Published in final edited form as:
Behav Neurosci. 2010 April ; 124(2): 225–233. doi:10.1037/a0018928.

Suppression of 3rd Ventricular NPY-Elicited Feeding Following Medullary Reticular Formation Infusions of Muscimol

Joseph B. Travers, **Kenneth Herman**, and **Susan P. Travers**
Division of Oral Biology, College of Dentistry, The Ohio State University, 305 W 12th Ave, Columbus, OH 43210

Abstract

The appetitive component of feeding is controlled by forebrain substrates but the consummatory behaviors of licking, mastication and swallowing are organized in the brainstem. The target of forebrain appetitive signals is unclear but likely includes regions of the medullary reticular formation (RF). The present study was undertaken to determine the necessity of different RF regions for mastication induced by a descending appetitive signal. We measured solid food intake in response to third ventricular (3V) infusions of the orexigenic peptide, neuropeptide Y 3-36 in awake, freely-moving rats and determined whether focal RF infusions of the $GABA_A$ agonist muscimol suppressed eating. Reticular formation infusions were centered in either the lateral tegmental field, comprised of the intermediate (IRt) and parvocellular (PCRt) RF, or in the nucleus gigantocellularis (Gi). Infusions of NPY 3-36 (5 ug/5ul) into 3V significantly increased feeding of solid food over a 90 minute period compared to the non-infused condition (4.3 ± 0.56 versus 0.57 ± 0.57g, $p < .001$). NPY 3-36 induced food intake was suppressed (1.7g ± 0.48) by simultaneous infusions of muscimol (0.6 mM/100 nl) into the IRt/PCRt ($p < .01$). Coincident with the decrease in feeding was a decrease in the amplitude of anterior digastric muscle contractions in response to intra-oral sucrose infusions. In contrast, infusions of muscimol into Gi had no discernible effect on food intake or EMG amplitude. These data suggest that the IRt/PCRt is essential for forebrain-initiated mastication but that the Gi is not a necessary link in this pathway.

Keywords

mastication; central pattern generator

Feeding is controlled by structures located in widespread regions of the brain (Broberger, 2005) (Berthoud, 2002; Morton, Cummings, Baskin, Barsh, & Schwartz, 2006) (Saper, Chou, & Elmquist, 2002). Within this distributed system are certain critical nodes that subserve specific functions. One node, the hypothalamus, is sensitive to humoral and neural signals reflecting homeostatic state and receives numerous other inputs; e.g., from the nucleus accumbens, a forebrain structure implicated in reward (Kelley, Baldo, & Pratt, 2005). Many of the integrative capacities of the hypothalamus are shared by the caudal nucleus of the solitary tract in the medulla (Grill, 2006), but an animal reliant solely on the brainstem does not feed

Correspondence to: Dr. Joseph B. Travers, College of Dentistry, 305 W 12 Ave, Columbus, OH 43210, Telephone: (614) 292 -6365, FAX: 614 2476945, Travers.1@osu.edu.

spontaneously when deprived (Grill & Norgren, 1978a). For this reason, the appetitive component of feeding is thought to be organized in the forebrain. Critically, however, the forebrain structures that monitor energy balance, generate craving, and sense the rewarding properties of food must at some point interface with substrates that actually produce the behavior of eating. Compared to the remarkable progress in unraveling the intricate circuitry of the hypothalamus, relatively little is known of these pathways. It is generally accepted that the lower brainstem contains the neural circuitry responsible for the generation of consummatory behavior but the precise locations of the substrate underlying the various components of this complex act are still debated.

Although there is general agreement that a central pattern generator (CPG) for swallowing is localized to the caudal nucleus of the solitary tract and the reticular formation near nucleus ambiguus (Jean, 2001), there is less consensus on the location(s) for the CPGs for licking and mastication. Based on studies of fictive mastication elicited by cortical (orbital cortex) stimulation, a pathway from nucleus gigantocellularis (Gi) to the lateral tegmental field, composed of the intermediate (IRt) and parvocellular (PCRt) reticular formation of the medulla, is hypothesized to be essential for mastication (Nakamura & Katakura, 1995). Other studies, however, have observed rhythmic motor trigeminal activity in an *en bloc* preparation devoid of the medulla, suggesting a pontine locus for a masticatory CPG (Kogo, Funk, & Chandler, 1996; Kogo, Tanaka, Chandler, & Matsuya, 1998; Lund, Kolta, Westberg, & Scott, 1998; Tanaka, Kogo, Chandler, & Matsuya, 1999). Indeed, these latter studies suggest that the medulla may be inhibitory to the expression of masticatory movements; i.e. blocs that included successively more medulla showed smaller-amplitude rhythmic activity (Tanaka *et al.*, 1999). Thus, it is actually unclear whether the medulla is excitatory or inhibitory to mastication.

To more directly assess the role of the medulla in mastication, we measured food intake in an awake, freely-moving preparation following infusions of the GABA agonist muscimol into selected regions of the medullary RF. Previous studies in awake animals suggested that laterally placed muscimol infusions in the IRt/PCRt suppressed licking induced by intraoral stimulation, but that inactivation of the Gi had no effect (Chen & Travers, 2003; Chen, Travers, & Travers, 2001). These studies are consistent with anatomical studies indicating that neurons in the IRt constitute a major source of projections to the hypoglossal nucleus (Holstege, Kuypers, & Dekker, 1977; Travers & Norgren, 1983). However, the functional inactivation studies with muscimol only measured licking of fluids, limiting generalizations regarding the mastication of solid food and because the neuroanatomical studies suggest that the IRt/PCRt is a major source of projections to the motor trigeminal nucleus as well, we wanted to clarify its role in mastication. In addition, because intraoral stimulation can induce consummatory behavior in decerebrate preparations, its use in awake, intact rats leaves open the question of whether the IRt/PCRt or GI are essential for consummatory behavior initiated by a forebrain “appetitive” stimulus. Thus, the present study combined infusions of muscimol into selected RF regions with 3rd ventricle (3V) infusions of NPY 3-36, a highly orexigenic compound (Flynn, Plata-Salaman, & Ffrench-Mullen, 1999; O’Shea *et al.*, 1997).

METHODS

Surgical procedure

Sixteen adult Sprague-Dawley rats (252 – 404 g) were anesthetized with pentobarbital sodium (Nembutal; 50 mg/kg ip) and fit with intraoral (IO) cannulae for delivery of fluid solutions into the oral cavity. The cannulae were guided into oral mucosa and exited via an incision on the skull (Grill & Norgren, 1978b). With a ventral approach, bipolar electromyogram (EMG) electrodes made of twisted fine wires (67 μm, NiCr) insulated except for 0.5 mm at the tip were implanted in the anterior digastric (AD; jaw opener) (Travers & Norgren, 1986). In several cases, we adapted the technique of Pearson and colleagues (Pearson, Acharya, & Fouad,

2005) for sewing the electrodes into the muscle, which we have now found to be a superior technique. With either method, leads from the EMG electrodes were guided through a subcutaneous path to the top of the head and attached to an Amphenol connector. Hindlimb areflexia was used as an index of the surgical level of anesthesia, and supplemental Nembutal was administered when needed. A DC electric body warmer maintained body temperature at 37°C throughout the surgery. After implantation of intra-oral cannulae and EMG electrodes, the head of the rat was fixed in a conventional stereotaxic instrument with blunt ear bars and the skull leveled with respect to bregma and lambda. After removal of a small portion of bone 4 mm posterior to lambda, the dura was removed and two stainless guide cannulas (26 gauge, 24 mm) were positioned either symmetrically in the lateral medullary RF at the level of the rostral nucleus of the solitary tract (rNST) or a single cannula was placed along the midline at the same depth. To aid in accurate cannula placement, the rNST was first identified by recording unit responses to a taste mixture applied to the tip of the tongue.

During subsequent placement of the guide cannula, a fine tungsten electrode extending through the cannula could record neural activity to re-identify landmarks such as the fourth ventricle, the surface of the brainstem, and the rNST to assure accurate placement into the RF. For injections into the 3rd ventricle (3V), a 3rd stainless steel guide cannula (26 gauge, 24 mm) was placed stereotaxically along the midline 2.3 mm caudal to bregma at a depth of 8 mm ventral to the brain surface. All locations were subsequently verified histologically. The guide cannulae, as well as the IO cannulae and Amphenol strip connector, were secured to the skull with dental acrylic. Thirty-three-gauge stainless steel tubing was used as a stylet. The incision was closed with wound clips, and rats were given penicillin G procaine (30,000 U im daily) for 3–4 postoperative days. During the recovery and subsequent testing period, rats were fed with standard rat chow and also had a mixture of powdered rat chow and Crisco (“mash”, see recipe below) available to enhance weight gain after surgery and to accustom them to the food that would be presented during test sessions. All procedures were approved by the Institutional Animal Care and Use Committee and conformed to the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society.

Testing

Testing occurred during the light phase of the light-dark cycle in a Plexiglas chamber (24.5-cm diameter 26.5 cm) that allowed the rat to move freely. After 4 days of recovery from surgery, rats were adapted to the observation chamber for 2–3 days prior to testing.

There were 4 test sessions that took place on consecutive days except for a 48 – 72 hr break between sessions three and four. A test session consisted of 8 fifteen minute blocks (fig. 1). Each block began with access to palatable wet mash (27 g crumbled rat chow plus 11 g Crisco) presented in a small dish. At the end of the 15 minute period, the dish was removed and weighed to determine the amount of food consumed and the rat was presented with intraoral (IO) taste stimulation consisting of a 50-μl infusion of sucrose (0.1 M) and 3 water rinses. After the water rinses the food dish was placed back in the test chamber for the next 15 minute block. The first test session was a baseline session with no drug infusions. Test sessions 2–4 included intracranial drug infusions given between blocks 2 and 3. The second test session consisted of infusing NPY 3-36 (5 μg/5μl) into 3V. NPY 3-36 was selected among several potential NPY agonists because of its high potency in increasing feeding when infused into the 3V (Flynn, Turrin, Plata-Salaman, & Ffrench-Mullen, 1999;O’Shea *et al.*, 1997). The third test session consisted of simultaneous infusions of NPY 3-36 into 3V and muscimol into the medullary RF. Infusions were made bilaterally into the IRt/PCRt (0.06nmol/100nl – .09 nmol/150 nl) or a single infusion into Gi (0.06nmol/100 nl – 0.12 nmol/300 nl) or, in one instance a bilateral midline infusion (0.09 nmol/150 nl). The fourth test session was again NPY 3-36 into 3V with a saline infusion into the medullary RF. With this protocol we could measure food intake and

EMG responses both within a session, i.e. compare pre- and post-drug, and across sessions, i.e. drug, no drug, saline.

Recording and measurement

The procedures for EMG recording were similar to those described previously (Chen *et al.*, 2001; Travers & Norgren, 1986). On the test day, the rat was placed in the observation chamber for 1 h before testing and the Amphenol connector on the head was mated to a cable that connected the EMG electrodes to conventional AC amplifiers. The EMG signals were monitored and recorded online through a CED interface (Power 1401, Cambridge Electronic Design) and stored in a microcomputer. After EMG responses and food consumption were obtained for the first two blocks, the stylets were removed and infusion cannulae containing drug or saline were inserted into the brain via the guide cannulae. Infusers constructed from 33-gauge stainless tubing extended 0.5–1.0 mm beyond the guide cannula, and the other end was fitted to PE-10 tubing. The PE-10 tubing was attached to a 10-μl syringe (Hamilton) that was driven by a syringe pump (KD Scientific). NPY 3-36 was delivered at a rate of 1 μl/min; muscimol at a rate of 100 nl/min. After infusion of the drug or saline into the brain, the infusers were left in place for 30 s and then removed. During the test session, the animals' behaviors were monitored and videotaped. At the termination of the experiment, Fluorogold (2%) was injected to mark the infusion sites. Under deep anesthesia with Nembutal (150 mg/kg), the rat was perfused transcardially with 0.9% saline followed by 10% formalin. The brain was removed, sectioned (50 μm) into two series, and mounted. One series was stained with cresyl violet for identification of neural structures, the other series was merely dehydrated and cleared to preserve the fluorescence. Injection sites were identified with fluorescent microscopy.

Data analysis

Using custom software written for the CED Spike2 system, EMG activity was rectified and filtered at 80 Hz, and baseline activity from 1 s before oral responses was calculated from a number of trials and used to set a threshold (Chen & Travers, 2003; Chen *et al.*, 2001). Crossing the threshold defined a contraction onset, and recrossing the threshold defined a contraction offset. Very short intervals between contractions were amalgamated with longer contractions, and very short contractions were eliminated. From the individual burst onsets and offsets we measured mean peak activity normalized to the pre-drug values obtained in block 1 for each test session. Drug effects were compared against saline using a repeated-measures ANOVA that included blocks 2–8.

RESULTS

Infusions of NPY 3-36 had a profound effect on food intake. Under ad lib conditions, rats consumed relatively little while in the test chamber in the no drug condition (Fig. 2). In contrast, infusions of NPY 3-36 alone or in combination with saline into the IRt/PCRt resulted in a significant increase in food consumption. When NPY 3-36 infusions were combined with muscimol infusions in the IRt/PCRt, however, there was a 69% reduction in food intake compared to the NPY/saline condition. A repeated measures ANOVA across the four conditions showed a significant drug effect ($p < .001$), and post hoc Bonferroni-adjusted comparisons indicated that both the baseline (no drug) and the NPY 3-36/muscimol conditions resulted in significantly less intake compared to either NPY 3-36 alone or NPY 3-36 combined with saline. Examples of EMG activity recorded under these different conditions are presented in figure 3. Although animals typically did not ingest the wet mash in the absence of NPY 3-36 stimulation, they were responsive to intraoral stimulation with sucrose (Fig. 3A). In the presence of NPY 3-36, rats consumed more and rhythmic AD activity captured these ingestive movements (Fig. 3B). Infusions of muscimol into the IRt/PCRt at the same time as 3V NPY 3-36 infusions suppressed both licking and masticatory movements. Figure 3C shows a trial

in which licking was totally suppressed; however, during the 15 minutes preceding the IO stimulation, the animal ingested a small amount of food associated with masticatory movements that were reduced in amplitude.

The total amount of mash consumed when NPY 3-36 was combined with IRt/PCRt muscimol was only reduced by 69% compared to NPY 3-36 combined with saline. However, an analysis of the time course of ingestion revealed a more pronounced effect in the blocks immediately after drug infusion (Fig. 4A). Infusions of NPY 3-36/saline resulted in an immediate increase in food consumption (block 3) that diminished over the subsequent blocks. In comparison, NPY 3-36/muscimol did not produce a spike in mash consumption but rather, consumption increased slightly over the course of the next 90 minutes. An ANOVA indicated a significant main effect of drug ($p < .001$) as well as a significant block X drug interaction ($p < .005$).

In parallel with the suppression of solid food intake following IRt/PCRt infusions of muscimol, the amplitude of the anterior digastric EMG diminished following intra-oral infusions of sucrose (Fig. 4B). The amplitude returned to baseline by block 7 in parallel with the increased food consumption evident in figure 4A. A repeated measures ANOVA indicated a significant drug ($p < .005$) and block effect ($p < .014$) but no block X drug interaction.

Compared to IRt/PCRt infusions, muscimol into Gi had little effect on food consumption evoked by NPY 3-36 (Fig. 5). An ANOVA revealed a significant drug effect ($p < .001$) but post-hoc Bonferoni-adjusted t-tests showed that only the baseline condition was significantly different from the other blocks; i.e., in the absence of any central drug infusions, these non-deprived rats ingested only a minimal amount of food but NPY 3-36 3V injections potently increased intake, regardless of whether or not the GI was inactivated by muscimol. This conclusion held up to the greater scrutiny permitted by analyzing the time course of ingestion following NPY 3-36 infusions after saline or muscimol infusions into Gi. The NPY 3-36 infusion combined with saline into the GI showed a peak in ingestion immediately after drug infusion (Fig. 6A), similar to the group receiving lateral tegmental field infusions. However, this condition did not differ from NPY 3-36 paired with the muscimol infusions into Gi (ANOVA, $p>.1$). Likewise, an analysis of the EMG contractions associated with IO stimulation indicated no difference between NPY 3-36 combined with saline and NPY 3-36 paired with Gi muscimol infusions (Fig. 6B).

Histology

A summary of the injection sites is shown in figure 7. Lateral infusions were centered subjacent to the rostral nucleus of the solitary tract at a level approximately 0.5 mm caudal to the rostral pole, in the PCRt/IRt lateral to Gi and medial to the trigeminal sensory complex Midline infusions were centered at the same rostral-caudal level. Inspection of the pattern of spread of 100nl of Fluorogold suggested that the effective injection sites were mostly limited to the PCRt/IRt for the lateral infusions and to the Gi for the medial infusions.

DISCUSSION

The present experiments demonstrate that the consummatory response of mastication induced by a forebrain appetitive signal is profoundly suppressed when neurons in the IRt/PCRt are inactivated with muscimol. These results extend previous observations showing that neurons in the IRt/PCRt are necessary for the consummatory response of licking induced with intra-oral stimulation (Chen & Travers, 2003; Chen *et al.*, 2001). It is unlikely that the suppression of consummatory responses was as a result of direct suppression of either the motor trigeminal or hypoglossal nucleus via the spread of the muscimol to these structures. Examination of the Fluorogold injections made at the conclusion of each experiment did not reveal any encroachment on either motor nucleus.

Together, these observations suggest the general importance of the IRt/PCRt in constituting a final common pathway for ingestive behavior. The essential role of the IRt/PCRt in the ingestive act is in conflict with models postulating that the medulla is mostly inhibitory to consummatory rhythm generation. These models are based on *in vitro* experiments demonstrating that glutamate-evoked rhythmic responses of motor trigeminal neurons become less pronounced when the medulla is included in the preparation (Tanaka *et al.*, 1999). However, the functional import of these *in vitro* observations is difficult to reconcile with the present *in vivo* data that imply a predominant excitatory drive from IRt/PCRt to these same cells in behaving animals. An excitatory role for the medullary IRt/PCRt is also more in line with *in situ* hybridization and immunohistochemical studies demonstrating that, despite a significant GABAergic minority, the majority of IRt/PCRt neurons with direct projections to the mV are glutamatergic (Travers, Yoo, Chandran, Herman, & Travers, 2005; Turman & Chandler, 1994).

As with previous reports, NPY 3-36 infused into 3V initiated a robust feeding response (Flynn, Plata-Salaman *et al.*, 1999; O'Shea *et al.*, 1997). Rats in the present study ate 4.85 g in a 90 min period, comparing favorably with a previous study demonstrating an intake ~ 4.5 g over 2 hrs with a comparable dose (Flynn, Plata-Salaman *et al.*, 1999). The precise pathway through which this response is effected is unknown, but likely involves the NPY Y5 receptor (MacNeil, 2007). Although NPY 3-36 has an affinity for the Y2 receptor that is primarily anorexogenic (Abbott *et al.*, 2005; Batterham *et al.*, 2002), it also has an affinity for Y5 receptors that are orexigenic (Gerald *et al.*, 1996). Highly selective Y5 agonists induce food intake (Cabrele *et al.*, 2000;E. M. Parker *et al.*, 2000), an effect blocked by Y5 antagonists (Criscione *et al.*, 1998; E. M. Parker *et al.*, 2000). Because Y5 receptors are found within the hypothalamus, including the arcuate nucleus, paraventricular nucleus and lateral hypothalamus (R. M. Parker & Herzog, 1999), it is likely that NPY 3-36 infused into 3V activates these structures to promote food intake.

There are multiple descending pathways through which a forebrain appetitive signal could engage brainstem areas that mediate consummatory responses (Berthoud, 2002; Kelley & Berridge, 2002; Morton *et al.*, 2006; Saper *et al.*, 2002; Travers, DiNardo, & Karimnamazi, 1997), including direct projections to the IRt/PCRt from the lateral hypothalamus (Luiten, ter Horst, & Steffens, 1987; Notsu, Tsumori, Yokota, Sekine, & Yasui, 2008; Shammah-Lagnado, Costa, & Ricardo, 1992). In addition to integrating signals related to energy balance, the hypothalamic projection may be particularly critical in serving as an interface with ventral forebrain regions implicated in motivation and reward (Kelley *et al.*, 2005). The present results indicate that the IRt/PCRt represents an essential link in transforming hypothalamic signals into feeding-related motor patterns. Other ventral forebrain structures that could impact the IRt/PCRt directly to trigger or modulate feeding include the central nucleus of the amygdala (Cassell, Gedney, & Agassandian, 2003; Jongen-Relo & Amaral, 1998) (which also contains some Y5 receptors) and the bed nucleus of the stria terminalis (Holstege, Meiners, & Tan, 1985).

Other data emphasize the role of the orbitofrontal cortex and its projections as a circuit for initiating mastication (Nakamura & Katakura, 1995) (Lund *et al.*, 1998). Stimulation of the cortical masticatory area in the orbitofrontal cortex produces masticatory movements in anesthetized preparations and these cortical outputs have been proposed to influence the brainstem via the pyramidal tract. According to one model, Gi neurons transform a "tonic" cortical signal via the pyramidal tract to the fundamental masticatory rhythm which is then relayed to pre-oromotor neurons in IRt/PCRt (Nakamura & Katakura, 1995). Although the present experiment did not directly address cortically-evoked chewing, we observed no effect of Gi inactivation on appetitive chewing elicited by 3V NPY 3-36. Thus, it remains unclear what role Gi plays in ingestive behavior under awake conditions. However, there are direct

projections to IRt/PCRt from the cortical masticatory area (Shammah-Lagnado *et al.*, 1992; Zhang & Sasamoto, 1990) and these projections could mediate the fine motor control of the masticatory apparatus that is compromised following cortical lesions (Hiraba *et al.*, 2007).

Although the present experiment highlights the critical role of the IRt/PCRt for consummatory behavior, it does not negate the likelihood of a more distributed brainstem consummatory substrate, one that includes the pons. Studies using cortical stimulation in anesthetized animals and *en bloc* preparations suggest that there is a masticatory rhythm generator in the pons (Lund & Kolta, 2006; Lund *et al.*, 1998). In this model, descending cortical outputs to the pontine nucleus caudalis influence motor trigeminal (mV) neurons via short axon pontine pathways to the RF immediately surrounding mV. Interestingly, the present study replicated a previous observation suggesting that the amplitude and rate of anterior digastric contractions are independent (Chen *et al.*, 2001). Thus, in cases where IRt/PCRt muscimol produced only a partial reduction in anterior digastric amplitude, allowing the residual mastication to be observed, contraction rate was essentially invariant (Fig. 8). Thus, it is possible that a pontine rhythm generator could influence interneurons and pre-oromotor neurons in the IRt/PCRt to produce the complete consummatory pattern. On the other hand, recent observations in our laboratory suggest that the rhythm-generating substrate itself might extend to the IRt/PCRt since infusions of a mu opiate agonist into this region profoundly slow lick rate (Kinzeler & Travers, 2008).

A number of experimental approaches lead to the conclusion that the IRt/PCRt represents an important node in the interface between brainstem and forebrain structures regulating homeostatic control of ingestion and the execution of the consummatory response (Fig. 9). Neurons in the IRt/PCRt that project to the oromotor nuclei are rhythmically active during both cortically induced (electrical) stimulation (Inoue, Chandler, & Goldberg, 1994;Sahara, Hashimoto, & Nakamura, 1996) as well as intraoral stimulation in the awake, freely moving preparation (Travers, DiNardo, & Karimnamazi, 2000). In addition to the forebrain projections discussed above, neurons in the IRt/PCRt receive input from the (gustatory) rNST (Nasse *et al.*, 2008) and parabrachial nucleus (Karimnamazi & Travers, 1998), as well as trigeminal somatosensory input (Dauvergne, Pinganaud, Buisseret, Buisseret-Delmas, & Zerari-Mailly, 2001;Zerari-Mailly, Pinganaud, Dauvergne, Buisseret, & Buisseret-Delmas, 2001). Thus, the IRt/PCRt may be an important integrative node for consummatory ingestive behavior that receives input from multiple descending forebrain as well as local orosensory sources.

Acknowledgments

This work was supported by NIH DC-00416 and NIH DC-00417.

References

Abbott CR, Small CJ, Kennedy AR, Neary NM, Sajedi A, Ghatei MA, Bloom SR. Blockade of the neuropeptide Y Y2 receptor with the specific antagonist BIIE0246 attenuates the effect of endogenous and exogenous peptide YY(3-36) on food intake. Brain Res 2005;1043(1–2):139–144. [PubMed: 15862527]

Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR. Gut hormone PYY(3-36) physiologically inhibits food intake. Nature 2002;418(6898):650–654. [PubMed: 12167864]

Berthoud HR. Multiple neural systems controlling food intake and body weight. Neurosci Biobehav Rev 2002;26(4):393–428. [PubMed: 12204189]

Broberger C. Brain regulation of food intake and appetite: molecules and networks. J Intern Med 2005;258 (4):301–327. [PubMed: 16164570]

Cabrele C, Langer M, Bader R, Wieland HA, Doods HN, Zerbe O, Beck-Sickinger AG. The first selective agonist for the neuropeptide YY5 receptor increases food intake in rats. J Biol Chem 2000;275(46): 36043–36048. [PubMed: 10944518]

Cassell MD, Gedney MT, Agassandian KA. Network architecture of central amygdala output shows strong connectional bias towards oro-lingual sensory and reflex circuits. Society for Neuroscience Abstracts 2003:594.10.

Chen Z, Travers JB. Inactivation of amino acid receptors in medullary reticular formation modulates and suppresses ingestion and rejection responses in the awake rat. Am J Physiol Regul Integr Comp Physiol 2003;285(1):R68–83. [PubMed: 12663257]

Chen Z, Travers SP, Travers JB. Muscimol infusions in the brain stem reticular formation reversibly block ingestion in the awake rat. Am J Physiol Regul Integr Comp Physiol 2001;280(4):R1085–1094. [PubMed: 11247831]

Criscione L, Rigollier P, Batzl-Hartmann C, Rueger H, Stricker-Krongrad A, Wyss P, Brunner L, Whitebread S, Yamaguchi Y, Gerald C, Heurich RO, Walker MW, Chiesi M, Schilling W, Hofbauer KG, Levens N. Food intake in free-feeding and energy-deprived lean rats is mediated by the neuropeptide Y5 receptor. J Clin Invest 1998;102(12):2136–2145. [PubMed: 9854049]

Dauvergne C, Pinganaud G, Buisseret P, Buisseret-Delmas C, Zerari-Mailly F. Reticular premotor neurons projecting to both facial and hypoglossal nuclei receive trigeminal afferents in rats. Neurosci Lett 2001;311(2):109–112. [PubMed: 11567790]

Flynn MC, Plata-Salaman CR, Ffrench-Mullen JM. Neuropeptide Y-related compounds and feeding. Physiol Behav 1999;65(4–5):901–905. [PubMed: 10073499]

Flynn MC, Turrin NP, Plata-Salaman CR, Ffrench-Mullen JMH. Feeding response to neuropeptide Y-related compounds in rats treated with Y5 receptor antisense or sense phosphothio-oligodeoxynucleotide. Physiology & Behavior 1999;66(5):881–884. [PubMed: 10405118]

Gerald C, Walker MW, Criscione L, Gustafson EL, Batzl-Hartmann C, Smith KE, Vaysse P, Durkin MM, Laz TM, Linemeyer DL, Schaffhauser AO, Whitebread S, Hofbauer KG, Taber RI, Branchek TA, Weinshank RL. A receptor subtype involved in neuropeptide-Y-induced food intake. Nature 1996;382(6587):168–171. [PubMed: 8700207]

Grill HJ. Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. Obesity (Silver Spring) 2006;14(Suppl 5):216S–221S. [PubMed: 17021370]

Grill HJ, Norgren R. Neurological tests and behavioral deficits in chronic thalamic and chronic decerebrate rats. Brain Res 1978a;143(2):299–312. [PubMed: 630411]

Grill HJ, Norgren R. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 1978b;143(2):263–279. [PubMed: 630409]

Hiraba H, Sato T, Saito K, Iwakami T, Mizoguchi N, Fukano M, Ueda K. Organization of cortical processing for facial movements during licking in cats. Somatosens Mot Res 2007;24(3):115–126. [PubMed: 17853054]

Holstege G, Kuypers HG, Dekker JJ. The organization of the bulbar fibre connections to the trigeminal, facial and hypoglossal motor nuclei. II. An autoradiographic tracing study in cat. Brain 1977;100(2): 264–286. [PubMed: 884485]

Holstege G, Meiners L, Tan K. Projections of the bed nucleus of the stria terminalis to the mesencephalon, pons, and medulla oblongata in the cat. Exp Brain Res 1985;58(2):379–391. [PubMed: 3996501]

Inoue T, Chandler SH, Goldberg LJ. Neuropharmacological mechanisms underlying rhythmical discharge in trigeminal interneurons during fictive mastication. J Neurophysiol 1994;71(6):2061–2073. [PubMed: 7931502]

Jean A. Brain stem control of swallowing: neuronal network and cellular mechanisms. Physiol Rev 2001;81(2):929–969. [PubMed: 11274347]

Jongen-Relo AL, Amaral DG. Evidence for a GABAergic projection from the central nucleus of the amygdala to the brainstem of the macaque monkey: a combined retrograde tracing and in situ hybridization study. Eur J Neurosci 1998;10(9):2924–2933. [PubMed: 9758162]

Karimnamazi H, Travers JB. Differential projections from gustatory responsive regions of the parabrachial nucleus to the medulla and forebrain. Brain Res 1998;813(2):283–302. [PubMed: 9838165]

Kelley AE, Baldo BA, Pratt WE. A proposed hypothalamic-thalamic-striatal axis for the integration of energy balance, arousal, and food reward. J Comp Neurol 2005;493(1):72–85. [PubMed: 16255002]

Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. J Neurosci 2002;22(9):3306–3311. [PubMed: 11978804]

Kinzeler, NR.; Travers, SP. Mu opiates infused into the NST significantly alter fluid-induced licking and gaping. Paper presented at the International Symposium on Olfaction and Taste; San Francisco, CA. 2008.

Kogo M, Funk GD, Chandler SH. Rhythmical oral-motor activity recorded in an in vitro brainstem preparation. Somatosens Mot Res 1996;13(1):39–48. [PubMed: 8725647]

Kogo M, Tanaka S, Chandler SH, Matsuya T. Examination of the relationships between jaw opener and closer rhythmical muscle activity in an in vitro brainstem jaw-attached preparation. Somatosens Mot Res 1998;15(3):200–210. [PubMed: 9874519]

Luiten PG, ter Horst GJ, Steffens AB. The hypothalamus, intrinsic connections and outflow pathways to the endocrine system in relation to the control of feeding and metabolism. Prog Neurobiol 1987;28 (1):1–54. [PubMed: 3547503]

Lund JP, Kolta A. Generation of the central masticatory pattern and its modification by sensory feedback. Dysphagia 2006;21(3):167–174. [PubMed: 16897322]

Lund JP, Kolta A, Westberg KG, Scott G. Brainstem mechanisms underlying feeding behaviors. Curr Opin Neurobiol 1998;8(6):718–724. [PubMed: 9914242]

MacNeil DJ. NPYY1 and Y5 receptor selective antagonists as anti-obesity drugs. Current Topics in Medicinal Chemistry 2007;7(17):1721–1733. [PubMed: 17979781]

Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443(7109):289–295. [PubMed: 16988703]

Nakamura Y, Katakura N. Generation of masticatory rhythm in the brainstem. Neurosci Res 1995;23(1): 1–19. [PubMed: 7501294]

Nasse J, Terman D, Venugopal S, Hermann G, Rogers R, Travers JB. Local circuit input to the medullary reticular formation from the rostral nucleus of the solitary tract. Am J Physiol Regul Integr Comp Physiol 2008;295(5):R1391–1408. [PubMed: 18716034]

Notsu K, Tsumori T, Yokota S, Sekine J, Yasui Y. Posterior lateral hypothalamic axon terminals are in contact with trigeminal premotor neurons in the parvicellular reticular formation of the rat medulla oblongata. Brain Res 2008;1244:71–81. [PubMed: 18948090]

O'Shea D, Morgan DG, Meeran K, Edwards CM, Turton MD, Choi SJ, Heath MM, Gunn I, Taylor GM, Howard JK, Bloom CI, Small CJ, Haddo O, Ma JJ, Callinan W, Smith DM, Ghatei MA, Bloom SR. Neuropeptide Y induced feeding in the rat is mediated by a novel receptor. Endocrinology 1997;138 (1):196–202. [PubMed: 8977404]

Parker EM, Balasubramaniam A, Guzzi M, Mullins DE, Salisbury BG, Sheriff S, Witten MB, Hwa JJ. [D-Trp(34)] neuropeptide Y is a potent and selective neuropeptide Y Y(5) receptor agonist with dramatic effects on food intake. Peptides 2000;21(3):393–399. [PubMed: 10793222]

Parker RM, Herzog H. Regional distribution of Y-receptor subtype mRNAs in rat brain. Eur J Neurosci 1999;11(4):1431–1448. [PubMed: 10103138]

Pearson KG, Acharya H, Fouad K. A new electrode configuration for recording electromyographic activity in behaving mice. J Neurosci Methods 2005;148(1):36–42. [PubMed: 15908013]

Sahara Y, Hashimoto N, Nakamura Y. Hypoglossal premotor neurons in the rostral medullary parvocellular reticular formation participate in cortically-induced rhythmical tongue movements. Neurosci Res 1996;26(2):119–131. [PubMed: 8953574]

Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. Neuron 2002;36(2):199–211. [PubMed: 12383777]

Shammah-Lagnado SJ, Costa MS, Ricardo JA. Afferent connections of the parvocellular reticular formation: a horseradish peroxidase study in the rat. Neuroscience 1992;50(2):403–425. [PubMed: 1279462]

Tanaka S, Kogo M, Chandler SH, Matsuya T. Localization of oral-motor rhythmogenic circuits in the isolated rat brainstem preparation. Brain Res 1999;821(1):190–199. [PubMed: 10064803]

Travers JB, DiNardo LA, Karimnamazi H. Motor and premotor mechanisms of licking. Neurosci Biobehav Rev 1997;21(5):631–647. [PubMed: 9353796]

Travers JB, DiNardo LA, Karimnamazi H. Medullary reticular formation activity during ingestion and rejection in the awake rat. Exp Brain Res 2000;130(1):78–92. [PubMed: 10638444]

Travers JB, Norgren R. Afferent projections to the oral motor nuclei in the rat. J Comp Neurol 1983;220 (3):280–298. [PubMed: 6315785]

Travers JB, Norgren R. Electromyographic analysis of the ingestion and rejection of sapid stimuli in the rat. Behav Neurosci 1986;100(4):544–555. [PubMed: 3741605]

Travers JB, Yoo JE, Chandran R, Herman K, Travers SP. Neurotransmitter phenotypes of intermediate zone reticular formation projections to the motor trigeminal and hypoglossal nuclei in the rat. J Comp Neurol 2005;488(1):28–47. [PubMed: 15912497]

Turman JE Jr, Chandler SH. Immunohistochemical localization of glutamate and glutaminase in guinea pig trigeminal premotoneurons. Brain Res 1994;634(1):49–61. [PubMed: 7512428]

Zerari-Mailly F, Pinganaud G, Dauvergne C, Buisseret P, Buisseret-Delmas C. Trigemino-reticulo-facial and trigemino-reticulo-hypoglossal pathways in the rat. J Comp Neurol 2001;429(1):80–93. [PubMed: 11086291]

Zhang GX, Sasamoto K. Projections of two separate cortical areas for rhythmical jaw movements in the rat. Brain Res Bull 1990;24(2):221–230. [PubMed: 2322856]

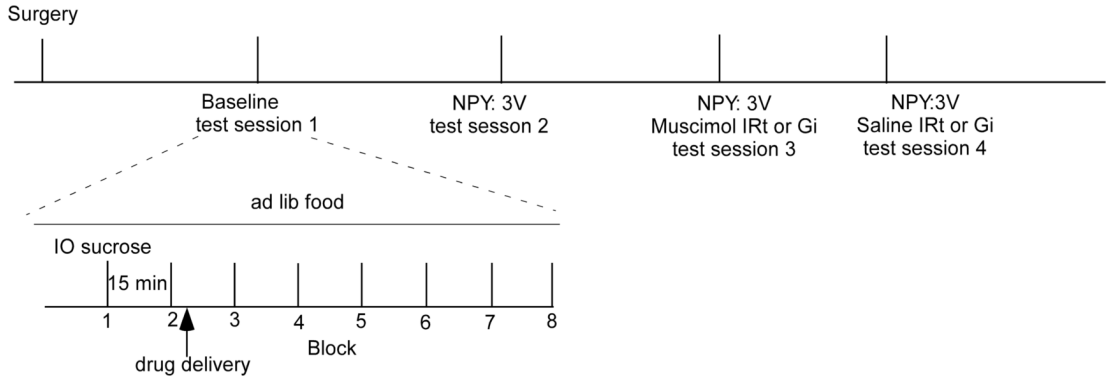

Fig. 1.

A timeline of the experimental protocol. Following recovery from surgery, animals were tested in four test sessions as indicated. Each test session consisted of access to wet mash which was available ad lib except for a brief period when the food was weighed every 15 minutes. When the food was removed, the animals were tested with a single intraoral stimulation with 0.1 M sucrose. Drug delivery on test sessions 2 – 4 were just after the second intraoral stimulation.

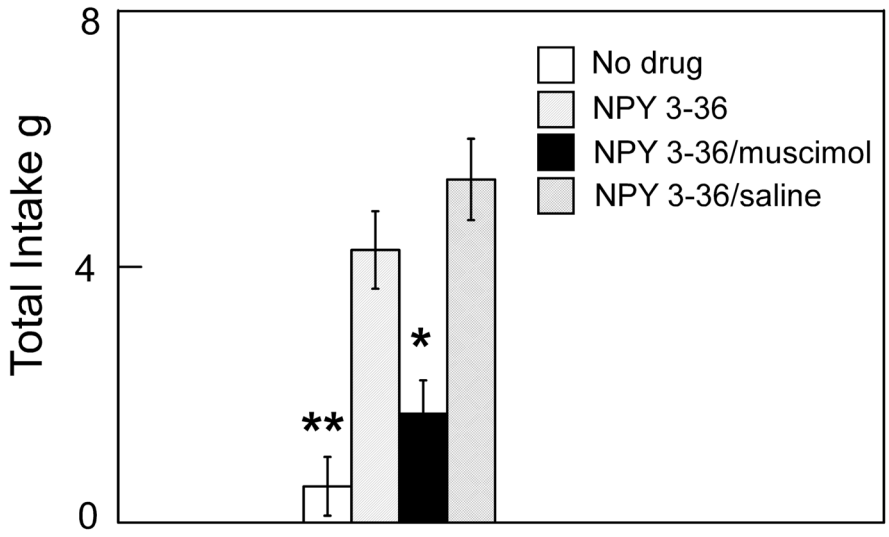

Fig 2.

Total amount of wet mash consumed over 8 blocks (90 min) for each of 4 different test sessions. NPY 3-36 was infused into the third ventricle, muscimol or saline was infused into the lateral tegmental field of the medullary RF. * p<.01; NPY 3-36/muscimol vs NPY 3-36 alone; ** p <.001, no drug vs NPY 3-36 alone; no drug vs NPY 3-36/saline; NPY 3-36/mucimol vs NPY 3-36/saline. Bonferroni adjusted post hoc comparisons.

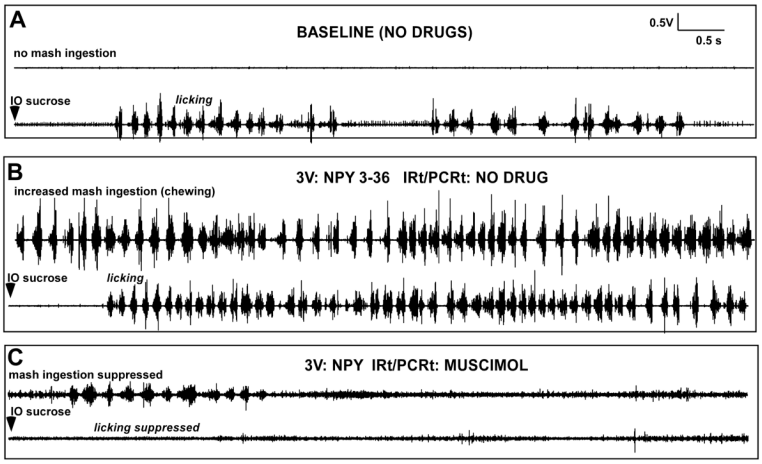

Fig 3.

Raw EMG records comparing anterior digastric activity during intra-oral induced licking and mastication of mash under 3 conditions in the same rat. A. baseline condition with no drugs; B. third ventricle NPY 3-36 only and C, third ventricle NPY 3-36 combined with muscimol infusion into lateral tegmental field (IRt/PCRt). For each block, anterior digastric EMG responses associated with mash ingestion are compared to subsequent EMG responses associated with intraoral licking.

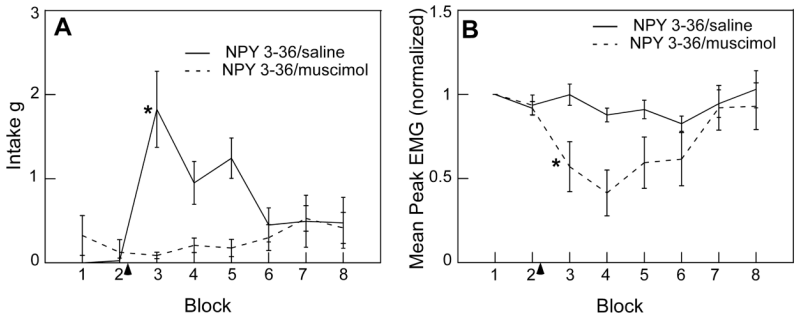

Fig 4.

A. Amount of mash consumed in successive blocks for two conditions: 3V NPY 3-36 combined with lateral tegmental field infusions of muscimol or saline. Arrow indicates drug delivery. * $p < .001$, drug effect using a repeated measures ANOVA and $p < .005$ drug X block interaction. B. Peak amplitude of anterior digastric EMG as a function of block for the same two conditions. Arrow indicates drug delivery. * $p < .005$, drug effect using a repeated measures ANOVA.

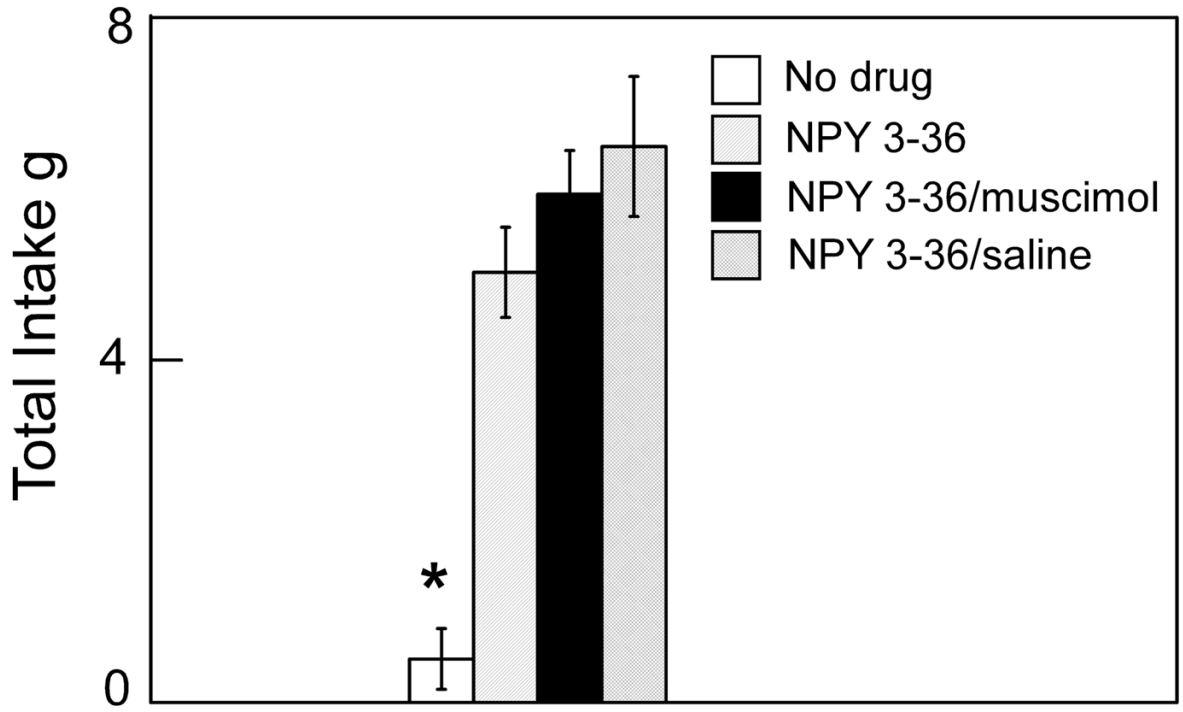

Fig 5.
Total amount of wet mash consumed over 8 blocks (90 min) for each of 4 different test sessions. NPY 3-36 was infused into the 3V, muscimol or saline into nucleus gigantocellularis. * p < . 001 Bonferroni adjusted post hoc comparison.

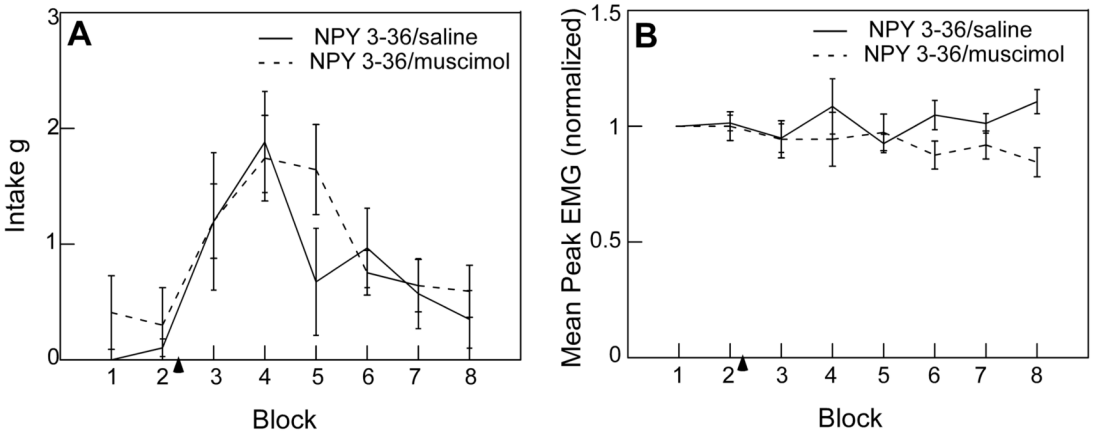

Fig 6.

A. Amount of mash consumed in successive blocks for two conditions: 3V NPY 3-36 combined with nucleus gigantocellularis infusion of muscimol or saline. Arrow indicates drug delivery. B. Peak amplitude of anterior digastric EMG as a function of block for the same two conditions. Arrow indicates drug delivery.

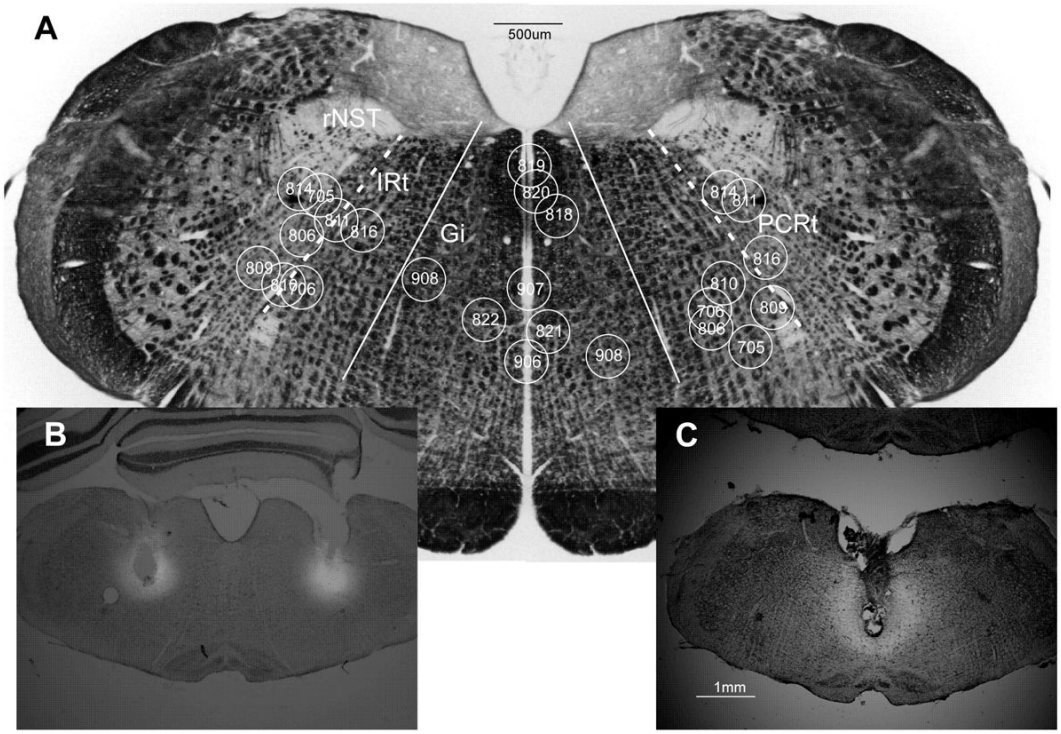

Fig 7.

A. Centers of infusion sites summarized over a Weil stained section through the rostral medulla. B Photomicrograph showing infusion site into lateral tegmental field (intermediate (IRt) and parvocellular (PCRt) and C. midline infusion into nucleus gigantocellularis.

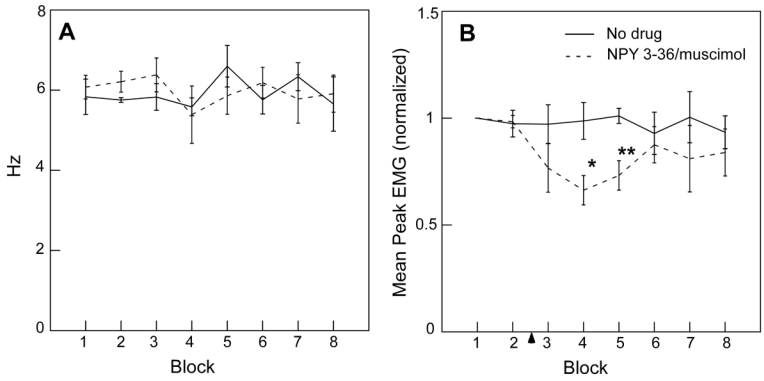

Fig 8.

Comparison of licking rate (A) and EMG amplitude (B) from only those cases showing partial suppression of anterior digastric EMG activity following an infusion of muscimol into the lateral tegmental field of the medullary RF. * p< .065; ** p < .035, paired t-test.

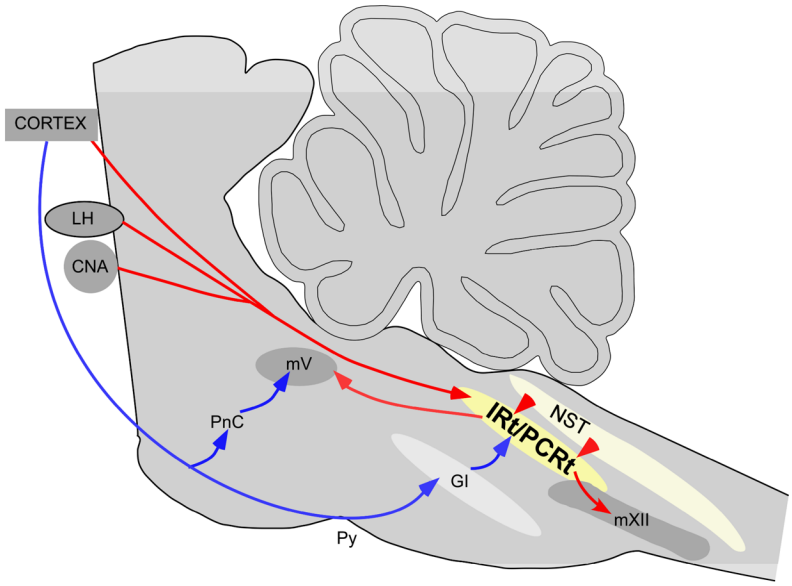

Fig 9.

A schematic diagram indicating several pathways through which forebrain signals for feeding influence oromotor neurons. Studies of fictive mastication emphasize descending cortical pathways that influence the oromotor nuclei via pyramidal tract input to either the pontine or medullary reticular formation (shown in blue). Data from the present study suggest that the parvocellular and intermediate zones of the medullary reticular formation also represent a point of convergence (node) through which descending forebrain input and local orosensory inputs converge to influence neurons with direct projections to the oromotor nuclei. Abbreviations: CNA, central nucleus of the amygdala; Gi, nucleus gigantocellularis; IRt, intermediate zone of the medullary reticular formation; LH, lateral hypothalamus; ; mV, motor trigeminal nucleus; mXII, hypoglossal nucleus; NST, nucleus of the solitary tract; PCRt, parvocellular reticular formation; PnC, nucleus pontine caudalis; Py, pyramidal tract.