Mapping a Novel Hedonic Hotspot in Insular Cortex

by

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Abstract

Insular cortex has been implicated in a wide array of functions, including integrating multiple sensory modalities, coding disgust for unpleasant foods, and processing reward and motivation. Specifically, anterior insula appears to be involved in mediating disgust while posterior insula may produce positive affect and code for palatable food stimuli. The current known regional functions within insular cortex support the prediction of a hedonic hotspot and coldspot in anatomically localized areas. To determine whether such pleasure/displeasure-generating zones actually exist in the region, anterior and posterior regions within insular cortex were selectively stimulated with DAMGO (mu-opioid agonist) or orexin-A, a peptide that is only produced in lateral hypothalamus. Posterior insular stimulation with either agonist amplified "liking" reactions to sucrose by three-fold, but failed to enhance intake of palatable M&M chocolate candies. In contrast, preliminary results from anterior insula stimulation with DAMGO or orexin shows suppressed "liking" of sucrose and no change in consumption of M&Ms. Taken together, these results support the discovery of a hedonic hotspot in posterior insula, and a potential hedonic coldspot in anterior insula. These results provide the first direct evidence for any cortical region amplifying or suppressing pleasure, and have important evolutionary implications on social and economic rewards in humans.

Keywords: Insula, Hedonic Hotspot, "Liking", Reward, Orexin, DAMGO, Taste Reactivity

Mapping a Hedonic Hotspot in Insular Cortex

Insular cortex has been studied broadly in humans, and is involved in a wide array of important cognitive functions. Patients suffering from major depressive disorder show insular activation patterns that deviate from the norm, indicating that the region may play a role in depression (Sprengelmeyer et al., 2011; Surguladze et al., 2010). Additionally, the insula has been implicated in cognitive rewards such as economic reward and gambling (Harle et al., 2012; Kringelbach & Berridge, 2009), the maintenance of abstinence after recovery from addiction (Clark et al., 2014), and even the phenomena of awareness and consciousness (Craig, 2009). Perhaps one of the most studied functions of insular cortex is its role in processing, coding, and combining food-related stimuli to produce the complex perception of flavor (Small, 2012). The primary gustatory cortex is situated between anterior insula and nearby opercular cortex, but nearly the entire length of the insula is activated in response to gustatory stimulation (Small, 2010; Verhagen, 2006). Various sections of the insula appear to be important for processing different tastes. Anterior insula is activated in response to sweet, unpleasant, and umami tastes, while only sweet stimuli are associated with posterior insula activation (de Araujo et al., 2003; Veldhuizen et al., 2010; Wicker et al., 2003). In addition to processing multiple types of taste, anterior insula appears to code the quality and strength of taste stimulation. For example, more intense concentrations of sweetness will produce higher amounts of insular activation than low concentrations (Stice et al., 2013; Veldhuizen et al., 2010).

In addition to its role in coding sensory taste, insula combines stimuli from multiple sensory modalities into a single holistic experience (Small, 2012). The gustatory and olfactory sensory modalities provide the insula with information about the taste and smell of a food through inputs to the region that are merged to create the experience of flavor. In addition, insula

also receives oro-somatosensory inputs, which provide information about the astringency (Cerf-Ducastel et al., 2001), temperature (Guest et al., 2007), and viscosity of food (de Araujo & Rolls, 2004). Insula discriminates between food and non-food cues, and will show activation in response to sweet food odors, like vanilla, but not in response to the sweet smell of a flower (Veldhuizen et al., 2010). Supra-additive activation is seen in the dorsal and ventral anterior insula when congruent olfactory and gustatory cues are presented (i.e. sweet taste and vanilla scent), but incongruent cues (i.e. salty taste and vanilla scent) produce no such effect (Small et al., 2004). The multiple overlapping food-related sensory inputs in insula, as well as the finding that insula only reacts strongly to cues representing a holistic food item indicate that the region plays a significant role in integrating food stimuli to produce the perceptive phenomenon of flavor (Small, 2012).

In addition to chemical senses, the insula has been implicated in producing and modulating affect, and in humans, it is also involved in processing the affective state of others. Much of the research in this domain has explored the basic human emotion of disgust, though some has focused on what appears to be disgust in non-human primates. Anterior insula is activated when processing unpleasant odors (Wicker et al., 2003), and intracranial microstimulation of the macaque anterior insula will cause a monkey to spit out and throw away a favorite food (Caruana et al., 2011). Together, these results indicate that activity in the region causes unpleasant sensations that may be interpreted as disgust. Rats with lesioned insular cortex do not show aversive reactions to tastes paired with unpleasant stimuli and are slower in learning to avoid those tastes than control rats, suggesting that the insula evolved to help individuals determine which foods may contain toxins (Kiefer & Orr, 1992). Additionally, anterior insula is activated when processing the disgusted expressions of other individuals who have smelled

unpleasant odors (wrinkled nose and upper lip), implying that viewing a disgusted facial expression instills a feeling of disgust in the viewer (Wicker et al., 2003). Interestingly, viewing expressions of disgust caused by disgusting foods (gaped mouth and tongue stuck out) are not processed by the viewer's insula, which seems maladaptive because it would be advantageous to be able to use others' expressions to avoid potentially toxic foods (von dem Hagen et al., 2009). One potential hypothesis to explain the discrepancy in the empathy of disgust is that the gaped mouth of distaste evolved as a functional motion meant to remove the food from the mouth, whereas the wrinkled nose of olfactory disgust evolved as a signal meant to be processed by others (von dem Hagen et al., 2009). This suggests that the human insula, as a cortical structure, has evolved the additional function of processing social cues pertaining to disgust (von dem Hagen et al., 2009). Although the role of the human insula in mediating social disgust is still being explored, it clearly plays an important role in generating and mediating disgust-related behavior.

Studies have also implicated insular cortex in positive affect. The aforementioned study using macaque monkeys also found that intracranial microstimulation of caudal and ventral insula will induce socially affiliative behaviors in monkeys (Caruana et al., 2011). At the onset of stimulation, monkeys will cease aggressive or submissive behaviors, and will make eye contact or approach the experimenter (Caruana et al., 2011). Additionally, high-sugar beverages elicit greater activation of bilateral insula compared to low-sugar beverages (Stice et al., 2013; Veldhuizen et al., 2010), and are also rated to be more pleasant to drink by study participants (Stice et al., 2013). Sugar is known to be a rewarding substance, so this indicates that insular cortex may be involved with reward processing (Stice et al., 2013). A caveat to this finding is that foods higher in fat, which are also known to have rewarding properties, were not associated

with higher insular activation compared to lower-fat foods (Stice et al., 2013). However, there is more evidence for the insula as a reward-related cortical area. Insular cortex has inputs to ventral striatum, which has been associated with hedonia and motivation for reward (DiFeliceantonio et al., 2012; Fudge et al., 2005; Richard et al., 2013). Kringelbach and Berridge (2009) suggest that while subcortical regions such as nucleus accumbens (NAc) and ventral pallidum (VP) increase or decrease the hedonic value of rewards, several cortical regions including insula may be involved in higher-level rewards processing, such as remembering, anticipating, evaluating, and experiencing reward.

The Insula in the Context of Reward and Motivation

In the reward circuits of the brain, there are related, but distinct processes of "liking" and "wanting". "Liking" is an operationalized term to describe core pleasure caused by a rewarding stimulus that is characterized by stereotyped behaviors and specific neural patterns, and can be amplified by neurochemical stimulation (Berridge et al., 2010; Pecina & Berridge, 2005; Steiner et al., 2001). In non-human animals, "liking" is studied in an experimental procedure known as taste reactivity, in which a pleasant taste such as sucrose, or an unpleasant bitter taste such as quinine, is delivered to the subject's mouth and orofacial reactions are recorded. Similar profiles of hedonic and aversive behavioral reactions have been found in humans, non-human primates, horses and rodents, indicating that "liking" is an ancestral trait shared by much of the mammalian class and that non-human mammals are a suitable model for understanding "liking" in humans (Jankunis & Whishaw, 2013; Steiner et al., 2001). In contrast, "wanting" is the motivation to obtain reward that takes the form of appetitive reward-seeking behavior, and can be triggered by reward-related cues even in the absence of a rewarding stimulus or "liking" (Mahler & Berridge, 2009; Robinson & Berridge, 1991). "Wanting" that has been projected onto

a reward-related cue from a rewarding stimulus is also known as incentive salience (Berridge et al., 2010; Robinson & Berridge, 1991).

"Liking" is produced by neurochemical signaling in small, localized brain regions known as hedonic hotspots, and neurochemical stimulation of these hotspots can increase the "liking" of reward (Berridge et al., 2010; Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005; Söderpalm & Berridge, 2000). The known hedonic hotspots are localized regions within the NAc, VP, and the parabrachial nucleus of the pons, where microinjections of mu-opioid agonist, GABA-A agonists, endocannabinoids, or orexin-A amplify "liking" of sucrose (Castro & Berridge, 2014; Ho & Berridge, 2013; Mahler & Berridge, 2007; Pecina & Berridge, 2005; Smith & Berridge, 2005; Söderpalm & Berridge, 2000). Stimulation in these regions can also increase "wanting", which is seen by dramatic increases in consumption of highly palatable foods. However, it is important to note that while hedonic and motivational hotspots overlap, areas capable of generating motivation are spread more broadly across a given brain region than areas that produce hedonia (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005). "Wanting" can often be induced in brain areas that cannot amplify "liking", providing an anatomical dissociation between the two neural processes (DiFeliceantonio et al., 2012; Pecina & Berridge, 2005; Smith & Berridge, 2005; Zhang & Kelley, 2000). In addition to hedonic hotspots, the NAc and VP contain hedonic coldspots that are anatomically distinct from hotspots (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005). When stimulated with the aforementioned neurochemicals, coldspots dampen the "liking" response to sucrose (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005), thereby making a pleasant taste less pleasant.

The anterior insula, given its known functions in coding unpleasant food-related stimuli and inducing disgust for pleasurable foods, may contain a hedonic coldspot. Because previously studied coldspots are located nearby hedonic hotspots, mid- or posterior insula may contain a hedonic hotspot. However, while multiple studies have implicated the insula in reward processing, most human studies are correlational fMRI experiments, and therefore fail to establish a causal link between insular cortex and reward. Stice et al. (2013) and Veldhuizen et al. (2010) showed that beverages with higher sugar content increase insular activation, but they do not indicate this activation causes the enhanced subjective pleasure derived from increased sugar. Moreover, Calder et al. (2007) showed that the hedonic coldspot in ventral pallidum and anterior insula have similar activation patterns to disgust cues, which suggests there might be a coldspot in insula, but the two regions might code independently. Caruana et al. (2011) conducted a caudal experiment studying the role of insula in generating positive or negative affect, but their findings are weakened by a sample size of two macaque monkeys. Thus, a comprehensive causal experiment is required to determine the role of insular cortex in amplifying and dampening hedonic impact. Using the previously mentioned taste reactivity paradigm with rats, I test the predictions that posterior insula contains a hedonic hotspot and anterior insula contains a hedonic coldspot.

Similar to previous experiments identifying hedonic hot and coldspots, I use targeted microinjections to stimulate discrete regions within insular cortex. DAMGO is used because mu-opioid receptors play a role in mediating reward and amplifying hedonic impact in many brain regions (Contarino et al., 2002; Duvauchelle et al., 1996; Kelley et al., 2002; Pecina & Berridge, 2005; Smith & Berridge, 2005). Orexin, an endogenous neuropeptide produced only in lateral hypothalamus (LH) (Sakurai et al, 1998), is also used because of its function in mediating

hunger, which is relevant to insula because of its role in processing food cues (Cai et al., 1999; Valdivia et al., 2014). Additionally, Ho & Berridge (2013) have recently shown orexin to increase hedonic impact in the ventral pallidum hotspot, so it may stimulate other hedonic hotspots, including the one predicted in insula. Microinjections of DAMGO or orexin are expected to increase "liking" of sucrose in caudal insula and decrease "liking" of sucrose in rostral insula. One should note that the present literature on insular function illustrates it as a food-cue coding region (Small, 2012; Veldhuizen et al., 2007), so it is possible that insula simply codes hedonic impact and may not contain a hot or coldspot. Previous studies have shown that motivated "wanting" to eat is more broadly dispersed compared to the localized hotspots (DiFeliceantonio et al., 2012; Pecina & Berridge, 2005; Smith & Berridge, 2005; Zhang & Kelley, 2000). Therefore, an additional prediction is that DAMGO or orexin microinjections will stimulate increased eating throughout insular cortex, across the hypothetical localized hot and coldspot (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005). This prediction will be tested in a food-intake paradigm in which rats are given access to palatable M&M candies following drug stimulation.

Method

Subjects

Male and female Sprague Dawley rats (male $n = 5$; female $n = 7$) were pair-housed at 21°C on a 12-hour light/dark cycle. Rats had *ad libitum* access to water and chow. Male rats weighed 300-600g and females weighed 250-400g. All experimental procedures were approved by the University Committee on the Use and Care of Animals of Michigan.

Surgery

After habituation to human contact, rats were anaesthetized with ketamine hydrochloride (80mg/kg, i.p.), xylazine (5mg/kg, i.p.), and were also given atropine (0.05mg/kg, i.p.). Bilateral oral cannulae [polyethylene (PE)-100] were implanted to enable oral infusions of sucrose and quinine solutions in taste reactivity testing. Oral cannulae were inserted into the cheek tissue lateral to the first maxillary molar, ascended beneath the zygomatic arch, and exited the skin above the dorsal head cap.

Rats were then placed in a stereotaxic apparatus (David Knof Instruments) with the incisor bar placed at 3.3mm below intra-aural zero to position the skull at a 0° horizontal angle for the surgical implantation of microinjection guide cannulae (12.5mm, 23 gauge, stainless steel) for drug infusions. Guide cannulae were aimed at locations throughout the rostrocaudal gradient of the insula (AP +2.7 to -2.5mm; ML ±4.0mm), resulting in 2 rats with rostral placements and 10 rats with caudal placements (Figure 1). Rostral targets were located between -3.0 and -3.6mm DV, while caudal targets were located between -4.4 and -4.6mm DV. Additionally, because of the extreme lateral position of insular cortex, placing cannulae at a vertical angle would have required such a lateral implantation site that the temporalis chewing muscle would be damaged significantly, increasing the recovery time and likelihood of infection. Thus, intracranial cannulae were implanted in an angled fashion at a more medial site for caudal targets (between 14° and 19°) to avoid such complications. The angle of caudal placements increased in progressively posterior locations because insular cortex is more laterally situated in caudal locations. Cannulae were attached to the cranium using dental cement and surgical screws. Stainless steel stylets (28 gauge) were inserted into cannulae to prevent obstruction, but were removed for microinjection delivery during behavioral testing. Rats were given subcutaneous injections of carprofen (5mg/kg) for analgesia and cefazolin (60mg/kg) as an

antibiotic immediately after surgery, and were given carprofen again after 24 hours. There was a one-week surgery recovery period before habituation or behavioral testing began.

Drugs and Insula Microinjections

Drug solutions (DAMGO and orexin-A) were prepared by dissolving drugs in an artificial cerebrospinal fluid (ACSF) vehicle and were kept in frozen storage. Prior to microinjection, DAMGO, orexin-A, and ACSF were brought to room temperature (~21°C). To deliver microinjections to insular cortex, stylets were removed from guide cannulae and 14.5mm stainless steel microinjection cannulae (29 gauge) connected to PE-20 polyethylene tubing were inserted into guide cannulae. Microinjections of 0.2μl were delivered over a period of 1 minute by a syringe pump at a rate of 0.2μl/min. Microinjectors were left in place for an additional 1 minute to allow the drug solution to fully diffuse, after which stylets were replaced in the guide cannulae and rats were placed in the testing chamber. To habituate rats to the microinjection process, rats were microinjected with ACSF, a non-psychoactive compound, two days prior to the first test day. The solutions microinjected on test days contained one of the following: DAMGO, a mu-opioid agonist (dose of 0.05μg/0.2μl); orexin-A (dose of 500pmol/0.2μl); or artificial cerebrospinal fluid, used as a baseline control for the drug conditions. Doses were determined from previous experiments using localized microinjections of these drugs (Smith et al., 2011; Thorpe & Kotz, 2005). Rats received one bilateral microinjection per test day, and the order of drug or vehicle infusions were counterbalanced across rats, eventuating in all rats being tested under all drug conditions. Testing occurred every other day for total of three test days.

Taste Reactivity Testing

Taste reactivity testing (Grill & Norgren, 1978; Steiner et al., 2001) was used to measure affective orofacial responses to sucrose or quinine solutions in rats. Testing occurred when the

pharmacological effects of the microinjected drugs peaked, which was 25 minutes for DAMGO and orexin-A (Pecina & Berridge, 2005; Thorpe & Kotz, 2005). Rats received 1-minute 1ml intraoral infusions of sucrose solution (1.0%; 0.029 M) or quinine solution ($3x10^{-3}$ M) via surgically implanted oral cannulae. Solutions were delivered by a syringe in a syringe pump attached to hollow tubing (PE-50 connected to PE-10 delivery nozzle). Each rat received sucrose first, followed by quinine, with a 1-minute break between infusions to allow the effects of sucrose to dissipate. Orofacial taste reactivity responses were video recorded via close-up lens and analyzed in slow motion.

Taste Reactivity Video Scoring

Video recordings of taste reactivity sessions were imported to Noldus Observer® and hedonic, aversive, and neutral taste reactivity patterns in response to sucrose or quinine were scored in slow motion ($1/5^{th}$ to ½ actual speed). Hedonic reactions consisted of rhythmic midline tongue protrusions, lateral tongue protrusions, and paw licking. Aversive responses were classified as head shakes, mouth gapes, face washes, forelimb flails, and chin rubs. Yawns, passively allowing solution to drip from the mouth, grooming, and rhythmic mouth movements were considered neutral responses. A time-bin scoring procedure was used to equalize representation of hedonic and aversive responses that occur at different frequencies in the final affective response totals. For example, lateral tongue protrusions, which occur relatively infrequently as discrete instances, were scored as-is, whereas midline tongue protrusions, which occur in bouts, were scored in 2-second time bins (e.g. two seconds of tongue protrusions was considered one bout) to reduce their overrepresentation in the final hedonic response total. Chin rubs, which occur in similarly timed bouts, were also scored in 2-second time bins. Rhythmic mouth movements, passive dripping, face washing, and paw licking occur in longer bouts and

were thus scored in 5-second time bins. Lateral tongue protrusions, gapes, yawns, head shakes, and forelimb flails occur as discrete events, and were scored as single occurrences. Individual totals of hedonic and aversive responses to sucrose or quinine were calculated for each trial. Hedonic response totals were the sum of midline tongue protrusion and paw licking bouts, and lateral tongue protrusion occurrences. Aversive response totals were the sum of occurrences of gapes, head shakes, and forelimb flails, and bouts of face washing and chin rubs.

Food Intake Testing

Prior to the first microinjection day, rats were habituated for three 1-hour sessions in food intake chambers (23 x 20 x 45cm) equipped with a water bottle, 1cm of corncob bedding, and ~20g of M&M candies that were available *ad libitum* throughout habituation. Voluntary consumption of palatable food and spontaneous eating behavior were observed and measured during 1-hour sessions immediately following taste reactivity testing on each test day. Approximately 20g of M&M chocolate candies were provided each test day and were weighed after testing to determine the amount eaten. All behavior was also recorded and later scored for time spent eating, drinking, and grooming, and for number of bouts of food sniffs, food carrying (grasping and transporting food 2 or more steps), cage crosses, and rearing.

Histological Analysis

Following behavioral testing, rats were euthanized with 0.9ml sodium pentobarbital injections (i.p.) and brains were extracted and stored in 10% paraformaldehyde for 1-2 days and in 25% sucrose buffer solution (0.1M NaPB) for three days. Brains were then frozen and sliced on a freezing microtome or cryostat at 40 or 60μm, mounted, and stained with cresyl violet. Microinjection sites were then plotted on coronal slices from a rat brain atlas (Paxinos & Watson, 2007) to determine whether drug microinfusions reached insula.

Statistical Analysis

Hedonic, aversive, and neutral reaction totals were analyzed for within-subject differences between drug conditions using a repeated measures ANOVA. Food intake testing was also analyzed within-subject with a repeated measures ANOVA. Comparisons between male and female behaviors were included as a between subjects factor, as was microinjection placement (rostral versus caudal). All statistical analyses were performed with Sidak corrections.

Results

Overall Results

Overall, neither DAMGO nor orexin stimulation had effects on taste reactivity responses to sucrose or quinine ($F(8, 32) = 0.671, p = .71$). However, there was a potential indication of a placement by drug interaction ($F(8, 32) = 1.559, p = .18$), indicating that drugs may have had differing effects on hedonic or aversive behaviors depending on the location of the microinjection (though this effect may appear smaller than it actually is due to a small sample size in rostral insula). Support for this hypothesis is shown with a significant interaction of placement by drug for specifically hedonic reactions to sucrose ($F(8, 32) = 7.968, p = .003$). There was no significant interaction between drug and sex, indicating no difference in drug effect between males and females ($F(8, 32) = 0.589, p = .78$). Therefore the remainder of the analyses will pool males and females together.

Evidence for a Hedonic Hotspot in Insular Cortex

Although DAMGO or orexin microinjections in caudal insula did not change overall taste reactivity responses to sucrose or quinine ($F(2, 18) = 1.774, p = .12$), a more thorough analysis revealed that DAMGO or orexin stimulation caused a three-fold amplification of hedonic reactions to sucrose compared to vehicle baseline (Overall drug effect: $F(2, 18) = 10.216, p =$

.001; DAMGO: $p = .001$; orexin: $p = .01$; Figures 2a & 3a). In contrast, these same drug stimulations had no effect on aversive reactions to sucrose ($F(2, 18) = 0.071, p = 0.93$; Figures 2b & 3b), hedonic reactions to quinine ($F(2, 18) = 0.00, p = 1.0$; Figures 2a & 3a), or aversive reactions to quinine ($F(2, 18) = 0.466, p = .64$; Figures 2b & 3b). Similarly, neither DAMGO nor orexin stimulation altered food intake of M&M candies ($F(2, 18) = 0.63, p = 0.54$; Figure 4). Altogether, these results show that mu-opioid or orexin receptor stimulation within the caudal portion of insular cortex selectively enhances the hedonic impact of a palatable taste, indicating the presence of a novel cortical hedonic hotspot.

A Potential Hedonic Coldspot in Rostral Insular Cortex

DAMGO or orexin stimulation in rostral insula produced no overall effects on taste reactivity reactions to sucrose or quinine ($F(4,4) = 0.984, p = .51$; Figures 2 and 3). A more thorough analysis indicated that DAMGO or orexin stimulation had no effects on hedonic responses to sucrose ($F(2,2) = 1.82, p = .36$), aversive reactions to sucrose ($F(2,2) = 5.44, p = .16$), hedonic reactions to quinine ($F(2,2) = 0.0, p = 1.0$), or aversive reactions to quinine ($F(2,2) = 0.794, p = 0.56$). Additionally, DAMGO or orexin stimulation did not change intake of M&M candies ($F(2,2) = 0.961, p = .51$; Figure 4). It is important to note that the statistical insignificance of these results does not necessarily reflect a lack of effect of drug stimulation in rostral insula, as the sample size in this region was two animals. In particular, both drug conditions appeared to suppress sucrose “liking” at rostral sites to just 50% of vehicle (Figures 2a & 3a). This suppressive effect is very similar to hedonic coldspots found to be anatomically near, but distinct from hedonic hotspots in NAc and VP and it is possible that with additional subjects, my analyses may reach statistical significance.

Discussion

The three-fold amplification of sucrose "liking" shown by DAMGO or orexin stimulation of caudal insula supports the original hypothesis that the region mediates hedonic impact in rats. This function of insular cortex appears to be consistent between males and females, as evidenced by the lack of difference in hedonic patterns shown in male and female rats during caudal insula stimulation. These data provide the first direct evidence of a hedonic hotspot in the neocortex. The original prediction of a hedonic coldspot in rostral insula was not supported, though these results may be confounded by the small sample size of two subjects tested in this region. However, preliminary results showing an average suppression of sucrose "liking" by 50% when stimulated by DAMGO or orexin warrant a continued investigation of rostral insula for a potential hedonic coldspot.

The discovery of a hedonic hotspot in insular cortex is corroborated by previous studies that have shown DAMGO stimulation of NAc and VP to selectively increase sucrose "liking" (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005), and orexin stimulation of VP to enhance hedonic reactions to sucrose in VP (Ho & Berridge, 2013). Within the NAc and VP hotspots, certain subregions appear to increase hedonic impact more than the surrounding hotspot tissue (Ho & Berridge, 2013; Pecina & Berridge, 2005; Smith & Berridge, 2005), and it is possible that a similar pattern of localization exists in caudal insula. To explore the boundaries of the hedonic hotspot, future testing should investigate the effect of stimulation throughout the rostrocaudal extent of insular cortex. Additionally, the use of the fos-plume technique to quantify the spread of a drug and its ability to activate cells within a region (Ho & Berridge, 2013; Pecina & Berridge, 2005; Smith & Berridge, 2005) will further illuminate the anatomy of the insula hotspot.

There are other potential interpretations of the data presented here; for example, drug stimulation of insular cortex may produce a psychomotor or sensory effect. A psychomotor interpretation of these results would argue that the enhanced orofacial responses to intraoral sucrose were caused by a motor effect mediated by mu-opioid or orexin receptor stimulation. This conclusion is supported by studies showing that mu-opioid receptors are important in locomotor activity and sensitization (Contarino et al., 2002; Smith et al., 2009), and orexin is associated with wakefulness and general motor activity (Anaclet et al., 2009). However, if these neurotransmitters produce general motor effects in caudal insula, the current experiment would have shown that mu-opioid and orexin stimulation would more broadly increase both hedonic and aversive orofacial responses to not just sucrose, but quinine as well. However our results show a selective amplification of sucrose "liking," suggesting that these neurochemical stimulations were not broadly affecting motor behaviors.

Previous studies of insular cortex implicate it in food quality coding, so another possible interpretation of these results is that the amplification of sucrose "liking" reactions was not due to increased hedonic impact, but rather, altering the sensory perception of the sweet taste to effectively make it "sweeter." The results of the present experiment cannot falsify this alternative hypothesis, but future testing using conditioned taste aversion (CTA) and insula stimulation can determine whether the region is a hedonic hotspot or a sensory coding region. The CTA paradigm can turn a pleasant taste into an unpleasant taste by pairing it with nauseating lithium chloride injections (Sewards, 2004). By using CTA to make sucrose an aversive taste for rats, caudal insula stimulation and taste reactivity testing can clarify the function of the region. If caudal insula is a sensory region, stimulation may enhance aversive reactions to sucrose via potentiation of its sensory intensity, or increase hedonic reactions to sucrose by changing the

taste to something that has not been paired with LiCl injections, thereby altering perception of its sensory properties. However, if there is a hotspot in insula, drug stimulation may mitigate the disgusting taste of sucrose, signifying a shift towards "liking", or will have no effect on conditioned sucrose disgust as mu-opioid or orexin stimulation had no effect on the similarly disgusting quinine.

These data also raise the question of why, if caudal insula contains a hotspot, DAMGO or orexin stimulation did not enhance intake of palatable M&M candies. While it is true that drug stimulation can amplify food intake in other hotspots, these results vary between hedonic regions, suggesting that the food intake test may not be an accurate indicator of hedonic impact. In NAc shell, DAMGO stimulation enhances eating in the anterior hotspot and posterior coldspot indiscriminately (Castro & Berridge, 2014; Pecina & Berridge, 2005), but DAMGO in VP only enhances eating in the hotspot (Smith & Berridge, 2005). Additionally, $GABA_A$ receptor blockage in VP produces eating in both the hotspot and the coldspot without amplifying pleasurable reactions to sucrose (Smith & Berridge, 2005) Moreover, orexin stimulation of the VP hotspot changed "liking" of sucrose (Ho & Berridge, 2013) without inducing food intake (Berridge, unpublished data). Castro & Berridge (2014) recently showed that mu-, kappa-, and delta-opioid receptor stimulation in NAc showed similar profiles of hedonic enhancement and suppression in the previously shown hotspot and coldspot, but stimulation of the three opioid receptors produced eating effects that were dissimilar from one another. These results, taken together, show that at the very least, the food intake test is not a reliable indicator of pleasure.

Instead, food intake may be indicative of motivation to obtain a stimulus. Previous studies have uncovered a vast mesolimbic circuit of brain structures involved in motivation and reward, in which small hotspots (cubic millimeter in the rat brain) such as VP and NAc produce

core pleasure and modulate hedonia, while a broad array of other regions code hedonic impact, learn to anticipate it, and produce appetitive "wanting" for reward or reward-related cues (reviewed in Berridge et al., 2010). "Wanting" without "liking" has been demonstrated in a number of regions, including dorsal neostriatum (DiFeliceantonio et al., 2012), central amygdala (Galaverna et al., 1993; Mahler & Berridge, 2009), and LH (Berridge & Valenstein, 1991). Even within hedonic areas such as NAc and VP, previously mentioned studies showed that drug stimulation in certain areas enhanced eating without enhancing pleasure (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005). Overall, these studies suggest that food intake shows motivation to obtain reward rather than the hedonic experience of the reward, and that areas that generate "wanting" are more broadly distributed than areas that generate "liking". However, though "liking" and "wanting" are dissociable from one another, these related neural processes show substantial interconnection—when one brain region produces core pleasure in response to a pleasant stimulus, other regions identify the stimulus as desirable and generate appetitive behaviors to obtain the stimulus in pursuit of pleasure (Berridge et al., 2010). The discovery of a hedonic hotspot in insular cortex, along with the finding that it does not produce motivation for food raises the question of how insular cortex functions within the greater mesolimbocortical reward circuits.

The Role of Insular Cortex Within the Reward and Motivation Circuits

Within the motivation and reward circuits, studies have uncovered substantial functional connections between various subregions. The hotspots in VP and NAc are functionally connected, and activation of one will recruit the other, as well as LH (Smith & Berridge, 2007; Smith et al., 2011). Anterior insular cortex projects to LH (Floyd et al., 2001), and Calder et al. (2007) showed increased BOLD signals to disgusting cues in anterior insula and ventral

pallidum, which could point to a functional connection between the two areas. Insular cortex is also neuroantomically connected to other subcortical regions such as amygdala (Flynn et al., 1999) and dorsal striatum (Fudge et al., 2005), but the functionality of these connections in the context of motivation and reward has not been investigated. An experimental paradigm employed by Smith & Berridge (2007), in which one hotspot is stimulated and another is examined for c-fos protein activation, could be used to explore the function of this hotspot within mesolimbocortical reward pathways. One might predict that activation of caudal insula would recruit other hedonic hotspots and vice versa, as is the case with NAc and VP. Further neuroanatomical studies of insula and its connected regions, along with selective stimulation of these neural pathways may also reveal communication between the caudal insula and mesolimbic regions. Additionally, there is a potential functional connection between the insula hotspot and LH, given that orexin production is localized to LH (Sakurai et al., 1998) and orexin receptor stimulation in insula modulates hedonic impact. Orexin production is triggered by low blood glucose levels (Sakurai et al., 1999), and appears to be involved with mediating ingestive behaviors, motivation for food, or sensations of hunger (Thorpe & Kotz, 2005; Valdivia et al., 2014). Thus, "liking" of gustatory stimuli produced by orexin receptor stimulation in hedonic hotspots may allow other mesolimbic regions to generate motivated behaviors to pursue food reward and food-related cues.

Evolutionary Implications of a Hedonic Hotspot in the Neocortex

Hedonic hotspots confer obvious selective advantages upon individuals. Natural selection, favoring traits that lead to an individual's survival and reproduction, would undoubtedly favor an intrinsic mechanism that gives food and sex powerfully rewarding qualities. Hedonic hotspots are finely tuned by evolution to help individuals achieve important

life history goals of energy consumption for somatic growth (Pecina & Berridge, 2005; Smith & Berridge, 2005), and reproductive success (Robbins & Everitt, 1996; Smith et al., 2011). It is unlikely to be a coincidence that out of all food reward, hedonic hotspots are most activated by sugars, given that sugar is the body's macronutrient of choice for energy production (Small, 2010). However, hedonic hotspots evolved at a time when calories were harder to come by than they are today, and perhaps they are no longer as selectively advantageous in this age of superabundant processed carbohydrates. Obesity and type 2 diabetes rates are spreading rapidly in children and these diseases may have deleterious effects on health in adulthood or even before children reach puberty (Pender & Pories, 2005). Though this hypothesis has not been tested, if children that are more susceptible to refined sugars and powerful marketing are less reproductively viable due to significant health issues in early life, natural selection may begin to favor less active hotspots or more active coldspots to mitigate consumption of unhealthy foods.

The subcortical structures mediating reward are found in such disparate taxa, including reptiles, birds, and mammals, that they are likely to have emerged early in the evolutionary history of vertebrates (O'Connell & Hofmann, 2011). Over the ages, the forces of evolution added more layers and regions to the brains of some species, creating more complex structures and behavior, all the while preserving the basal reward structures and their functions (O'Connell & Hofmann, 2011; Reiner, 2000). The origins of the neocortex are unclear, and discussions are hampered by a debate between those who believe reptilian and avian brains possess neocortical homologues and those who believe it evolved *de novo* within the mammalian order (Reiner, 2000). Regardless, researchers in both schools of thought agree that the astounding complexity of the neocortex evolved more recently than the midbrain, hindbrain, and other subcortical structures. This fact produces a juxtaposition in which a recently evolved brain structure

performs an archaic function, and raises the fundamental question of why the caudal insula houses a hedonic hotspot.

There are multiple ways to answer this question. Following evidence that the neocortex is existent in distantly related taxa, the hotspot within insula may have been part of an evolutionary suite alongside the NAc, VP, and parabrachial nucleus hotspots. Alternatively, if the neocortex appeared *de novo* within mammals, then the insula hotspot may have evolved to serve uniquely mammalian purposes. It is possible to combine both explanations to form the hypothesis that the insula hotspot evolved around the time of the other subcortical hotspot and, being anatomically linked to other cortical regions, was catapulted to complexity alongside the structures that became the mammalian neocortex. Under this hypothesis, the hedonic function of the insula evolved early on, but the evolution of complexity allowed it to take on additional roles, making it distinct from other hotspots. These hypotheses might be testable by further parsing the unique functions of insular cortex, but such studies would explain nothing with great confidence other than the *current* functions of the insula, and evolutionary conclusions drawn from them should be taken with a grain of salt. Instead, these hypotheses may be tested by stimulating insular cortex in other taxa and conducting taste reactivity testing can reveal whether the region produces hedonic impact in non-mammal species. Further experimentation is needed to uncover the origins of hedonia in the insula, but regardless of which hypothesis is correct, the very presence of a hotspot in the neocortex most likely impacted mammalian evolution. Therefore, the evolutionary implications of a hotspot in insula may be better understood by asking how the presence of a cortical hedonic hotspot has affected mammalian evolution rather than why it exists.

The reasons behind the recent evolution of the entire neocortex within mammals have themselves been the subject of mystery and speculation. Dunbar (1992) identified group living and complex sociality as one of the most reliable predictors of large brain size in monkeys and apes in the Social Brain Hypothesis (SBH). Across these taxa, species that live in more complex social groups have a larger neocortex relative to body size, and larger neocortex relative to hindbrain, compared to the average ratios found across monkeys and apes (Dunbar, 1992). The SBH explains these trends by positing that some species, under the pressure of predation, began living in groups to better protect against predators (Dunbar & Schultz, 2007). Alternatively, the cognitive demands of pairbonding may have been a selective pressure for increased brain volume in other mammal taxa, including carnivores, artiodactyles, and bats (Dunbar & Schultz, 2007). In both possible scenarios, the increase in social complexity subsequently acted as a selection pressure for enhanced cortical neuron density. For the social brain, brains needed to cope with the dramatic increase in interactions with new individuals (Dunbar, 1992), while pairbonded animals needed form strong bonds with a mate and provision for offspring (Dunbar & Schultz, 2007). These larger and more complex brains may have enabled the individuals carrying them to engage in more complex social behavior, including the ability to form alliances and engage in cooperation through reciprocity (Trivers, 1971). The presence of a hedonic hotspot in the cortex may have greased the wheels for this surge in cooperative social systems.

Humans, a species with astonishing social complexity, are extremely motivated by social interactions. People become happier when they feel liked by their peers, and experience sadness when they believe they are disliked (Davey et al., 2010; Hsu et al., 2013). Reward derived from social interactions is likely to have a selective advantage, as individuals driven to participate in a social group may better be able to find a mate and can enjoy the benefits of reciprocal social

interactions, compared to those who are not motivated to interact with others. Therefore, as neocortex increased in size to keep up with ever-growing social groups, the insula may have acted as a mechanism to increase social complexity by making social interactions "liked" and "wanted". This hypothesis is supported by a study by Caruana et al. (2011) that showed that stimulating the caudal insula of macaques, a group-living primate, was able to produce affiliative behaviors and social affinity. Curiously, fMRI studies of the human brain have shown rostral insula activation, but not caudal insula activation, to be associated with social reward (Bereczkei et al., 2013; Davey et al., 2010; Hsu et al., 2013). However, fMRI studies only show what brain regions are activated during a certain task, and rarely indicate whether a particular region is necessary or sufficient to produce a given behavior. To further understand the role insular cortex may have played in mammalian social evolution, causal tests are imperative. Mu-opioid receptors are found to be involved with social reward (Hsu et al., 2013), so a preliminary hypothesis is that DAMGO stimulation of rostral or caudal insula can modulate playful social interactions between rat conspecifics. Additionally, because drug microinjections degrade the targeted brain tissue with multiple test days, the use of optogenetic methods to stimulate rostral or caudal insular cortex can allow for more extensive testing within subjects. This future direction is particularly exciting because it can add depth to discussions on why humans and non-human primates have evolved to live in large social groups, and ultimately lead to a more intimate understanding of the nature of human sociality and social cognition.

Conclusion

In this experiment, I sought to determine the role of insular cortex in modulating hedonia and anhedonia for gustatory stimuli, and to test if the region is involved in motivated eating behaviors. The results show that mu-opioid and orexin receptors are involved in pleasure

amplification in caudal insula, and preliminary results indicate that these same receptors may also suppress hedonic impact in rostral insula. These results support the conclusion that caudal insula contains a hedonic hotspot and that rostral insula may contain a hedonic coldspot. Though insular cortex does not appear to code or modulate "wanting" of food, the discovery of a hotspot in the neocortex suggests that the region may be involved in social reward or motivation. Future research should seek to elucidate the role of insular cortex within the greater mesolimbocortical hedonic and motivational circuits, and determine the impact of the region on the evolution of sociality or vice versa.

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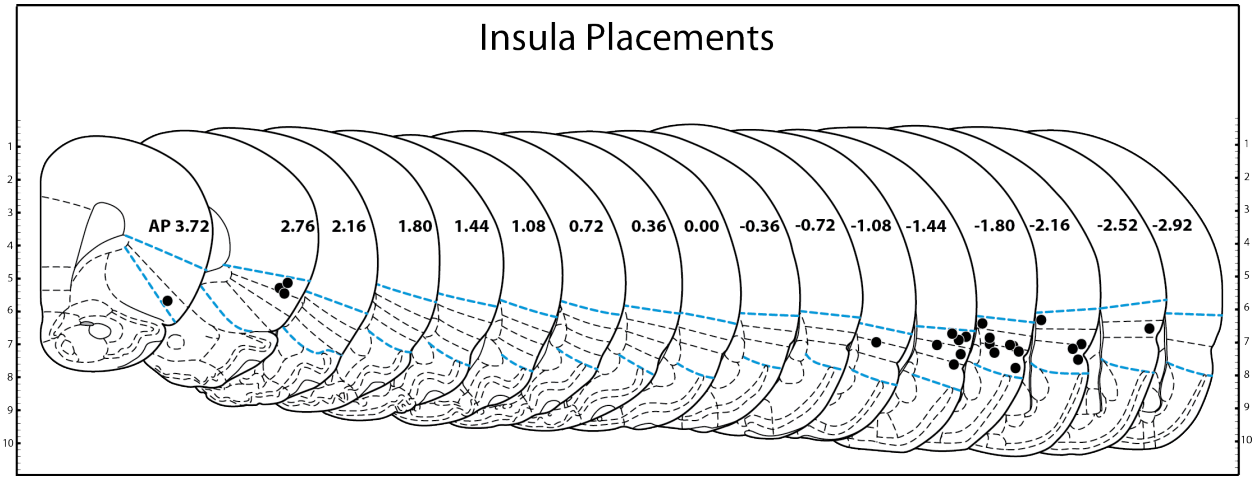

Figure 1 – **Coronal map of microinjection sites in rostral and caudal insula.** Bilateral microinjection sites were plotted on this unilateral coronal map, compiled from Paxinos and Watson (2007). Due to the extensive rostrocaudal spread of insular cortex, one in every three brain plates are included here. Slices are represented on a constant dorsoventral plane to maintain a consistent view of insular cortex, and each brain slice is labeled by its AP distance from bregma.

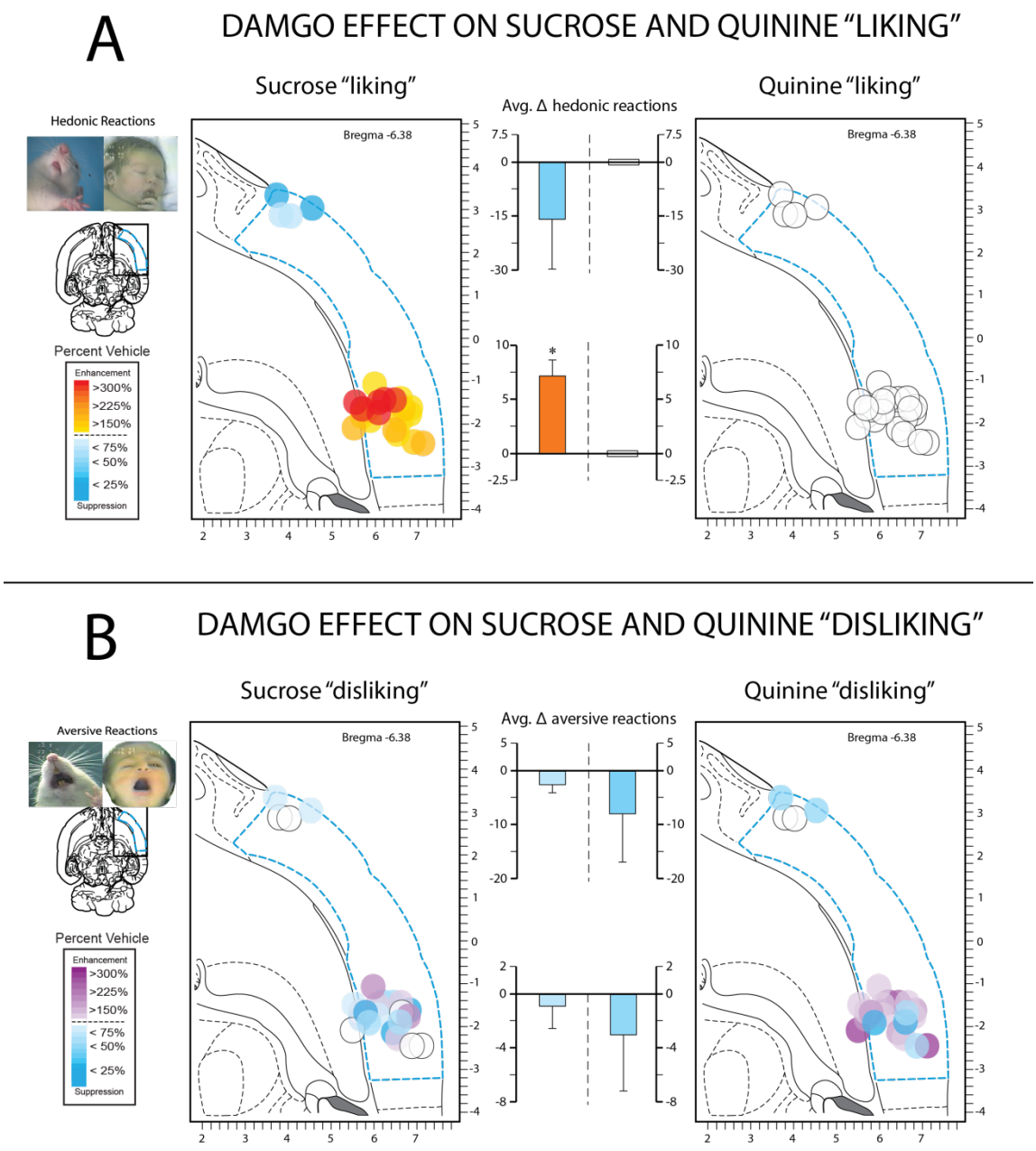

Figure 2 – **DAMGO effects on sucrose or quinine "liking" and "disliking"**. *A*. Horizontal maps of insular cortex show DAMGO microinjection sites in rostral or caudal insula, color-coded according to enhancement or suppression of hedonic reactions, with individual circles representing distinct microinjection sites. The yellow-red gradient shows "liking" enhancement, while the white-blue gradient indicates "liking" suppression. Central bar graphs show average change in hedonic reactions during DAMGO stimulation, compared to vehicle. *B*. Horizontal

maps of insular cortex show DAMGO microinjection sites in rostral or caudal insula, color-coded according to enhancement or suppression of aversive reactions. The white-purple gradient denotes increases in “disliking”, while the white-blue gradient indicates suppression of “disliking”. Central bar graphs show average change in aversive reactions caused by DAMGO in rostral and caudal insula, compared to vehicle.

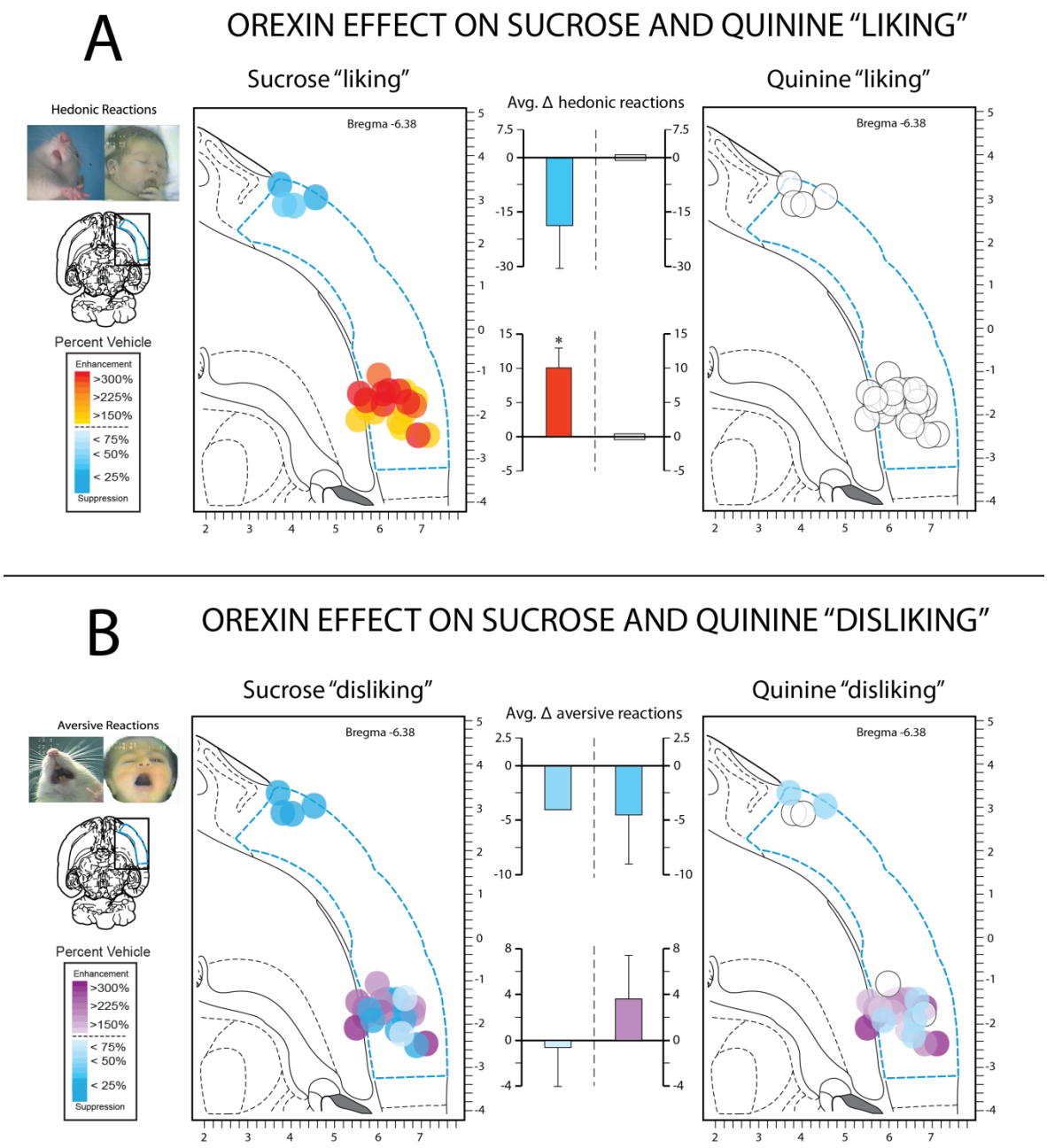

Figure 3 – **Orexin effects on sucrose or quinine "liking" and "disliking"**. *A*. Horizontal maps of insular cortex show orexin microinjection sites in rostral or caudal insula, color-coded according to enhancement or suppression of hedonic reactions, with individual circles representing distinct microinjection sites. The yellow-red gradient indicates "liking" enhancement, while the white-blue gradient indicates "liking" suppression. Central bar graphs

show average change in hedonic reactions during orexin stimulation, compared to vehicle. *B*. Horizontal maps of insular cortex show orexin microinjection sites in rostral or caudal insula, color-coded according to to enhancement or suppression of aversive reactions. The white-purple gradient denotes increases in “dislike”, while the white-blue gradient indicates suppression of “dislike”. Central bar graphs show average change in aversive reactions caused by orexin in rostral and caudal insula, compared to vehicle.

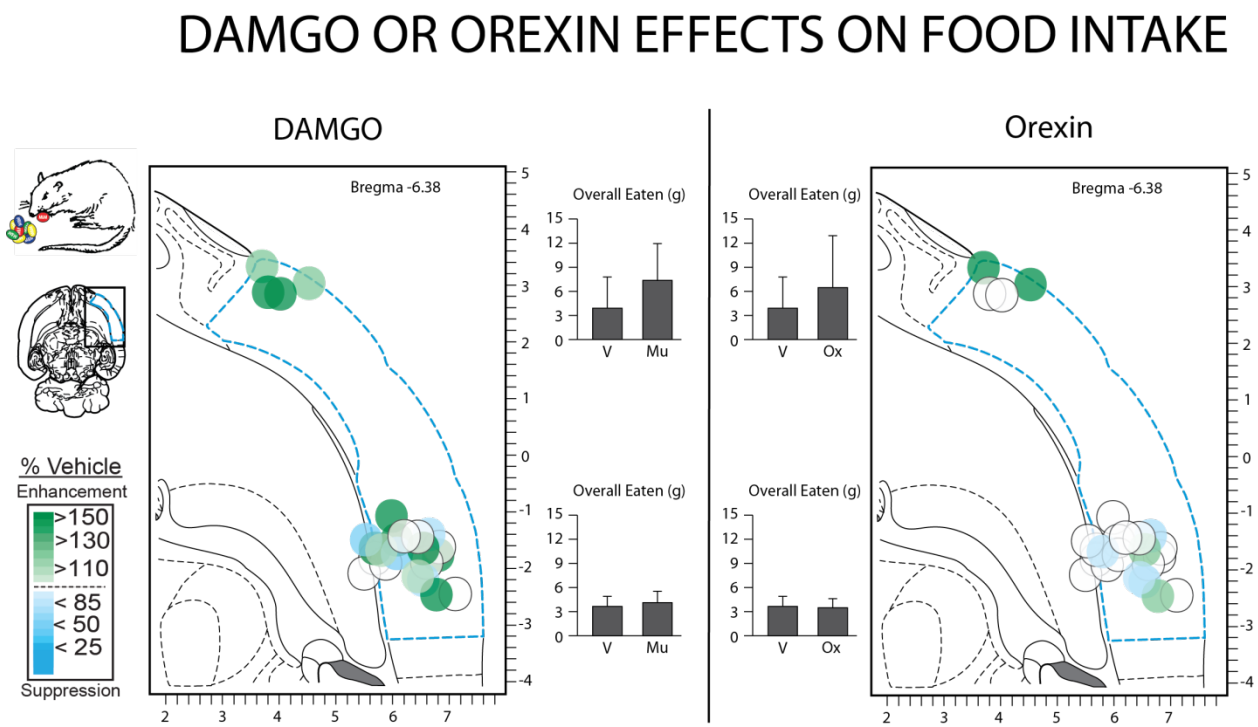

Figure 4 – **Effects of DAMGO or orexin stimulation on food intake.** Horizontal maps of insular cortex show DAMGO or orexin microinjections in rostral or caudal insula, color-coded for effects on food intake. The white-green gradient indicates enhancement of eating while the white-blue shows suppression of eating. Central bar graphs represent amount of M&Ms eaten during vehicle or drug stimulation.