

Effects of Deep Brain Stimulation on Taste Reactivity
in the Central Medial Nucleus of the Amygdala

by

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Abstract

Alterations to the hedonic value of food could be an underlying cause of obesity. To assess the hedonic impact, a taste reactivity test, which is sensitive to the palatability of food and dissociable from sensory processes, was used. In this preliminary study, we measured hedonic and aversive taste reactions to oral infusions of sucrose, water and quinine during deep brain stimulation of the central medial nucleus of the amygdala (CeM). It was hypothesized that stimulation of the CeM will decrease the hedonic impact to a sucrose solution while increasing the aversive impact to a quinine solution. Since this was a preliminary study with a small sample size, no strong conclusion can be made. However, the results hint that an aversive state was experienced by the rats. An increase in aversive taste reactions and a decrease in hedonic reactions were observed. During taste infusions, an increase in aversive responses to sucrose was seen. Even the small number of positive facial reactions to water decreased. These findings suggest deep brain stimulation in the central amygdala may be creating an overall aversive state in the rat. With further work, an experiment such as this can be useful in understanding how brain stimulation could be used as a treatment for morbid obesity.

Keywords: deep brain stimulation, taste reactivity, central medial nucleus of the Amygdala

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Brain Reward System and Obesity

Obesity has become a national epidemic in the past three decades, where one in four Americans may be considered obese (Centers for Disease Control and Prevention, 2009). Incidences of chronic diseases, such as diabetes, colon cancer and heart disease; reduction of quality of life and a decrease in life expectancy are all associated with the increase of weight on an individual (Field et al., 2001). Many theories have been proposed to explain this increase in body weight. For one, the availability and abundance of palatable foods and cues associated with food in a food-rich environment has contributed to a state where anyone can eat at any time and overeat during mealtimes. Secondly, modern foods contain higher levels of fat, sugar and salt thus making them more pleasurable. The brain's response to these foods and cues may induce an increased desire and motivation to overeat (Berridge, Ho, Richard, & DiFeliceantonio, 2010). The brain's reward circuit ("wanting" and "liking" systems) is thought to be wired as a "go" system, which is stimulated by appetizing foods and related cues (Berridge, 2010). When an individual feels satiated, the "go system" is attenuated but never creates a strong "stop" signal. Berridge and Valenstein (1991) showed that super-satiated rats, who consumed 10% of their body weight in a half-hour session, reduced their "liking" to sweetness but the sweet taste was never converted to a negative taste nor did they cease to respond positively to this stimulus. Thus, the sweet taste was still appealing. With the availability and abundance of food, the more the "go" system is stimulated, the greater the likelihood of overeating (Berridge, 2010). However, not all individuals overeat. Individual differences in the reward circuit may account for why some over-consume while others do not.

In the reward circuit “hedonic hotspots”, which magnify the “liking” reaction to a sensory pleasure, can be found (Kringelbach & Berridge, 2010). The distinction between “liking” and “wanting” must be made in order to understand the reward circuit. “Liking” refers to the objective hedonic response in behavior to a reward. “Wanting” refers to the motivation for reward and makes rewards “more attractive, sought after and likely to be consumed” (Berridge, 1996). “Liking” and “wanting” may occur together but this may not always be the case. Using the above example, the sweet taste may still be “liked” by the super-satiated rat but it is not “wanted” since the rat is full. Dysfunctions in reward processing can arise. This may help explain an underlying cause of obesity which may be due to alterations to the hedonic value of food. Excessive activation of a “hedonic hotspot” may enhance the “liking” response to pleasurable food thus making an individual like the food more. This may lead to overeating (Berridge, 2009; Davis & Carter, 2009, Pecina, Smith, & Berridge, 2006).

Taste Reactivity: A Measure of Hedonic Impact

To assess the hedonic impact, a taste reactivity test can be used, which is sensitive to the palatability of food and dissociable from sensory processes (H. J. Grill & Norgren, 1978). The taste reactivity test entails delivering a small amount of a taste solution directly into the oral cavity of a freely moving animal through intraoral fistulae (H. J. Grill & Norgren, 1978). The facial and body responses are analyzed and differentiated into three categories: positive hedonic, aversive and neutral responses. Positive hedonic responses include rhythmic midline tongue protrusions, lateral tongue protrusions and paw licking (Berridge, 2000; Tindell, Smith, Pecina, Berridge, & Aldridge, 2006). Aversive responses include gapes, headshakes, forelimb flails, face washing and chin rubs (Berridge, 2000; Tindell et al., 2006). Neutral reactions include rhythmic mouth movements, passive dripping, locomotion and grooming (Berridge, 2000; Tindell, Smith,

Berridge, & Aldridge, 2009). Using physiological, psychological, pharmacological, and neural manipulations, studies of taste reactions have shown these behaviors reflect affective processes and not sensory processes (Berridge, 2000). By separating sensory properties from affective properties, it has been shown that the responses seen reflect the pleasure or aversion of a taste rather than the intensity of the taste (Berridge, 2000). Even if a stimulus has not changed, the taste reactivity test can detect manipulations that change the palatability of a taste (H. J. Grill & Berridge, 1985). In this study, deep brain stimulation will be used to modulate the neural activity of the central medial nucleus of the amygdala (CeM) while the animal is given a hedonic, aversive, and neutral taste. The taste reactivity test will assess if deep brain stimulation has an effect on the palatability of the given taste.

Deep Brain Stimulation

Deep brain stimulation (DBS) is currently used as a treatment option for neurological movement disorders such as Parkinson's disease and dystonia, and is being investigated as a potential treatment for depression, obsessive compulsive disorder and morbid obesity (Halpern et al., 2008). In DBS, a continuous electrical stimulation, at high frequencies typically between 130-200 Hz, is delivered to a region of the brain. Current studies in animal models are investigating the effect of DBS on behavior, cognition, neurophysiology and reward. Sani, Jobe, Smith, Kordower, and Bakay (2007) found that bilateral continuous stimulation of the lateral hypothalamus (LH), an appetite center, in rats decreased weight gain when compared to the non-stimulated control group. However, the weight loss was not due to less food consumption but may be due to changes in the rat's metabolism (Sani et al., 2007). The research group concluded that stimulatory inhibition of the LH was effective in causing weight loss in rat. While Sani et al (2007) found that inhibiting the appetite center resulted in weight loss; Halpern et al. (2008)

suggested that feeding behavior was also influenced by the palatability of the food, irrelevant of appetite levels. Chronic DBS in the nucleus accumbens (a reward nucleus associated with the palatability of foods) is suggested to modulate dietary preferences and reward (Halpern et al., 2008). However, how information and processing is affected by extracellular stimulations is still debated (Warren M. Grill & McIntyre, 2001).

W. M. Grill, Snyder, and Miocinovic (2004) have proposed a theory that high frequency stimulation (HFS) generates an “informational lesion” in the affected region. HFS may increase the regularity of the neuronal firing pattern and mask the intrinsic cell activity, thus the output of the neuron resembles that of the high frequency signal. If the output is drastically different from a typical output signal, normal communication between cells is disrupted. Information is lost and communication may even be blocked (Grill et al., 2004). Low frequency stimulation may cause the neuron to increase its firing output but not enough to overwhelm it, thus activating the region (Alesch et al., 1995; Grill et al., 2004; Kuncel, Cooper, Wolgamuth, & Grill, 2007). As stated above, the “go” system is stimulated by appetizing foods and may lead to overeating. Using HFS, a blockage in communication may occur thus attenuating the system in this rich-food world. Also, HFS in a “hedonic hotspot” may block communication among neurons thus decreasing the hedonic response to pleasurable food. This theory may provide a potential therapeutic treatment to morbid obesity.

Role of Amygdala in Reward

Currently the nucleus accumbens, ventral pallidum and brainstem have been identified as “hedonic hotspots” (Kringelbach & Berridge, 2010; Pecina et al., 2006). Lateral hypothalamus, mesolimbic dopamine projections, and bombesin hindbrain system have been identified as “wanting” neural substrates (Berridge, 2010). Another reward-activated site has been implicated

in the amygdala. The amygdala could be a “hedonic hotspot” and may participate in a larger “liking” circuit but it has not been confirmed yet (Berridge, 2010). However, lots of evidence has pointed that the amygdala could mediate the “wanting” circuit and be a “false hedonic candidate” (Berridge, 1996, Berridge, 2000, Mahler & Berridge, 2011). Amygdala lesions have changed food preferences in monkeys, reduced taste aversion in rats and abolished the reward value of salt in rats (Berridge, 2000). Seeley, Galaverna, Schulkin, Epstein, and Grill (1993) found rats with lesions of their central nucleus of the amygdala (CeA) did not increase their intraoral intake of salt while being in sodium-depleted states. Generally, rats in sodium-depleted states would prefer salt solutions, thus the lesion in the CeA has blocked this preference. They concluded that CeA lesions altered the reward impact of salt in sodium-depleted rats.

Galaverna et al. (1993) found that even though CeA-lesioned rats did not increase their intake of salt, the rats’ hedonic reactions were similar to their sodium-depleted counterparts. Normally, physiologically normal rats produce aversive reactions to salt solutions while sodium-depleted rats produce hedonic reactions to salt solution. CeA-lesioned rats would produce hedonic reactions to salt solutions (Galaverna et al., 1993). This suggested that the “liking” circuit may not have been affected since both lesioned and non-lesioned rats produced hedonic reactions. However, the “wanting” circuit may have been affected since lesioned rats did not “want” the salt solution even if they “liked” it. Meanwhile, Simbayi, Boakes, and Burton (1986) found that lesions to the basolateral amygdala may block changes in taste palatability by blocking shifts from hedonic to aversive reactions, which indicates other areas of the amygdala may play a role in “liking”. All these results suggest that the amygdala may mediate the reward impact of food (Berridge, 2000). However, more research needs to be done to determine the amygdala’s role on the reward impact of food.

Exploring Deep Brain Stimulation in CeM during Food Consumption

A recent study conducted in the Aldridge lab measured the effects of continuous DBS on food consumption using a self-administration task (unpublished data, Ross, Lehmann, & Schoen, 2011). Rats were given access to two levers. Pressing one lever would deliver a sucrose pellet, while the other lever was inactive. The test conditions consisted of rats receiving high frequency (130 Hz), low frequency (20 Hz) or no stimulation. Seven rats were implanted with electrodes in the central medial nucleus of the amygdala (CeM).

This study has found that these rats decreased the amount of sucrose pellets they delivered and consumed in an instrumental responding task during high and low frequency DBS. There was no significant difference between high and low frequency. So both frequencies had a “blocking” effect on the consumption of food. During control conditions, the rats would consistently lever press throughout the session and consumed all the pellets delivered. However, during low and high frequency stimulation, the majority of the lever presses occurred early in the test session and faded within a few minutes. This suggests a decrease in motivation to obtain sucrose pellets. The active lever presses outnumbered the inactive lever presses revealing the rat correctly associated the active lever with food delivery. In some instances, the rats were seen to insert the pellet in their mouth but immediately expel it. Aversive facial reactions such as gapes were also displayed. A facial reaction test was performed on one rat where the facial responses during high, low and no stimulation were quantified. During this period, the rat was not given any foods or liquids. In HFS conditions, this rat exhibited increased aversive reactions. When stimulation was discontinued, no aversive facial reactions were observed. Thus, only during DBS did the rewarding aspect of the food change. Stimulation of the CeM blocked feeding. However, it is unknown if DBS affected the “liking” or “wanting” system (or perhaps both).

To gain insight into which system DBS may have affected, a study was designed to test the idea that stimulation alters the hedonic and/or aversive impact of food. We measured facial and body reactions to oral infusions of sucrose, water and quinine during DBS in the CeA (specifically the medial nucleus of the CeA). We hypothesize that stimulation of the medial nucleus of the CeA will decrease the hedonic impact of a sucrose solution while increasing the aversive impact of a quinine solution. Stimulation frequency will alter the affective impact of taste responses. Low frequency stimulation will have a greater effect on the affective impact when compared to high frequency stimulation.

Method

Animals

Three adult male Sprague-Dawley rats (Charles River Laboratories, MA) were individually housed in a 14 h light: 10 h dark cycle with free access to rat chow and water, unless otherwise specified. All procedures were carried out under a protocol approved by the University of Michigan Committee for the Use and Care of Animals.

Habituation- Chamber

Prior to surgery, rats were handled for 10-15 minutes and placed in the chamber for 10 minutes to acclimate to the chamber set-up. This was done for 2 days.

Habituation- Sucrose Solution

Rats had daily access to 20-mL of sucrose (0.5M) solution in their home cage and the amount of liquid consumed was quantified for 4 days. Rats that did not consume at least 15-mL of sucrose solution on day 4 were not included in this study.

Surgery- Oral Cannula

Bilateral intraoral cannulae were implanted in rats anesthetized with ketamine (100mg/kg) and xylazine (10mg/kg). The intraoral cannulae were inserted in the mouth lateral to the first molar and exited the head near the skull screws, where they were attached to a stainless steel guide cannulae (Tindell et al., 2006).

Surgery- Stimulating Electrode

In the same surgery, the anesthetized rats were implanted with a stimulating electrode aimed at the central medial nucleus of the amygdala or CeM (AP -2mm, ML +/-3.5mm, DV 7.5-8.5mm) on each side of the brain. Electrodes were constructed with two bundles of wires containing four (25 μ m, tungsten) recording wires and two (75 μ m, stainless steel) stimulating wires in each bundle. Animals were allowed to recover for seven days after surgery. Detailed surgical procedures can be found in Tindell et al. 2006.

Training and Taste Infusion Protocol

Rats underwent three days of taste-infusion training. Animals were placed in the recording chamber illuminated under white light needed for video recording. Sessions consisted of a two-minute habituation period, followed by a sequence of intraoral infusions. Each rat received intraoral infusions of 17 % sucrose solution, tap water, and 0.01% quinine solution, in this sequence. Each solution was presented in 10 repetitions with 0.1 ml of tastant delivered over a period of 1 second. Infusions were delivered using a 3 ml syringe connected to hollow tubing (PE-50 fixed to a PE-10 delivery nozzle) that was attached to a single oral cannula. Infusions were delivered using a computer-controlled pump. A variable interval (45-75 seconds) separated infusions. Because quinine leaves an aftertaste, it was always presented last after the water and sucrose infusions. Between the sucrose and tap water block, two infusions of water were given to the rat to wash-out any sucrose solution left in the rat's mouth.

Testing

Rats underwent six days of testing. Video recordings of taste reactions were made with the camera zoomed and focused on the mouth and forelimb region to visualize and record facial and body reactions. Testing conditions were similar to training conditions with the exception that rats receiving brain stimulation on four out of the six testing days. For stimulation sessions, the stimulation was turned on from the second minute of habitation and remained on till the end of the session. Rats received either high frequency (130 Hz), low frequency (20 Hz) or no stimulation while receiving all three taste infusions - sucrose, water and quinine. The testing session order began with no stimulation on day one, followed by two days of high frequency stimulation, continued with one more day of no stimulation and ended with two days of low frequency stimulation. Rats experienced three blocks of taste under one type of stimulation per day.

Brain Stimulation Procedure

A continuous monopolar and biphasic current (250-300 μ A), with a pulse width of 100 μ s per phase, was delivered bilaterally into the CeM. For high frequency stimulation, a frequency of 130 Hz was used. For low frequency stimulation, 20 Hz was used. Control consisted of periods with no stimulation.

Histology

Rats were anesthetized with isoflurane gas and then a 0.1 mA lesioning DC current was passed for 10 seconds to mark the electrode location in the brain. After euthanizing the rats with a drug overdose of pentobarbital, the brains were removed, frozen in an isopentane and isopropyl alcohol solution, sliced into 40 μ m sagittal sections and finally stained with cresyl violet. The electrode placement was confirmed by observing the histologic slices under a light microscope.

Behavioral and Data Analysis

Video recordings of the facial and body responses were scored frame-by-frame using the Datarat software (developed by the Aldridge Lab). Three time periods in relation to each trial (each 10 seconds in duration) were examined for all trials. Taste reactions to the taste infusion were assessed in a 10 second period beginning at the onset of the infusion. A background period 10 seconds in duration immediately before each infusion was used for comparison along with an additional 10 second period beginning 30 seconds before the infusion. In this analysis, the two background time periods were combined into one, creating one background period to assess if stimulation alone produced any vacuum facial and body reactions (specifically mouth, tongue, or forelimb movements) without any actual taste present (Berridge & Valenstein, 1991). If this is the case, it could influence the interpretation of reactions seen in response to a taste during stimulation. Positive (hedonic) and aversive reactions were recorded during taste infusions with and without CeM stimulation. Positive hedonic responses were signified by lateral tongue protrusions. Aversive responses consisted of gapes, forelimb flails and headshakes. Other hedonic and aversive responses, such as paw lick bouts and tongue protrusion bouts were not included in this preliminary study. This data is currently being analyzed and will be included in future results.

Each occurrence of these aversive and hedonic responses were counted as one event and the total number of events was summed to provide overall hedonic and aversive reactions. The average count of reactions per trial was calculated for each session. Five trials of each taste infusion were analyzed. Rats' reactions were averaged to provide an overall hedonic and aversive response to each test condition. Brain stimulation occurred in two sessions (130 or 20

Hz). A control session with no stimulation was also recorded. After data from a larger sample size is collected, statistical assessment will be made using an ANOVA general linear model.

Results

The Effects of CeM Stimulation on Taste Reactions

This preliminary study assesses the results from three animals (additional testing is underway). Results are reported as mean \pm standard error. All measures are average counts of facial and body responses per taste infusion trial. Taste reactions during the taste infusion were compared to the reactions before the taste infusion across all taste infusion (sucrose, water and quinine). We observed no difference between high and low frequency stimulation so periods with stimulation, high or low frequency, were averaged together (labeled “stim in the figures).

When measuring hedonic responses, a deep brain stimulation-induced decrease in hedonic responses was noted with taste infusions (Figure 1; 0.37 ± 0.09 (stim) vs. 0.79 ± 0.22 (no stim), $n=3$). As one might expect, the numbers of taste reactions during the periods prior to taste delivery had very low numbers of taste reactions. The purpose of measuring them here was to ensure that brain stimulation itself did not evoke spontaneous taste reactions even in the absence of actual tastes. During periods preceding the infusion, no change was seen in the extremely small numbers of hedonic reactions (Figure 1; 0.09 ± 0.08 (stim) vs. 0.13 ± 0.05 (no stim), $n=3$). Thus, brain stimulation on its own does not appear to be invoking taste reactions. This indicates that to see the decreased hedonic responses during stimulation, a taste needs to be introduced.

Although the numbers were small, more aversive reactions were observed during CeM stimulation before taste delivery (Figure 2, 0.64 ± 0.04 (stim) vs. 0.20 ± 0.04 (no stim), $n=3$). Taste infusions increased the average numbers of aversive reactions (averaged across all tastes:

sucrose, water, quinine) and stimulation seemed to make little difference although more testing will be needed to assess any significant differences (Figure 2; 1.54 ± 0.58 (stim) vs. 1.00 ± 0.52 (no stim), $n=3$). Further study is needed to confirm the suggestion from these preliminary tests that stimulation of the CeA may provoke aversive taste reactions even when no taste is presented to the rats. Overall, rats exhibited more aversive responses and less hedonic responses.

Specific Tastes and Stimulation

Three types of tastes were introduced to rats- sucrose, water and quinine. Our observations suggest that the numbers of hedonic facial responses decreased with stimulation to water and quinine taste solutions, although the magnitude of change may be less with sucrose (Figure 3; sucrose- 0.67 ± 0.28 (stim) vs. 1.22 ± 0.55 (no stim), water- 0.27 ± 0.09 (stim) vs. 0.67 ± 0.15 (no stim), quinine- 0.02 ± 0.08 (stim) vs. 0.5 ± 0.06 (no stim), $n=3$). This suggests that stimulation may be taste specific; however no clear conclusions can be formed with the current data set.

When evaluating aversive facial and body responses to specific tastes following a taste infusion, an increase in aversive responses was only seen with sucrose tastes and not to water or quinine (Figure 4; sucrose- 0.60 ± 0.23 (stim) vs. 0.13 ± 0.10 (no stim), water- 0.74 ± 0.44 (stim) vs. 1.65 ± 0.77 (no stim), quinine- 2.38 ± 0.73 (stim) vs. 2.12 ± 1.01 (no stim), $n=3$). This suggests that stimulation may affect aversive responses, but only to specific tastes. With a decrease in hedonic responses to water and quinine and an increase in aversive responses to sucrose, the net affective effect appears to be in the aversive end of the spectrum.

CeM stimulation resulted in greater numbers of aversive reactions before taste delivery (Figure 5; sucrose- 0.86 ± 0.19 (stim) vs. 0.19 ± 0.09 (no stim), water- 0.51 ± 0.03 (stim) vs. 0.23 ± 0.03 (no stim), quinine- 0.56 ± 0.16 (stim) vs. 0.18 ± 0.08 (no stim), $n=3$). Interestingly, of the

three tastes, there was a greater increase prior to sucrose infusions. Why these rats were reacting negatively before taste delivery (and more specifically prior to sucrose infusions) needs to be investigated further. Under no stimulation conditions, the aversive reaction levels are similar across tastes.

Histology

Only three animals were included in the final analysis of this preliminary study. The left side of the electrode for rat 1 was implanted at the edge of the central medial nucleus of the amygdala (CeM) and the extended amygdala (Figure 6; AP -1.8 mm, ML 3.00 mm, DV 8.5 mm). Lesion damage from the right side was not visible. The left side of electrode for rat 2 was implanted in the CeM (Figure 7; AP -2 mm, ML 3.70 mm, DV 7.8 mm). Lesion damage from the right side was not visible. The electrode location for rat 3 was in the CeM (Figure 6; right side: AP -1.8 mm, ML 3.40 mm, DV 8.4 mm, left side: AP -1.8 mm, ML 3.90 mm, DV 8.1 mm). Locations were confirmed using the Paxinos and Watson 6th ed. (2007) atlas.

Discussion

Since this was a preliminary study with a small sample size, many of the observations seen could be due to the inherent variability in the data set, thus no strong conclusions can be made. However, the results from the current study do suggest that both aversive and hedonic facial and body responses are affected by stimulation. An overall aversive state is seen by an increase in aversive reactions and a decrease in hedonic reactions. The frequency of stimulation did not appear to affect hedonic or aversive taste reactions. We had predicted that stimulation would decrease the hedonic impact to the sucrose solution and increase the aversive impact to the quinine. What we found was a seemingly decrease in hedonic responses only to water and quinine and an increase in aversive responses to sucrose. This suggests that both hedonic and

aversive response systems may be working in tandem or, a dual-valenced system may be activated to create an overall aversive state in the rat, that is, increased aversion to sweet tastes and decreased hedonics to neutral and bitter tastes. Even during the periods before tastes were delivered, aversive responses increased across all three tastes which further suggest that stimulation may be placing the rat in an overall negative state.

The idea of an interrelationship between hedonic and aversive subcomponents of “liking” was discussed by Berridge (1996). The orthogonal hypothesis states that the underlying mechanism of palatability may not be uni-dimensional with hedonic and aversive responses on polar ends of one continuum but an orthogonal process with different neural components (Berridge, 1996). Large shifts in the hedonic dimension will result in an indirect change in aversive responses so that a decrease in hedonic response will increase aversive response (Berridge, 1996). However, smaller shifts in the hedonic dimension will not change aversive responses. This independent shift in hedonic or aversive responses is called the “zone of hedonic independence” (Berridge, 1996). The current results appear to have fallen within this “zone of hedonic independence” since under water and quinine taste tests, hedonic responses decreased without changing the aversive responses. With sucrose tastes, aversive responses increased without changing the hedonic responses. This suggests that different neural mechanisms may be affected by stimulation due to the separate hedonic and aversive responses to taste. This is useful in better understanding how stimulation could be used as a possible treatment for morbid obesity. Neural manipulation could create independent changes to the hedonic or aversive responses by decreasing the hedonic reactions to palatable tastes while not increasing aversive reactions to the same taste (Berridge, 1996).

Berridge and Valenstein (1991) found similar effects to facial and body responses as we did when they stimulated the lateral hypothalamus (LH) in rats. When stimulation was turned on, aversive affective reactions increased while not changing hedonic reactions. The aversive reaction showed a greater increase with high and low concentration of sucrose and high concentration of hydrochloric acid. The taste responsiveness seen in the LH was similar to the responses seen in the CeM. When stimulation was present in each structure, an increase in aversive responses was seen with no change in hedonic responses. This suggests that hedonic enhancements are not being activated by electrical stimulation of the LH or CeM. Berridge and Valenstein (1991) argued that feeding increases due to stimulation was not mediated by hedonic enhancements but by activating the incentive salience attribution or the “wanting” system. Since consumption did increase and it was not due to hedonic enhancements, this prediction is logical. However, this reasoning cannot not be directly translated to the CeM since stimulation of the CeA decreased consumption while decreasing hedonic responses thus the “liking” system may still be involved. More research needs to be conducted to determine if the “wanting” system is being activated alongside the “liking” system or if “liking” system is not being activated at all so the changes seen are only due to changes to the “wanting” circuit.

Kemble, Studelska, and Schmidt (1979) studied the effect of central amygdaloid nucleus lesions on ingestion and they found rats consumed less quinine after the lesion. However this decrease in consumption was not due to aphagia or adipisa. They predicted that the central amygdaloid nucleus may play a role in the relationship in taste-mediated behaviors. To further explain these observations, the current results suggest that an overall aversive state may be observed in the rats. The stimulation the rats received may have generated an “informational lesion” and temporarily blocked the neuronal communication in the central medial nucleus of the

amygdala (CeM) (Grill et al. 2004). One possible explanation for why rats consumed less quinine after the lesion may be due to damage to the reward circuit. The lesion may have amplified the aversive response the rat experienced with quinine thus the rat consumed less. The Aldridge lab found that stimulating the CeM caused rats to consume less sucrose pellets (unpublished data, Ross, Lehmann, & Schoen, 2011). This Aldridge study further shows that blocking the CeM does indeed affect consumption behavior. The current results suggest that this decrease in consumption may be mediated through a heightening of aversive responses and a dampening of hedonic responses to the tastes being consumed.

One of the goals of the current study was to determine the cause to the decreased pellet consumption during stimulation of the CeM and the results suggest that the “liking” pathway may be affected by stimulation since the rats’ aversive and hedonic facial responses did change during the taste reactivity test. However, the “wanting” circuit may also play a role in the decrease in consumption. Mahler and Berridge (2011) have suggested that the central nucleus of the amygdala (CeA) is involved in the “wanting” pathway. They found that opioid stimulation of the CeA enhanced cue-triggered sucrose seeking in Pavlovian to instrumental transfer tests (PIT) and also food intake. Although an increase in “wanting” was seen, a decrease in hedonic responses for sucrose was seen in a taste reactivity test. However in this present study, no effect was seen to the aversive response for sucrose. By stimulating specifically the medial aspect of the CeA, we found the opposite result since no change was seen in hedonic responses to sucrose while an increase in aversive responses was seen. This provides evidence that stimulation may be creating an “information lesion” since it produced results that are opposite to the activation of the CeA. Mahler and Berridge (2011) suggest that the decrease in “liking” with an increase in “wanting” in the CeA provided evidence for the dissociation between motivation and hedonic

impact of the CeA. They predict that the CeA is involved in the “wanting” pathway. However, the “liking” circuit may still be involved in the CeM by dampening the hedonic impact and enhancing the aversive impact which could have caused the decrease in consumption. This suggests that the effect of stimulation on the “liking” and “wanting” circuit is complex and more research needs to be done in order to better understand stimulation’s role in the CeA. Due to the low sample size, statistical analysis could not be done and this is a large limiting factor in this study. The overall aversive effect could be due to chance and the “liking” pathway may not have been affected at all.

Other limiting factors include the lack of randomization to the taste infusion schedule and the narrow range between each taste infusion (45-75 seconds). All rats received sucrose infusions first, followed with water and finished with quinine. The reason for not randomizing the taste infusions was due to the lingering aftertaste effects of quinine. However, this lack of randomization could have led the rats to learn the taste sequence and anticipate specific tastes at certain time periods. Stimulation could have been involved in reward anticipation in the period before tastes were delivered. Studies have shown that rats can anticipate reward (Burgdorf, Knutson, & Panksepp, 2000). Burgdorf et al (2000) found that rats’ ultrasonic vocalizations are indicators of reward anticipation. Anticipation of rewarding electrical stimulation of the brain in the ventral tegmental area (VTA) and lateral hypothalamus (LH) can evoke these vocalizations. LH is involved in food consumption while VTA is part of the reward circuit. These results suggest that rats can anticipate reward associated to the feeding and reward circuit. This can be an alternative explanation to why an increase in aversive responses was seen before the taste infusions. To further explore this topic, facial and body response immediately before the taste infusions can be compared to the facial and body responses during the background time period

beginning 30 seconds before the infusion. If increases in aversive responses only occurred immediately before the taste infusion and not in the background period, then anticipation may have a role in the increase in aversive response. Figure 5 revealed that aversive reactions before taste infusions increased during stimulation when compared to no stimulation, which indicates that anticipation may be playing a role. It has also been observed that rats do exhibit more rearing and grooming behaviors before receiving the water or quinine taste infusions; however these behaviors were not quantified in this study. In future studies, taste reactions, rearing and grooming behaviors can be quantified and the ratio of occurrence in each time period (before and after taste infusions) can be compared to see if anticipation is playing a role in the increase of aversive responses.

Another explanation to the increase in aversive reactions is that stimulation may be affecting the motor circuit. Vacuum reactions are motor responses that occur in the absence of an actual taste or external stimulus, especially movements of the mouth, paw, or tongue (Berridge and Valenstein, 1991). If these vacuum responses exist, then a motor effect could be present which will affect how the taste reactivity changes are to be interpreted. Berridge and Valenstein (1991) found that only locomotion increased as a vacuum reaction elicited by electrical stimulation of LH. No changes in aversive responses (gapes, head shakes and forelimb flails) were seen. To further explore this topic, facial and body responses before the taste infusion can be compared to the responses during the taste infusions. For this purpose, two periods before the taste delivery are currently being analyzed-one immediately before the taste delivery and the other 30 seconds before the infusion. These two baselines will be used to assess if the rats are experiencing vacuum reactions versus anticipation. If increases in responses occur by the same amount for all three time periods analyzed (that is, 10 seconds background period, the 10

seconds just before taste, and the 10 seconds after taste infusion) then stimulation may be evoking motor responses. For hedonic responses, stimulation decreased hedonics only during taste infusions (figure 1), indicating that vacuum reactions did not appear. However, aversive responses to sucrose increased before and during the taste infusion (figure 4, figure 5), suggesting that vacuum responses or anticipation may be occurring. Analysis is still in progress to determine if stimulation is eliciting vacuum reactions.

Conclusion

Since this was a preliminary study with three rats, strong conclusions cannot be made. Overall, the rats exhibited an increase in aversive responses and a decrease in hedonic responses under stimulation. This information will be useful in understanding how stimulation can be used as a treatment to morbid obesity. Under stimulation, hedonic and aversive responses appear to be working independently suggesting that selective neural manipulation of each mechanism can be used to specify the type of treatment an individual can receive. As to which reward system, “wanting” or “liking”, stimulation is affecting, more research needs to be conducted. This study suggests that the “liking” system may be activated through the dampening of hedonic reactions and enhancement of aversive reactions. This could explain why a decrease in consumption occurred. However, the “wanting” system may also be activated, alongside the “liking” system. The current study has found that the central medial nucleus of the amygdala is affected by stimulation and this provides exciting prospects for its implication in morbid obesity.

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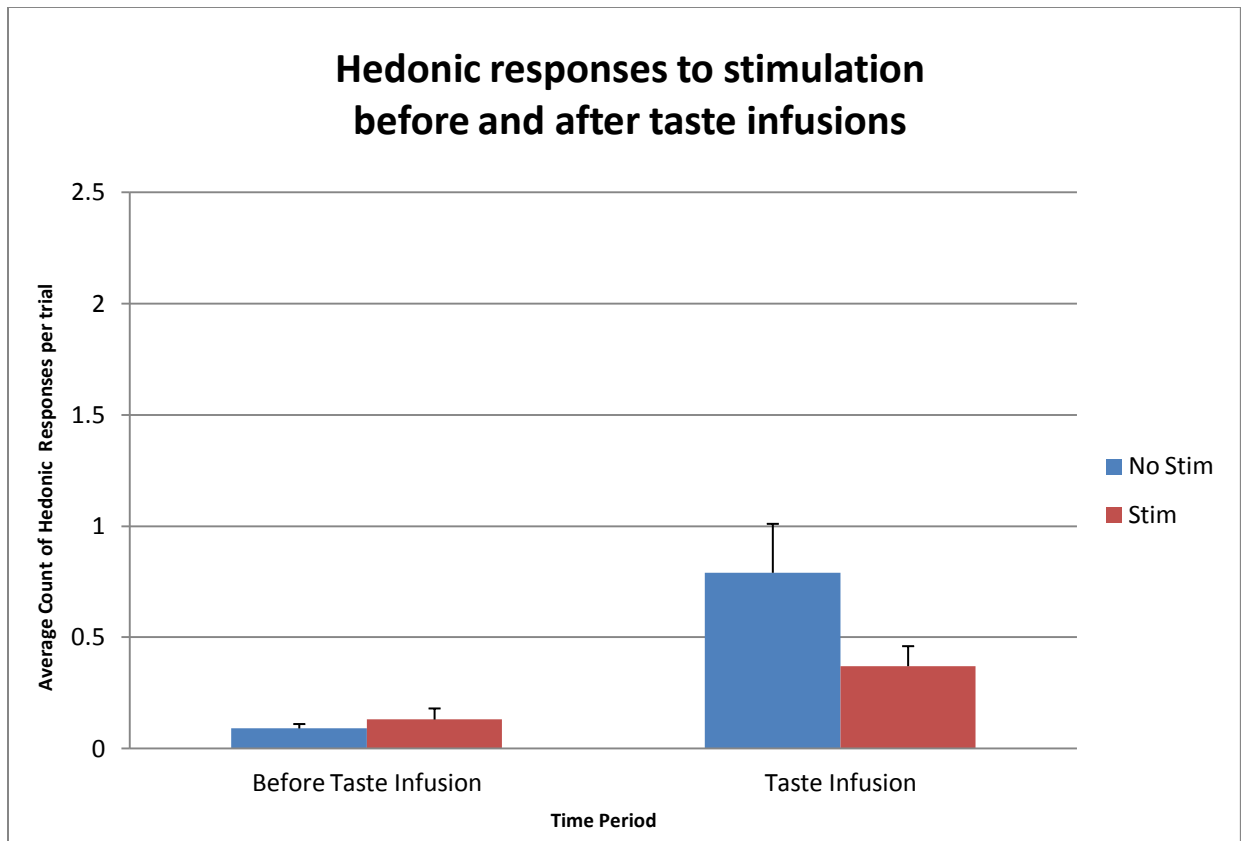


Figure 1. Average count of hedonic responses per trial under all taste types (sucrose, water and quinine taste). Before taste infusions include 10 seconds immediately before the taste onset and the 10 second background period beginning 30 seconds before the infusion. 20 and 130 Hz stimulation frequencies were combined in “stim”. “No stim” consists of 0 Hz stimulation frequency. Error bars represent standard error.

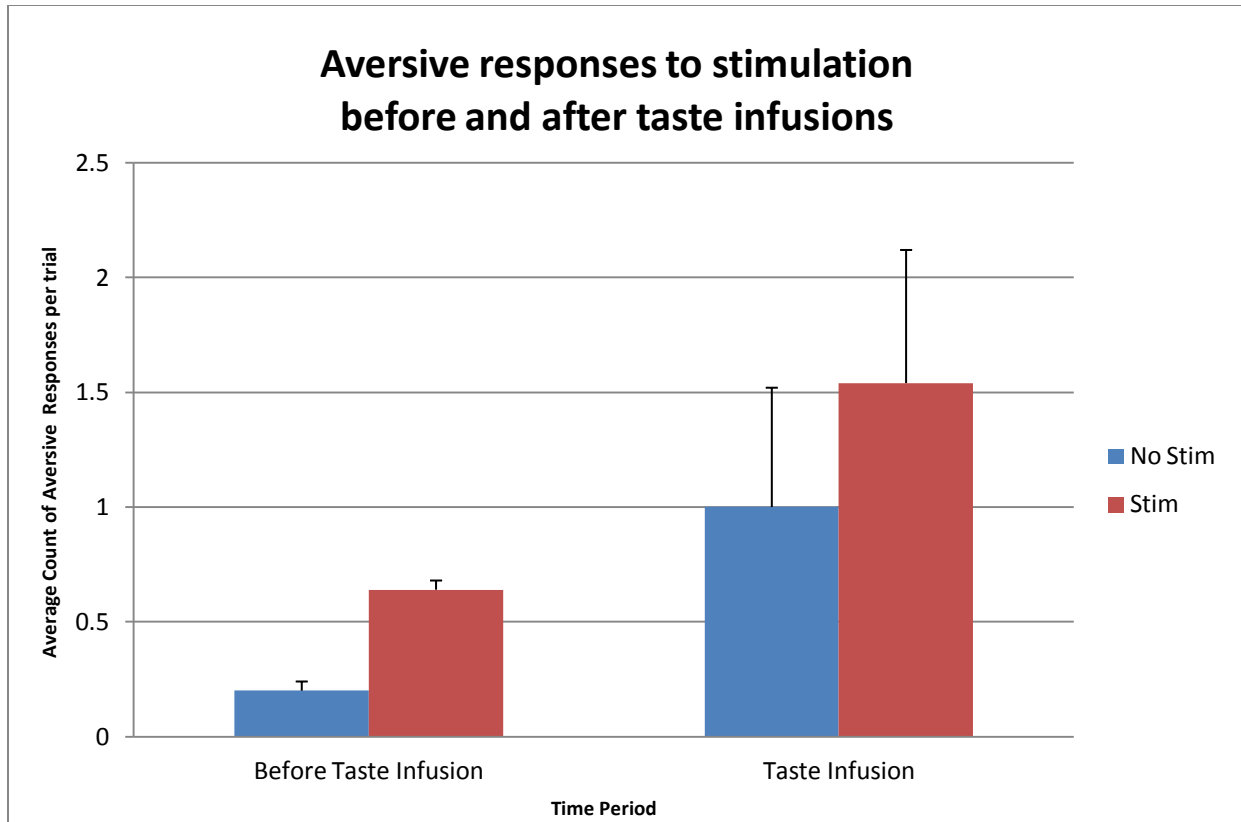


Figure 2. Average count of aversive responses per trial under all taste types (sucrose, water and quinine taste). Before taste infusions include 10 seconds immediately before the taste onset and the 10 second background period beginning 30 seconds before the infusion. 20 and 130 Hz stimulation frequencies were combined in “stim”. “No stim” consists of 0 Hz stimulation frequency. Error bars represent standard error.

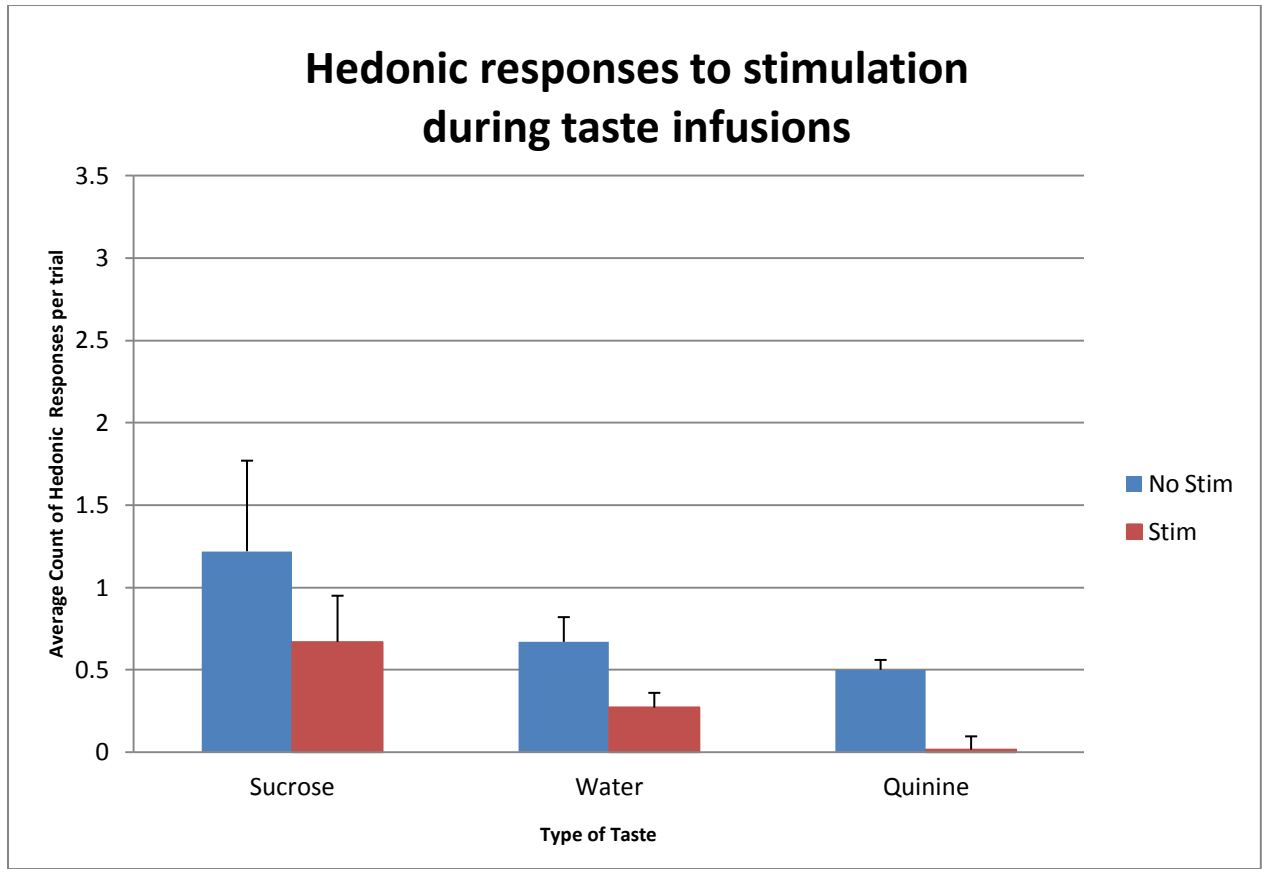


Figure 3. Average count of hedonic responses per trial during taste infusions (sucrose, water and quinine). 20 and 130 Hz stimulation frequencies were combined in “stim”. “No stim” consists of 0 Hz stimulation frequency. Error bars represent standard error.

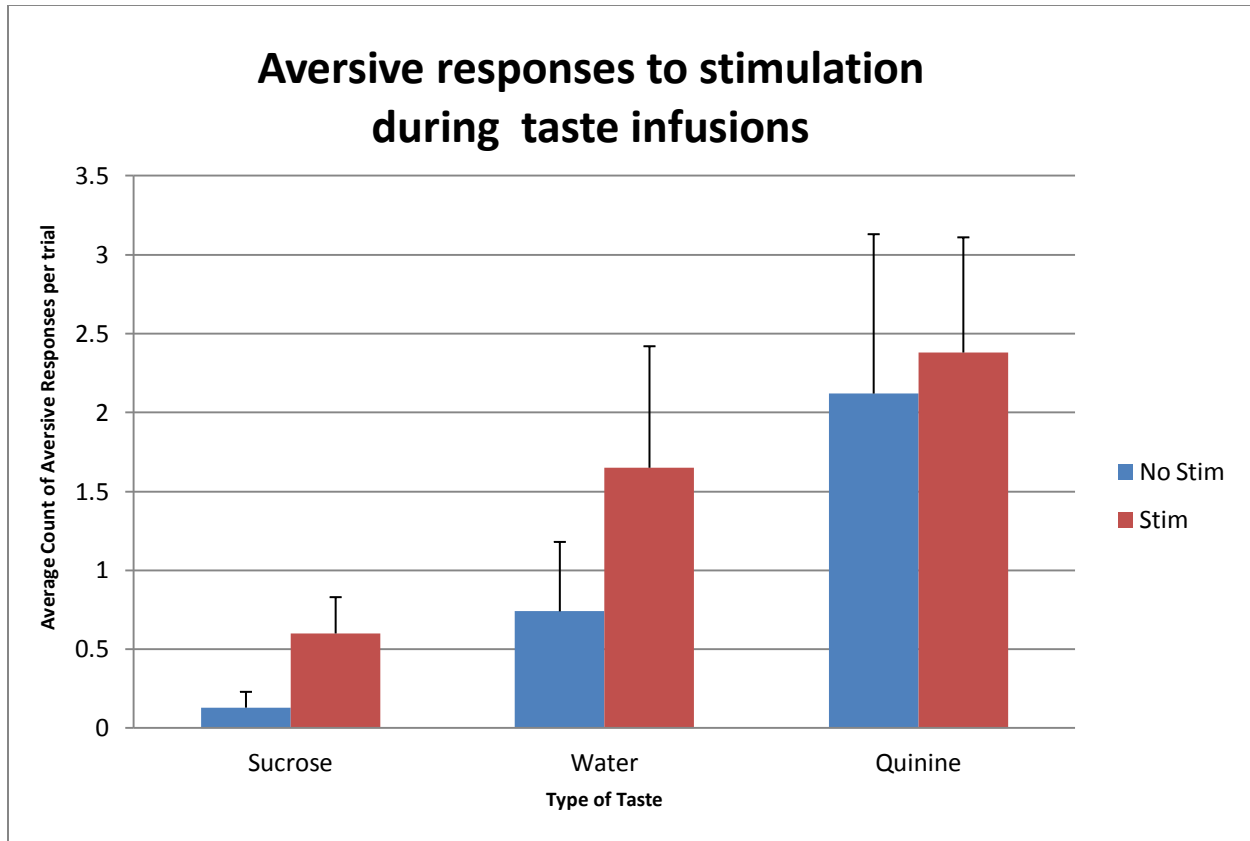


Figure 4. Average count of aversive responses per trial during taste infusions (sucrose, water and quinine). 20 and 130 Hz stimulation frequencies were combined in “stim”. “No stim” consists of 0 Hz stimulation frequency. Error bars represent standard error.

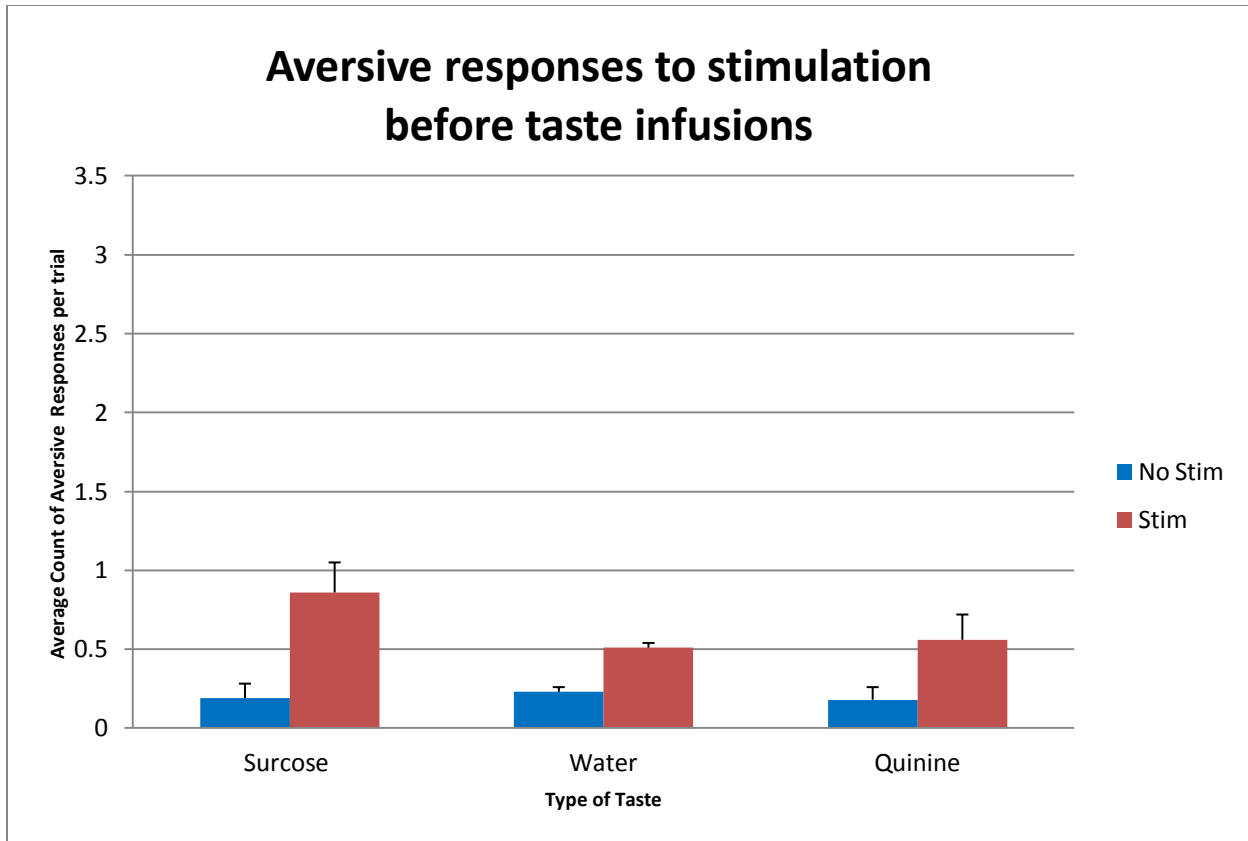


Figure 5. Average count of aversive responses per trial before taste infusions (sucrose, water and quinine). Before taste infusions include 10 seconds immediately before the taste onset and the 10 second background period beginning 30 seconds before the infusion. 20 and 130 Hz stimulation frequencies were combined in “stim”. “No stim” consists of 0 Hz stimulation frequency. Error bars represent standard error.

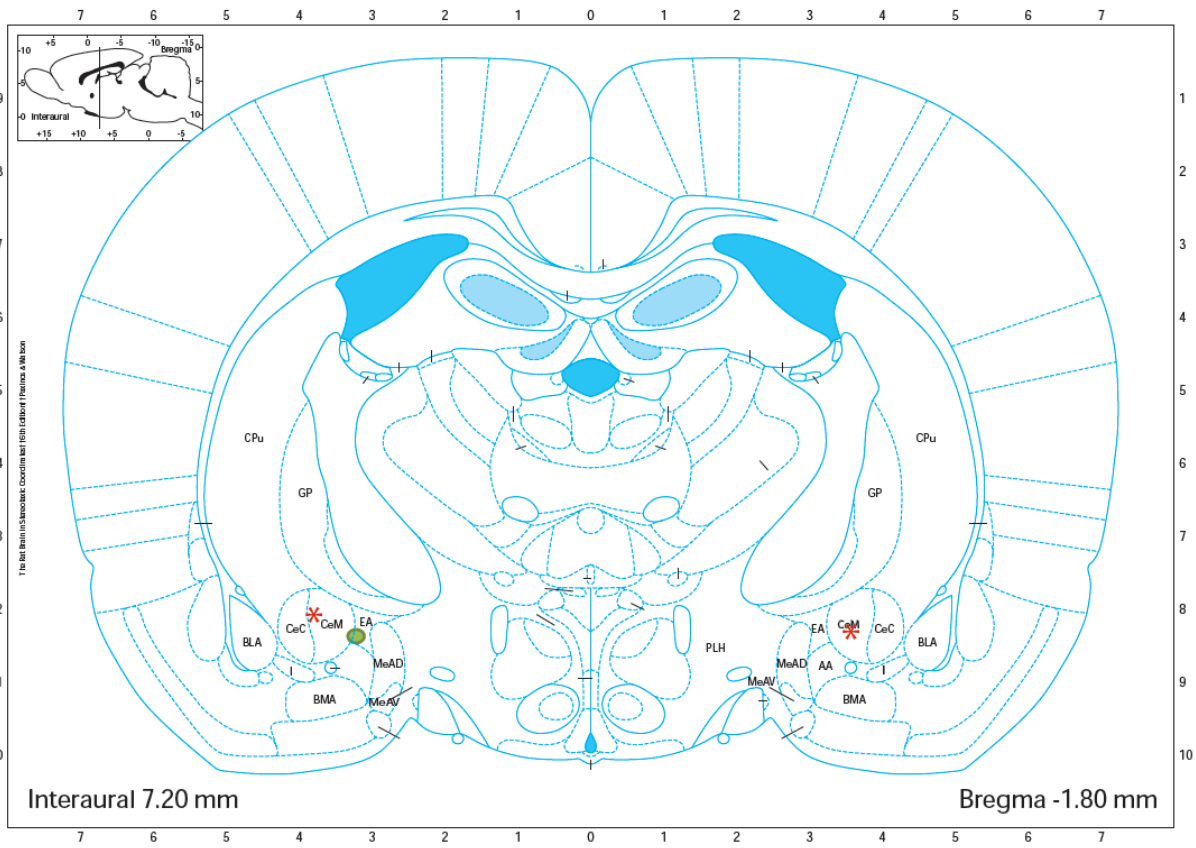


Figure 6. Histological reconstruction of bilateral electrode sites in rat 1 and 3. The small circle indicates the location of rat 1 (AP -1.8 mm, ML 2.90-3.20 mm, DV 8.5 mm). Lesion damage from the right side of rat 1 was not visible. Rat 1 was implanted in the boundary between the central medial nucleus of the amygdala (CeM) and the extended amygdala (EA). Asterisks indicate the location of rat 3 (right side: AP -1.8 mm, ML 3.40 mm , DV 8.4 mm, left side: AP -1.8 mm, ML 3.90 mm, DV 8.1 mm). Rat 3 was implanted in the CeM.

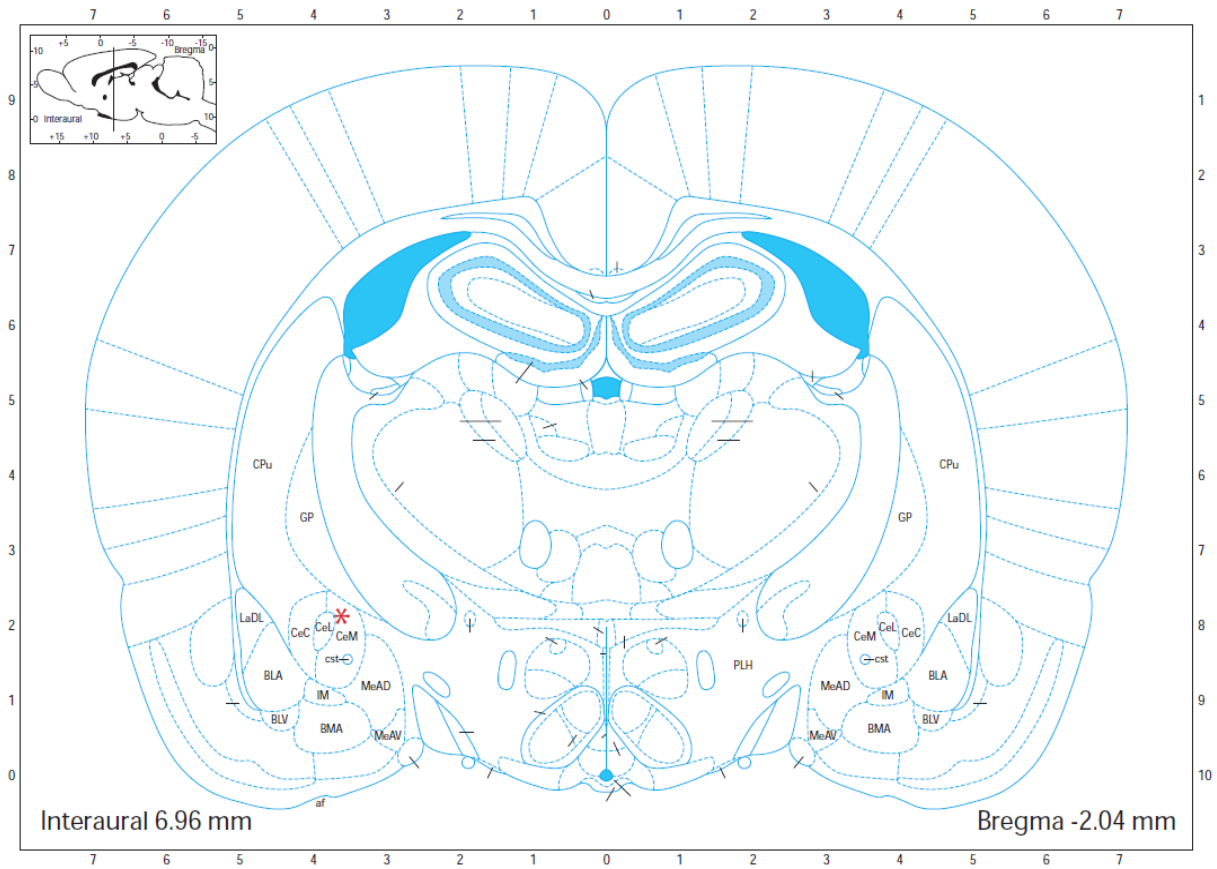


Figure 7. Histological reconstruction of bilateral electrode sites in rat 2 (AP -2.0 mm, ML 3.70 mm, DV 7.8 mm). The asterisk indicates the electrode was located in the central medial nucleus of the amygdala (CeM). Lesion damage from the right side was not seen.