Mapping a Novel Hedonic Hotspot in the Orbitofrontal Cortex

by

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Abstract

Prevailing research suggests a complex hedonic reward network exists in the brain that is coordinated in generating hedonic experience. Previous sites of hedonic hotspots have been identified within subcortical structures such as the nucleus accumbens and the ventral pallidum. However, little is known about the identity and role of other brain substrates that are part of this reward circuit. Here, we investigated the role of orbitofrontal cortex (OFC), a region located within the prefrontal cortex known to be involved in sensory experiences of pleasure, to determine whether activation of this region can independently produce/ampify hedonic impact. Using the affective taste reactivity test with Sprague-Dawley rats, we can objectively assess the degree of hedonic amplification in taste reward following (1) opioid or (2) orexin stimulation within the OFC. Our results show a significant increase in hedonic reactions with either drug microinjection in the rostral portion (+3.50–4.80 mm) of OFC. In contrast, we also have evidence for the existence of a hedonic 'coldspot' within the caudal portion (+2.76–3.50 mm) of OFC, subject to further investigation. Lastly, in agreement with previous work, we find that opioid or orexin stimulation throughout OFC robustly enhances food intake of palatable M&Ms. These results extend the hedonic network to now include a cortical pleasure generator.

Keywords: orbitofrontal cortex, opioid, hedonic, reward, affect, pleasure, motivation

Mapping a Novel Hedonic Hotspot in the Orbitofrontal Cortex

Previous studies have shown that hedonic hotspots exist within specific areas of the brain, most notably the nucleus accumbens and ventral pallidum; opioid-like neurochemical signals microinjected into these areas are able to directly magnify hedonic reactions to food rewards (Berridge, Lawrence, van Ditzhuijzen, Davis, Woods, & Calder, 2006; Peciña & Berridge, 2005; Smith & Berridge, 2005). Nonetheless, there exists evidence of a larger functional circuitry formed by the interaction between multiple hedonic hotspots within the brain (Peciña, Smith, & Berridge, 2006; Richard & Berridge, 2013; Richard, Plawecki, & Berridge, 2013; Smith & Berridge, 2007). Here we attempt to identify and characterize the possible presence of a hedonic hotspot within the orbitofrontal cortex through taste reactivity experiments on Sprague-Dawley (SD) rats.

One of the major goals of affective neuroscience is to identify the brain substrates that are associated with pleasure (Berridge & Kringelbach, 2013). In this vein, neuroimaging studies have found that a large number of brain structures are activated in response to rewarding stimuli (Beaver, Lawrence, van Ditzhuijzen, Davis, Woods, & Calder, 2006; Kringelbach, 2005; Pessiglione, Schmidt, Draganski, Kalisch, Lau, Dolan, & Frith, 2007; Small, Veldhuizen, Felsted, Mak, & McGlone, 2008). Nonetheless, there remains the question of which, if any, of these structures are directly responsible for and thus cause the pleasure associated with the reward, as opposed to being simply correlates of externally generated hedonic activity, due to spreading network activation or as neurological responses to the pleasure event (Berridge, Robinson, & Aldridge, 2009). In order to identify these areas of the brain that are responsible for pleasure causation, research studies specifically look at brain regions that, when manipulated (e.g. pharmacological activation), are able to directly amplify hedonic impact (Castro &

Berridge, 2014; Peciña & Berridge, 2005; Smith & Berridge, 2005; Söderpalm & Berridge, 2000; Tindell et al., 2006); this would imply that these brain sites are capable of independently producing or amplifying the experience of pleasure.

In our discussion of hedonic impact, an important distinction exists between the psychological components of reward: namely "liking" and "wanting" effects. "Liking" refers to sensory pleasure that may be experienced, consciously or unconsciously, as a reaction to hedonic stimuli. The extent of "liking" reactions may be measured objectively through behavioral studies, such as through orofacial expressions in the case of taste arousal. On the other hand, "wanting" refers to incentive salience, a type of incentive motivation that promotes consumption of and approach towards rewards (Berridge et al., 2009). Concordantly, "wanting" effects can be measured using food intake volume as an indicator of internal motivation. Typically, due to their roles in reward acquisition, "liking" and "wanting" co-occur naturally in a brains; a dissociation of their effects is commonly observed when we perform specific investigations into hedonic hotspot regions (Berridge et al., 2009). Hence in these cases, stimulation of specific brain areas may result in enhancement of "wanting" but not "liking". Our investigation therefore is to identify if an area of the orbitofrontal cortex can specifically enhance "liking" when subject to appropriate neurochemical manipulations.

In the aforementioned studies, researchers employ the use of opioid, endocannabinoid, or GABA-benzodiazepine neurotransmitter systems in their investigations (Berridge, Ho, Richard, & DiFeliceantonio, 2010; Mahler et al., 2007; Richard, Plawecki, & Berridge, 2013); these are a few of the known neurochemical systems that are able to enhance "liking" within specific sites in the brain. These specific sites are known as 'hedonic hotspots'; anatomical subregions within specific brain structures that, when activated, result in an amplification of sensory pleasure

sensation (Berridge et al., 2009; Peciña et al., 2006). The current candidate locations of these hotspots include a cubic millimeter (1.0 mm^3) region in the rostrodorsal quadrant of the medial nucleus accumbens shell and a 0.8 mm^3 region in the posterior ventral pallidum (Berridge et al., 2009; Peciña & Berridge, 2000; Smith & Berridge, 2005; Smith & Berridge, 2007). These hedonic hotspots also demonstrate reciprocal interactions with each other; for instance, an opioid transmission in the NAc hotspot can cause activation in the VP hotspot, vice versa (Peciña et al., 2006; Smith & Berridge, 2007). Thus, it has been proposed that there may exist a network of hedonic hotspots, other than the ones previously identified, throughout the brain that are coordinated in producing hedonic experience (Peciña et al., 2006). In particular, several cortical regions show promise for housing a hotspot, which will be discussed below.

In the prevailing research surrounding hedonic substrates of the brain, the opioid peptide neurotransmitter system is one of the best-studied. There exist three major opiate receptor subtypes: μ, δ and κ, all of which are present and localized within the medial prefrontal cortex (Steketee, 2003). Previous studies have shown that opioid stimulation increases food intake, which by inference is an enhancement of "wanting" (incentive salience), and causes conditioned place preference effects (DiFeliceantonio, Mabrouk, Kennedy, & Berridge, 2012; Glass, Billington, & Levine, 1999; Grandison & Guidotti, 1977; Mucha & Iversen, 1986; Wise, 1989). Accordingly, microinjections of an opioid agonist (morphine) into the nucleus accumbens shell produces a general increased eating effect in rats. However, in addition to food consumption, opioid stimulation produces selective increases in positive hedonic patterns of behavioral affective reaction elicited by oral sucrose, in a localized region of the nucleus accumbens; this region has hence been defined as a hedonic hotspot (Peciña & Berridge, 2000). Identical results of localized hedonic enhancement were observed when the selective μ-opioid agonist DAMGO

([D-Ala2, N-MePhe4, Gly-ol]-enkephalin) was microinjected into the posterior ventral pallidum, the other identified hedonic hotspot region (Smith & Berridge, 2005). Most recently, our lab has shown that δ and κ receptor stimulation can similarly enhance hedonic reactions, as long as they are activated within the confines of the respective hotspots (Castro & Berridge, 2014). Conversely, there is also evidence for opioid stimulation that suppresses hedonic reactions, in regions anatomically distinct from the hotspots; these are thereby known as hedonic coldspots (Castro & Berridge, 2014; Pecina & Berridge, 2005; Smith & Berridge, 2005). Hence, the evidence shows that opioid stimulation can induce dissociable effects of "wanting" and/or "liking", the latter occurring in localized regions of specific brain substrates. The presence of localized "liking" effects from opioid stimulation would thereby serve as a reliable indicator for the presence of any currently unidentified hedonic hotspots or coldspots.

A secondary neurotransmitter system that had been implicated in hedonic reward is orexin, which also has receptors expressed in the prefrontal cortex (Marcus et al., 2001). Orexin neurons have projections from the lateral hypothalamus, a brain region regularly implicated in food reward and hunger, to many forebrain targets including the nucleus accumbens and ventral pallidum (Baldo, Daniel, Berridge, & Kelley, 2003; Fadel & Deutch, 2002; Harris & Aston-Jones, 2005). A recent study has shown that an orexin hotspot exists within the VP in essentially the same location as the previously identified opioid hotspot (Ho & Berridge, 2013), and related preliminary research suggests that an orexin hotspot in the nucleus accumbens may also exist. In addition, as with the case of opioids, orexin stimulation also appears to enhance food intake in parts of the brain including the lateral hypothalamus, paraventricular nucleus, and nucleus accumbens (Dube, Kalra, Kalra, 1999; Edwards, Abusnana, Sunter, Murphy, Ghatei, & Bloom, 1999; Thorpe & Kotz, 2005).

The primate orbitofrontal cortex (OFC) is a subregion of the prefrontal cortex that receives sensory inputs from a number of sensory modalities such as taste, olfaction, vision and somatic sensation (Carmichael & Price, 1995; Critchley & Rolls, 1996; Öngür & Price, 2000; Rolls, 2000). Specifically, it contains the secondary taste cortex, the secondary and tertiary olfactory cortical areas, and the inferior temporal cortical visual areas, which code for the reward value of their respective sensations; the neurons within these areas respond only to food (through taste, smell or sight) when the primate is in a state of hunger. Accordingly, these areas also exhibit a decrease in neuronal response to a food when it is eaten to satiety, while retaining the respective reward values of other foods; a mechanism known as sensory-specific satiety (Rolls et al., 1981; Rolls, 1986). Sensory-specific satiety refers not only to alterations in neural activity, but also entails a decline in the hedonic and motivational value from consuming a food that the subject was previously exposed to, evidenced through taste reactivity and food intake tests in rats as well as in humans (Balleine & Dickinson, 1998; Berridge, 1991; Havermans, Janssen, Giesen, Roefs, & Jansen, 2009). The existence of this mechanism as mediated by the OFC suggests an important and significant role in the affective brain representations of food; specifically, that the OFC is capable of modifying reward values (hedonic impact) of experienced stimuli. Lesions of the OFC also eliminated the ability of monkeys to modify their behavior to changes in the incentive value of food (Butter, Mishkin, & Rosvold, 1963) and altered food preferences (Baylis & Gaffan, 1991). These evidences implies that OFC activation in the presence of hedonic activity is not merely representative of a simple neurological response, but indicative of a source of hedonic amplification or attenuation. These results are consistent with human neuroimaging studies, which similarly show that OFC activations are associated with subjective pleasantness

produced by sensory arousal (Kringelbach, O'Doherty, Rolls, & Andrews, 2003), therefore affirming its involvement and importance in hedonic reward across species.

In terms of anatomy, the orbitofrontal cortex contains significant densities of opioid peptides and its receptors (Lerich, Cote-Vélez, & Méndez, 2007; Steketee, 2003); concordantly, research studies have shown that bilateral infusions of DAMGO in circumscribed regions of the OFC leads to increased food intake in rats, similar to its action within the aforementioned known opioid hotspots (Mena, Sadeghian, & Baldo, 2011). Likewise, orexin receptors have been found to be expressed within the OFC (Marcus et al., 2001). In addition, a recent study has found that the medial orbitofrontal cortex exerts a top-down corticolimbic control of appetitive eating behavior induced by nucleus accumbens microinjections; OFC activation also increased Fos activity within the nucleus accumbens hotspot (Richard & Berridge, 2013). This is congruent with the notion that corticolimbic projections from the orbitofrontal cortex form part of a larger neural network that guides motivation. However, while we know that the orbitofrontal cortex is implicated in appetitive eating behavior, little is known about its role in hedonic "liking" of food reward. Thus, informed by a reliable and significant amount of evidence within the current body of research, we seek to determine the possible existence of a hedonic hotspot within the region of the orbitofrontal cortex.

Method

Subjects

Female Sprauge-Dawley rats (n = 13; 250–350 g at surgery), were housed on a 12 hr light/dark reverse cycle (~21°C) with ad libitum food (Purina Rat Chow) and water (tap water). All experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

Oral Tube and Cranial Cannulation Surgery

All rats were anesthetized with intraperitoneal injections of a mixture of ketamine (80 mg/kg) and xylazine (5 mg/kg), and treated with atropine (0.05 mg/kg) to prevent respiratory distress. Rats also received subcutaneous injections of cefazolin (75 mg/kg) to prevent infection and carprofen (5 mg/kg) for analgesia. After anesthesia induction, polyethylene oral tubings [PE-50] were inserted bilaterally, just lateral to the first maxillary molars and ran subcutaneously along the zygomatic arch to the top of the skull, where they exited through an incision. The tubings were secured with wiring and dental cement. Rats were then placed in a stereotaxic apparatus (David Kopf Instruments), with the mouth bar set to -3.3 mm below intra-aural zero. Bilateral stainless steel guide cannulae (14 mm, 23 gauge) were aimed 2 mm above points throughout the medial prefrontal cortex (orbitofrontal), between coordinates anteroposterior (AP) +3.24–4.68 mm ahead of bregma, mediolateral (ML) ±1.0–2.0 mm from the midline, and dorsoventral (DV), -5.7–6.0 mm below skull. Cannulae were anchored to the skull using surgical screws and secured with dental cement; stainless steel obturators (28 gauge) were inserted to prevent occlusion of the cannulae. Post-surgery, rats were carefully monitored for 2 h for signs of distress and topical antibiotic was applied to the surgical area to prevent infection. Rats were again administered carprofen 24 h after surgery, and were allowed to recover for at least 7 days before testing.

Habituation and Testing Apparatus

Prior to the first test day, rats were habituated to handling and procedures for 7 days: rats were handled for 10 min per day for 3 days, and then habituated to the testing procedure and apparatus for 1 h each on four additional days. On the 4th day of habituation, rats received ‘mock’ microinjections (described below) of vehicle before being placed in the testing chambers.

On drug test days, each rat received one of three drug microinjections (DAMGO; orexin; vehicle) and were placed immediately in a taste reactivity testing chamber.

The taste reactivity chambers had a transparent floor, under which an angled mirror reflected an image of the rat's ventral face and mouth into a digital video camera. The food intake chamber was transparent (23 × 20 × 45 cm), contained pre-weighed food (~20 g M&Ms), ad libitum water, and granular cob bedding (~2 cm deep).

Intracerebral Microinjections

Drug microinjections were administered bilaterally in a 0.2µl volume on test days spaced at least 48 h apart. On test days, drug solutions were brought to room temperature (~21 °C), inspected to confirm the absence of precipitation, and bilaterally infused at a speed of 0.2 µl/min using a syringe pump attached via PE-20 tubing to stainless steel injectors (16 mm, 29 gauge) which extended 2 mm beyond the end of the guide cannulae into the orbitofrontal cortex. Injectors were left in place for 1 min to allow for drug diffusion, after which obturators were replaced, and rats were immediately placed in one of the taste reactivity testing chambers.

Behavioral Testing of Hedonic Behaviors

Rats were left in their respective taste reactivity testing chambers (described above) for 25 min to allow for the respective drug to take effect. At 25 min and 30 min post-microinjection, a solution containing sucrose or quinine respectively (0.1 M sucrose or $3x10^{-3}$ M quinine) was infused in 1 ml volume over a 1 min period via syringe pump connected to the oral delivery tube. The sucrose mixture was used to elicit positive hedonic reactions while the quinine mixture was used to elicit negative aversive reactions. The taste reactivity behavior of each rat was video-recorded for subsequent analysis.

Behavioral Testing of Unconditioned Motivated Behaviors

Following the taste reactivity testing, rats were placed in their respective food intake testing chamber (described above). Rats remained in the chamber for 60 min while their eating behavior was video-recorded for later analysis. Immediately following the testing period, the remaining food was removed and weighed to determine volume of consumption.

Behavioral Coding of Videorecorded Behaviors

Hedonic, aversive, and neutral response patterns were scored in slow motion (1/5 - 1/2 actual speed) by a trained observer blind to the drug condition and cannulae placement, using procedures developed to compare hedonic and aversive taste reactions (Berridge, 2000). Hedonic or positive "liking" responses included tongue protrusions, lateral tongue protrusions, and paw licking. Aversive or negative "disliking" responses included gapes, head shakes, face washing, forelimb flails, and chin rubs. Neutral responses include relatively non-valenced behaviors or passive dripping of solution out of the mouth, rearing or mouth movements. All video analysis was conducted using Observer software (Noldus Information Technology, 2008). A time bin scoring procedure was used to ensure that taste reactivity components of different relative frequency were balanced in their contributions to the final affective hedonic/aversive totals (Berridge, 2000). Individual totals were calculated for hedonic and aversive categories for each rat by adding all response scores within an affective category for that rat.

Eating patterns were scored in the same manner described above, by a trained observer blind to the drug condition and cannulae placement. The behaviors scored included appetitive behavior (food carrying, food sniffs), eating behavior (consumption), drinking behavior (licking from water spout), general motor behavior (rearing, cage crossing), fearful defensive treading/burying behavior (pushing of bedding) and grooming behavior (Aldridge, Berridge,

Herman, & Zimmer, 1993). Individual totals were calculated for each rat with regards to the time they spent exhibiting each type of behavior.

Histology

After testing was complete, rats were deeply anesthetized with an overdose of sodium pentobarbital, and their brains were removed and fixed in 10% paraformaldehyde for 1–2 days followed by 25% sucrose solution for at least 3 days. To assess microinjection site locations, brains were sliced at 60 μm on a freezing microtome, and stained with Cresyl violet. Microinjection sites were mapped onto coronal slices from a rat brain atlas (Paxinos & Watson, 2007). Functional effects on appetitive and aversive behaviors were mapped using color-coding to express intensity of changes in motivated behaviors for individual behaviorally tested rats. A site was considered to be in the rostral orbitofrontal cortex if it was located between +3.50 – 4.80 mm AP and caudal if it was located between +2.76 – 3.50 mm AP.

Results

We used an alpha level of .05 for all statistical tests. Overall, there was no main effect of drug on hedonic reactions to sucrose. However, an anatomical analysis revealed both DAMGO and orexin stimulations caused opposing processes within the rostral or caudal sections of the OFC. When microinjection sites were located within the rostral section of the OFC, the number of positive hedonic orofacial reactions elicited by the taste of sucrose was enhanced two to three-fold for microinjections of opioid agonist or orexin, compared with within-subject control levels measured after vehicle microinjections in the same rats ($F_{(2,20)} = 6.16$, $p < .01$). In comparison, when microinjection sites were located within the caudal section of the OFC, the number of positive hedonic reactions to sucrose were suppressed to less than half that of within-subject control levels ($F_{(2,6)} = 1.47$, $p = .30$).

Specifically, mu receptor stimulation by DAMGO microinjections within the rostral section of the OFC tripled the number of hedonic reactions elicited by the taste of sucrose, compared with vehicle levels in the same rats (average = 312%; $p = .02$). Orexin receptor stimulation by orexin microinjections within the rostral section of the OFC doubled the number of hedonic reactions elicited by the taste of sucrose, compared with vehicle levels in the same rats (average = 216%; $p = .02$). Finally, both drug conditions, after microinjection, elicited a two to three-fold increase in food intake independent of stimulation site within the OFC ($F_{(2,26)} = 3.73$, $p = .04$).

Drug Stimulations Produce Selective Hedonic Enhancement

The effect of drug stimulation on overall (hedonic/aversive; sucrose/quinine) taste reactions between-subjects, with the factor difference being site location (rostral vs. caudal), was marginally significant ($F_{(10,46)} = 1.71$, $p = .11$). In particular, drug stimulation effects between sites significantly altered positive hedonic orofacial reactions elicited by the taste of sucrose ($F_{(2,26)} = 6.29$, $p < .01$). No change was statistically detectable for drug stimulation effects on aversive orofacial reactions to sucrose ($F_{(2,26)} = .44$, $p = .65$), hedonic reactions to quinine ($F_{(2,26)} = .26$, $p = .77$), or aversive reactions to quinine ($F_{(2,26)} = .76$, $p = .48$). The consistency of these non-hedonic reactions protects against the possibility that the increase in hedonic reactions may be attributed to alternative locomotor or sensory explanations.

Unlike the localized changes in hedonic reactions, μ-opioid or orexin stimulation produced a significant increase in food intake ($F_{(2,26)} = 3.73$, $p = .04$; DAMGO: $p = .02$; orexin: $p = .03$) throughout all sites within the orbitofrontal cortex, similar to the anatomically distributed eating effects observed in NAc medial shell (Peciña & Berridge, 2005; Castro & Berridge, 2014).

Localization of Hedonic Enhancement Within Rostral Orbitofrontal Cortex

At sites located within the rostral section of the OFC (+3.50–4.80 mm), the number of positive hedonic orofacial reactions elicited by the taste of sucrose was more than doubled by DAMGO or orexin microinjections ($F_{(2,20)} = 6.16$, $p < .01$; DAMGO: $p = .02$; orexin: $p = .02$). Food intake levels were also significantly increased following either receptor stimulation ($F_{(2,20)} = 10.29$, $p < .01$; DAMGO: $p < .01$; orexin: $p < .01$).

Localization of Hedonic Suppression Within Caudal Orbitofrontal Cortex

At sites located within the caudal section of the OFC (+2.76–3.50mm), the number of positive hedonic orofacial reactions elicited by the taste of sucrose was cut in half by DAMGO or orexin microinjections ($F_{(2,6)} = 1.47$, $p = .30$). Though the data was not statistically significant, this may be attributed to the low *n* for the subset of rats with caudally located microinjection sites. Further investigation with more animals would enable a better understanding of the functional properties of this subregion. Regardless, this section reported no enhancement of hedonic reactions to sucrose, thereby suggesting the specific localization of the hotspot to only the rostral section of the OFC.

Discussion

Our results point to the existence of a hedonic hotspot within the rostral section of the orbitofrontal cortex. μ-opioid and orexin stimulation within this area generates 200-400% enhancements of hedonic reactions to sweetness. Contrarily, we also discovered a potential region of suppressive coldspot in the caudal section of the OFC; mu and orexin stimulation within this area resulted in a 20-50% suppression of hedonic reactions to sweetness; however, the data is not significant, attributable to the limited number of subject trials. In addition, all drug placements located within the OFC elicited a 150-300% increase in food intake; this data

corresponds to similar previous studies of hedonic hotspots (Peciña & Berridge, 2000; Smith & Berridge, 2005; Castro & Berridge, 2014), as well as previous investigations into the OFC (Mena et al., 2011).

The small, selective region of hedonic enhancement within the rostral section contrasts with the lack of enhancement across the rest of the OFC, as the opposing behavioral effects would wash out any localized effect. This data confirms a selective role in hedonic reward for the rostral OFC, which as shown, is capable of independently producing and amplifying hedonic experience. With regards to the psychological components of reward "wanting" and "liking", the OFC displayed an anatomical localization of "liking" function to the aforementioned rostral section; in comparison, "wanting" effects from drug stimulation were detected throughout the OFC.

Our study is limited due to the low number of trial animals; as such, more trials would enable a clearer elucidation and mapping of the anatomical locations of the identified rostral OFC hotspot. Future Fos plume studies would also help to clearly define the actual boundaries of this novel hotspot. Furthermore, as apparent from the statistically insignificant distinction of the caudal OFC coldspot, more subject trials may provide enough data to establish significance in confirming the existence of a coldspot. Conditioned taste aversion experiments would help to further verify that our experimental findings demonstrate an alteration in hedonic value of taste stimuli, rather than simply an alteration of their sensory quality. Though we have shown that opioid or orexin receptor function are sufficient to produce the effects of the hotspot, it would also be insightful to determine their necessity. Finally, further research could also be done to investigate stimulation effects of other opioid receptors (delta, kappa), which had recently been

found to also act as hedonic generators (similar to mu and orexin) within the nucleus accumbens hotspot (Castro & Berridge, 2014).

Our findings are consistent with the original expectation of locating an area of hedonic hotspot within the specific region of the orbitofrontal cortex. It is also significant that, unlike previously discovered subcortical brain structures containing hedonic hotspots, the OFC hotspot is uniquely located within the cortical region. The presence of a hedonic generator within the cortical region lays down a foundation for a new, informed understanding of the hedonic circuit as an extensive network that stretches throughout the entire brain; from the parabrachial nucleus of the brainstem pons (Berridge, 1996), to subcortical structures as the nucleus accumbens and ventral pallidum, to this newly discovered hedonic OFC hotspot in the cortex.

With the current understanding of the OFC as a sensory integration nexus involved in affective brain representations and reward processing (Berridge & Kringelbach, 2008; Critchley & Rolls, 1996; Öngür & Price, 2000; Rolls, 2000), our findings support this role of the OFC by establishing it as a brain structure capable of independently generating hedonic pleasure. Multiple neuroimaging studies had implicated the OFC in reward representations elicited by an assortment of sensory cues (De Araujo, Rolls, Kringelbach, McGlone, & Phillips, 2003; Gottfried, O'Doherty, & Dolan, 2003; Hinton, Parkinson, Holland, Arana, Roberts, & Owen, 2004; Kringelbach et al., 2003); our discovery offers concrete evidence that the neural activity of the OFC does not simply represent a network correlation but rather an active generation of hedonic experience.

The significance of the OFC lies not only in its interpretation of primary sensations, but also in its unique involvement in the representations of the reward value of abstract reinforcers. Human neuroimaging studies point to the OFC as a region activated during social judgment

(particularly empathic and forgivability judgments) (Farrow et al., 2001), musical experience (Blood, Zatorre, Bermudez, & Evans, 1999), as well as monetary transaction (Bechara, Damasio, Damasio, & Anderson, 1994). Accordingly, humans with bilateral damage to the OFC region demonstrate a deficiency in their ability to use positive and negative outcomes (incentives) to guide choice behavior (Baxter, Parker, Lindner, Izquierdo, Murray, 2000), and exhibit severe impairments in real-life decision making despite preserved general intellect (Bechara et al., 1994).

Consequently, our findings characterizing the OFC as a hedonic generator paves the way for novel and effective treatments in the field of abnormal brain function. Through appropriate manipulations of brain structures such as the OFC, we may seek to remedy or improve patient outcomes for disorders such as major depression, binge eating, addiction and other behavioral disorders (Covington et al., 2010; Drevets, 2007; Stanfield et al., 2009; Volkow & Fowler, 2000; Woolley, Gorno-Tempini, Seeley, Rankin, Lee, Matthews, & Miller, 2007). Proposed therapeutic strategies can include deep brain stimulation of the OFC in the treatment of anhedonia, as has been shown to be effective with studies targeting the nucleus accumbens (another hedonic hotspot), even for patients with resistant forms of clinical depression (Bewernick et al., 2010; Schlaepfer et al., 2008). Alternatively, optogenetic approaches have also demonstrated promising results in modulating brain activity (selective or inhibitory effects), for the treatment of aforementioned disorders (Diester et al., 2011; Lobo, Nestler, & Covington, 2012).

Successful manipulations of the brain's hedonic centers may be beneficial in treating patients with deficits of positive hedonic impact, such as in major depressive or bipolar disorders. Unrestrained and functionally problematic motivational "wanting", as exemplified in compulsive disorders, eating disorders and addiction, can potentially be curbed by clinical

applications informed by a growing understanding of the brain's underlying reward circuitry. The role of the unique cortically-located orbitofrontal cortex in reward circuits can prove useful in informing future therapeutic strategies for cases of psychopathology, paving the way for more effective treatments.

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Figures

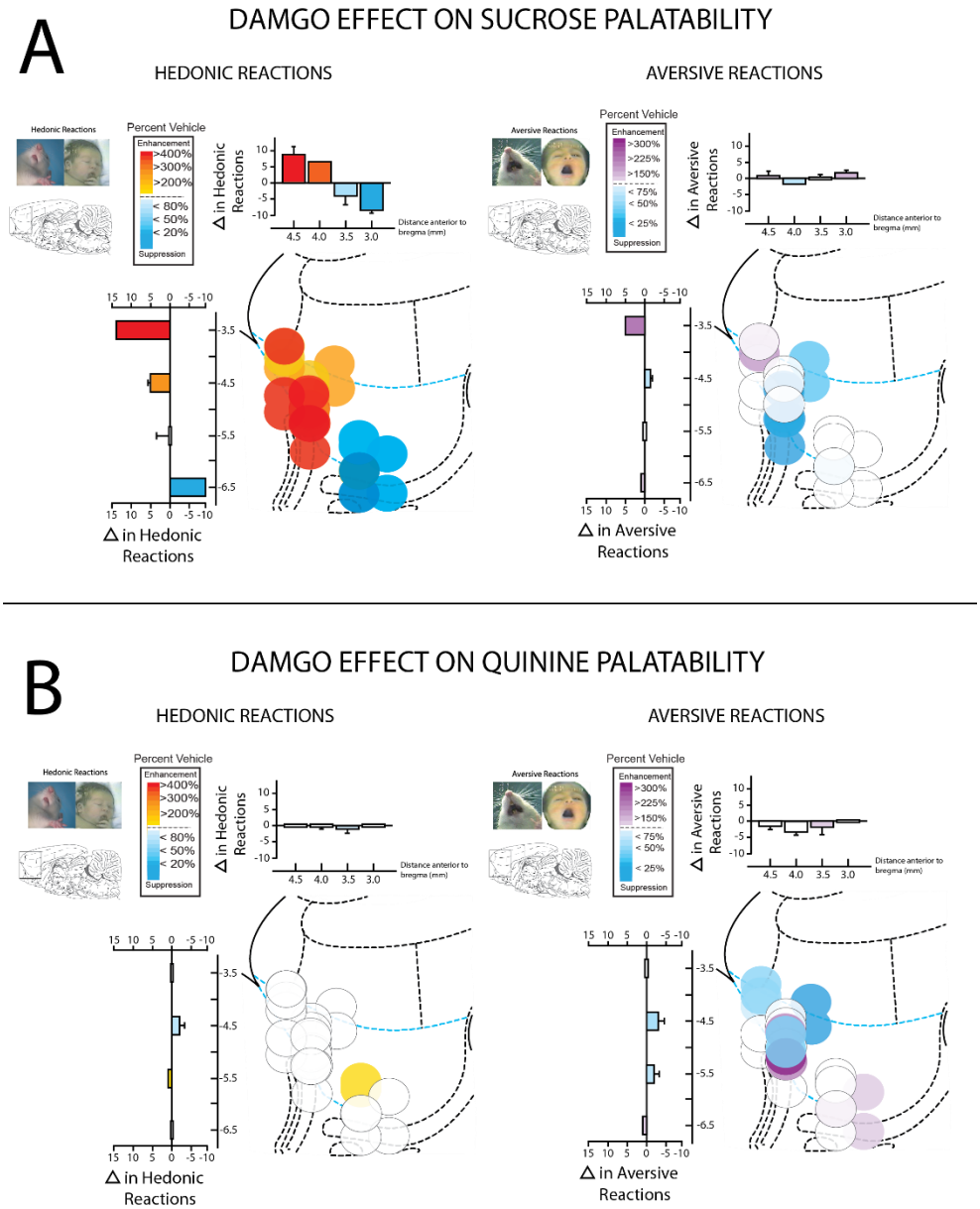

Figure 1. **DAMGO drug microinjection effects on palatability of sucrose or quinine taste stimuli.** Sagittal slice maps of the orbitofrontal cortex brain region show locations of drug microinjection sites and corresponding effects on palatability of respective taste stimuli. All value comparisons are made against a within-subject vehicle condition. Each color-coded circle represents a distinct microinjection placement site; symbol colors are on a gradient denoting percentage enhancement/suppression of hedonic (left column) or aversive (right column) orofacial reactions to a given taste stimuli (A: sucrose; B: quinine). Histogram bar graphs along the anterior-posterior (3.0–4.5mm) and the dorsal-ventral axes (3.5–6.5mm) denote mean change in number of observed hedonic/aversive reactions within each corresponding level (AP: ±0.3mm; DV: ±0.6mm). DAMGO microinjections in rostral section of orbitofrontal cortex show significant enhancement of hedonic reactions to sucrose but not any other types of reactions.

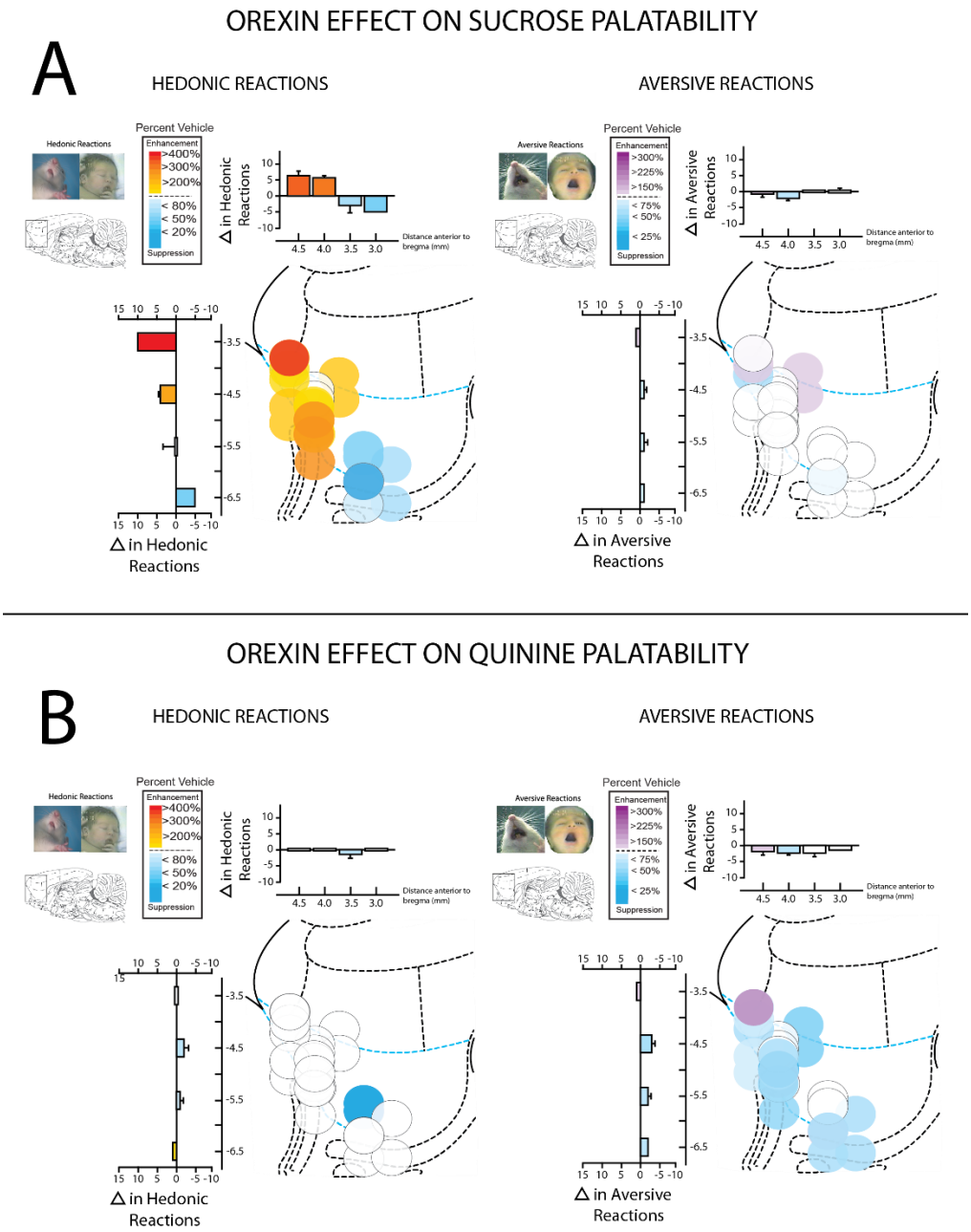

Figure 2. **Orexin drug microinjection effects on palatability of sucrose or quinine taste stimuli.** Sagittal slice maps of the orbitofrontal cortex brain region show locations of drug microinjection sites and corresponding effects on palatability of respective taste stimuli. All value comparisons are made against a within-subject vehicle condition. Each color-coded circle represents a distinct microinjection placement site; symbol colors are on a gradient denoting percentage enhancement/suppression of hedonic (left column) or aversive (right column) orofacial reactions to a given taste stimuli (A: sucrose; B: quinine). Histogram bar graphs along the anterior-posterior (3.0–4.5mm) and the dorsal-ventral axes (3.5–6.5mm) denote mean change in number of observed hedonic/aversive reactions within each corresponding level (AP: ±0.3mm; DV: ±0.6mm). Orexin microinjections in rostral section of orbitofrontal cortex show significant enhancement of hedonic reactions to sucrose but not any other types of reactions.

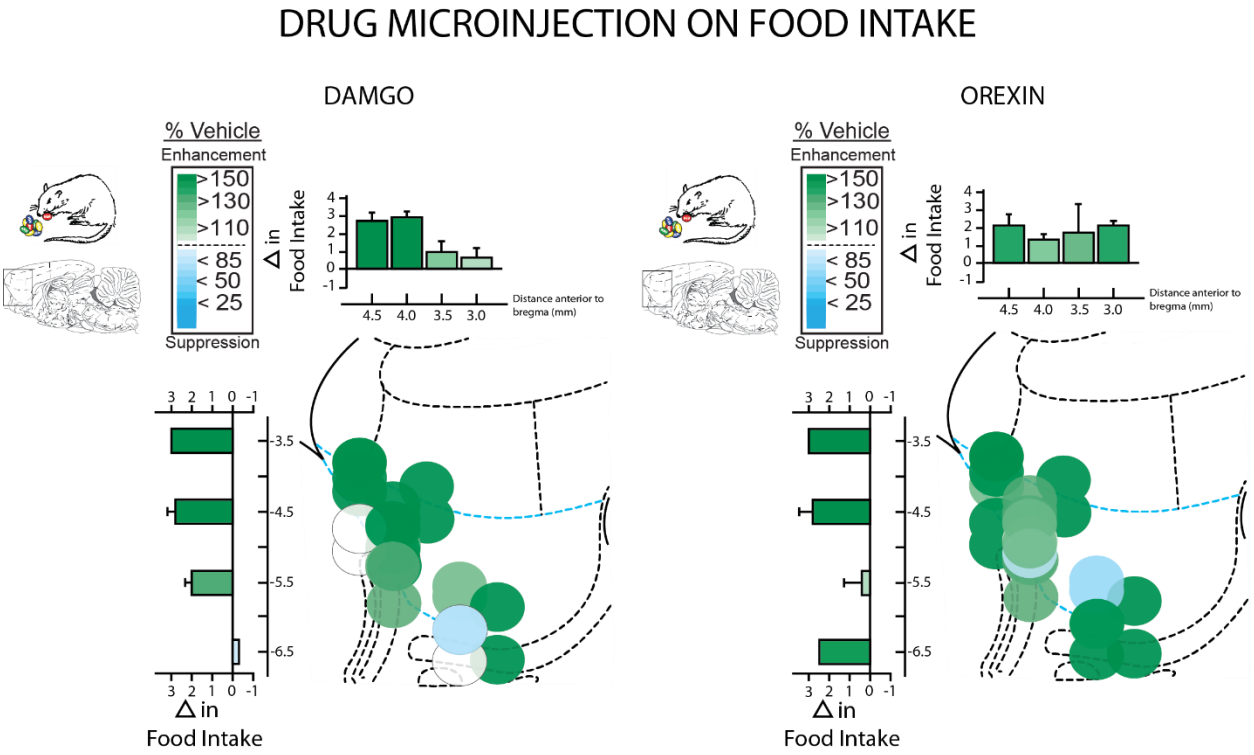

Figure 3. **DAMGO and orexin drug microinjection effects on subject food intake.** Sagittal slice maps of the orbitofrontal cortex brain region show locations of drug microinjection sites and corresponding effects on food intake. All value comparisons are made against a within-subject vehicle condition. Each color-coded circle represents a distinct microinjection placement site; symbol colors are on a gradient denoting percentage enhancement/suppression of food intake volume (left column: DAMGO; right column: orexin). Histogram bar graphs along the anterior-posterior (3.0–4.5mm) and the dorsal-ventral axes (3.5–6.5mm) denote mean change in food intake volume (in grams) within each corresponding level (AP: ±0.3mm; DV: ±0.6mm). DAMGO and orexin microinjections show significant enhancement of food intake irrespective of microinjection location within the orbitofrontal cortex.