From the Department of Clinical Neuroscience, Occupational Therapy, and Elderly Care Research (Neurotec), Section of Applied Neuroendocrinology, Karolinska Institutet, Huddinge, Sweden

Appetitive and consummatory ingestive behavior - role of taste, dopamine and NPY

Fredrik Sederholm

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To my parents

När råttan är mätt smakar mjölet beskt

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1 Abstract

The present thesis has examined the role of taste, dopamine and neuropeptide Y (NPY) in the regulation of the two phases of ingestive behavior: appetitive (approaching and handling of food) and consummatory ingestive behavior (chewing and swallowing). Behavioral studies were performed in male and female rats.

THE ROLE OF TASTE: *Background.* A sweet stimulus loses its pleasantness to humans preloaded with glucose, but not if the subjects are food deprived. Thus, physiological state modulates the perception of taste. We hypothesized that taste perception should change during intraoral intake (consummatory ingestive behavior) because physiological state does. Hence, a series of experiments was performed to study aversive taste-related behavior during intraoral intake of a sucrose solution.

Results. Behavioral markers of an aversive taste present in the oral cavity, gapes and chin rubs, were found to increase at the end of intraoral intake. This indicates that the taste perceived by the rat is turning aversive towards the end of the infusion and that this contributes to the termination of ingestive behavior.

THE ROLE OF DOPAMINE: *Background.* Previous work in this laboratory has suggested that dopamine, cholecystokinin and glutamate interact in the inhibition of ingestive behavior at the level of the brainstem. Subsequently, it was discovered that dopamine D2 receptors are present in the rostral part of the nucleus of the solitary tract (NTS), a region in the brainstem that receives primary taste input. In addition to the brainstem, the nucleus accumbens has been implicated in the regulation of ingestive and taste-related behavior. A series of experiments including peripheral and central administration of dopamine D1 and D2 receptor drugs was performed to examine the role of the brainstem and the nucleus accumbens in intake inhibition and aversive responses.

Results. It was found that brainstem D2 receptors mediate suppression of consummatory ingestive behavior. Behaviors related to an aversive taste were also found to be mediated by D2 receptors but at a different neural site, the shell nucleus accumbens. However, none of these receptor populations are involved in taste evaluation.

THE ROLE OF NPY: *Background.* NPY is often referred to as a powerful stimulant of food intake and leptin is referred to as an inhibitor. A series of experiments studying the effect of central administration of NPY and leptin on ingestive, taste-related and sexual behavior was performed.

Results. NPY was found to stimulate appetitive ingestive behavior and, interestingly, decrease consummatory ingestive behavior. Leptin had the opposite effects. NPY suppressed sexual behavior in the presence of a sucrose-filled bottle, but had only a minor effect in the absence of a bottle and leptin facilitated sexual behavior. The suppression of consummatory ingestive behavior after NPY treatment was not blocked by an aversive taste that suppressed appetitive behavior. The NPY-induced suppression of sexual behavior was unaffected by blocking appetitive ingestive behavior. Furthermore, the effects of NPY on ingestive behavior is independent of taste evaluation since NPY treatment did not affect taste responses to a palatable or an aversive taste.

SUMMARY: The taste of an ingested stimulus elicits aversive responses at the end of an intraoral infusion of sucrose. This increase in aversiveness contributes to the termination of ingestive behavior. Stimulation of D2 receptors in the brainstem inhibits ingestive behavior and stimulation of accumbal D2 receptors enhances the display of aversive taste-related behavior. While different consummatory behaviors can be displayed simultaneously, appetitive ingestive behavior interferes with the display of sexual behavior. NPY selectively stimulates appetitive ingestive behavior. Fat depletion and the ensuing decrease in leptin levels may be a peripheral stimulus for directing the attention of a starving animal (or human patient) towards food and to develop strategies to search for, although not necessarily eat, the food.

2 Abbreviations

aCSF	artificial cerebrospinal fluid
Arc	arcuate nucleus of the hypothalamus
BST	bed nucleus of the stria terminalis
cAMP	cyclic adenylyl mono-phosphate
CCK-8	cholecystokinin octapeptide
CeA	central nucleus of the amygdala
CMC	carboxymethyl cellulose
CTA	conditioned taste aversion
D1	dopamine D1 receptor family
D2	dopamine D2 receptor family
DMX	dorsal motor nucleus of the vagus
HYP	hypothalamus
ip	intraperitoneal
IP_3	inositol triphosphate
L-DOPA	L-dihydroxyphenylalanine
LTP	lateral tongue protrusion
M	molar
MTP	midline tongue protrusion
NPY	neuropeptide Y
NAc	nucleus accumbens
NTS	nucleus of the solitary tract
PBN	parabrachial nucleus
PL	paw-lick
PVN	paraventricular nucleus of the hypothalamus
quinine	quinine hydrochloride
VPMpc	parvicellular part of the ventroposteromedial nucleus of the thalamus

3 List of publications

This thesis is based on the following papers.

I **Sederholm F** and Södersten P. Aversive behavior during intraoral intake in male rats. Physiol Behav 2001;74:153-168

II **Sederholm F**, Johnson AE, Brodin U and Södersten P. Dopamine D2 receptors and ingestive behavior: brainstem mediates inhibition of intraoral intake and accumbens mediates aversive taste behavior in male rats. Psychopharmacol *in press*.

III Ammar AA, **Sederholm F**, Saito TR, Scheurink AJ, Johnson AE, Södersten P. NPY-leptin: opposing effects on appetitive and consummatory ingestive behavior and sexual behavior. Am J Physiol 2000;278:R1627-33.

IV **Sederholm F,** Ammar AA and Södersten P. Intake inhibition by NPY: role of appetitive ingestive behavior and aversion. Physiol Behav *submitted.*

4 Introduction

4.1 Animal Behavior

Behavior is a means by which an animal interacts with the environment. A behavioral act has a cause, ie, there is always a stimulus that triggers the behavior. The stimulus may either be external or internal and is perceived and processed by the nervous system that in turn regulates the expression of the behavior through muscular activity.

4.1.1 *Appetitive behavior and consummatory reactions*

Early in the 20th century two scientists presented similar ideas on how to categorize animal behavior. Sherrington (1906) thought of precurrent and consummatory reactions. Precurrent reactions being those that precede the final or consummatory reaction. For example, an animal approaches and brings a food item into the mouth and chews it, precurrent reactions, whereupon a final or consummatory act follows – swallowing. Likewise, a movement away from an irritant stimulating the skin is also termed a consummatory reaction because the behavior is "calculated to be final".

Some years later, Craig (1918) presented a similar categorization.

> An appetite (...) is a state of agitation which continues so long as a certain stimulus (...) is absent. When the appeted stimulus is at length received it stimulates a consummatory reaction, after which the appetitive behavior ceases and is succeeced by a state of relative rest.

For example, a human being searches for, handles and brings food to the mouth, appetitive behavior, whereupon she chews and swallows it, consummatory reactions. Analogous to the appetitive behavior, Craig viewed an aversion as a state of agitation that continues so long as the irritant is present.

Precisely where, in a behavioral sequence, the appetitive behavior ends and the consummatory reaction is initiated is arbitrary. In the present thesis I will rely on the categorization of Craig.

4.1.2 *Ingestive behavior*

Naturally, there is more to ingestive behavior than the dicotomy above. Ingestive behavior consists of a series of behavioral events eventually leading to the consumption of the food or fluid and a subsequent cessation of ingestion, ie, satiety. Ingestive behavior may start and terminate for a variety of reasons. Initiation may occur for homeostatic reasons, because of a diurnal pattern of behavior or simply because the environment allows the animal to eat (Stricker 1990, Woods and Strubbe 1994, Woods and others 2000). First, the behavior must be organized in a way that increases the likelihood of contacting food, ie, appetitive behavior. Once contacted, the food needs to be sampled for its adequacy and, if adequate, consumed. In the case of food excess, the animal need to detect repletion and

terminate ingestive behavior. Hence, ingestive behavior is replaced by “a state of relative rest”, ie, satiety.

What induces satiety? It may be elevated concentrations of blood borne metabolites of the ingested food, stomach distention (Stricker 1990, Deutch 1990) or nutrients in the gastrointestinal tract may evoke neurohormonal activity. Thus, the presence of nutrients in the duodenum stimulates the release of the octapeptide of cholecystokinin (CCK-8) which in turn activates signalling in the vagus nerve that leads to the cessation of ingestive behavior (Gibbs and others 1973a; 1973b).

Commonly, ingestive behavior is studied by measuring the weight of the food that an animal eats. This measure confounds the appetitive and the consummatory part of ingestive behavior since the food is approached, handled, chewed and swallowed. Appetitive behavior may be measured using an operant (see section 4.3.3.1.1), whereas consummatory behavior may be measured using a methodology in which a solution is infused into the mouth of a rat, thus bypassing the appetitive phase (see section 6.4.1.2).

4.1.3 *Taste-related behavior*

Behavioral reactions to taste stimuli in rats were first investigated systematically by Grill and Norgren (1978a). Sapid solutions were infused into the oral cavity and the behavioral reactions were videotaped and analyzed in detail. Behaviors associated with the acceptance of the stimulus were termed ingestive behaviors and included mouth movements, lateral and midline tongue protrusion and paw licks. Aversive behaviors, by contrast, were associated with the rejection of the infused stimulus. These behaviors included gapes, chin rubs, headshakes and paw treadings. (Descriptions of the ingestive and aversive behaviors are found in section 6.4.2). This method was initially developed to study the neural substrates of taste, but has also come to play a major role in the study of ingestive behavior.

Similar to rats, humans react to taste stimulation with facial expressions that reflect the nature of the stimulus (Steiner 1979). It has been suggested that human affective facial expression is analogous to the orofacial behaviors in rats (Berridge 2000). Thus, one may hypothesize that the orofacial behaviors in rats reflect pleasure and displeasure as they do in humans.

4.2 The sense of taste

The main function of the sense of taste is to guide an animal to select adequate food items and fluids (Grill and others 1987, Scott and Verhagen 2000). That is, to avoid non-nutritious and potentially toxic substances and to favor those with nutritious value. This is accomplished together with the sense of smell and vision as well as with somatosensation. Importantly, the sense of taste, as opposed to

the other sensory modalities, is thought to be designated exclusively to ingestive behavior.

What is a tastant? Aristotle argued that a tastant is tangible and that the sense of taste is similar to the sense of touch (Johansen 1998). Sherrington (1906) distinguished a tastant from an odorant in that the odorant can be detected from a distance and at lower concentrations than can a tastant.

In humans, the adjectives salty, sweet, sour, bitter and umami are used to describe the percieved taste of a tastant. However, recent psychophysical experiments question the completeness of this categorization and argue for that taste may be better characterized by a continuum (Schiffman 2000).

Tastants are all able to stimulate taste receptor cells to release neurotransmitters that interact with receptors on cranial nerves that in turn relay the message to the brain. The involved mechanisms and neural pathways are briefly outlined below.

4.2.1 *A tastant acts through receptors or ion channels*

Salty taste is typically evoked by sodium chloride but also by potassium chloride. These salts can trigger an action potential with a subsequent neurotransmitter release through entering the taste receptor cell via ion channels in the apical or basolateral membrane of the taste receptor cell (Lindeman 1996). Blockade of the ion channels in the apical membrane with amiloride renders a rat unable to distinguish between sodium and potassium chloride. Interestingly, in an operant task, amiloride treated rats have a tendency to respond to sodium salt as if it were potassium chloride (Spector and others 1996). This suggests that sodium taste is evoked by sodium entering the cell through ion channels at the apical membrane whereas potassium and sodium can evoke the taste of potassium by entering the cell through channels at the basolateral membrane of the taste receptor cell.

Sweet compounds, sugars and non-sugars, bind to G-protein coupled receptors. Sugars are thought to initiate action potentials by closing potassium channels through an intracellular pathway including the activation of the G-protein gustducin, adenylyl cyclase and cAMP (Scott and Verhagen 2000). Non-sugars also act through gustducin, but then through the activation of phospholipase C and IP_3 to evoke the release of intracellular calcium that enables the discharge of neurotransmitters (Scott and Verhagen 2000). Impairments in sweet taste responsiveness are apparent in mice lacking a functional gustducin protein and there is evidence for that there are other G-proteins that transduce sweet taste (Ruiz-Avila and others 2001).

Sour taste may be evoked by protons entering sodium or potassium channels located at the apical membrane of the taste receptor cell, thereby causing an action potential (Scott and Verhagen 2000, Herness and Gilbertson 1999).

Bitter taste transduction is thought to occur through three mechanisms including up to 80 different receptors (Chandrashekar

and others 2000, Scott and Verhagen 2000). Bitter compounds, such as quinine, may initiate an action potential through the blockade of apical potassium channels. Also, bitter compounds acting at receptors may initiate transmitter release through the activation of gustducin and phosphodiesterase with a subsequent decrease in cAMP. Finally, bitterness may be evoked through a G-protein coupled receptor inducing the synthesis of IP_3 (Scott and Verhagen 2000). As for sweet taste transduction, gustducin-deficient mice show impaired responses to bitter tastants, but there seems to be other G-proteins that transduce bitter taste information (Ruiz-Avila and others 2001).

The salt of glutamate, monosodium glutamate (MSG) is thought to evoke the umami taste. Glutamate activates *N*-methyl-D-aspartate (NMDA) ion channels (Brand 2000), which leads to the depolarization of the taste receptor cell. Glutamate may also depolarize taste receptor cells through the activation of metabotropic glutamate receptors (mGluR4) (Brand 2000).

Additionally, there are indications that free fatty acids are involved in taste transduction. Free fatty acids may modulate the activity of the taste recepor cell through the blocking of potassium channels or by the activation of transporter proteins (Gilbertson 1998b, Herness and Gilbertson 1999).

4.2.2 *Receptor families for sweet and bitter tastants*

Two families of receptors, expressed in discrete taste receptor cells, have recently been cloned (Hoon and others 1999, Adler and others 2000, Chandrashekar and others 2000, Nelson and others 2001). The T1R family is thought to relay sweet taste. Among the three T1Rs, cells coexpressing the T1R2 and 3 subtypes have been demonstrated to respond to some sweet stimuli, eg sucrose, fructose and saccharine (Nelson and others 2001). A mouse strain lacking the T1R3 receptor displays a reduced, albeit not abolished, preference for sucrose and saccharine (Nelson and others 2001). The other receptor family, the T2R family, consists of 40-80 receptor subtypes and is hypothesized to receive bitter taste. Out of the 11 T2R receptors thus far tested, three receptors, T2R4, 5 and 8, respond specifically to some bitter stimuli (denatonium, cycloheximide and 6-n-propyl-2-thiouracil, Chandrashekar and others 2000).

4.2.3 *Taste receptor cells are found in taste buds*

Tastants act through taste receptors or ion channels located on taste receptor cells. Taste receptor cells are found in onion-shaped formations, taste buds, which are primarily located in the surface epithelia of the tongue (Herness and Gilbertson 1999). Taste buds are also found in the epithelia the soft palate, incisal palate, epiglottis, esophagus, nasopharynx, sublingual organs and the buccal wall (Travers and Nicklas 1990, Herness and Gilbertson 1999). A vertebrate taste bud consists of four different cell types, the taste receptor cell, the supporting cell, the precursor cells and in amphibians and fishes there are cells termed Merkl-like basal cells (Lindeman 1996). Further, taste buds are located in papillae which

appear in three different major subpopulations, fungiform, foliate and vallate papillae. Fungiform papillae are found on the anterior two thirds of the tongue, foliate papillae are found on both sides of the mid portion of the tongue and vallate papillae are located on the posterior tongue (Gilbertson 1998a).

Receptors for salty-, sweet- and umami taste are also found at other bodily sites (Scott and Verhagen 2000). Here it may not be adequate to talk about taste reception, chemoreception would be preferable.

The receptor cells release neurotransmitters that interacts with postsynaptic receptors that initiate an action potential in the cranial nerve. These neurotransmitters are thought to include GABA, serotonin and norepinephrine (Nagai and others 1996).

4.2.4 *Cranial nerves convey taste information to the brain*

Three cranial nerves, the facial (VII), the glossopharyngeal (IX) and the vagus (X) nerve transmit taste information from the taste receptor cells to the central nervous system (see Figure 1). In humans, all taste qualities are detected at all taste sensitive regions of the tongue (see Lindeman 1999). Differences and specializations are, however, apparent. Substantial inter-species differences are also evident. In this text rodent systems are described if not otherwise stated.

The chorda tympani branch of the facial nerve innervates the anterior tongue and its fungiform papillae as well as the anterior portion of the foliate papillae on the sides of the tongue (Norgren 1995). The chorda tympani responds potently to salts and sour taste stimuli but less to sweet stimuli (Harada and others 1997). If the chorda tympani nerve is cut, a rat has a reduced capacity to distinguish between sodium and potassium chloride and between quinine and potassium chloride (Spector and Grill 1992, St John and Spector 1998). This nerve branch, therefore, plays an important role in the discrimination of tastes (St John and Spector 1998).

Another branch of the facial nerve, the greater superficial petrosal branch, conveys taste information from the soft palate (Norgren 1995). It responds solidly to sweet stimuli but poorly to salt stimuli (Harada and others 1997). Accordingly, sectioning of this nerve decreases the rate of licking of a sucrose solution (Krimm and others 1987), but, paradoxically, tends to increase sucrose intake (Vigorito and others 1987).

The linguotonsilar branch of the glossopharyngeal nerve innervates the posterior part of the foliate papillae and the vallate papillae on the posterior tongue (Norgren 1995). This nerve responds firmly to the bitter taste of quinine (Dahl and others 1997, Frank 1991) and is essential for the display of aversive behaviors, gapes and chin rubs, that follow intraoral stimulation with quinine (Grill and others 1992, King and others 2000). These behaviors are important for the rejection of an aversive tastant (Grill and Norgren 1978). Taste evoked activity in cells in the nucleus of the solitary tract (NTS, see below) is blocked by the transsection of this nerve (King and others 2000). Somewhat paradoxically, rats do not need

this nerve to avoid bitter tastants (Grill and others 1992). When combining the sectioning of the glossopharyngeal and the chorda tympani nerve, the ability to detect quinine is attenuated (Grill and Schwartz 1992).

Taste buds located in the pharynx and larynx are innervated by the superior laryngeal branch of the vagus nerve (Norgren 1995) and have been shown to respond to taste stimulation in the hamster (Smith and Hanamori 1991) and rat (Miyaoka and others 1998). These taste buds are however thought to play a minor role in taste discrimination (see Norgren 1995, St John and Spector 1998) but may play a role in the intake of water (Miyaoka and others 1987) and in the control of diuresis (Shingai and others 1988).

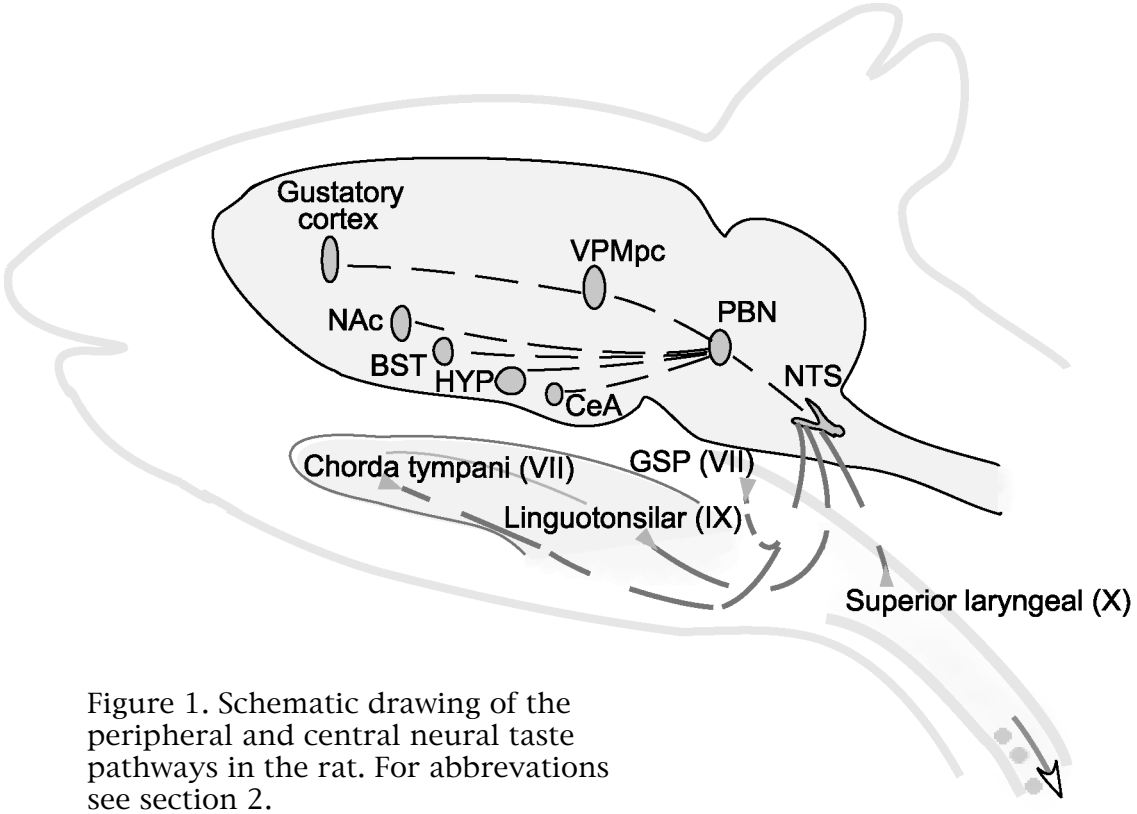

Figure 1. Schematic drawing of the peripheral and central neural taste pathways in the rat. For abbrevations see section 2.

4.2.5 *Taste areas in the brain*

The first central relay for peripheral gustatory information is the NTS. The NTS is a Y-shaped nucleus extending caudo-rostrally in the lower brainstem. The two branches of the facial nerve terminate in the rostral tips of the NTS, the glossopharyngeal nerve has a termination area caudal to the chorda tympani and the vagus nerve is represented caudal to the area of the glossopharyngeal nerve (Norgren 1995).

From the NTS most gustatory neurons project to the second major

taste relay, the parabrachial nuclei (PBN). But NTS neurons also project to areas within the NTS, to the adjacent dorsal motor nucleus of the vagus (DMX), to brainstem neurons relaying information to oromotor nuclei and to brainstem neurons projecting to peripheral salivary glands (Norgren 1978; 1985; 1995, Matsuo 1999, Travers and Hu 2000). The gustatory information in the PBN is further relayed in two major pathways, one leading to cells in the parvicellular part of the ventroposteromedial nucleus (VPMpc) in the thalamus (Norgren 1995). These thalamic cells send further projections to the agranular insular cortex, referred to as the gustatory cortex (Kosar and others 1986). The other pathway from the PBN projects ventrally to ventral forebrain areas, eg the central nucleus of amygdala (CeA), the hypothalamus (HYP), the bed nucleus of the stria terminalis (BST) and the nucleus accumbens (NAc) (Brog and others 1993, Norgren 1995, Delfs and others 1998). The thalamocortical route is more associated with gustatory information than is the ventral projection (Norgren 1995). The above taste pathways are schematically depicted in Figure 1.

In addition to the ascending pathways described above, there are extensive ascending projections from all levels of the taste system. Both branches of the central gustatory pathways, the thalamocortical and the ventral forebrain pathway, project back to the brainstem nuclei (Norgren 1995). Interestingly, there is some evidence for projections from the brainstem, via cranial nerves, to taste receptor cells (Yoshi and others 1996).

4.2.5.1 *Taste areas in the brain of primates*

The above taste pathways are applicable to rodents. Primate gustatory information travels by partially different routes (Norgren 1984, Rolls and others 1989). Peripheral nerve supply terminates in the NTS and is further relayed to the thalamus (VPMpc), rather than to the PBN as in rodents. Further, the thalamus connects to the taste cortex in the frontal operculum and insula, which connect to a second cortical area in the caudolateral orbitofrontal cortex (Rolls and others 1989).

4.2.6 *Taste and ingestive behavior*

Taste processing and ingestive behavior are closely related. Without doubt, taste cues guide an animal in food selection (Scott and Verhagen 2000). Also, the gustatory system may escort the animal in the termination of ingestive behavior. Accordingly, peripheral taste input is modulated by physiological state. For example, humans report that the pleasantness a sweet stimulus decreases after a stomach load of glucose, a phenomenon referred to as negative alliesthesia (Cabanac and others 1971). A similar result is obtained in rats. Thus, taste-related behaviors are shifted from ingestive to aversive after a glucose load in the duodenum (Cabanac and others 1992).

Measurements of neural activity support the hypothesis of negative alliesthesia. Stomach distention in frogs and rats alters neural activity

in gustatory afferents in response to oral taste stimulation (Brush and Halpern 1970, Glenn and Erickson 1976). Moreover, in the NTS, glucose evoked activity is decreased by intravenous injection of glucose (Giza and Scott 1983). Similarly, gustatory activity in the NTS, induced by sodium chloride, is decreased in sodium deficient rats (Contreras and others 1984, Nakamura and Norgren 1995). This plasticity may be regulated by efferent neural stimulation of taste receptor cells or by circulating factors (Hellekant 1971, Wang and others 1995, Yoshie and others 1996, Gilbertson 1998b, Herness and Gilbertson 1999).

4.2.6.1 *Neural substrates for taste and ingestive behavior*

A rat without a forebrain reacts to tastants almost as a neurologically intact rat. Thus, if the forebrain is surgically disconnected from the brainstem, the rat, ie, a decerebrate rat, responds to an intraorally infused tastant as a rat with an intact brain, with only minor differences (Grill and Norgren 1978a; 1978b, Flynn 1995). The decerebrate rat, of course, is unable to approach food. However, if a solution of sucrose is infused into the oral cavity the rat will regulate its intake, ie, it will initiate ingestive responses when the infusion starts and reject the solution when sated (Grill and others 1987). Moreover, if satiety is evoked by CCK-8, the rat will decrease its intake (Grill and Smith 1988). If the sucrose concentration is elevated the rat increases its intake as well as the number of taste reactivity responses, ie, the orofacial and forelimb behavior evoked by a taste, as does an intact rat (Flynn 1995). However, the intake is somewhat lower and the number of taste responses is slightly higher in the decerebrate rat. Moreover, the decerebrate rat upregulates its intake in response to food deprivation, insulin administration and when the ingested food is drained out from the stomach through a gastric fistula (Grill and Norgren 1978a, Flynn and Grill 1983, Grill and Kaplan 1992). The decerebrate rat is however unable to associate a taste with a nauseating event, ie, to form a conditioned taste aversion (CTA) (Grill and Norgren 1978).

With 70% of the rostral gustatory part of the NTS lesioned, the taste reactivity to sucrose and quinine is impaired (Flynn and others 1991b). Surprisingly, the capacity of forming a CTA is intact, as is the ability to increase the intake of a solution of sodium chloride after sodium deprivation, ie, sodium appetite (Grigson and others 1997a).

When lesioning the PBN, taste reactivity responses to sucrose and quinine are slightly blunted (Flynn and others 1991b). These rats fail to form a CTA and sodium appetite (Flynn and others 1991a, Scalera and others 1995, Grigson and others 1997b, see Spector 1995, Reilly 1999 for reviews) an effect that does not depend on impairment in taste detection (Spector 1995). Accordingly, peripheral and gustatory signalling have been demonstrated to converge in the PBN (Hermann and Rogers 1985) implicating an integrative role. PBN- and NTS-lesioned rats remember a CTA formed before the lesion (Grigson and others 1997b), suggesting that the identification of tastants is

preserved, but again, the PBN-lesioned rats are unable to make a taste-nausea association (Spector and others 1992).

Together with the information from decerebrate rats, which have the NTS and the PBN intact, it seems as if forebrain structures interacting with the PBN are necessary for associative processes involving taste whereas the central identification of tastants take place in the NTS. Thus, the brainstem, and regions caudal to it, embodies a sufficient neural substrate for regulation of ingestive behavior, or more specifically the consummatory part of ingestive behavior. In a crude manner one could state that the forebrain is needed for the initiation of ingestive behavior whereas the brainstem is sufficient to maintain and terminate ingestive behavior.

Contrary to the brainstem taste relays, rats lacking the third gustatory relay, the VPMpc, display normal taste detection, formation of CTA and sodium appetite (Reilly 1998). However, they have impaired capacity to form taste associations that are independent of nausea and are suggested to be involved in gustatory guided search for food (Reilly 1998; 1999).

The cortical taste area responds to taste stimulation together with stimuli from other sensory modalities (Kosar and others 1986, Hanmori and others 1998, Katz and others 2001). Rats with lesion in the gustatory cortex acquire a CTA somewhat slower than intact rats and display minor deficits in aversive taste-related behaviors (Kiefer and Orr 1992).

The amygdala is long implicated in ingestive behavior (Klüver and Bucy 1939), perhaps more because of its visceral than its gustatory input. Accordingly, taste reactivity is unaffected by lesion of the central nucleus of the amygdala (Galaverna and others 1993). Sodium depletion in the same rats results in an increased number of palatable taste reactivity responses, indicating that the rats find sodium chloride more palatable when sodium chloride deficient. Paradoxically, these rats do not increase intake of sodium chloride. Neither bottle nor intraoral intake increases (Seeley and others 1993), indicating that a need-induced increase in taste palatability is separable from an increase in ingestive behavior. Destruction of another part, the posterodorsal part of amygdala, produces an overeating rat that gains excessive weight (Rollins and others 2001). Subregions of amygdala are also important in the formation of CTA (Schafe and others 1998).

There are old reports of an increase in taste-related aversive responding following lateral hypothalamic lesions. These rats display increased aversive responding to food applied in or outside the oral cavity (Teitelbaum and Epstein 1962). The increased aversion probably depends on destruction of the adjacent ventral pallidum, which if lesioned stimulates aversive behaviors in response to a normally pleasant taste (Cromwell and Berridge1993).

In primates, the taste of a specific food item elicits neural activity in the orbitofrontal cortex. This activity decreases as the monkey eats and as it eventually stops eating the signal is zero. Interestingly, other food stimuli triggered neural activity in the sated monkey

(Rolls and others 1989, see also Rolls 1999b). This result is consistent with the concept of sensory-specific satiety. In this, humans rate the ingested food as having lost its original pleasantness, whereas the rating of other foods remains unchanged (Rolls 1986). Hence, neural gustatory activity in the NTS in rats and the orbitofrontal cortex in macaques is altered by physiological state.

4.2.7 *Nuclei regulating oromotor behavior*

Before continuing I believe that it is appropriate to briefly look into the muscles and the motor nuclei that gear oromotor behavior. Oromotor behavior is governed by the interaction of the trigeminal motor nucleus, the facial nucleus and the hypoglossal nucleus and their respective muscles (Travers 1995, Fay and Norgren 1997).

The trigeminal motor nucleus provides information to jaw opening and jaw closing muscles. The closers are the superior masseter, anterior deep masseter, temporalis, medial pterygoid, lateral pterygoid and the openers are the anterior digastric and mylohyoid. Input to the trigeminal motor nucleus comes mainly from the mesencephalic trigeminal nucleus but also from the A5 and A7 cell groups, the nucleus subcoeruleus and from the reticular formation (Ter Horst and others 1991, Travers 1995, Fay and Norgren 1997). Notably, treatment with the catecholamine precursor L-DOPA facilitates masseter contractions induced by electrical stimulation of the mesencephalic trigeminal nucleus (Morilak and Jacobs 1985) where dopamine D1 and D2 receptors are present (Lazarov and Pilgrim 1997).

The facial nucleus innervates the digastric and stylohyoid muscles that are involved in oral behavior. Muscles in the neck and ear also receive innervation. Neural input to the facial nucleus comes from the retrorubral nucleus (A8) in the mesencephalon, the PBN, the NTS and from the reticular formation in the brainstem (Ter Horst and others 1991, Travers 1995).

The hypoglossal nucleus innervates muscles in the tongue. These include the retractors hyoglossus and styloglossus, the protruding muscles geniglossus and genihyoid as well as intrinsic muscles that change the form of the tongue (Altschuler 1994). Afferent information to the hypoglossal nucleus comes from the spinal trigeminal nucleus, the nucleus subcoeruleus, the PBN, the NTS and from the reticular formation in the brainstem (Ter Horst 1991, Travers 1995).

Few, if any direct connections exist between the forebrain and the oromotor nuclei (Travers 1995). In-vitro examination of the minimal neural circuit essential for rythmic oral movements (Tanaka and others 1999) suggests that it resides in the caudal brainstem and initiates trigeminal nerve impulses when stimulated by *N*-methyl-D,L-aspartate (excitatory amino acid agonist) and bicuculline ($GABA_A$ receptor antagonist). Therefore, the decerebrate rat displays rythmic oromotor behavior (Grill and Norgren 1978b).

4.2.8 *Somatosensation and ingestive behavior*

In addition to gustatory properties, food also has general somatosensory properties that are detected by the trigeminal orosensory nerves (Ziegler 1985, Norgren 1995). Trigeminal and gustatory afferents travel in adjacent or overlapping pathways in the brain (Norgren 1995, Hanmori and others 1998, Katz and others 2000, Katz and others 2001) indicating interdependence. Temperature, texture and volume all contribute to the regulation of ingestive behavior (Sato 1962, Ziegler 1985, Le Magnen 1992, Rolls 1999a).

Trigeminal orosensory deafferentation severely disrupts appetitive ingestive behavior as well as operant responding to food and water. Thus, deafferented rats respond adequately to intraoral food stimulation but are unable to move their jaw and tongue adequately for food acquisition (Ziegler 1985). Also, taste responses to sweet stimuli are impaired whereas aversive taste reactivity is not (Berridge and Fentress 1985). The impairments of ingestive behavior after trigeminal orosensory deafferentation are far more severe than after total gustatory deafferentation (Ziegler 1985, Vigorito and others 1987).

4.3 Dopamine

Dopamine is a catecholamine present in both the periphery and in the central nervous system. Dopamine synthesizing cells are found in the mesencephalon, the diencephalon, in the olfactory bulb and in the retina (Björklund and Lindvall 1984). Early on, dopamine was thought of as merely being a step in the formation of noradrenaline and adrenaline. However, in the mid 1950ies dopamine and noradrenaline were found in discrete neuronal populations and it was therefore suggested to have a biological function of its own (Carlsson 1959, Björklund and Lindvall 1984). Dopamine is generally thought to play a modulatory role in the control of behavior (Le Moal and Simon 1991) through the action on two families of receptors, the D1 and the D2 (Terry 1996).

An enormous amount of work has been performed on dopamine and ingestive behavior (see Le Moal and Simon 1991, Smith 1995, Salamone and others 1997, Berridge and Robinson 1998). Below I will briefly review the principal neuroanatomy of the dopaminergic system, the dopamine receptors and the proposed models attempting to picture the role of dopamine in ingestive behavior, central taste processing and taste-related behavior.

4.3.1 *Dopamine in the brain*

The mesencephalic dopamine cell system embodies the majority of dopamine containing cells in the brain. This cell system includes subgroups in the retrorubral nuclues (A8), the substantia nigra (A9) and the ventral tegmental area (A10). These neurons extend rostrally

in the nigrostriatal pathway and the medial forebrain bundle to striatal, limbic and cortical termination areas (Björklund and Lindvall 1984).

In addition to the massive forebrain projections, dopaminergic neurons in the mesencephalon also project to the pituitary gland as well as caudally to areas in the spinal cord and the locus coeruleus. Also, projections travel from the arcuate nucleus in the hypothalamus to the lateral habenular nucleus (Björklund and Lindvall 1984).

4.3.2 *Dopamine receptors*

The classification of dopamine receptors is functional. It is based on transduction mechanisms initiated by the receptor-ligand interaction. Thus, the dopamine D1 receptor family increases the synthesis of cAMP whereas the D2 family decreases the synthesis of cAMP in response to dopamine agonist stimulation (Terry 1996). The D1 family consists of the D1, D4 and D5 receptor subtypes whereas the D2 and D3 receptor subtypes form the D2 receptor family (Terry 1996). Both the D1 and the D2 receptor families are mainly found in the caudate putamen, the NAc and in the olfactory tubercle (Sibley and others 1993). In the NTS, however, there are only D2 receptors (Qian and others 1997).

4.3.3 *Dopamine and ingestive behavior*

Destruction of the lateral hypothalamus produces aphagia (Teitelbaum and Epstein 1962). Later, using 6-hydroxydopamine which is a neurotoxin that destroys catecholaminergic neurons, it was revealed that the hypophagia was largely due to the destruction of dopaminergic fibers in the medial forebrain bundle travelling from the mesencephalon to the striatum (Ungerstedt 1971, Zigmond and Stricker 1972, Le Moal and Simon 1995). These seminal studies initiated the search for the role of dopamine in the control of ingestive behavior. In particular, the projections to the NAc in the ventral striatum have attracted attention. Thus, in many studies, dopamine utilization and release in the ventral striatum has been correlated to the intake of food (Le Moal and Simon 1995, Bassareo and Di Chiara 1999, Hajnal and Norgren 2001).

Dopamine or dopamine receptor agonists administered peripherally and into the NAc are able to increase ingestive behavior at low doses whereas higher doses decrease ingestive behavior (Evans and Vaccarino 1986, Evans and Vaccarino 1987, Sills and Vaccarino 1996). Interestingly, amphetamine, which increases the synaptic availability of dopamine, infused into the accumbens in doses that decreased ingestive behavior simultaneously increased physical activity (Evans and Vaccarino 1986). This effect might be analogous to hoarding, which is physical activity with the aim of gathering food, ie, appetitive ingestive behavior. Importantly, the primary response to the loss in body weight is hoarding, not food intake (Cabanac and Swiergiel 1989).

An effect similar to the above mentioned effect of 6-

hydroxydopamine depletion of dopamine is present in a genetic model of dopamine deficiency. Thus, mice lacking the capacity to produce dopamine due to the inactivation of an enzyme needed for dopamine synthesis - tyrosine ß-hydroxylase - are unable to initiate adequate ingestive behavior. Unless treated with the dopamine precursor L-DOPA the mice will die shortly after birth (Szczypka and others 1999, Kim and others 2000). However, restoring dopamine synthesis by local gene transfer into the caudate putamen, but not into the NAc, fully restores food intake. Restoration of dopamine synthesis in the NAc stimulated explorative behavior (Szczypka and others 2001).

Two main models have been forwarded in attempts to explain the role of dopamine in the regulation of ingestive behavior, as well as other behaviors. The sensorimotor and the anhedonia hypothesis.

4.3.3.1 *Dopamine: hedonics or motor control?*

Dopamine depletion renders a rat aphagic. Is that because the pleasure of ingesting food is absent – the anhedonia hypothesis - or is the rat simply unable to move – the sensorimotor hypothesis?

The anhedonia hypothesis depicts the role of dopamine as a mediator of pleasure (Wise and others 1978, see Wise 1994 and Smith 1995 for reviews). Without dopamine transmission the pleasure is decreased and hence, the behavior is not maintained. A number of experimental results support this hypothesis, including decreased responding to obtain a stimulus in the apparent absence of motor incapabilities (Wise 1994, Smith 1995). The suggested site for the reward is the terminal field of the mesostriatal dopaminregic fibers in the NAc (Smith 1995).

The sensorimotor hypothesis assigns to dopamine a role in the motor control of behavior (Le Moal and Simon 1991, Salamone and others 1997). Crudely, the motoric capacity of a rat devoid of dopamine transmission is attenuated and the rat can, therefore, not display ingestive behavior.

Additionally, dopamine has been suggested to be important for the process “wanting” but not for “liking” (Berridge and Robinson 1998). “Wanting” refers to a process in which a stimulus gains motivational salience, ie, the process between the perception of the stimulus and the ensuing action. “Liking” refers to the animals liking of the stimulus as measured by the taste reactivity test (Berridge and Robinson 1998). “Wanting“ and “liking” are separable processes. Roughly, a rat obstructed to move around may “like” a tastant but cannot “want” it because it cannot move.

4.3.3.1.1 *Lever pressing for sucrose depends on dopamine*

To measure how eager a rat is to obtain a certain stimulus many investigators use an operant. That is a task, often to press a lever, the rat needs to perform to gain a food pellet or a sip of water or sucrose. Rats press a lever more frequently to obtain a sip of a sucrose solution with a higher concentration than one with a lower (Baily and others 1986). If amphetamine is administered to a rat, it also presses

the lever more often, an effect mediated by the NAc (Taylor and Robbins 1984, Taylor and Robbins 1986). Accordingly, adding a bitter taste to the sucrose produces a reduction in lever pressing. A similar reduction is observed after blockade of dopamine receptors with pimozide (Baily and others 1986), an effect that is not dependent on motor incapacity.

One might be tempted to conclude that the taste of a sucrose solution is less rewarding to the rat treated with pimozide. However, this testing procedure also requires the rat to perform a task. It is not a pure test of taste responsiveness. A more specific test for taste reactivity is provided by the intraoral infusion paradigm (Grill and Norgren 1978a), which is discussed below (see section 4.3.4).

Another line of research argues that appetitive and consummatory behavior should be considered separately when investigating the influence of dopamine on ingestive behavior. Haloperidol, a D2 receptor antagonist, can block lever pressing for water without affecting the actual intake of water (Ljungberg 1990). Thus, after blockade of the D2 receptors the rat will not work for water, yet it will drink when the water is available. This suggests that lever pressing and ingestive behavior is governed differently by dopamine. In this situation the rat does not dislike the water, arguing against the anhedonia hypothesis.

In this context it should be noted that dopamine also is implicated in learning processes (Robbins and Everitt 1996) and prediction of reward (Waelti and others 2001).

4.3.3.2 *Dopamine influences meal termination and initiation*

Peripheral administration of a high dose of a dopamine D2 receptor antagonist decreases the intake of food pellets (Bednar and others 1995). The drug interferes with the motor capacity of the rat thus suppressing appetitive ingestive behavior. However, the consummatory ingestive behavior is not suppressed. If the rat receives the food directly through an intraoral cannula, thus enabling consummatory behavior without the need for appetitive behavior, the dopamine antagonism does not affect intake (Bednar and others 1995). By contrast, stimulation of dopamine receptors suppresses intraoral intake, an effect effectively reversed by the pretreatment with an antagonist (Bednar and others 1995).

After a meal or peripheral treatment with the satiety peptide CCK-8, the concentration of dopamine and glutamate increases in the NTS (Bednar and others 1994). Here, dopamine has been suggested to stimulate D2 receptors (Qian and others 1997) and together with glutamate participate in the control of meal termination (Södersten and others 1996). The decreased intraoral intake after treatment with apomorphine or CCK-8 in the decerebrate rat supports this hypothesis (Grill and Smith 1988, Kaplan and Södersten 1994). Likewise, the intraoral intake and the responsiveness to CCK-8 are intact after depletion of forebrain dopamine pathways (Qian and others 1998).

To summarize, a decerebrate rat, a rat lacking forebrain dopamine

and a mouse devoid of dopamine synthesis all have severe deficits in approaching food (Grill and Norgren 1978, Qian and others 1998, Szczypka and others 2001). The decerebrate rat and the rat lacking forebrain dopamine are able to initiate, maintain and terminate consummatory ingestive behavior. Also, in the total absence of dopamine, the capacity to display consummatory ingestive behavior seems to be intact although this has not yet been formally demonstrated (Szczypka and others 2001). Consequently, the consummatory ingestive behavior is controlled by a mechanism separable from the appetitive ingestive behavior. The dopamine system seems to be required for the performance of appetitive behavior, but not for consummatory ingestive behavior.

The results described in the sections above suggest that the appetitive and consummatory aspects of ingestive behavior should be investigated separately.

4.3.4 *Dopamine and taste*

The presence of D2 receptors in the gustatory part of the NTS (Qian and others 1997) and terminal chorda tympani synapses on NTS neurons, that most probably are dopaminergic (Davis 1998), argue that dopamine is involved already at an early stage in central taste processing. However, most behavioral results argue against a gustatory function of dopamine, as reviewed below.

As mentioned above, depletion of dopamine pathways in the forebrain produces aphagia. If the dopamine-depleted rat is infused intraorally with tastants and observed for its orofacial responses, ie, a taste reactivity test (Grill and Norgren 1978a), the rat displays essentially normal taste-related behavior (Berridge and others 1989). Interestingly, there was a trend of an increased responsiveness in the dopamine-depleted rats. The ability to form a CTA is intact in the 6-OHDA lesioned rat (Berridge 1996). Likewise, using a pharmacological approach, taste-related behavior was found unaltered after dopamine agonist and antagonist treatment (Treit and Berridge 1990, Pecina and others 1997) even though the results are somewhat unclear (Berridge 2000). Also, sweet taste discrimination remains intact after D2 antagonist treatment (Willner and others 1990). From these studies one may conclude that neural taste processing is dopamine-independent. Instead, dopamine has been suggested to be involved in sensorimotor or “wanting” aspects of taste (Berridge 2000).

However, the literature is not entirely clear on the involvement of dopamine in taste responses. Oral dyskinesias, commonly jaw movements, occur after dopamine receptor manipulations (Koshikawa and others 1989; 1996) and dopamine receptor stimulation elicits or enhances the display of aversive taste behaviors. Thus, in one study, a dopamine agonist infused into the NAc increased the display of a behavior that fits precisely to the definition of a gape (Prinssen and others 1994, Grill and Norgren 1978a). Interestingly, in that experiment the behavior was not evoked by a taste stimulus and, hence, the behavior was not recognized as a taste-related behavior (Prinssen and others 1994). Secondly, stimulation of

the NAc with amphetamine increases aversive taste behaviors to a sucrose-quinine solution (Wyvel and Berridge 2000), but in this case the effect was considered weak. Thus, there are indications in the literature that aversive taste responding is modulated by dopamine transmission.

4.4 Neuropeptide Y

In 1982, a 36 amino acid peptide was isolated from porcine brain tissue. Because of its high tyrosine (Y) content it was called neuropeptide Y (NPY, Tatemoto and others 1982). NPY is a common peptide in the brain (Allen and others 1983, Everitt and Hökfelt 1989) and in the periphery (Lundberg and others 1984) belonging to the pancreatic polypeptide family. Thus far, six receptor subtypes (Y1-Y6) are known to mediate its effects (Gehlert 1998) in various regulatory processes (see Woods and others 1998).

4.4.1 *NPY and ingestive behavior*

Among its numerous functions (Woods and others 1998), NPY is considered to be a potent stimulator of ingestive behavior, an effect thought to be mediated by the hypothalamus (for review see Kalra and others 1999). Food deprivation, ie, a situation where an increased food intake is required, increases the concentration of NPY in various hypothalamic regions (Sahu and others 1988b). Thus, food deprivation increases NPY synthesis in the arcuate nucleus of the hypothalamus (Arc, Ahima and others 1996, Shimizu-Albergine and others 2001). Neurons in the Arc project to and release NPY in the paraventricular nucleus (PVN) of the hypothalamus (Baker and Herkenham 1995, Dube and others 1992). Infusion of NPY into the PVN increases food intake (Stanley 1986), hence, this is one of the plausible sites of the intake enhancing effect of NPY.

In addition to the hypothalamus, the brainstem is sensitive to NPY. Thus, fourth ventricular infusion of NPY enhances ingestive behavior (Corp and others 1990). Brainstem NPY producing neurons project to various brain regions including the PVN (Sahu and others 1988c, Everitt and Hökfelt 1989). Interestingly, transection of neurons connecting the brainstem and the hypothalamus potentiates the NPY evoked food intake (Sahu and others 1988a).

Although there is ample evidence that NPY is an orexigenic peptide, this view may be overly simplistic. First, and perhaps most important, intraoral intake, ie, consummatory behavior, is not enhanced by NPY (Seeley and others 1995). Second, NPY stimulation of the prepiriform cortex decreases intake of an amino acid imbalanced diet (Cummings and others 1998). Third, NPY has aversive properties. For example, NPY facilitates the formation of CTA and the consumption of kaolin (Woods and others 1998). Increased kaolin (clay) intake is associated with the nausea produced by the prototypical aversive drug lithium chloride and is expected from a nauseating or otherwise noxious

compound, not a peptide that is merely orexigenic.

4.4.2 *NPY and taste*

NPY has been located in vertebrate taste papillae (Kusakabe and others 1998), the NTS (Härfstrand and others 1987) and the PBN (Kobashi and Bradley 1998), but the possible relation to taste processing is, so far, unknown. As stated above NPY facilitates the formation of CTA. That is, if a taste is associated with NPY, that particular taste will be avoided on future encounters (Woods and others 1998).

4.4.3 *NPY and sexual behavior*

NPY has been reported to suppress male (Clark and others 1985, Poggioli and others 1990) and female (Corp and others 2001) sexual behavior. This is what would be expected from a peptide associated with scarce food supply and low body weight, a situation when reproduction is not a priority.

4.5 Leptin

The genetic code of the so called “obese gene”, the ob-gene, was published in 1994 (Zhang and others 1994). The product of this gene is a 167 amino acid peptide called leptin (from Greek, leptos = thin). Leptin is produced in white adipose tissue (Zhang and others 1994) and can enter the brain through an active uptake mechanism (Banks and others 1996). Through its action on numerous and widespread receptors subtypes (Mercer and others 1996, Fei and others 1997) leptin is involved in the regulation of energy homeostasis, reproduction and neuroendocrine function (Ahima and others 1996; 1999).

4.5.1 *Leptin and ingestive behavior*

Two animals can be surgically joined in parabiosis so that they exchange part of their circulation. If one of the animals is obese the ingestive behavior in the other is suppressed (Coleman 1973). This finding suggests the existence of a circulating signal that inhibits food intake. These studies provided the impetus for the search for and eventual discovery of leptin. Leptin inhibits ingestive behavior and is correlated to the level of adipose tissue, a lean individual has low levels of leptin and an obese has high levels. Thus, leptin is likely one of the peripheral signals that informs the brain about the fat depot (for review see Woods and Seeley 2000). This is further supported by results obtained in experiments with genetically modified mice in which a part of the leptin signalling chain is missing. Thus, mice deficient of the *ob*-gene, the *ob/ob* mice, and mice lacking the leptin receptor, the *db/db* mice, eat more and become overweight. Administration of leptin suppresses ingestive behavior in the *ob/ob* and in genetically normal mice, but not in the receptor-lacking *db/db*

mice (Stephens and others 1995). Moreover, there is evidence that leptin, by stimulating leptin receptors in the Arc suppresses the synthesis of NPY (Stephens and others 1995, Satoh and others 1997, Ahima and others 1999), thereby reducing ingestive behavior.

4.5.2 *Leptin and taste*

Leptin administration suppresses sucrose evoked responses in peripheral gustatory nerves. This might be due to leptin acting on Ob/Rb receptors that are present in mice taste cells. Mice lacking this receptor are more sensitive to, and display a preference for sweet stimuli (Ninomiya and others 1995, Kawai and others 2000).

4.5.3 *Leptin and sexual behavior*

Leptin deficient mice, *ob/ob*-mice, do not undergo puberty unless treated with leptin. Thus, leptin restores reproductive capacity in these mice and accelerates the onset of puberty in genetically normal mice (for review see Flier 1998). In hamsters, leptin facilitates sexual behavior in non-fasted females but, paradoxically, potentiates fasting induced inhibition of sexual behaviors (Wade and others 1997).

This introduction has hopefully provided the reader with a background to the experiments in this thesis.

5 Aims

The aim of the present thesis was to investigate the following.

1 The role of taste in the control of consummatory ingestive behavior

2 The role of dopamine in the control of consummatory ingestive behavior and aversive taste responses

3 The role of NPY in the control of appetitive and consummatory ingestive behavior

6 Materials and methods

6.1 Animals and housing

Male and female Wistar rats (Möllegaard Breeding Laboratories, Möllegaard, Denmark), weighing 220-300 g were used. They were housed in an air-conditioned, temperature controlled room in regular plastic cages with wooden shavings. Water was available at all times but the food pellets were removed 6 h before tests for ingestive behavior. Before the surgery rats were kept four to a cage and after surgery they were kept in individual cages.

6.2 Surgery

Surgery was conducted under deep pentobarbital anæthesia (1 ml/ kg, intraperitoneally, 60 mg/ml, Apoteksbolaget AB, Umeå, Sweden). A 2–4 week period of recovery was allowed.

6.2.2 *Intraoral cannulation*

In order to infuse sapid solutions into the mouth, the rats were implanted with an intraoral cannula as described by Grill and collegues (1987). Briefly, the cannulation procedure is as follows. The head of the rat is fixed to a stereotactic instrument, an incision is made at the top of the head and three small stainless steel screws are fixed to the skull. A 50 mm piece of polyethylene tubing (PE-100) is heat-flanged at one end and a needle is inserted to the other end. A circular piece (3 mm diam) of a 1 mm thick silicone sheet is then threaded on the tubing and placed at the flanged end. The cannula is then led subcutaneously starting by the first molar tooth on the right side in the mouth and ending at the incision at the top of the head. The tube is cut off and a 20 mm steel tube (1 mm OD) is inserted and the ensemble is anchored with acrylic cement to the screws.

6.2.3 *Gastric cannulation*

Sucrose solutions were also infused directly to the stomach via an intragastric cannula. This cannula consisted of a silicone tube (0.51 mm ID, 0.94 mm OD, Degania, Bethlehem, Israel) with a small rim of silicone glue (Degania) 5 mm to the ending. The ending of the tubing was inserted through a minimal stab wound by the greater curvature of the stomach. A loosely tightened purse-string suture surrounding the stab wound secured the tubing to the stomach. Omental fat was applied to the wound to facilitate healing. The tubing was then led subcutaneously to the head and pulled over a 20 mm piece of steel tubing (1.0 mm OD). The cannula was anchored with acrylic cement (Svedia Dental, Stockholm, Sweden) at the site of the intraoral cannula. Daily flushing with water kept the cannula open.

A second type of gastric cannula was used to drain the stomach of its content during feeding. It consisted of a 30 mm long stainless steel tubing (3.0 mm ID, 5.0 mm OD) with a removable screw at one end, a

circular silicone sheet (20 mm diam, 1 mm thick, Degania), and a 20 mm long piece of silicone tubing (3.0 mm ID, 5.0 mm OD, Degania). The silicone tubing and the silicone sheet were glued together with silicone glue and 15 mm of the silicone tubing was pulled over the steel tubing. This produced a tight and durable connection that outlasted the experiment. The silicone tubing protruding the sheet was then inserted through a small hole made by blunt dissection at the level of the greater curvature of the stomach. The sheet was tightly secured to the stomach wall with sutures. Pieces of omental fat were applied to the wound to facilitate healing. A small stab wound in the abdominal wall and skin was made to externalize the cannula.

6.2.4 *Duodenal cannulation*

Two rims of silicone glue were added to a piece of silicone tubing (0.31 mm ID, 0.64 mm OD, Degania). One 5 and the other 40 mm from the ending. The tubing was inserted through a minimal stab wound made in the duodenum 20 mm caudal to the pylorus so that the first rim was inside the duodenum. The tubing was secured to the duodenal wall whereupon it was led and anchored to the head as described for the gastric infusion cannula. The second silicone rim was secured to the stomach wall with two loosely tightened ligatures.

6.2.5 *Chronic brain cannulation*

To administer drugs into the brain, cannulæ (26 gauge, Plastics One, Roanoke, VA, USA) were implanted aiming at the lateral or fourth brain ventricle or at the NAc. The head of the rat was fixed in a stereotactic instrument and an incision was made on the top of the skull. The stereotactic coordinates of Paxinos and Watson (1998) were used. After a hole had been drilled in the skull, the cannula was lowered and anchored to the skull with acrylic cement and stainless steel screws. A dummy cannula was inserted into the brain cannula to prevent clotting. The coordinates used were the following. The lateral ventricle: bregma –1.0 mm, 1.4 mm lateral and 3.5 mm ventral to the skull surface; the fourth ventricle: bregma –13.0 mm and 7.0 mm ventral; the shell region of the NAc: bregma +1.5 mm, bilateral +/-3.0 mm and ventral 5.2 mm; the core region of the NAc: bregma +2.0 mm, bilateral +/-3.0 mm and ventral 4.8 mm. The cannulæ aiming for the NAc were angled lateromedially at an angle of 10° (core) or 20° (shell) to minimize ventricular entry of the infused drugs.

6.2.6 *Jugular and hepatic portal vein cannulation*

The jugular and hepatic portal vein were equipped with a permanent cannula as described by Steffens (1969) and Strubbe and Steffens (1977).

In short, the right jugular vein was exposed and clamped. A sterile silicone tubing (0.51 mm ID, 0.94 mm OD, Degania) was inserted and fastened to the vein with loosely tightened ligatures. The tubing was led and anchored to the head as the gastric infusion cannula. The cannula was filled with a solution consisting of 5.5 g polyvinylpyrrolidone (MW 25 000, Merk) and 10 ml heparin (500 U/

ml) to prevent clotting. The hepatic portal vein was cannulated using a thinner silicone tubing (0.31 mm ID, 0.64 mm OD, Degania) and secured to the vein and head as the jugular vein cannula.

6.3 Test solutions

Sucrose was dissolved in tap water to concentrations ranging from 0.5 to 2 M, ie, 70-280 kcal. Quinine hydrochloride (quinine) was dissolved in deionized water to concentrations ranging from $3x10^{-6}$ to $3x10^{-3}$ M. Sweetened condensed milk (Rainbow, Leuwaarden, Holland) was diluted with tap water (1:1).

6.4 Behavioral tests

Behavioral testing started 1 h after the lights were switched off. All behavioral testing took place in a semi-dark room just next to the colony room. Testing was preceded by a 6 h fast.

6.4.1 *Intake*

6.4.1.1 *Bottle intake*

Observing a rat ingesting from a bottle provides information on appetitive and consummatory ingestive behavior. The number of times the rat visits the bottle is a measure of appetitive ingestive behavior. A visit was defined as a circular movement away from the bottle and a subsequent return to the bottle.

Circular (35 cm diam) Plexiglas test arenas equipped with a bottle-holder, 50 ml bottles and an angled spout were used. One min after the rat had been placed in the test arena the bottle was introduced through a small hole 5 cm from the floor. Intake from the spout is initiated gradually and after 6-7 days all rats lick from the spout and the intake stabilizes at about 8 ml (1 M sucrose). The test period was 15 min since most rats stop visiting the bottle after about 12-13 min.

6.4.1.2 *Intraoral intake*

The intraoral infusion method was introduced by Grill and Norgren (1978a; b). At that time the method was used to monitor taste reactivity in rats with cerebral lesions. Later the method has also been used to study consummatory ingestive behavior (Grill and others 1987).

The test equipment includes a peristaltic pump (Alitea XV, Ventur Alitea, Stockholm, Sweden), Plexiglas test arenas (35 cm diameter), lids with swivels to cover the arena and to connect the polyethylene tubing to the rat, polyethylene tubing (PE 100) and connecting steel tubes (1 mm O.D.). The rat is placed in the test arena and the PE tubing is connected to the intraoral cannula, which was cleaned before each test. As the pump is turned on, consummatory ingestive behavior (see section 6.4.2.2) are initiated if the solution is palatable to the rat. Aversive behaviors (see section 6.4.2.1) are displayed if

the solution has an aversive taste. Ingestion of palatable solutions continues until the rat rejects the solution, either passively or actively. Passive rejection occurs if the rat lets the solution drop from the mouth. When the rat clears its mouth and perioral region of the solution using aversive behaviors, rejection is termed active. When rejection occurs, the infusion is interrupted and restarted after 30 s. If the rat rejects the solution within 60 s after the restart, the test is terminated. The intake equals the time the rat has spent ingesting the solution. Hence, an infusion at a rate of 1 ml/min during a period of 15 min equals an intake volume of 15 ml. The intake on the first infusion day is about 5 ml and intake stabilizes at about 14-20 ml after 7-8 days of testing. When intake has stabilized the experiment is started.

6.4.1.3 *Combined bottle and intraoral intake*

A test was developed to measure appetitive and consummatory ingestive behavior simultaneously. As the rate of intraoral infusion of sucrose is decreased the rat starts to visit a bottle filled with sucrose. Hence, a measure of appetitive behavior (visits to the bottle) and consummatory behavior (intraoral intake) is obtained.

The rats were trained to ingest a sucrose solution intraorally during 3-4 days and then trained to ingest from a bottle during 3-4 days. Subsequently, the intraoral (0.5 ml/min) and bottle tests were combined and the rats were trained for an additional 3 days. The intraoral intake stabilizes at about 17-20 ml and the bottle intake at about 2-3 ml under these conditions.

6.4.2 *Taste reactivity*

The behavioral responses to a typical aversive taste, quinine, which has a bitter taste to humans, and the responses to a typical palatable taste, sucrose, which humans rate as having a pleasant taste, were measured.

6.4.2.1 *Aversive responses*

Responses evoked by solutions of quinine were recorded by direct observation. Mirrors behind the test arena allowed scoring when the rat turned away from the observer. The rats were allowed to adapt for 10 min to the testing conditions on two days prior to the experiment. On the first day they were infused intraorally for 20 s with deionized water and on the second day for 20 s with a $3x10^{-5}$ M solution of quinine. This infusion period was selected because a solution drops from the mouth after about 20 s in a rat that displays neither ingestive nor aversive behaviors. Hence, this volume, 0.33 ml, was considered sufficient to stimulate the entire oral cavity.

In the first study, the behaviors recorded were gapes, chin rubs, headshakes, forelimb flails and paw-pushes (see Fig 2). A gape was defined as a distinct, rapid opening and closing of the mouth. Usually, a gape occurs in bursts with about two to four repetitions. A chin rub was defined as a rub of the chin against the floor or wall of the test arena. The definition of headshakes was a burst of rapid,

high frequency, side to side movements of the head. Forelimb flail was scored when a burst of high frequency shaking of at least one forelimb was observed. Orofacial grooming was scored when the rat performed an uninterrupted sequence of rubs and strokes with its forepaws against the perioral region of the face. Finally, a paw-push was scored when the rat displayed a sequence of treading with the forepaws on the floor. These behaviors were chosen on the basis of the work of Grill and Norgren (1978a).

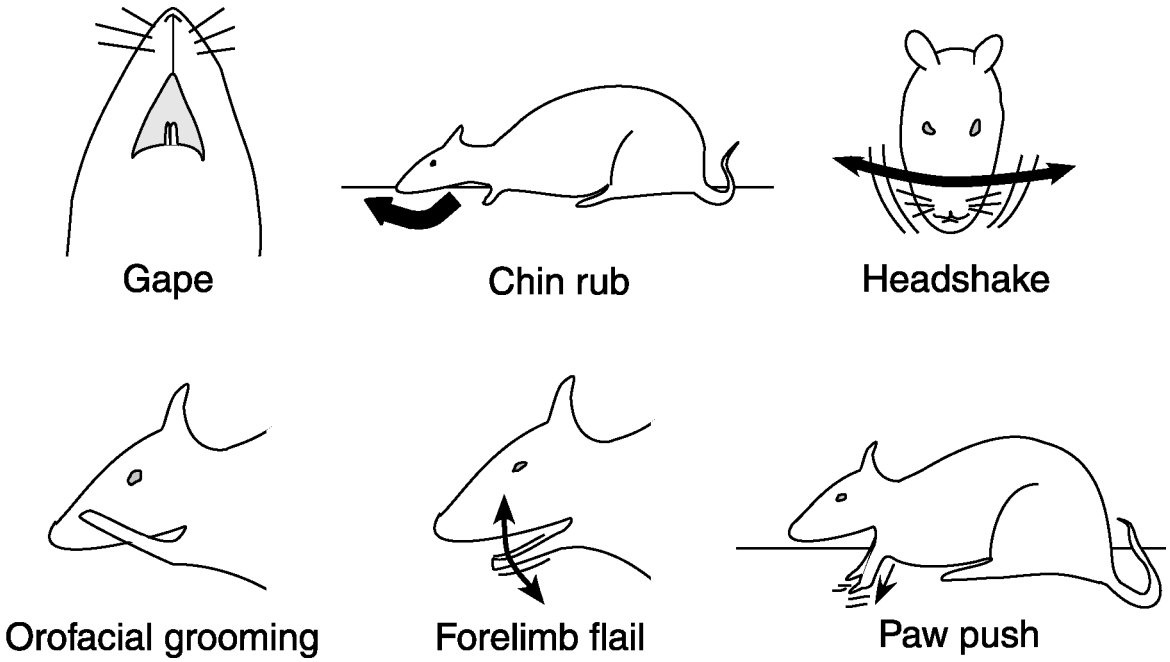

Figure 2. Aversive responses in the rat. See section 6.4.2.1 for descriptions.

6.4.2.2 *Ingestive responses*

Responses to sucrose are difficult to quantify by direct observation. Therefore, the rats were placed in a Plexiglas tube (5.5 cm diam) on a glass floor beneath which a mirror was angled so that the head of the rats could be filmed using a digital video camera (Canon XM-1, Canon Inc, Tokyo, Japan). The rats were adapted to being restrained in the Plexiglas tube for 10 min on 3 successive days. On the first day the rats were not infused. On the second day deionized water was infused for 20 s at a rate of 1ml/min, and on the third day a 0.3 M solution of sucrose was infused at the same rate. The Plexiglas tube prevented the rats from moving around and, hence, enabled continuous recording of the head. The films were subsequently analyzed frame-by-frame using a computer connected to the camera.

The following behaviors were recorded. Midline tongue protrusions (MTP), lateral tongue protrusion (LTP) and paw lick (PL) (see Fig 3). MTP is a series of rhythmic forward tongue protrusions and subsequent withdrawals. An LTP is a lateral extension and subsequent withdrawal of the tongue. A PL is scored as the rat licks on its forepaws. MTPs and PLs were recorded in bins of 2 and 5 s,

respectively, whereas each occurrence of an LTP was recorded. These values were combined to a measure of ingestive responses. The behaviors and the recording protocol were adopted from the work of Berridge and Robinson (1998).

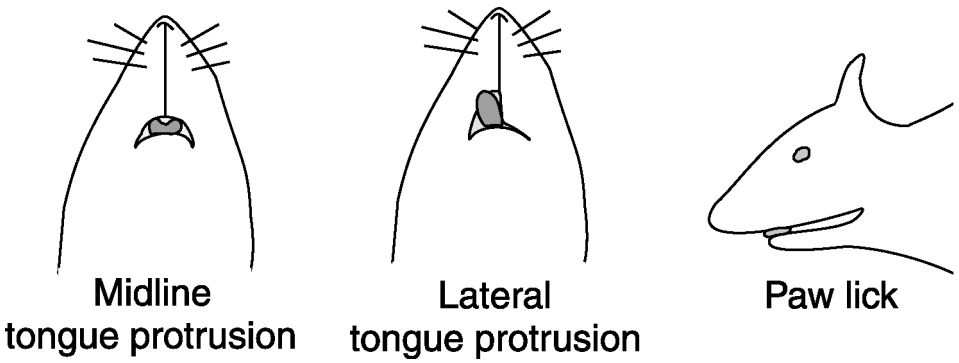

Figure 3. Ingestive responses in the rat. See section 6.4.2.2 for descriptions.

6.4.3 *Test for sexual behavior in male rats*

Male rats were tested for sexual behavior in the same testing arenas used as those used for testing ingestive behavior. After placing the male in the arena a sexually receptive female rat was introduced and mounts, intromissions, intromission latency, ejaculation latency and post ejaculatory interval (Larsson 1956) were recorded. A 7-day period of daily testing was allowed for these behaviors to stabilize. The females were injected subcutaneously with estradiol benzoate (5 µg) and progesterone (0.5 mg) 48 and 6 h before testing, respectively. The hormones were diluted in 0.1 ml sesame oil.

Male sexual behavior was also recorded during simultaneous intraoral infusion of tastants and in a choice situation. In the choice situation, the male rat could chose between copulating with a female and ingest sucrose from a bottle.

6.5 Extraoral infusion and blood sampling

6.5.1 *Intragastric and intraduodenal infusions*

Rats with intragastric silicone cannulæ were infused with sucrose at a rate of 1 ml/min whereas the intraduodenal infusion rate was 0.33 ml/min. The slower rate was selected because rats infused intragastrically empties about one third of the gastric content into the duodenum during a 12 min glucose infusion (Kaplan and others 1992).

6.5.2 *Intravenous sampling and infusion*

Based on the method described by Steffens (1969) blood samples were taken from rats during intraoral intake. Briefly, 0.15 ml samples where withdrawn at regular intervals starting 2 min before the test.

In another test, blood samples were withdrawn during intrajugular vein infusion of glucose. The infusion and blood sampling were performed through the same cannula. To avoid glucose contamination the cannula was flushed with saline before each sample was taken. The samples were centrifuged and the plasma was stored at –70° C until analyzed for glucose concentration using a colometric kit (Waco Chemicals GmbH, Neuss, Germany).

6.6 Determination of brain cannula position

The position of the lateral and 4th ventricular cannulæ were verified by slowly infusing 2 µl of 0.9% saline with a 25 µl Hamilton syringe connected to an injection needle. If the saline infusion was accomplished without resistance the cannula was considered to be in the ventricular space. The position of the cannulæ aiming at the subregions of the NAc was verified after the completion of the experiments. The brains were removed and stored at –70° C for subsequent sectioning with a cryostat. Twenty µm sections were stained with cresyl violet to visualize the brain structures and tissue damage produced by the cannula. The sections were compared to the Paxinos and Watson (1998) brain atlas.

6.7 Drugs and drug administration

The following drugs were used. Dihydrexidine HCl (dopamine D1 agonist, Tocris Cookson, Ltd, Bristol, UK), SCH-23390 (dopamine D1 antagonist, Research Biotechnical Inc (RBI), Natick, MA, USA), quinpirole (LY-171555, dopamine D2 agonist, Sigma-Aldrich Sweden AB, Stockholm, Sweden), raclopride (dopamine D2 antagonist, RBI), porcine NPY (Bachem, Bubendorf, Switzerland).

The drugs were administred by intraperitoneal (ip) injection, intraventricular infusion or intracerebral infusion. Drugs injected ip were dissolved in 0.9% saline and injected in a volume of 1 ml/kg. Drugs infused into the ventricles were dissolved in artificial cerebrospinal fluid (aCSF: 8.98 g NaCl, 0.25 g KCl, 0.14 g CaCl2, 0.11 g MgCl2, 0.07 g NaH2PO4, 0.13 g urea, and 0.61 g glucose, dissolved in triple-distilled water to 1000 ml) or 0.9% saline in a volume of 2 or 5 µl. Infusion was performed slowly by hand using a 25 µl Hamilton syringe and a 28 gauge injection cannula (Plastics One) protruding 1 mm from the guide cannula. Drugs infused into the NAc were dissolved in 1 µl 0.9% saline. Bilateral infusions were performed with an infusion pump (CMA 100, CMA Microdialysis AB, Stockholm, Sweden) at an infusion rate of 0.5 µl/min. A volume of 0.5 µl/side was infused. The 28 G injection cannula protruded 1.8 mm from the guide cannula. The antagonists and agonists were administered 15 and 10 min before the test, respectively. NPY and leptin were administered 10 min and four hours before testing, respectively.

6.8 Analysis of data

In the first study in the present thesis the data were presented as percentage of rats displaying the different behaviors whereas the behavioral frequencies were presented as means and standard errors of the mean (SE). Within-group effects on the number of rats displaying a behavior were analysed using the Cochrans Q-test whereas between-group effects were analyzed using Chi^2 analysis. Within-group effects on the frequencies of the aversive behaviors were analyzed using Friedmans ANOVA followed by Wilcoxon signed rank test whereas between-group effects were analyzed with Kruskal-Wallis ANOVA followed by the Mann-Whitney U-test. Within-group effects on intake volumes and blood-glucose concentration were analyzed using parametric one factor ANOVAs for repeated measures or two factor ANOVAs for repeated measures on one factor. Between-group effects were analyzed with factorial ANOVA. Subsequent pairwise comparisons for the different parametric ANOVAs were made with contrast comparisons.

In the second and fourth study, the results were presented from individual rats to allow inspection of individual values. Medians and ranges were used to describe the results when presented in the text. Gapes and chin rubs were combined as a measure of aversive taste responses, and MTP, LTP and PL were combined to a measure of ingestive taste responses. Before analyzing the data-sets they were tested for homogeneity of variance with Bartlett´s test. In the case of heterogeneity, data-sets were log-transformed and analyzed with a one factor ANOVA for repeated measures or a two-factor ANOVA with repeated measures on one factor followed by contrast comparisons. In cases where log-transformation did not produce homogeneity, data-sets were analyzed with Friedmans or Kruskal Wallis ANOVA and subsequent pair-wise comparisons were made with Wilcoxon signed rank test or Mann-Whitney U-test, respectivley. Differences in proportions of ejaculating rats were analyzed with the Fisher test.

In the third study the results were presented as means and SE and analyzed using a one-factor ANOVA for repeated measures. The subsequent pairwise comparisons were performed with the Scheffé comparisons.

Software for the Macintosh computer: GB-STAT v6.03 Dynamic microsystems Inc, Silverspring, MD,USA; Super-Anova v1.11, Abacus Concepts Inc, CA,USA and Stat-View v5.0 SAS Institute Inc, NC, USA, were used.

7 Results

7.1 Aversive taste responses during intraoral intake.

In exploring the role of taste in the termination of consummatory ingestive behavior we first investigated behavioral markers for an aversive taste. The display of gapes and chin rubs increased with the increasing concentration of an intraorally infused quinine solution. On the contrary, the frequency of headshakes, forelimb flails and orofacial grooming did not (Fig 4). Paw pushes were almost never observed under the conditions of the present study and will therefore not be further considered.

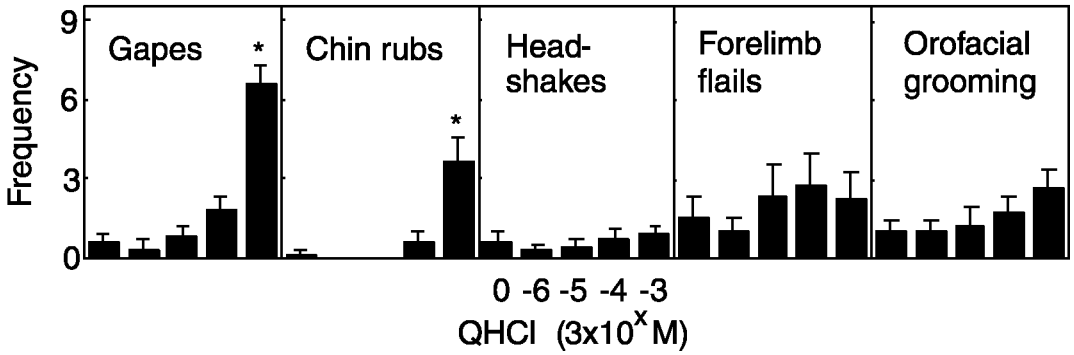

Figure 4. The display of aversive responses elicited by a 20 s intraoral infusion of quinine in male rats. *P<0.02 to all other concentrations. Mean ± SE, n=9.

A sucrose solution did not stimulate these behaviors if briefly infused into the mouth. However stimulating the head with sucrose induced headshaking and orofacial grooming whereas stimulation of the limbs stimulated forelimb flails. By contrast, gapes and chin rubs were only observed when the oral cavity was stimulated with an aversive taste.

These results suggest that gapes and chin rubs are markers of an aversive taste present in the oral cavity. These aversive responses were therefore selected for further studies.

During intraoral infusion of sucrose, rats showed gapes and chin rubs more frequently when a 2-M solution of sucrose was infused than when a 0.5 or a 1-M solution was infused. Gapes and chin rubs were displayed predominantly during the last part of the infusion (Fig 5). These findings generated the hypothesis that the taste of an initially palatable stimulus turns gradually aversive and that this contributes to termination of intake.

The display of gapes and chin rubs was not a consequence of the higher viscosity of the 2-M solution. These responses were not stimulated by a 0.5-M solution rendered as viscous as a 2-M solution by the addition of carboxymethyl cellulose (CMC). Likewise, the addition of low amounts of quinine to a 0.5 M solution did not stimulate gapes or chin rubs, however, intraoral intake was

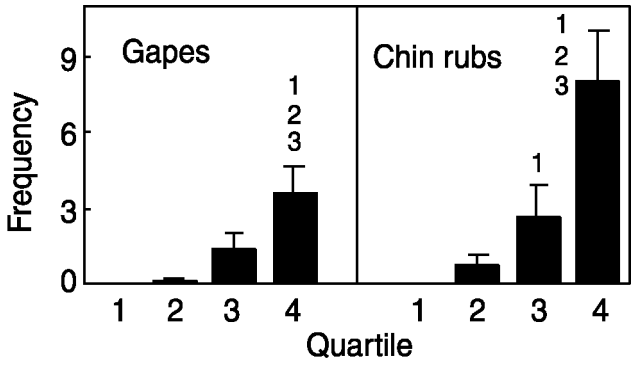

Figure 5. The display of gapes and chin rubs during each quartile of an intraoral infusion of a 2 M sucrose solution in male rats. 1,2,3P<0.04 compared to respective quartile. Means ± SE, n=12.

suppressed. This implicates that aversive properties of the infused solution can inhibit ingestive behavior without overt signs of aversion.

The display of gapes and chin rubs was stimulated by a brief pulse of quinine during ongoing intraoral intake of sucrose. Intake was suppressed if the quinine was presented late during the test. By contrast, the quinine pulse did not abbreviate intake if presented at an early stage of the test, yet it stimulated the display of gapes and chin rubs. This dissociates the display of these responses from meal termination. Conversely, saccharine, a nutritionally inert taste stimulus prolonged intraoral intake if mixed into the sucrose solution and presented after meal termination.

In response to intraoral quinine stimulation and during intraoral sucrose intake gapes and, to a lesser extent, chin rubs were suppressed by pretreatment of the oral cavity with the local anaesthetic Xylocain. Interestingly, intraoral sucrose intake was unaffected by the Xylocain treatment. Adulterating the sucrose solution with quinine suppressed intake by about 50%. This suppression was attenuated by Xylocain treatment.

Intragastric infusion of sucrose produced about the same number of gapes as during the intraoral infusion. Chin rubs were not observed. Intraduodenal infusion of glucose resulted in the display of both gapes and chin rubs. However, neither method of infusion produced an increased frequency of these responses at the end of the test. Sucrose sham feeding, ie, the drainage of the ingested sucrose from the stomach via a gastric cannula, and glucose infusion into the circulation did not stimulate the display of gapes and chin rubs.

Thus, behavioral markers of taste aversion are seen by the end of a period of intraoral infusion of sucrose. This is the consequence of a continuous stimulation of the oral taste receptors and filling of the gastrointestinal tract. Importantly, however, stimulation of the oral taste receptors is not an absolute requirement for the display of gapes or chin rubs.

7.2 Dopamine D2 receptor manipulations: intraoral intake and aversive responses

Dopamine plays a role in terminating consummatory ingestive behavior and possibly also in display of aversive behavior. A study

was first made to determine the relative importance of D1 and D2 receptors in the inhibition of intraoral intake. Another study was then made to investigate the role of D2 receptors in the display of gapes and chin rubs.

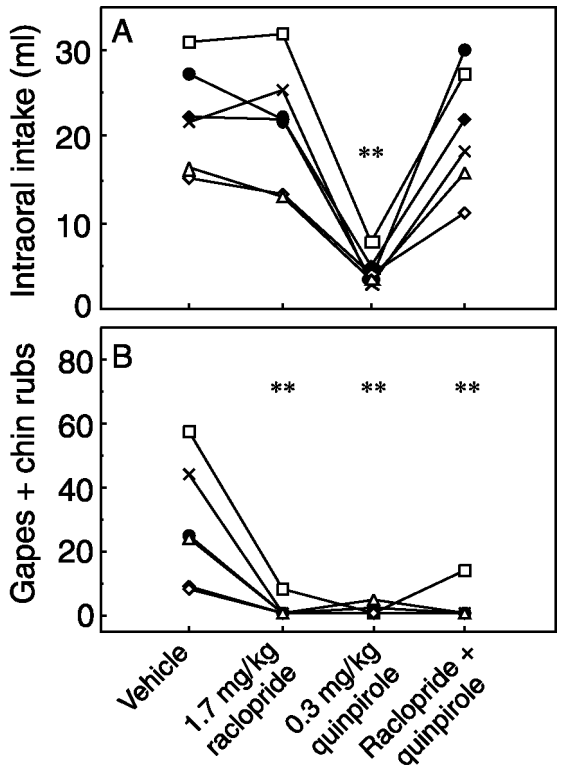

Figure 6. Intraoral intake of a 2 M solution of sucrose (A) and aversive responses during intraoral intake (B) in male rats. Effect of the dopamine D2 receptor antagonist raclopride, injected IP 15 min pretest, and the D2 receptor agonist quinpirole, injected IP 10 min pretest. Symbols represent individual values, **P<0.001 compared to vehicle, n=6.

Quinpirole injected peripherally suppressed intraoral sucrose intake and the display of gapes and chin rubs. Peripheral administration of the D2 receptor antagonist raclopride did not affect intraoral intake but blocked the display of gapes and chin rubs (Fig 6A,B). Intake suppression after quinpirole treatment was blocked by raclopride treatment (Fig 6A).

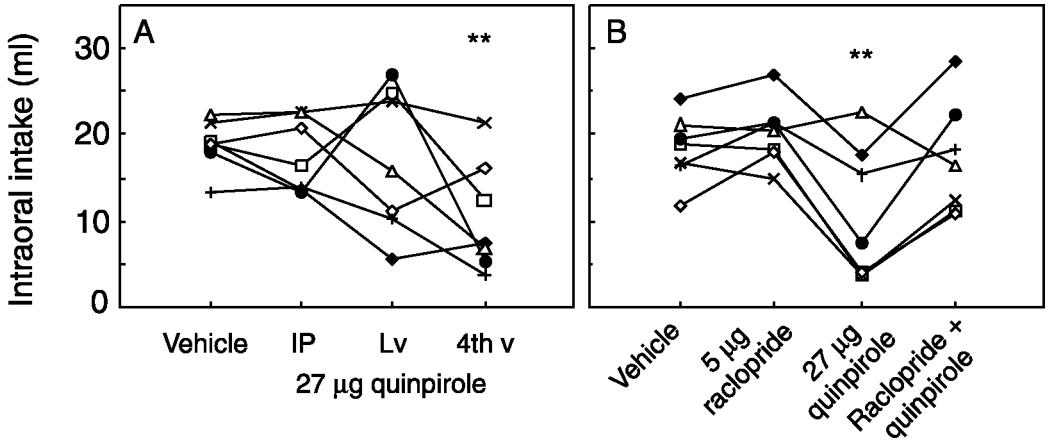

Figure 7. Intraoral intake of a 2 M solution of sucrose. Effect of quinpirole administred IP or into the lateral or 4th brain ventricle (A, n=7). Effect of quinpirole and/or raclopride infused into the 4th ventricle (B, n=7). Symbols represent individual values, **P<0.001 compared to vehicle.

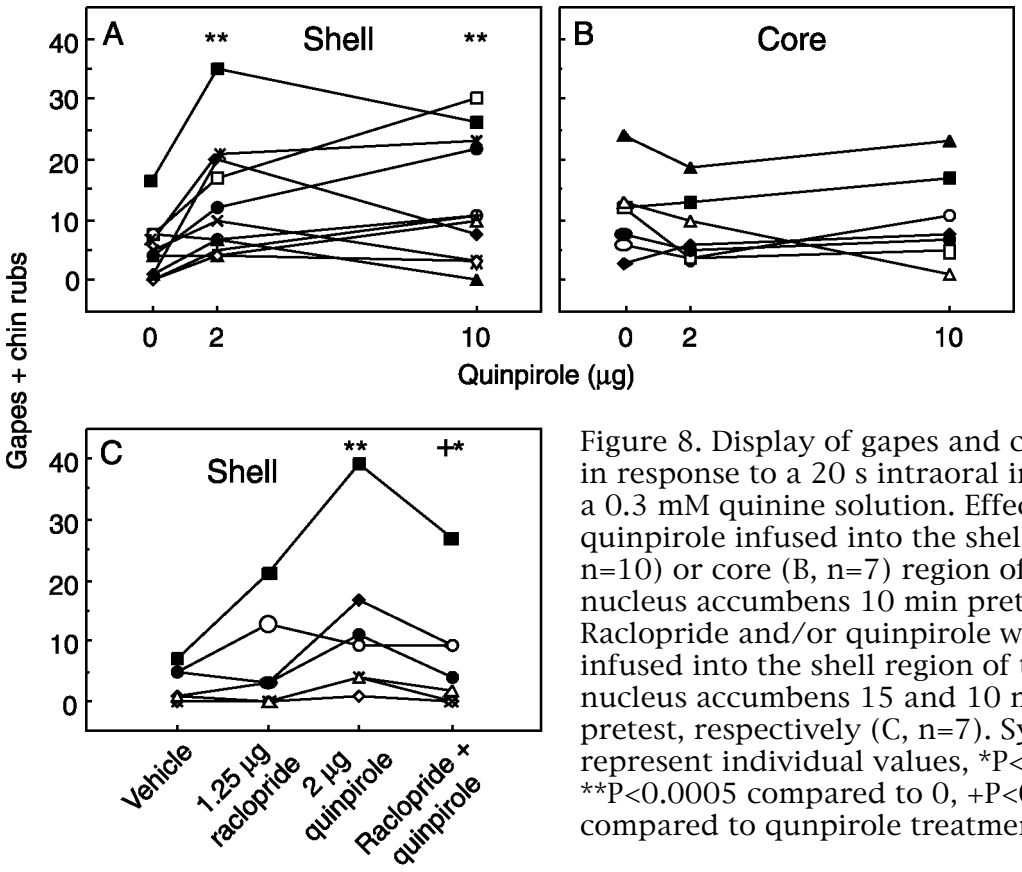

Figure 8. Display of gapes and chin rubs in response to a 20 s intraoral infusion of a 0.3 mM quinine solution. Effect of quinpirole infused into the shell (A, n=10) or core (B, n=7) region of the nucleus accumbens 10 min pretest. Raclopride and/or quinpirole were infused into the shell region of the nucleus accumbens 15 and 10 min pretest, respectively (C, n=7). Symbols represent individual values, *P<0.04 and **P<0.0005 compared to 0, +P<0.01 compared to qunpirole treatment.

Intraperitoneal injection of the D1 receptor agonist dihydrexidine decreased intraoral intake of sucrose. However, pretreatment with raclopride, but not the D1 receptor antagonist SCH 23390, prevented this suppression. The D1 agonist and antagonist treatments did not markedly affect the display of gapes and chin rubs.

Quinpirole infused into the 4th brain ventricle, but not into the lateral ventricle or peripherally, suppressed intraoral intake (Fig 7A). This suppression was blocked after pretreating the rats with raclopride (Fig 7B). The display of gapes and chin rubs did not vary uniformly under these conditions.

After having investigated the role of D2 receptors in the brainstem we turned to the forebrain and the NAc because of its relation to feeding and aversive responses. Quinpirole infused into the shell region of the NAc did not affect intraoral intake of sucrose. On the other hand, quinpirole infused into the shell, but not core, subregion increased the display of gapes and chin rubs stimulated by quinine (Fig 8A, B). Raclopride treatment prevented this effect (Fig 8C).

As mentioned above, peripheral treatment with raclopride and intraaccumbal infusion of quinpirole affected the display of aversive responses but not intraoral intake. To investigate if the drug treatments affected the ability to react to an aversive taste, quinine was added to the sucrose solution. The reduction in intake due to the quinine was unaffected by the drug treatments (Fig 9).

These results show that brainstem dopamine D2 receptors mediate inhibition of consummatory ingestive behavior. The findings also show that the proposed dopaminergic brainstem mechanism is

unrelated to the mechanisms whereby aversive taste-related behaviors are controlled. The expression of these behaviors may instead be modulated by a D2 mechanism in the NAc. None of the proposed mechanisms is likely to play a role in taste evaluation.

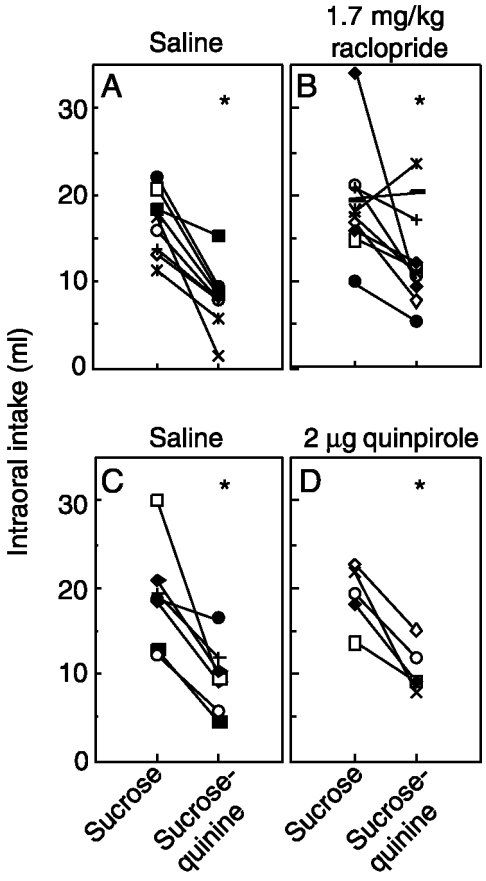

Figure 9. Intraoral intake of a 2 M sucrose solution or a 2 M sucrose-0.15 mM quinine solution. The rats were injected with saline (A) or the dopamine D2 antagonist raclopride (B) IP or with saline (C) or the D2 agonist quinpirole (D) into the shell region of the nucleus accumbens, n=5-10 rats/group. Symbols represent individual values. A, B: main effect of quinine: $F(1,17)=23.68$; $P=0.0001$. C, D: main effect of quinine: $F(1,10)=38.82$; $P=0.0001$). There were no significant effects of the drugs or significant drug X quinine interactions.

7.3 Neuropeptide Y: display of appetitive and consummatory ingestive behavior

Next, the possible role of NPY in appetitive ingestive behavior was examined.

Ingestion from a bottle filled with a 1-M sucrose solution during simultaneous intraoral infusion of the same solution was initiated as the infusion rate was 0.5 ml/min. In this situation, NPY treatment increased the number of visits to, and intake from the bottle and suppressed intraoral intake (Fig 10A). As a consequence, total intake was suppressed.

The rats also visited the bottle if it was empty. In this situation the intraoral intake was suppressed to a similar extent as when the bottle was filled with sucrose (Fig 10B), and the effect was potentiated after NPY treatment.

Leptin treatment had the opposite effects on ingestive behavior compared to those of NPY (Fig 11).

To evaluate the behavioral specificity of these effects, male rats were simultaneously tested for the display of consummatory sexual

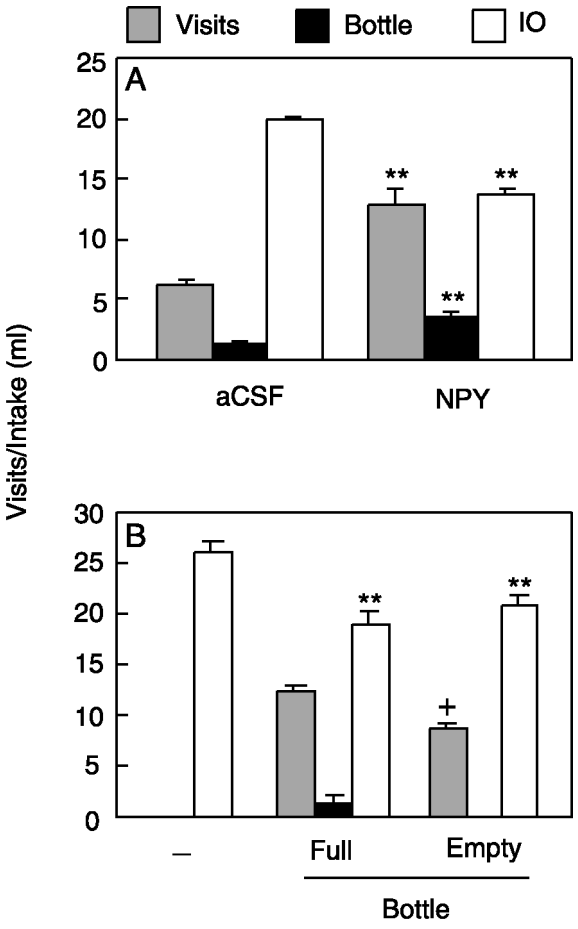

Figure 10. Visits during simultaneous intraoral (IO) and bottle intake of a 1 M solution in female rats. Effect of NPY on intraoral intake (infusion rate, 0.5 ml/min), visits to the bottle, and intake from the bottle (A, n=6). Effect of an empty bottle on intraoral intake and visits (B, n=7). Means ± SE, **P<0.01 compared to aCSF, +P<0.01 compared with full bottle.

behavior and intake from a bottle. When the bottle was absent, NPY treatment had a minor effect on sexual behavior. When the bottle was introduced, however, the rats ignored the sexually receptive female and ingested from the bottle. Leptin treatment, by contrast, suppressed bottle intake and enhanced the display of sexual behavior independent of the presence of the bottle.

These results show that NPY treatment increases intake only in a test that includes appetitive ingestive behavior and that it decreases intake in a test for consummatory ingestive behavior.

7.4 Intake inhibition by neuropeptide Y and the display of appetitive behavior

The testing paradigm introduced above allows differential study of appetitive and consummatory ingestive behavior. Also, treatment with NPY makes selective enhancement of appetitive ingestive behavior possible. On this background, the role of appetitive ingestive behavior and taste in the effect of NPY on consummatory ingestive behavior was examined.

The enhanced number of visits after NPY treatment was blocked if the sucrose solution in the bottle was replaced by a 1-M sucrose-3mM quinine solution. Although this manipulation caused an increase in the simultaneous intraoral intake of sucrose, intraoral intake was still

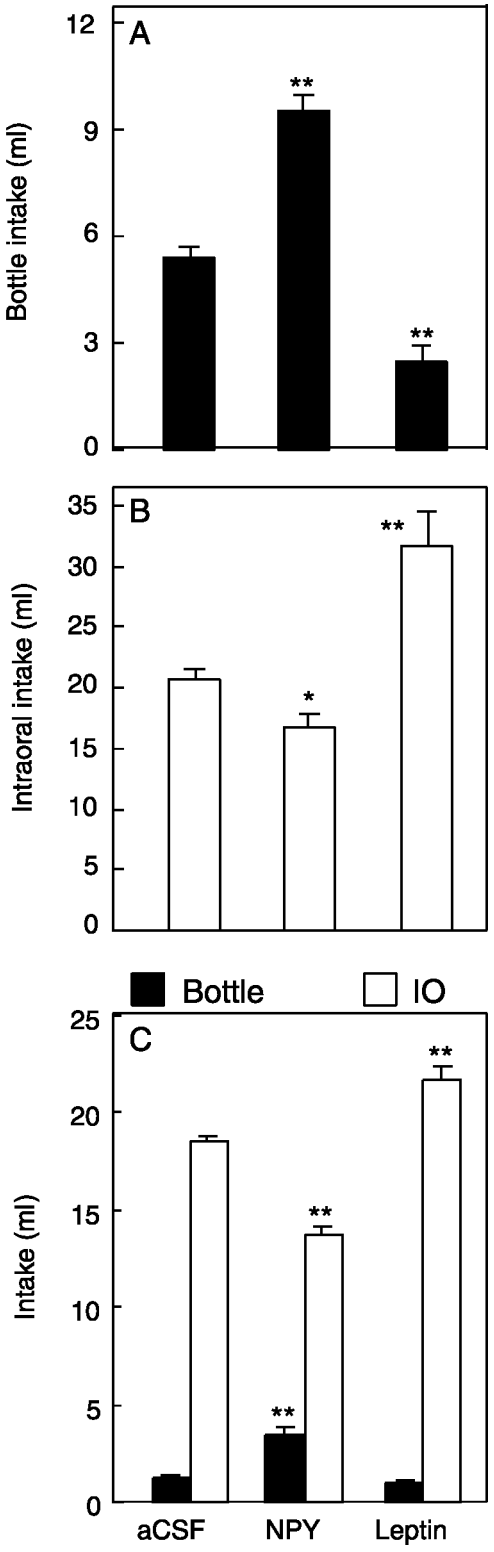

Figure 11. Intake of a 1 M solution of sucrose available from a bottle or infused intraorally (infusion rate, 0.5 ml/min) in 8 rats. Effect of NPY (10 µg icv, 10 min pretest) and leptin (10 µg icv, 4 h pretest) on intake from the bottle (A), intraoral (IO) intake (B), and simultaneous intraoral and bottle intake (C). Means ±SE. **P<0.01 and *P<0.05 compared with aCSF.

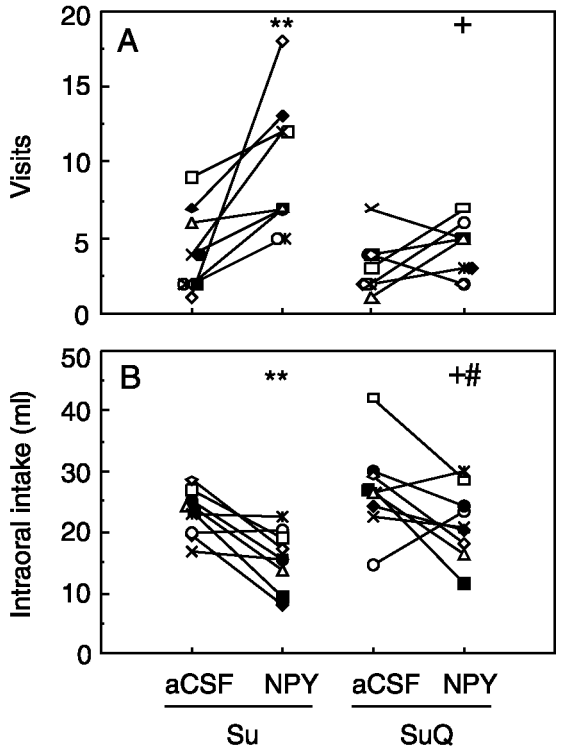

Figure 12. Number of visits (A) to a bottle filled with a 1 M solution of sucrose (Su) or with sucrose and 3 mM quinine HCl (SuQ) and intake from the simultaneous intraoral infusion of sucrose (B, 1 M, infusion rate 0.5 ml/min) in nine female rats. The rats were infused with aCSF or 10 µg NPY intraventricularly 10 min before testing. **P<0.003 compared to aCSF Su, +P=0.011 compared to NPY Su, #P<0.016 compared to aCSF SuQ.

suppressed by the NPY treatment compared to the aCSF treatment (Fig. 12).

Likewise, the blockade of the NPY induced visits to the bottle by quinine adulteration did not reinstate consummatory sexual behavior in male rats.

Treatment with NPY had no significant effect on the display of either ingestive or aversive responses to a brief intraoral infusion of sucrose or quinine. However, there was a suppressive trend on the ingestive responses at the highest sucrose concentration.

8 Discussion

8.1 Aversive taste responses are displayed at the end of intraoral intake

The finding that the display of gapes and chin rubs increase with the concentration of the intraoral quinine stimulus is consistent with a previous report (Schwartz and Grill 1984). Headshakes, forelimb flails and orofacial grooming have been suggested to be taste dependent (Berridge and Robinson 1998). Here, however, these responses did not correlate with the quinine concentration. Moreover, they were displayed in response to extraoral stimulation with sucrose, and sucrose did not elicit these behaviors if infused into the mouth. Therefore, we suggest that headshakes, forelimb flails and orofacial grooming are markers of an aversive stimulus, but not necessarily a taste stimulus. Gapes and chin rubs, on the other hand, were only observed when a quinine solution stimulated the oral cavity. Hence, gapes and chin rubs are likely to be specific behavioral markers of an aversive taste in the mouth. Together, these findings gave us reason to include only gapes and chin rubs when investigating aversive taste aspects during intraoral sucrose intake.

Gapes and chin rubs are displayed during the last part of an intraoral infusion of a 2-M solution of sucrose. This solution is equally potent in stimulating ingestive behaviors as solutions with lower molarities (Flynn 1995) suggesting that this high molarity is not initially aversive to the rat. Neither does the display of gapes and chin rubs depend on the higher viscosity of the 2 M solution since the increased viscosity produced by the addition of CMC to a 0.5 M solution failed to stimulate these responses. Intake of the 0.5-M solution terminated without a display of aversive responses. Interestingly, the intake of this molarity was suppressed by the addition of quinine, but also here, in the absence of aversive responses. This suggests that the suppression of intraoral intake induced by an aversive taste is not necessarily accompanied by the display of overt signs of taste aversion.

Intraoral Xylocain treatment seemingly suppressed the aversive taste properties of quinine but did not influence the intake of sucrose. The reason why sucrose intake remained unaffected may be because the rats were used to receiving daily sucrose infusions, ie, they had learned to ingest a certain amount (Weingarten 1984). Alternatively, it has been shown that local anaesthetics applied in the oral cavity are more likely to affect responses to quinine than to sucrose (von Skramlik 1962).

The finding that gapes and chin rubs were displayed during intragastric and intraduodenal infusions shows that oral taste receptors are not necessary for the display of these responses. Interestingly, however, during gastrointestinal infusion the responses did not increase toward the end of the infusion. Continuous stimulation of the oral taste receptors and the gastrointestinal tract is

therefore required to produce an increase in the display of both gapes and chin rubs at the end of the infusion. Also, the aversive responses during intragastric and intraduodenal infusions may correspond to a phenomenon referred to as “dumping” in humans (Roberts 1967). “Dumping” is the nausea that is associated with the rapid emptying of the stomach.

Based on these results, we suggest that the taste of an intraorally ingested solution of sucrose becomes aversive during the meal and that this contributes to the regulation of meal size. While it is tempting to speculate that this also occurs during free feeding conditions, this possibility remains to be investigated.

8.2 Dopamine D2 receptors regulate meal termination and aversive responses at different neural sites

The finding that there is an increase in the display of aversive behavior related to taste by the end of an intraoral infusion of a sucrose solution generated the following hypothesis. Rats stop ingesting food because of negative feedback from gastrointestinal secretions, CCK-8 in particular (Smith and Gibbs1998). Bednar and collegues (1991; 1992a; b; 1994; 1995) developed the hypothesis that CCK-8 terminates intake by causing release of dopamine and glutamate in the NTS. The presence of D2 receptors in the NTS including its gustatory part (Qian and others 1997) opened the possibility that D2 receptor in the NTS mediate inhibition of intraoral intake.

However, in a previous study (Bednar and others 1995), both D1 and D2 receptor stimulation suppressed intraoral intake of a sucrose solution. It was therefore of interest to re-investigate the influence of D1 receptors in the inhibition of intraoral intake using a more specific agonist and antagonist. To this aim, dihydrexidine was found to suppress intake but treatment with SCH-23390 did not block this effect. On the contrary, raclopride treatment partially blocked the dihydrexidine-induced suppression. This implies, therefore, that the effect of dihydrexidine is, at least partially, due to an action on D2 receptors. This is consistent with the suggested D2 receptor mediation of the behavioral effects of dihydrexidine treatment reported by Darney and collegues (1991).

The decrease of aversive responding after peripheral D2 receptor agonist/antagonist treatment indicates that these receptors are crucial for the expression of aversive responses and possibly also for taste processing. The presence of D2, but not D1 receptors, in the NTS (Qian and others 1997) together with their overlap with the termination area of primary taste input led us to investigate the effects of D2 receptor drug treatment of this region on intraoral intake. In line with our expectations, 4th ventricular infusion of quinpirole produced a raclopride-reversible suppression of intraoral intake. As quinpirole administered at other bodily sites had no effect, these findings suggest that the effect is mediated specifically via D2

receptors in the brainstem. Naturally, the precise site of action of quinpirole within the brainstem remains to be determined since fourth ventricular infusion merely provides preliminary evidence. Contrary to the intraoral intake, the display of aversive responses during intraoral intake was not affected consistently by the D2 receptor manipulations.

These observations suggested that inhibition of intraoral intake and display of gapes and chin rubs are controlled at separable neural loci. An attempt was thereafter made to elicit aversive taste behaviors by stimulating the forebrain with quinpirole. We turned to the shell region of the NAc, because reports had indicated that this brain area may be a target for dopamine agonists in the control of aversive behavior (Prinssen and others 1994, Wyvell and Berridge 2000). In line with this, quinpirole stimulating the shell region of the NAc was demonstrated to amplify the number of gapes and chin rubs in response to oral stimulation with quinine. The increased responding after quinpirole treatment is probably due to a sensorimotor effect since this treatment did not potentiate intake suppression after quinine-adulteration of a sucrose solution. The same result was obtained after peripheral raclopride treatment. If these treatments had changed the perception of quinine, intake of a sucrose-quinine solution should be affected.

A possible neural substrate relaying the enhanced motor output was outlined by Swanson and collegues (1984) who suggest that the NAc is influencing motor behavior through a pathway traveling via the substantia innominata to the “mesencephalic locomotor region”. This region may include or be connected to the mesencephalic trigeminal nucleus, which gears orofacial motor behavior (Travers 1995, Fay and Norgren 1997).

Release of dopamine in the NAc in association with ingestive behavior has been demonstrated many times (Le Moal and Simon 1995, Bassareo and Di Chiara 1999). More interesting than these correlations, it was recently demonstrated that enhancement of dopamine release in the accumbens increased food intake (Hajnal and Norgren 2001). However, the effect could not be attributed either to the D1 or the D2 receptor subtype and was suggested to be a pharmacological rather than a physiological one. Correspondingly, we found no effect of quinpirole stimulation of the shell subregion on intraoral intake.

Taken together, the results suggest that a D2 receptor dependent mechanism in the brainstem contributes to the termination of consummatory ingestive behavior and that a D2 receptor population in the shell region of the NAc mediates the display of gapes and chin rubs. Neither mechanism is likely to play a role in taste evaluation.

8.3 Neuropeptide Y differentially affects appetitive and consummatory ingestive behavior

Having examined the role of taste and dopamine in the regulation of consummatory ingestive behavior, we here turned to studying the role of NPY in the control of appetitive and consummatory ingestive behavior. To study this role, a procedure to investigate appetitive and consummatory behavior simultaneously was first established. Inspiration to these investigations were provided by an interesting study by Seeley and others (1995) which indicated that NPY stimulates food intake only in tests which require that the rat shows appetitive ingestive behavior.

Rats treated with NPY increased the display of appetitive behavior and as a consequence, intake from a bottle was increased. Interestingly, simultaneous intake of an intraorally infused sucrose solution was decreased. This was not merely a compensation for the increased bottle intake since the total intake was suppressed. We suspected that the reduced intake was dependent upon the increased display of appetitive behavior *per se* since the mere visiting of an empty bottle decreased intraoral intake to an extent similar to that when the bottle was filled with sucrose. Moreover, as visiting the empty bottle was enhanced by treatment with NPY, the suppression of intraoral intake was potentiated. This observation indicates that appetitive behavior inhibits consummatory behavior.

We then examined the role of leptin, because leptin is thought of as having behavioral effects opposite to those of NPY, most likely because the synthesis NPY by the brain is controlled by leptin (Stephens and others 1995, Satoh and others 1997, Ahima and others 1999). Treating rats with leptin produced a small suppression of bottle intake and, somewhat unexpectedly, a significant simultaneous increase in intraoral intake. The enhancement of intraoral intake was not just a compensation for the decreased bottle intake since total intake increased.

Thus, NPY and leptin have opposing effects on appetitive and consummatory ingestive behavior. A recent study suggests that the suppression of intraoral intake after NPY treatment is dependent on the noradrenaline synthesizing neurons in the locus coeruleus (Ammar and others 2001).

We then asked the question whether the effects of NPY are specific for behavioral responses related to ingestion. To investigate this issue, we examined sexual behavior, which is often the mirror image of ingestive behavior (Kaplan and others 1992). Interestingly, NPY and leptin were found to have opposing effects on sexual behavior. Hence, male sexual behavior was suppressed by NPY, but only when the rats had the opportunity to ingest a solution of sucrose from a bottle. Leptin, on the other hand, potently increased the display of sexual behavior independently on the presence or absence of a bottle.

8.4 Intake inhibition by neuropeptide Y is independent of appetitive behavior and taste

The simultaneous measurement of appetitive and consummatory ingestive behavior outlined above offer the opportunity to investigate interactions between the appetitive and consummatory phases in the control of ingestive behavior. Having examined the role of taste in consummatory ingestive behavior we here turned to its possible role in appetitive ingestive behavior.

Treatment with NPY did not stimulate visiting of a bottle if the content of the bottle was aversive. As a consequence, the simultaneous intraoral intake increased. However, intraoral intake was reduced by the NPY treatment compared to the vehicle condition even when appetitive responding had been attenuated. Thus, NPY reduces consummatory ingestive behavior independently of its stimulatory effect on appetitive ingestive behavior.

It was demonstrated above that male sexual behavior was suppressed by NPY treatment if the rats had the opportunity to ingest from a bottle. In this situation sexual behavior was not reinstated by making appetitive ingestive behavior an unpleasant behavioral option by adding quinine to the sucrose in the bottle. Thus, while blockade of appetitive ingestive behavior causes an increase in consummatory ingestive behavior it does not indiscriminately reinstate any consummatory behavior.

Berridge (1996) has suggested that motivated behavior is initiated if the animal “wants” a stimulus. However, an animal may “want” something yet not “like” it. In this framework, NPY enhances “wanting”, ie, the actions to obtain a stimulus (the sucrose in the bottle). Using the categorization of Berridge, the results presented here suggest that NPY treatment increases the “wanting” but not “liking”. This since NPY treatment did not increase the ingestive responses of the sucrose stimuli. Contrarily, the number of ingestive responses tended to decrease in response to the most concentrated sucrose solution after NPY treatment, however, this effect was not statistically significant.

While investigating the taste-NPY interactions in the regulation of ingestive behavior our most remarkable finding was that the proposed orexigen NPY acts as an intake inhibitor. NPY exerts this effect in the absence of an influence on taste-related behavior. Equally interesting, however, we found that NPY potently stimulates appetitive ingestive responding. The possibility that NPY, a messenger in the brain that is increased in situations of food depletion, specifically affects appetitive ingestive behavior is an attractive alternative to “NPY-orexigen” hypothesis. The latter hypothesis fails to explain why some human patients, eg anorexics, with a minimum of stored fat and, therefore, low circulating leptin and supposedly high cerebral levels of NPY (Gendall and others 1999) do not eat.

8.5 Methodological considerations

The advantages of the methods used in the present thesis have been argued for above. However, naturally, they have drawbacks as well. The intraoral infusion test, for example, delivers a nutrient at a constant rate, which rarely would occur outside the laboratory. Adjusting the rate of infusion to the ad lib-rate would perhaps produce a more realistic meal, however, it would simultaneously limit the rats capability to adjust its intake after experimental manipulations. It would, of course, be possible to introduce an operant, but then the test would also be a test for appetitive behavior.

The display of gapes and chin rubs is highly variable, both within and between subjects. This urges the use of within group-designs and to study effects with large response amplitudes. Future studies on taste reactivity may benefit from parametric evaluations of their stimulus specificity, both for the aversive and for the ingestive responses.

The local cerebral infusions may intuitively be appealing, but the control over the spread to neighboring regions is limited. In particular, as the cannulæ to the shell region of the NAc is in close proximity to the ventricle, the possibility of spread to more distant regions should not be discarded. In this case, an additional control infusion into the lateral ventricle might have been useful. Also, pharmacological strategies that increases the intrinsic neurotransmitter availability in the synapse, eg amphetamine, and block these effects with selective antagonists may provide physiologically relevant results (see Hajnal and Norgren 2001). As an alternative to the pharmacological approach we performed small-scale attempts to modify behavior through the use of more modern techniques modulating gene-expression. However, while failing to produce behavioral effects this does not mean that the same approach will be unsuccessful in future studies.

8.6 Concluding remarks and some speculations

The suggestion that taste contributes to meal termination is consistent with the theory that nutrient repletion produces satiety in a sensory specific manner (Rolls 1986, Rolls and others 1989) and with the concept of alliesthesia (Cabanac and others 1971; 1991; 1992). This implies that taste processing is dynamic and adjusts rapidly to a changing physiological state during the course of a meal. Modulation of this sort may be accomplished by circulating factors (Giza and Scott 1983, Gilbertson 1998, Herness and Gilbertson 1999) or efferent neural influence (Brush and Haplern 1970, Hellekant 1971, Wang and others 1995) that in turn may be influenced by visceral afferent activity (Norgren 1983). This hypothesized mechanism would form an important and adaptive proximate cause for the termination of ingestive behavior. Studies on peripheral

gustatory nerve activity, both efferent and afferent, may provide further information regarding this issue.

Leptin transmission has been reported to affect peripheral gustatory activity (Ninomiya and others 1995, Kawai and others 2000). Thus, investigating taste responses after leptin treatment appears worthwhile. In parallel, it would be interesting to investigate the effect of leptin in situations were a minimum of appetitive effort is required. That is, to mimic the effect of leptin on intraoral intake in free feeding conditions, perhaps including the ingestion of palatable diets, eg cafeteria feeding.

The present findings on the involvement of brainstem D2 receptors in the inhibitory control of consummatory ingestive behavior extend previous results on intake suppression after apomorphine treatment in decerebrate rats (Kaplan and Södersten 1994, Södersten and others 1996). Here we added that forebrain D2 receptors in the shell region of the accumbens apparently do not contribute to meal size regulation, which is consistent with the recent results of Hajnal and Norgren (2001). In accordance with others (Berridge and others 1989, Berridge 2000), none of the dopaminergic mechanisms - the control of consummatory ingestive behavior and the expression of aversive responses - were found to be involved in taste processing. It is an interesting topic for further study to integrate these various aspects of ingestion with the emerging knowledge on the neural concommittants of taste evaluation (Söderpalm and Berridge 2000, Spector 2000, Zhang and Kelley 2000).

Craig (1918) viewed aversive behaviors as analogous to appetitive behaviors. Also, more recent authors have suggested that appetitive and aversive responses may be controlled by a common mechanism in that they both promote life-sustaining activities (Ikemoto and Panksepp 1999). In this perspective, aversive responses resulting from dopamine receptor stimulation in the accumbens might correspond to the dopamine stimulated increase in operant responding (Taylor and Robbins 1984). Such enhanced appetitive responding has never been coupled to an increase in actual intake. In most operant situations the rat eagerly pressing a lever for a food reward is merely allowed to consume a small pellet or two. It would be interesting to examine if such enhanced appetitive display might also decrease the total amount ingested in rats offered more than a pellet or two.

In an evolutionary perspective the opposing effects of NPY and leptin on ingestive and sexual behavior is not surprising. When food supply is scarce and body-fat content is suppressed, ie, low leptin and elevated NPY levels, the primary goal may not be to consume the food the animal encounters. Rather, it is more adaptive to hoard food and chose not to reproduce. Interestingly, hoarding, rather than eating, food may be the primary response to a loss of body weight (Cabanac and Swiergiel 1989). We suggest, therefore, that these peptides are involved in a process by which an animal selects its strategy for feeding and reproduction.

It seems likely that the regulation of appetitive ingestive behavior

rather than that of the consummatory ingestive behavior is under evolutionary pressure. It appears axiomatic that consummatory behavior does not occur in the absence of an appetitive phase, ie, consummatory behavior follows more or less passively on appetitive behavior. Hence, in conditions where appetitive behavior is stimulated, there would be no benefit for the animal to upregulate its consummatory responses. Perhaps, although highly speculative, it might even be maladaptive.

The outcome of the experimental treatments in the present thesis

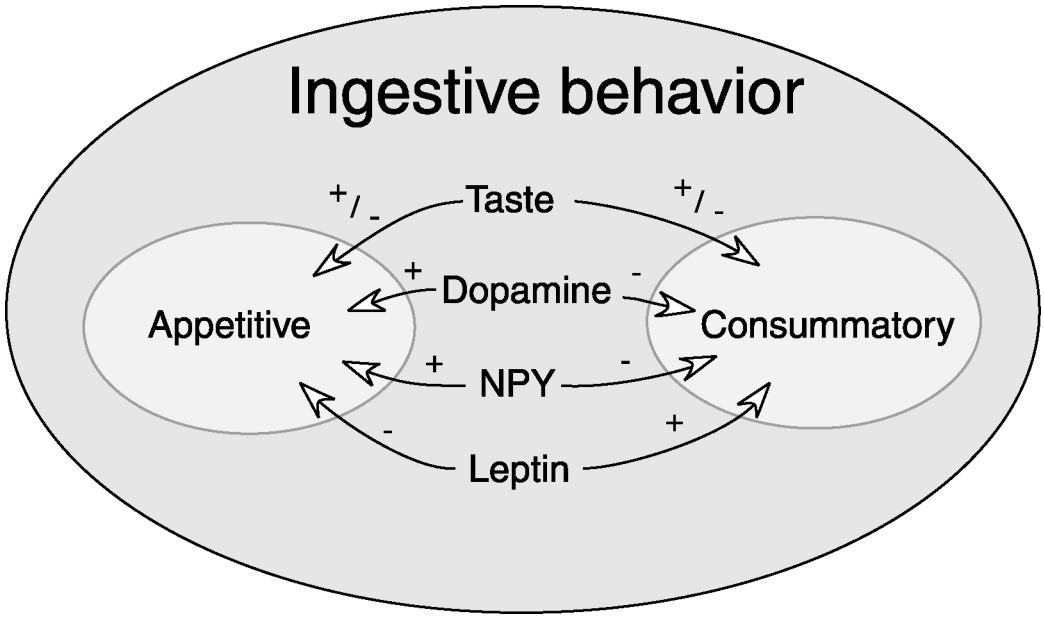

Figure 13. The influence of taste, dopamine, NPY and leptin of the appetitive and consummatory aspects of ingestive behavior.

has been largely dependent on the testing paradigm (see Fig 13). To consider the appetitive-consummatory categorization in future studies of ingestive behavior will most certainly be fruitful.

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