. Eur J Neurosci 1:53-60.

Schoenbaum G, Chiba AA, Gallagher M (1998) Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nat Neurosci 1:155-159.

Schultz W (1998) Predictive reward signal of dopamine neurons. Journal of Neurophysiology 80:1-27.

Schultz W (2000) Multiple reward signals in the brain. Nature Reviews Neuroscience 1:199-207.

Shimura T, Imaoka H, Yamamoto T (2006) Neurochemical modulation of ingestive behavior in the ventral pallidum. Eur. J. Neurosci. 23:1596–1604.

Shizgal P (1999) On the neural computation of utility: Implications from studies of brain stimulation reward. In: Well-being: The foundations of hedonic psychology (Kahneman D, Diener E, Schwarz N, eds), pp 500-524. New York: Russell Sage Foundation.

Sienkiewicz-Jarosz H, Scinska A, Kuran W, Ryglewicz D, Rogowski A, Wrobel E, Korkosz A, Kukwa A, Kostowski W, Bienkowski P (2005) Taste responses in patients with Parkinson's disease. J Neurol Neurosurg Psychiatry 76:40-46.

Simon SA, de Araujo IE, Gutierrez R, Nicolelis MA (2006) The neural mechanisms of gustation: a distributed processing code. Nat Rev Neurosci 7:890-901.

Small DM, Zatorre RJ, Dagher A, Evans AC, Jones-Gotman M (2001) Changes in brain activity related to eating chocolate - From pleasure to aversion. Brain 124:1720-1733.

Smith KS, Berridge KC (2007). Opioid limbic circuit for reward: Interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. The Journal of Neuroscience, 27(7), 1594-1605.

Smith KS, Berridge KC, Aldridge JW. (2011). Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. Proc Natl Acad Sci U S A, 108(27), E255-E264.

Taha SA, Fields HL (2006) Inhibitions of nucleus accumbens neurons encode a gating signal for reward-directed behavior. J Neurosci 26:217-222.

Tindell AJ, Berridge KC, Aldridge JW (2004) Ventral pallidal representation of pavlovian cues and reward: population and rate codes. J Neurosci 24:1058-1069.

Tindell AJ, Smith KS, Berridge KC, Aldridge JW (2005a) VP Neurons Integrate Learning and Physiological Signals to Code Incentive Salience of Conditioned Cues. Society for Neuroscience Abstracts.

Tindell AJ, Berridge KC, Zhang J, Pecina S, Aldridge JW (2005b) Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. Eur J Neurosci 22:2617-2634.

Usuda I, Tanaka K, Chiba T (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. Brain Research 797:73-93.

Volkow ND, Wang GJ, Fowler JS, Logan J, Jayne M, Franceschi D, Wong C, Gatley SJ, Gifford AN, Ding YS, Pappas N (2002) "Nonhedonic" food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. Synapse 44:175-180.

Wise RA (1985) The anhedonia hypothesis: Mark III. Behavioral and Brain Sciences 8:178-186.

Wise RA (1998) Drug-activation of brain reward pathways. Drug & Alcohol Dependence 51:13-22.

Wyvell CL, Berridge KC (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. Journal of Neuroscience 20:8122-8130.

Zahm DS (2000) An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. Neuroscience & Biobehavioral Reviews 24:85-105.
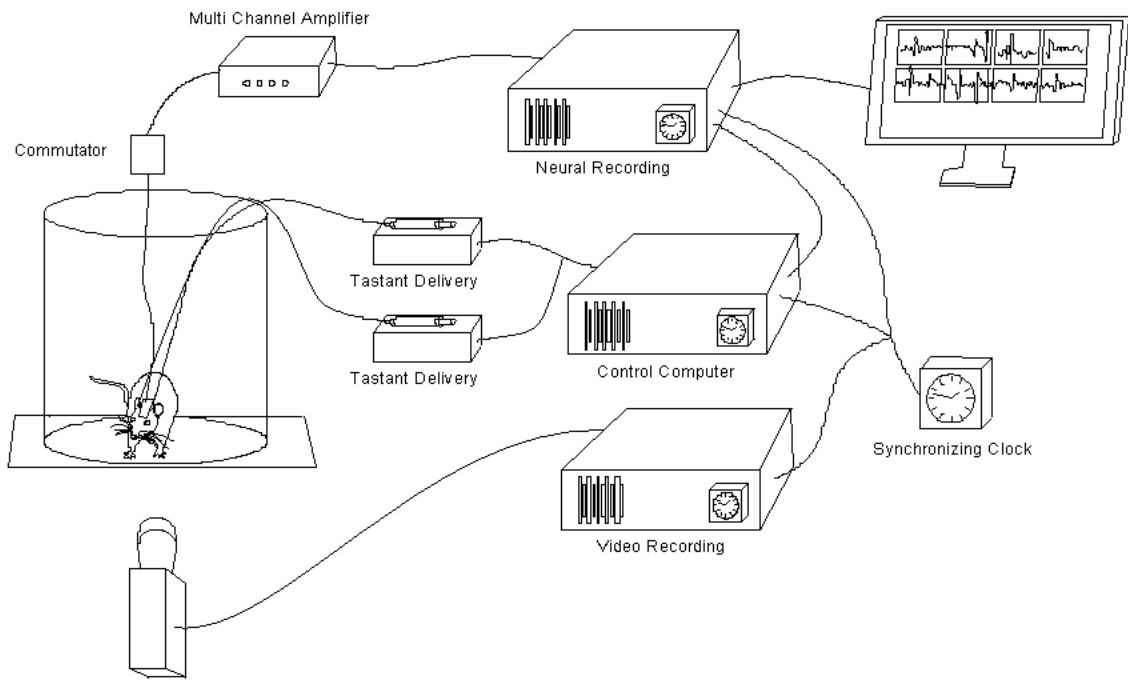
APPENDIX A

Figure A.0.1 Diagram of chamber set-up. Animals were tested with either one or two taste delivery tubes connected, depending on the experiment. The rat was placed inside a clear plastic tube to restrict them to the field of view of the video camera. The syringe(s) were connected to a computer-controlled pump which delivered tastes to the rat via a flexible clear plastic tubing that connected to an oral cannula on the rat's head. VP cells were recorded via a commutator, allowing free movement within the chamber. The tastant deliveries, neural recordings and behavioral responses were all synchronized at the start of each session, allowing neural and behavioral data to later be aligned to taste onset.