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Parabrachial coding of sapid sucrose: Relevance to reward and obesity

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Abstract

Cumulative evidence in rats suggests that the pontine parabrachial nucleus (PBN) is necessary for assigning hedonic value to taste stimuli. In a series of studies, our laboratory has investigated the parabrachial coding of sapid sucrose in normal and obese rats. First, using chronic microdialysis, we demonstrated that sucrose intake increases dopamine release in the nucleus accumbens, an effect that is dependent on oral stimulation and on concentration. The dopamine response was independent of the thalamocortical gustatory system, but was blunted substantially by lesions of the PBN. Similar lesions of the PBN but not the thalamic taste relay diminished cFos activation by sucrose ingestion in the nucleus accumbens. Recent single neuron recording studies demonstrated that processing of sucrose-evoked activity in the PBN is altered in the Otsuka Long Evans Tokushima Fatty (OLETF) rats that develop obesity due to chronic overeating and express increased avidity to sweet. Compared with lean controls, taste neurons in OLETF rats had reduced overall sensitivity to sucrose and altered concentration responses: decreased responses to lower and augmented responses to higher concentrations. The decreased sensitivity to sucrose was specific to NaCl-best neurons that also responded to sucrose, but the concentration effects were carried by the sucrose-specific neurons. Collectively, these findings support the hypothesis that the PBN enables taste stimuli to engage the reward system and, in doing so, influences food intake and body weight regulation. Obesity, in turn, may further alter the gustatory code via forebrain connections to the taste relays or hormonal changes consequent to weight gain.

Keywords

taste; gustatory plasticity; pons; dopamine; appetite; obesity; diabetes

Introduction

Taste helps guide food selection and intake, and gustatory plasticity in response to homeostatic changes occurs at several levels of the taste system. Nevertheless, the neural mechanisms that support these changes in sensory or hedonic evaluations of tastants remain unknown. These mechanisms are pivotal to food preferences and intake control in dietary obesity.

After a brief background discussion we will review our own research investigating how gustatory afferent activity may engage the reward system and the opposite relationship, how increased motivation in obese rats alters coding for sucrose. Although arguments can be

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made for studying other areas, we chose the parabrachial nuclei (PBN), the second central taste relay in rat, because the data suggest that these areas play a role in hedonic assessments of taste stimuli (for a review, see ¹) and because these nuclei have bidirectional connections with limbic structures that are essential in controlling feeding behavior ².

In order to investigate the relationship between the taste and reward systems, we had to identify appropriate oral stimuli and neural measures. For sapid stimuli we used sucrose solutions, a prototype for food reward in behavioral studies. Sucrose is the most effective sugar both in driving peripheral and central taste-evoked responses and, in the short term, in evoking licking (for a review, see ³). Because the mesoaccumbens dopamine (DA) system is implicated in food reward (for reviews, see ⁴⁻⁶), and because our microdialysis studies revealed strong correlation between oral sucrose stimulation and nucleus accumbens (NAcc) DA release ⁷, we use activation of this system as a tool to track the capacity of taste stimuli to engage the motivational system. In order to determine whether the thalamocortical or limbic gustatory projections support the release of DA in the NAcc during sucrose licking, we conducted two sets of experiments that used different dependent variables: NAcc DA flux ⁸ and immunohistochemical staining of the protein product of the early intermediate gene Fos (cFos) ^{9, 10}. With this evidence in hand we then performed single neuron extracellular recording in the PBN to assess sucrose concentration responses in lean and obese rats ¹¹. Accordingly, this summary of our findings follows the same order. The subsequent discussion addresses potential mechanisms by which the PBN may contribute to affective modulation of the taste code and factors related to obesity that may further alter this process.

Taste, food reward and obesity

The incidence of obesity is on the rise (see TFA Health Report 2007 ¹²) and contributes to a number of serious health problems including type-2 diabetes ¹³. Although the etiology of obesity is complex, most obesity is caused by overeating that is apparently stimulated by the increased palatability of the Western diet ^{14, 15}. This increase seems to be independent of other environmental and social factors because diets rich in highly preferred foods (carbohydrates and fat) also can establish obesity in animals (for a review, see ¹⁶).

Both the short term control of eating (meal-size) and the long term regulation of energy homeostasis (body weight) require integration of metabolic signals with central systems that control the motivation for eating ¹⁷⁻¹⁹. In obesity, this intricate relationship is perturbed: increased motivation overrides homeostatic regulation resulting in sustained overeating despite normal or excessive energy storage. Thus, in addition to homeostatic mechanisms that regulate consumption of food based on metabolic need ^{17, 19-21}, reward systems contribute critically to food intake in both human and laboratory mammalian species ^{22, 23}. In this context, it is feasible that the development of diet-induced obesity is associated with malfunctions in systems that assign hedonic value to a meal.

In addition to their visual and olfactory components, the orosensory characteristics of meals stimulate ingestion, whereas gastrointestinal factors are more often inhibitory ²⁴⁻²⁶. In rats, the orosensory effects of sucrose solutions correlates well with intake as well as reinforcing value as long as the postabsorptive effects are eliminated or minimized by using a gastric fistula preparation ²⁶. Specifically, rats sham-feed normally preferred high concentrations of sucrose ⁷, and also work harder on a progressive ratio reinforcement schedule in a linearly increasing fashion ²⁷. These findings suggest that sucrose's taste is sufficient to activate the reward systems that increase motivation to eat. This relationship, however, does not contradict the importance of postingestive reinforcing effects of carbohydrates ²⁴. An initially neutral or even mildly aversive stimulus (e.g. sucrose octaacetate) paired with a

nutritive gastric load increases preference for that fluid²⁴ and has been shown to increase NAcc DA release²⁸. Araujo et al.²⁹ confirmed that the post ingestive stimulatory effects of ingested sucrose can release DA in the NAcc independently of oral factors by using *trpm5*–/– mice that lack functional sweet taste receptors³⁰. The enhancement of perceived pleasantness for sweets by caloric deprivation (“alliesthesia”³¹⁻³³) demonstrates another critical feature of orosensory-motivation integration: not only does taste influence motivation, but motivation influences taste.

In obese individuals, hunger may enhance stimulatory effects of palatable meals. Bartoshuk et al.³⁴ demonstrated that obese individuals prefer sweet and fatty meals more than lean individuals. Similarly, after caloric deprivation, a glucose load fails to reduce the pleasantness ratings of sweet stimuli in either normal or obese humans, but if the sated, the same load still lowers pleasantness in lean people, but not in the obese³⁵. In conditioned taste preference tests, we found a similar effect in the genetic obese OLETF rat. The obese rats tended to form their preferences from the orosensory stimulation of a meal rather than from the reduced drive resulting from ingestion of the same meal when food deprived³⁶. Thus, it appears that, in obese subjects, not only orosensory signaling but also postingestive feedback operates differently on the affective component of a taste stimulus resulting.

Dopamine and sweet reward

PET imaging studies in humans show involvement of DA both in food reward³⁷ and in the motivation to procure food^{38,39}. In humans, striatal DA release directly correlates with the perceived hedonic value of food stimuli^{37,39}. During ingestion, DA release in the NAcc is a function of the relative salience of oral reward^{40,41} and motivational state^{1,4,42,43}. Most relevant feeding experiments were performed under deprivation conditions that by themselves augment the incentive value of external stimuli^{44,45} as well as taste reactivity⁴⁶. Deprivation also prevents habituation of NAcc DA release after repeated exposure to ingestive stimuli (i.e. latent inhibition)⁴². One potential mechanism is elevated presynaptic DA storage in deprived states and, in turn, a larger release upon stimulation by either natural rewards or drugs^{45,47}. Nevertheless, rats fed ad libitum on standard food still exhibit an increase of NAcc DA when given a more preferred diet⁴⁸⁻⁵². In fact, highly preferred food itself reliably elicits an increase in the extracellular DA in the NAcc^{51,53,54}. Mark and his colleagues⁵⁵ demonstrated that saccharin intake increases DA in the NAcc; an observation recently replicated in our lab⁴¹. More importantly, the potency of the sweet taste (saccharin) to stimulate DA release is controlled associative processes. Acquisition of a conditioned taste aversion to saccharin⁵⁵ or reduction of its hedonic properties by association with stronger reward such as morphine⁴¹ may reverse or diminish the DA response to subsequent presentations of saccharin. This observation further demonstrates that NAcc DA tracks the actual hedonic properties of an oral stimulus. Whereas sweet taste may be inherently rewarding and hence stimulate ingestion and DA releases, experience can alter both the ingestive behavior and the DA response in the opposite direction.

Systemically applied DA antagonists provide further evidence for the involvement of DA in sucrose reward⁵⁶⁻⁶¹. Selective VTA lesions that destroy the source of the mesoaccumbens DA projections also result in impaired sucrose preference⁶². Conversely, manipulations that increase DA levels enhance both sucrose preference and intake⁶³⁻⁶⁶. Electrophysiological studies demonstrate that neurons in the NAcc and the ventral pallidum explicitly encode for hedonic value^{40,67-69}. Dopamine deficient (DD) mice can eat but are not motivated to do so⁷⁰, suggesting that they are unable to integrate the neuronal signals necessary to stimulate and maintain feeding. Indeed, DA replacement reestablishes preference for sucrose in DD mice⁷¹. Additional evidence for NAcc DA modulating reward arises from the cross-sensitization effects of sucrose and the indirect DA agonist amphetamine. Compared with

controls, rats previously sensitized to amphetamine increased their locomotor activity after brief access to sucrose⁷². Similarly rats receiving restricted sucrose access exhibited more locomotor activity when subsequently injected with amphetamine^{73, 74}. In addition, rats that spontaneously ingest more sucrose show increased amphetamine-induced DA overflow in the caudal NAcc⁶⁵. Conversely, greater self-administration of addictive drugs predicts great intake of sweet fluids in rats⁷⁵. Sucrose-fed rats display changes in the brain that are similar to those observed with drugs of abuse⁷⁶⁻⁷⁸, as well as withdrawal-like behavioral effects when sucrose is removed⁷⁹. Not surprisingly, sucrose-preferring OLETF rats release more DA to amphetamine or cocaine⁸⁰ or high potassium stimulation in the NAcc⁸¹. Together these findings support the contention that accumbens DA levels reflect the affective component of sweet tastes that, in turn, is the basis for the exaggerated intake and preference and preference for these fluids.

Despite this strong correlation, this evidence does not mean that DA was exclusively responsible for mediation of palatability or hedonic aspects of a taste called 'sweet' by humans. For example, endogenous opioid peptides also are released by sucrose or saccharin intake⁸². Their activity in the brain, particularly in the NAcc^{83, 84} and the PBN⁸⁵ likely play a role in taste hedonics.

Gustatory sensory-motivation integration in the hindbrain

Primary sensory systems regulating food intake interact with the neural mechanisms that elaborate reward and aversion and those that monitor homeostatic variables^{19, 86}. This interaction might occur in forebrain reward areas, such as the mesolimbic DA system, or in the sensory nuclei themselves. For taste, there is evidence for both¹. Both vagal afferent axons from the GI tract and neurons carrying taste information from the oral cavity make their first central synapses within the NTS. The terminal area for the gustatory system is in the rostral half of the NTS, an area that also receives primary afferent intraoral trigeminal axons⁸⁷. In rodents, second order taste neurons ascend from the NTS largely ipsilaterally to the PBN⁸⁸⁻⁹⁰. From the medial PBN, third order taste neurons take two routes to the forebrain⁹¹. One projection terminates bilaterally in a medial extension of the thalamic trigeminal relay, in the ventral posteromedial nucleus (VPM). Thalamic gustatory neurons then ascend to the cortical taste area, the agranular or dysgranular insular cortex⁹². The second branch from the parabrachial nuclei projects to the central gray, hypothalamus, cAMY, and bed nucleus of the stria terminalis (BNST)⁹¹. It has been called the ventral or limbic gustatory pathway.

Gustatory responses in the hindbrain are sensitive to the animal's metabolic state. Salt-deprivation and sodium repletion both influence taste coding^{93, 94}. Satiety factors affect taste activity in the NTS⁹⁵. Specifically, stomach distension⁹⁶ and increases in plasma levels of glucose⁹⁷, insulin⁹⁸, and glucagon⁹⁹ reduce the NTS response to orally applied sugars. Similarly, intraduodenal nutrient infusions resulted in altered neuronal responses in the PBN of hungry rats to oral sucrose stimulation¹⁰⁰. In addition to peripheral nutrient and metabolic signals, central anorexigenic and orexigenic factors also modulate hindbrain functions. For example, the PBN receives afferent projections from hypothalamic NPY and melanin-concentrating hormone (MCH) producing neurons^{101, 102}. In addition, similar to the NTS, neurons of the lateral PBN express melanocortin 4 receptor (MC4-R)¹⁰³ which plays a pivotal role in maintaining energy homeostasis. In fact, MC4-R or POMC deletion or mutation results in obesity, hyperphagia, and insulin resistance^{104, 105}. Recently, we have found reduced expression of the anorexigenic CART peptide in the rostromedial NTS that is the major projective field to the gustatory PBN¹⁰⁶. CART plays an important role in the integration of energy homeostasis and motivation¹⁰⁷⁻¹¹⁰, and its satiating effect appears to be mediated by the hindbrain¹¹¹. Both leptin and insulin also are expressed in the PBN and

throughout the NTS suggesting that the energy homeostasis system extends well beyond the hypothalamus and may directly regulate viscerosensory integration.^{112,113}

In addition to being sensitive to acute and tonic metabolic signals, the PBN appears to support associative processes related to taste-visceral integration, such as acquisition of conditioned taste aversion¹¹⁴⁻¹¹⁶. Lesions of the medial (gustatory) PBN¹¹⁵⁻¹¹⁹, but not the NTS^{120, 121} disrupt the association of a taste CS with the malaise produced by the US. This suggests that the pons is critical for gustatory associative processes. This may only be the case for aversive learning since animals with PBN lesions are able to form a conditioned flavor preference¹¹⁴⁻¹¹⁶. Importantly, decerebrate rats with supracollicular transection of the brainstem rostral to the PBN are not able to acquire either conditioned taste aversions or preferences¹²², despite nearly normal behavioral reactions of acceptance or rejection of sapid fluids¹²³. These findings imply that the forebrain is necessary for the motivation and reward systems to bring about complex taste-guided behaviors, and also suggest that taste processing in the PBN involves central modulation of the afferent neural activity that conveys the hedonic value of a taste stimulus.

Activation of descending projections from reciprocally connected forebrain regions such as the lateral hypothalamus (LH), cAMY, the bed nucleus of stria terminalis, and the gustatory cortex (GS) alter taste responses in the PBN¹²⁴⁻¹²⁷. The NTS also receives descending inputs from the same forebrain areas¹²⁸⁻¹³³. In most cases, forebrain activation inhibited taste activity in the hindbrain. Injections of GABA into the PBN increase input resistance by acting on GABAA receptors¹³⁴. One obvious mechanism of action for this centrifugal control is mediation by GABAergic transmission. This possibility seems less likely as the forebrain neurons projecting to the PBN lack glutamic acid decarboxylase¹³⁵. Nevertheless, injections of benzodiazepines (e.g. diazepam, chlordiazepoxide) -- which act as indirect GABAA receptor agonists -- in the PBN increases hedonic responses to oral sucrose stimulation¹³⁶. These findings collectively suggest that local neuronal circuitry in the PBN operating on GABA-A receptors are involved in affective processing of taste responses to sweet. Thus it appears that the intrinsic properties of PBN neurons and their bidirectional connections with the forebrain (and brainstem) leave these nuclei uniquely positioned to integrate systems controlling energy balance and the sensory-motivational aspects of eating¹³⁷⁻¹³⁹.

Pontine parabrachial nuclei mediate the dopamine stimulating effect of sapid sucrose

In an earlier experiment⁵³ we established the effect of sucrose ingestion on DA in the NAcc using chronic microdialysis. Licking 0.3 M sucrose for 20 min produced a 300% increase in DA overflow in the NAcc. Although deprived overnight (~18 h), ingesting water failed to alter DA levels in the same rats tested on an alternate day. The effect was impressive, but linking it directly to the reward value of sucrose was not that straightforward. The rats ingested considerably more sucrose solution than water and, even in 20 min, the sucrose would have produced metabolic feedback to which the brain was sensible. To control for these possibly confounding effects, we did two further experiments.

We repeated the previous microdialysis study using a gastric fistula preparation to assess the role of orosensory factors alone in the NAcc DA activation. In the first of these experiments, accumbens DA levels concentration-dependently increased in non-deprived rats that sham licked different concentrations of sucrose (Fig. 1A). Since sucrose intake was dose-dependent, and in turn, DA release also correlated with intake, we repeated the study in a separate set of rats using a similar protocol but clamped intake to control for this differential ingestion. Sham licking fixed amounts of 0.03M and 0.3M sucrose solution still resulted in a

differential increase in extracellular DA levels in the NAcc (Fig. 1B) [$F(1,8) = 6.855$; $p < 0.03$, $n = 5$]. The more concentrated sucrose solution caused a significantly higher DA overflow (0.03M: $126.47 \pm 12.83\%$; 0.3M: $156.05 \pm 111.78\%$; $p < 0.02$, $n = 5$). As with the previous experiment with unrestricted access, the correlation between sucrose concentration and the DA response was statistically significant [$r = 0.639$, $F(1,8) = 5.546$; $p < 0.05$]. Thus, even with ingested volume controlled, NAcc DA release rose as a function of increasing sucrose concentration⁷. If we operationally define hedonic value by relative preference, then sucrose reward varies directly with its concentration because, in brief access tests, rats prefer stronger solutions to weaker ones¹⁴⁰⁻¹⁴². Because accumbens dopamine release also varies directly with sucrose concentration, we elected to take these values as a forebrain index of gustatory reward. With this assumption, we used this NAcc DA index to determine which forebrain taste pathways supported activation of the reward system during sucrose licking¹⁴³.

Licking of sucrose stimulated accumbens DA flux in all rats. The magnitude of the effect, however, varied between groups (Fig. 1C). Rats with bilateral ibotenic acid (IBO) lesions in the PBN showed significantly reduced dopamine release in comparison with the sham-operated controls [$123.16 \pm 8.01\%$ vs. $160.99 \pm 13.64\%$; $p < 0.01$]. In contrast, the THLX and control release did not differ from one another, but both were greater than that of the PBNX group [$F(2, 20) = 4.93$, $p < 0.02$]. Although animals with IBO lesions consumed generally less of a novel 0.3M sucrose solution than the sham-operated controls, intake of the PBNX rats did not differ from that of the THLX rats. Thus, lesions of the limbic, but not the thalamo-cortical gustatory pathway attenuate the stimulatory effect of sucrose on the mesoaccumbens dopamine system suggesting that the incentive motivational or hedonic components of gustatory stimuli reach the forebrain via this ventral projection.

We performed another, similar experiment using immunohistochemical staining of Fos, the nuclear phosphoprotein product of the early-immediate gene c-Fos^{9, 10}. After having similar, bilateral lesions the PBN or the thalamic taste relay, rats were allowed to sham-feed sucrose or water for an hour. Their brains were then cut and stained for the Fos protein. In the controls and the rats that received thalamic lesions (“CTRL” and “TTax” groups in Fig. 2), sham licking sucrose produced more Fos in the shell of the NAcc than did ingestion of water. In contrast, lesions of the gustatory PBN reduced the overall level of Fos staining and eliminated the differential effect of licking sucrose (“mPBNx” in Fig. 2). These complementary data represent the first demonstration that the affective character of a sensory stimulus might separate from the thalamocortical stream as early as the second central synapse.

Parabrachial coding of sucrose is altered in obese rats

Although our DA studies used normal (lean) rats, they identified the PBN as critical substrate that may contribute to altered sweet preferences in obesity. We presented a general rationale for this assumption in the Introduction. In short, irrespective of its etiology, in obese animals preference is biased towards higher concentrations of sucrose^{36, 144, 145}. Although this exaggerated preference probably results from both orosensory and postingestive factors, the taste component is sufficient for expression of the effect^{144, 146, 147}. Furthermore, we reviewed functional and anatomical data implicating the PBN in the hedonic processing of taste information. Therefore, the discussion that follows is restricted to specific points related to the particular model we used.

Recent research in our laboratory has focused on taste preference and sucrose reward functions in a rat model of obesity, the Otsuka Long Evans Tokushima Fatty (OLETF) rat. The OLETF rat has a congenital cholecystokinin (CCK) - 1 receptor deficiency, resulting

from a 6847-base pair deletion spanning the promoter region and the first and second exons of the CCK-1 receptor gene¹⁴⁸. The OLETF rats express reduced satiety, increased meal size; they gradually become obese and develop type 2 diabetes^{149, 150}. In addition to diminished sensitivity to postingestive satiation signals^{151, 152} and vagal responses¹⁴⁵, OLETF rats show increased avidity for palatable sweet solutions^{145, 146} and increased sensitivity to sucrose reward¹⁵³. Specifically, OLETF rats perform more licks in brief-intake tests to various carbohydrate and non-carbohydrate tastants¹⁴⁵ and expend more effort for sucrose reinforcement in a progressive ratio operant task⁸⁹. These effects become obvious at higher concentrations and they are augmented by the development of obesity and metabolic conditions.

As in humans, obesity and diabetes can be ameliorated by caloric restriction¹⁵⁴ or exercise^{155, 156} in this strain. This suggests that these ailments in OLETF rats are secondary to their hyperphagia. Therefore, the OLETF rats are more relevant to the most common form of obesity in humans than other rodent models in which the primary defects (e.g. leptin signaling in fatty Zucker rats and ob/ob mice) result in obesity even in absence of overeating. Although the hyperphagic phenotype of the OLETF rats could be accounted for by the lack of peripheral CCK-1 receptors, central orexigenic systems also are altered. Specifically, in the OLETF rat, neuropeptide Y (NPY) expression in the dorsomedial nucleus of hypothalamus (DMH) is higher than in LETO controls¹⁵⁷ and this difference could contribute to hyperphagia¹⁵⁸. Recently, we have found that anorexigenic cocaine and amphetamine regulated transcript (CART) expression is decreased in the rostral NTS and the NAcc in OLETF rats¹⁰⁶. Additional studies from our lab revealed altered synaptic DA regulation in the NAcc of the OLETF rats⁸¹. The same set of experiments demonstrated a functional relationship between these alterations and DA receptor manipulations of preference and operant performance for sucrose solutions^{153, 159, 160}.

Based on these findings we have used the OLETF rats to investigate how obesity may affect sucrose coding in the PBN. In this study¹¹, we recorded extracellular single neuron activity using a semi-chronic preparation^{124, 125}. This procedure is essentially a modified chronic recording¹⁰⁰, in which a rat is operated to localize the taste area and set up with an acrylic head-piece with a reclosable opening above the target brain area. This allows repetitive recording with a minimally invasive approach in a lightly anesthetized condition. The anterior tongue was stimulated using a computer-controlled 16-channel fluid delivery system (Octaflow, ALA Scientific Instruments Inc., Westbury, NY, USA). In addition to standard stimuli, we tested neuronal taste responses to a sucrose concentration series (0.01, 0.03, 0.1, 0.3, 1 and 1.5 M). The analyses included a total of 179 taste-responsive neurons in age-matched prediabetic, obese OLETF (n = 4) and lean LETO (n = 3) controls. At the time of the recording sessions (25-27 weeks), OLETF rats were significantly heavier than age-matched LETO cohorts (580 ± 31 g vs. 481 ± 21 g; $F_{1,5} = 8.288$, $P < 0.05$) and body composition analysis in a separate set of age-matched litter-mates demonstrated significantly higher body fat content in OLETF compared to LETO rats (31.13 ± 0.59 % vs. 25.48 ± 0.62 %, $p < 0.01$). In addition, glucose tolerance was significantly impaired in OLETF rats compared with age-matched LETOs [area under curve (AUC) +210% in OLETF ($F_{1,5} = 7.187$, $P < 0.05$)], but did not meet the criteria for clinical diabetes.

There were more sodium-, and fewer sucrose-responsive neurons in the OLETF rats than in LETOs (67% vs. 47% and 14% vs. 32%, respectively). Compared with LETOs, taste neurons in the OLETF rats had higher baseline activity (8.88 ± 0.53 Hz vs. 7.19 ± 0.34 Hz; $P < 0.01$; $F_{1,787} = 8.368$, $p = 0.004$). A further analysis revealed that this effect derived from the “specialist” neurons that responded exclusively to one taste stimulus (Ts, Fig 3.). There was no strain difference in spontaneous firing in “generalist” neurons that responded to more than one taste stimulus (Tx, Fig 3.). When the proportion of total responses or

power (P_i) of sucrose exhibited by all taste units was compared between strains, PBN neurons in the OLETF rats were 27.7% less responsive to sucrose than in the LETO controls (P_s : 0.34 ± 0.03 vs. 0.47 ± 0.04 , $P < 0.05$). Furthermore, in the obese rats there was a rightward shift in sucrose concentration-response functions relative to lean controls resulting in a higher response threshold ($0.37 \pm 0.05M$ vs. $0.23 \pm 0.2M$, $P < 0.05$) and maximal neural response to high sucrose concentrations ($0.96 \pm 0.07M$ vs. $0.56 \pm 0.5M$, $P < 0.001$) (Fig. 4). The overall decreased sensitivity to sucrose was produced by sodium-responsive neurons that also responded to sucrose, but the concentration effects were carried solely by the sucrose-specific neurons (Fig. 5). Since neuronal responses to NaCl either by N-best or S-best units (i.e. on the main, and side band, respectively) did not differ between strains, the proportion of sucrose responses carried by the broadly tuned (generalist) neurons was further reduced in the OLETF rats.

Discussion

Our electrophysiological study ¹¹ demonstrates that increased behavioral avidity for sugar solutions in OLETF obese rats is correlated with rightward shift in the sucrose concentration response function in a subset of PBN gustatory neurons that respond best to sucrose. This might be interpreted as a 'labeled line' effect rather than a general change across the entire population of PBN taste neurons. Nevertheless, the more general reduction in sucrose sensitivity resulted from a lower proportion sucrose activity being carried by N-best units that were also responsive to sucrose. These changes reflect the way a sucrose stimulus is coded across the population of neurons. Thus, in obese animals, an altered across-neuron pattern for sucrose may increase the distinction between different classes of taste stimuli and different sucrose concentrations.

A similar mechanism may alter gustatory code for sucrose as a function of hunger and satiety. In a previous study ¹⁰², we have recorded from PBN gustatory neurons before, during, and after duodenal infusions of a lipid emulsion that induces behavioral satiety ^{161, 162}. Following lipid infusions, the response to sapid NaCl was depressed about 35% in sodium-best neurons. In units that responded best to sapid sucrose, the response inhibition was greater, reaching 55%. The contrast was even greater (77%) in a subset of these neurons that responded significantly to only sucrose (i.e. sucrose-specific neurons). Thus, when a rat is food deprived, ingesting a normally-preferred food stimulus, sucrose, may produce a substantial response in gustatory neurons that respond preferentially to just that chemical. Conversely, in the process of satiation the relative proportion of the specific responses to all concurrent taste activity gradually diminishes. In the present experiment, we found exactly opposite effects by sucrose in OLETF rats: overall reduced responsiveness in N-best units to sucrose coupled with increased responsiveness by sucrose-specific neurons to higher sucrose concentrations. Thus, the signal-to-noise ratio for sucrose with respect to the entire population of PBN neurons tested was increased. Because the OLETF rats in these experiments were ad libitum-fed, one may suppose that altered sucrose code characteristic to energy deprivation may be a result of a perceived state of hunger (increased alliesthesia ³¹) rather than actual energy deficit. Thus, taste functions in some form of obesity may also be altered due to a persistent and exaggerated motivational state to eat. The present observation together with the known satiety deficit ^{145, 146, 151, 152, 163} and increased sweet preference of the OLETF rats further supports this notion and suggest that taste processing in the PBN has particular relevance to the motivational control of meal-size.

As discussed earlier, the PBN possess bidirectional connections with various forebrain areas ² that may alter taste responses ¹²⁴⁻¹²⁶. Simultaneous recordings from gustatory cortex, orbitofrontal cortex, amygdala, and the lateral hypothalamus indicate that neurons in these different areas compose a circuit in which changes in firing are closely related to sucrose

licking as a rat cycles through satiety and hunger¹⁶⁴. More specifically, electrical stimulation of the central nucleus of the AMY has an overall inhibitory effect on gustatory sensitivity, yet produces a dramatic shift in the across-neuron pattern for sucrose, increasing the proportion of the total sucrose response carried by sucrose-best neurons from 62% to 91%. A similar effect of sharpening the across-stimulus response profiles of PBN cells, particularly with regard to the NaCl- and citric acid best cells, occurs during stimulation of the AMY, the gustatory cortex, and the lateral hypothalamus^{124, 125}. Based on this, the observed effects in the OLETF rats could be related to modulation by the forebrain of the PBN gustatory neurons. One potential mechanism is a change in descending inhibitory tone from the forebrain on the PBN neurons^{165, 166} that, in turn, could alter input resistance and the signal-to-noise ratio in neuron subtypes receiving differential proportion of the afferent stimulation from the taste receptor cells via the NTS¹³⁴. In fact, in our study we observed significantly higher spontaneous firing rate in the sucrose-specific units of the OLETF rats, whereas the basal activity of generalist neurons was unaltered relative to lean cohorts. Interestingly, our preliminary studies in non-mutant, high fat diet-induced obese rats show a similar change in spontaneous firing rate of gustatory neurons of the PBN¹⁶⁷. This suggests a common effect of obesity (independent of its etiology) rather than a perceived state of hunger. In normal-weight rats, food deprivation decreased the mean spontaneous firing rate of NTS gustatory neurons¹⁶⁸. Although the underlying anatomical and neurochemical aspects remain obscure, such a mechanism (setting basal firing properties) may provide a means of temporal coding¹⁶⁹ based on the simultaneous activity of descending forebrain¹⁷⁰⁻¹⁷⁴ and ascending inputs from the NTS to the PBN^{132, 175-177}.

Modulation of central taste processing could also be affected by short-term postingestive signals such as stomach distention¹⁷⁸. Stimulation of the gastric vagal and greater splanchnic nerve results in activation of PBN neurons¹⁷⁹. Gastric distension, an inhibitory feedback signal that enhances satiation and diminishes taste reactivity^{180, 181}, also has been shown to reduce sucrose-evoked neuronal activity in the PBN while NaCl-responses remain unaltered¹⁷⁸. OLETF rats exhibit reduced sensitivity to gastric mechanoreception. Using intraduodenal nutrient infusions, compared with LETO controls, OLETF rats not only fail reducing meal size, but they also decrease the intermeal interval following isocaloric infusion¹⁴⁶. Thus, it is plausible that in addition to within-meal signals (meal-size), deficits in between-meal regulation (frequency) may also alter the taste code.

The PBN neurons express insulin and leptin receptors¹¹³ suggesting that the energy homeostasis regulating system is more wide-spread and, via extrahypothalamic sites, may directly regulate viscerosensory integration. Insulin acts via arcuate nucleus peptide systems as a tonic adiposity feedback signal to reduce food intake. Behavioral data in normal-weight rats also suggest that insulin operates through central mechanisms to reduce food reward^{182, 183}. Direct neuronal applications of insulin are inhibitory in brain regions such as the hypothalamus and hippocampus^{184, 185}. On this basis, insulin might suppress sucrose responses carried by sucrose-best neurons reducing response magnitude to the higher concentrations. It has also been proposed that obesity-prone individuals exhibit a higher threshold for sensing a variety of hormonal and metabolic signals which normally inhibit weight gain^{186, 187}. This raised threshold minimizes the impact of inhibitory signals which inform the brain when there is an excess of energy stores. Thus, an opposite effect by elevated insulin levels (increased responses to higher sucrose concentrations) observed in diabetic OLETF rats can be explained with development of insulin resistance that may also affect insulin's neuronal functions. Alternatively, insulin-dependent peripheral-to-central signaling pathways may undergo changes with advancing stages of obesity or diabetes, or both. A recent study showed that during euglycemic-hyperinsulinemic clamping insulin effects on spontaneous cortical activity correlated negatively with body mass index and percent of body fat, and positively with insulin sensitivity of glucose disposal¹⁸⁸. To

investigate this possibility, in a separate set of animals, we used a similar procedure to record from the PBN of OLETF rats multiple times over an extended period during development of obesity and diabetes. Although the sample size in that study was rather limited (a total of 44 neurons), we did see significantly reduced sucrose sensitivity in the OLETF rats' N-best neuronal subgroup, and an augmentation of this effect with the progression of reduced oral glucose tolerance¹⁸⁹.

Conclusions

The parabrachial nuclei appear to take part in the processes underlying hedonic evaluation of gustatory information by enabling taste stimuli to engage the reward system. This occurs in the context of integrating hormonal, metabolic, and afferent sensory signals under descending forebrain control. Thus, the gustatory PBN may contribute to the regulation of meal-size by increasing or reducing the orosensory positive feedback. In order to change meal-size, the affective component of the oral afferent sensory activity could be modulated by experience, perhaps via the reciprocal forebrain projections, or by current hormonal and physiological states, or both. One potential mechanism to effect such changes is altering the gain for one stimulus at the expense of others. This amplifying or filtering function may require multiple changes (basal firing rate, spatial convergence, temporal coding, etc), to ultimately shape motivational processes such as selective attention, stimulus selection, reward and aversion, cue and incentive assignment, and reinforcement. The present data demonstrate that, in obese animals, at least sucrose coding is perturbed in a manner that could contribute to behavioral phenotype. It remains to be determined, however, whether these observed parabrachial changes in gustatory responses lead or follow the changes in ingestive behavior. In order to address this relationship further advancement in using chronic recording techniques in the hindbrain and adaptation of neuron specific molecular manipulations are warranted.

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Fig. 1.

Dopamine release from the nucleus accumbens shell before, during, and after licking 0.3 M sucrose expressed as a percentage of prestimulus baseline. Dopamine was collected by microdialysis in 20-min samples and measured with high performance liquid chromatography. The dotted lines indicate the sample taken during sucrose licking. Only one solution was tested per day. Statistically significance differences from baseline ($p < 0.05$) are indicated by asterisks. A. Concentration-response functions using 0.03 M, 0.1 M, and 0.3 M sucrose during sham feeding. B. Sham feeding a fixed volume of either 0.03 M or 0.3 M sucrose. The volume was 75% of the average that the rats ingested of the 0.03 M stimulus during a 20 min sham feeding session. C. The effect of central gustatory lesions on

dopamine release in nucleus accumbens while ingesting 0.3 M sucrose. Abbreviations: PBNx – Bilateral lesions of the parabrachial nuclei; Sham. Op. - Combined data from 2 groups of full surgical controls; TTAx –Bilateral lesions centered on the thalamic taste relay. The figure is published elsewhere (Norgren et al. 2006) and reprinted with the permission of Publisher.

Fig. 2.

The number *c-fos* positive neurons in the nucleus accumbens elicited by 1-h sham drinking of distilled water (dH₂O) or 0.6 M sucrose (SUC). CTRL, sham-operated controls; TTAx, lesions in the thalamic taste relay; mPBNx, lesions in the medial (gustatory) PBN; IPBNx, lesions in the lateral PBN. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; SUC vs. dH₂O.

Fig. 3. Spontaneous firing activity (spikes/sec) of PBN taste neurons in obese OLETF and lean LETO rats. Numbers indicate number of recorded neurons; Ts, “specialist” units; Tx, “generalist” units. * $P < 0.05$

Fig. 4.

Mean corrected neuronal response magnitudes (5-s minus prestimulus water baseline, spike/sec) to various concentrations of sucrose. A, sucrose-specific units (Ss); B, NaCl-best neurons also responding to sucrose (NS). \$, statistical differences between strains based on overall ANOVA; * statistical differences between strains for a particular concentration; # statistical difference between the actual and the former sucrose response magnitude within the same strain. *First significant response concentration*: the lowest sucrose concentration that results in a significant neuronal taste response determined by significant t-test ($p < 0.05$) compared to the 5-s prestimulus water baseline. *Maximum response concentration*: the stimulus (sucrose) concentration that causes the highest magnitude taste response in the neuronal activity (one response magnitude is higher than the other if there is at least a 10% increase in the normalized firing rate). *Maximum effective concentration*: the highest applied sucrose concentration that results in significant taste response. *Dynamic sucrose concentration range*: a particular range within the tested sucrose concentrations (0.01, 0.03, 0.1, 0.3, 1 and 1.5 M), in which the consecutive higher concentrations are potent to cause at least 10% increase in the normalized neuronal firing rate over the effect of the one lower concentration. *Non-dynamic sucrose concentration range* or “plateau”: a particular range within the tested increasing sucrose concentrations, in which the consecutive higher concentrations do not cause increase in the normalized neuronal firing rate. The figure is published elsewhere (Kovacs and Hajnal, 2008) and reprinted with the permission of Publisher.

Fig. 5. Concentration effects on mean response magnitude in all sucrose-responsive PBN neurons. For definitions of measures, see the text. * $P < 0.05$. The data are published elsewhere in a different form (Kovacs and Hajnal, 2008).