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Terminal field specificity of forebrain efferent axons to brainstem gustatory nuclei

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Abstract

Rostral forebrain structures like the gustatory cortex (GC), bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and lateral hypothalamus (LH) send projections to the nucleus of solitary tract (NST) and the parabrachial nucleus (PBN) that modulate taste-elicited responses. However, the proportion of forebrain-induced excitatory and inhibitory effects often differs when taste cell recording changes from the NST to the PBN. The present study investigated whether this descending influence originates from a shared or distinct population of forebrain neurons. Under electrophysiological guidance, the retrograde tracers fast blue (FB) and fluorogold (FG) or green (GFB) and red (RFB) fluorescent latex microbeads were injected iontophoretically or by pressure pulses (10 ms at 20 psi) into the taste-responsive regions of the NST and the ipsilateral PBN in six rats. Seven days later, the animals were euthanized and tissue sections containing the LH, CeA, BNST, and GC were processed for co-localization of FB and FG or GFB and RFB. The results showed that the CeA is the major source of input to the NST (82.3 ± 7.6 cells/section) and the PBN (76.7 ± 11.5), compared to the BNST (31.8 ± 4.5 ; 37.0 ± 4.8), the LH (35.0 ± 5.4 ; 33.6 ± 5.7), and the GC (27.5 ± 4.0 ; 29.0 ± 4.6). Of the total number of retrogradely labeled cells, the incidence of tracer co-localization was $17 \pm 3\%$ in the GC, $17 \pm 2\%$ in the CeA, $15 \pm 3\%$ in the BNST and $16 \pm 1\%$ in the LH. Thus, irrespective of forebrain source the majority of descending input to the gustatory NST and PBN originates from distinct neuronal populations. This arrangement provides an anatomical substrate for differential modulation of taste processing in the first and second central relays of the ascending gustatory system.

Keywords

Taste; Parabrachial; NST; Amygdala; Cortex; Hypothalamus

1. Introduction

In rodents, the nucleus of the solitary tract (NST) is the first central relay for taste information processing. Ascending gustatory information is then carried to the parabrachial nucleus (PBN) of the pons. From there, gustatory projections diverge with one pathway sending axons to the

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gustatory cortical area (GC) via the thalamus and the other a direct route into the ventral forebrain providing taste information to areas like the lateral hypothalamus (LH), central nucleus of the amygdala (CeA), and bed nucleus of the stria terminalis (BNST) (Norgren, 1976; Nishijo et al., 1998; Li and Cho, 2006). These same forebrain regions that receive gustatory information send axonal projections back to the NST and PBN (Veening et al., 1984; van der Kooy et al., 1984).

Reciprocal communication between the forebrain and brainstem gustatory nuclei plays a critical role in learned and some forms of unlearned control of taste-guided behavior (Grill and Norgren, 1978; Grill et al., 1986). For instance, chronically decerebrate rats, in which all neural connections between the forebrain and the brainstem are severed, are unable to learn a conditioned taste aversion (CTA) or express a sodium appetite. Generally, induction of a CTA occurs following experience with negative gastrointestinal consequences of ingesting a taste stimulus (e.g. nausea, sickness, or vomiting), which produces a switch from acceptance to avoidance of that and any like tasting stimulus. In contrast, a switch from avoidance of concentrated sodium salt to avid ingestion characterizes sodium appetite, the behavioral manifestation of a negative sodium balance. Similar deficits are observed following bilateral lesions of the PBN, but not after lesions one synapse further along in the gustatory thalamus (Spector et al., 1992; Reilly et al., 1993; Scalera et al., 1995, 1997; Grigson et al., 1998; Reilly and Trifunovic, 2000).

Electrophysiological studies demonstrate that CTA and sodium appetite selectively alter taste elicited responses in the NST and PBN (Chang and Scott, 1984; Nakamura and Norgren, 1995; Shimura et al., 1997a,c). Of import, the change in PBN neural responsiveness to an aversively conditioned taste stimulus following CTA acquisition was abolished by disrupting communication between the brainstem and forebrain (Tokita et al., 2004). One interpretation is that centrifugal activity normally modulates gustatory processing in the brain stem, which is critical for adjusting gustatory preference-aversion behavior. In fact, stimulation of the GC, BNST, CeA, and LH has been shown to modulate taste-evoked neural activity in the NST and PBN.

In the hamster and rat NST, more taste cells are excited by LH stimulation than inhibited (Matsuo et al., 1984; Cho et al., 2003), while in the PBN more taste cells are inhibited than excited by stimulating the same forebrain site (Lundy and Norgren, 2004; Li et al., 2005). Although the influence of CeA stimulation has yet to be examined on rat NST taste cells, PBN taste cells are more often inhibited than excited (Lundy and Norgren, 2004). In hamster, CeA modulation of NST and PBN taste cells has been examined, and similar to LH activation more NST neurons were excited by CeA stimulation than inhibited, while the opposite was observed for PBN taste cells (Cho et al., 2003; Li et al., 2005). In the case of GC, data from hamster NST indicates that equal numbers of taste cells are under inhibitory and excitatory control (Smith and Li, 2000), although comparable data from the PBN in this species does not exist. In rat, PBN taste cells are more often inhibited by GC activation than excited (Lundy and Norgren, 2004), while in the NST temporary inactivation of GC produced more inhibitory effects than excitatory (Dilorenzo and Monroe, 1995). This suggests that GC activation might produce more excitatory effects than inhibitory, which again would contrast with the primarily inhibitory influence of this forebrain site on PBN taste neurons. Thus, the available data from two species that have been studied strongly suggests that centrifugal inputs differentially modulate taste processing in the first and second central synapses of the ascending gustatory system. The exception appears to be the BNST, which only has been tested in hamster. Taste neurons both in the hamster NST and PBN are more often inhibited than excited (Smith et al., 2005; Li and Cho, 2006).

The present study investigates whether separate populations of neurons in the LH, CeA, BNST and GC project to the NST and PBN. We electrophysiologically isolated the ipsilateral gustatory NST and PBN then injected a different retrograde tracer into each area. Brain tissue containing the above forebrain structures was subsequently examined for neurons positive for uptake of only one tracer and those positive for both tracers.

2. Results

2.1. Injection sites

In each animal, the taste responsive regions of the NST and PBN were relocated with the tracer filled injection pipette. Fig. 1A shows an example of the electrophysiological response from the NST to 0.1 M NaCl applied to the anterior tongue through a tracer filled pipette. Representative fluorescent photomicrographs of tracer injections in the NST (top, GFB) and the PBN (bottom, RFB) are shown in panels 2 and 4 of Fig. 1B. Panels 1 and 3 show the fluorescent image merged with the brightfield image of the same section. Microscopic examination of each injection site revealed that NST injections were concentrated in the rostral central, rostral lateral and ventral subdivision. In the PBN, the central medial, ventral medial, and ventral lateral portions were targeted with minimal spread into rostromedial non-gustatory areas. Fig. 2 shows a summary of the six tracer injections into the gustatory responsive NST and PBN.

2.2. Retrograde labeling

Fig. 3 shows a schematic of the areas in which retrogradely labeled neurons were quantified (case #0785). No attempt was made to signify double-labeled neurons or provide an exact representation of the total number of cells in each section. Panels A–D depict the general location of BNST and GC neurons projecting to the NST (green) and PBN (red), while E–H shows that for the CeA and LH. Although each forebrain area contained numerous retrogradely labeled neurons, the number of CeA neurons projecting to the brainstem gustatory nuclei was consistently greater compared to the other forebrain regions. In fact, a two-way ANOVA varying injection and forebrain site revealed that the number of fluorescent-labeled cells differed between forebrain sites ($F_{3,15}=37.62$, $P<0.001$). Fig. 4 shows that the CeA was the major source of input both to the NST (82.3 ± 7.6 cells/section) and the PBN (76.7 ± 11.5). The BNST (NST: 31.8 ± 4.5 ; PBN: 37.0 ± 4.8), the LH (NST: 35.0 ± 5.4 ; PBN: 33.6 ± 5.7) and the GC (NST: 27.5 ± 4.0 ; PBN: 29.0 ± 4.6) contained a similar number of fluorescent-labeled cells per section (P 's <0.1). For a specific forebrain site, a comparable number of neurons projected to the PBN and NST ($F_{1,5}<0.01$, $P=0.98$).

The photomicrographs of Fig. 5 show examples of GFB (NST injection) and RFB (PBN injection) labeled neurons in the BNST, CeA, GC and LH. Examination of the merged images (far right) indicates that some forebrain neurons in each region project both to the NST and PBN. Nevertheless, it appears that an even greater number of neurons projects to only one of the brainstem gustatory nuclei. Higher magnification images of labeled neurons in each forebrain site are shown in the panels of Fig. 6.

Expressed as a function of terminal field (NST or PBN), the percentage of singly labeled cells was similar across forebrain sites ($F_{1,5}=0.04$, $P=0.84$). Overall, the mean number of forebrain neurons that projected only to the NST was $65\pm1\%$ and only to the PBN $68\pm2\%$. Although the actual numbers of labeled cells per section varied depending on the size of the injection into the NST (GC, 16.1–44.8; BNST, 21.8–51.2; CeA, 63.7–109.6; LH, 18.4–49.5) and PBN (GC, 17–45.1; BNST, 16.8–51.5; CeA, 35.8–111.8; LH, 15.8–51), a consistently small number of forebrain neurons were double labeled. When expressed as a function of the total number of retrogradely labeled cells (NST and PBN projecting neurons combined), the incidence of tracer

co-localization was $17\pm3\%$ in the GC, $15\pm3\%$ in the BNST, $17\pm2\%$ in the CeA and $16\pm1\%$ in the LH. Significant differences between forebrain sites were not evident ($F_{3,20}=0.29$, $P=0.82$). Thus, there appears to be three separate populations of forebrain neurons, two large groups of cells that project axons to the NST or PBN and a third smaller group sending axon terminals to both gustatory nuclei.

3. Discussion

The present results demonstrate that the gustatory NST and PBN receive afferent input largely from separate populations of neurons within the GC, BNST, CeA and LH. Irrespective of brainstem target, the largest source is from the amygdala. Prior studies demonstrate that stimulation of the GC, BNST, CeA and LH modulates taste-evoked neural activity in the NST and PBN (Dilorenzo and Monroe, 1992, 1995; Lundy and Norgren, 2001; Cho et al., 2003; Lundy and Norgren, 2004; Li et al., 2005; Smith et al., 2005; Li and Cho, 2006). The influence of descending input on taste processing in the first and second central synapses of the ascending gustatory system is due, in large part, to ongoing activity of distinct populations of forebrain neurons. Nonetheless, the present study also shows that a smaller subset of forebrain neurons gives rise to efferent axon collaterals that target both the NST and PBN.

3.1. Coordinated modulation of taste processing

About 20% of the forebrain neurons that send axons to brainstem gustatory nuclei target both the NST and PBN. Theoretically, this divergent forebrain output could simultaneously modulate taste elicited neural activity in the NST and PBN. Prior electrophysiological studies also show that multiple forebrain areas can influence individual taste cells within the NST or PBN. In 46% (52/113) of forebrain responsive NST neurons, neural activity was influenced by stimulation of both the LH and CeA (Cho et al., 2003). For the PBN, 70% (37/50) of the forebrain responsive neurons were influenced by stimulation of the CeA, LH, and/or GC (Lundy and Norgren, 2004). Together with the present anatomical data, it appears that descending forebrain inputs can independently modulate individual brainstem taste cells, converge to produce coordinated modulation of the same cell, or diverge to modulate taste cells simultaneously in the NST and PBN. Divergence of forebrain output to both brainstem gustatory nuclei, however, seems to be the exception, not the rule.

3.2. Differential modulation of NST and PBN gustatory neurons

Prior investigations in rats and/or hamsters demonstrate that the GC, BNST, CeA, and LH modulate taste responsive neurons in the NST and PBN, which is often differential. In hamsters, the most common effect of CeA and LH stimulation on NST taste cells was excitatory; while GC activation induced a more equal distribution of excitation and inhibition (Smith and Li, 2000; Cho et al., 2003). One synapse further along in the ascending gustatory system, forebrain activity produced a somewhat different pattern of effects on taste cells in the PBN. Here inhibition was the most common influence of CeA and LH stimulation; the influence of GC activation on hamster PBN cells has yet to be tested (Li et al., 2005). Similar observation of differential modulation of NST and PBN taste cells has been reported in rats during GC and LH stimulation (Matsuo et al., 1984; Dilorenzo and Monroe, 1992, 1995; Lundy and Norgren, 2001, 2004; Li et al., 2005; Li and Cho, 2006). Although some gaps remain in determining the influence of specific forebrain inputs both on NST and PBN taste neurons, the available data from both species points to differential forebrain modulation of taste processing in these brainstem nuclei.

Given the terminal field specificity of most descending axons, one hypothesis is that separate neuronal pools in a particular forebrain region utilize common neurotransmitters, and differential forebrain modulation of taste processing in the NST compared to the PBN might

rely on differences in local brainstem/forebrain synaptic connections. Terminals in both brainstem gustatory nuclei are immunoreactive for some of the same neurochemicals like somatostatin, neurotensin, enkephalin, substance P, and GABA (Mantyh and Hunt, 1984; Moga and Gray, 1985; Moga et al., 1989, 1990; Leonard et al., 1999), some of which have been shown to influence the neural discharge of gustatory NST cells (Smith et al., 1994; Davis and Smith, 1997; Li et al., 2003). Another possibility is descending axons innervating only the NST utilize different neurotransmitters compared to those destined solely for the PBN. Clearly, additional research is needed to understand the neurochemicals and neural circuitry that mediates top-down modulation of central taste processing.

3.3. Implications for ingestive behaviors

The lateral parvocellular reticular formation in the medulla (PCRt) is thought to process orosensory information in a gustatory-motor circuit related to ingestion (Travers et al., 1997; Karimnamazi and Travers, 1998). The gustatory region both of the NST and PBN project to the PCRt as do neurons in the LH, CeA, BNST, and GC. Thus, increases and decreases in activity of target specific populations of forebrain neurons likely modulate mastication and licking, at least in part, through control of gustatory neural activity.

For instance, bilateral ibotenic acid lesions of LH disrupt concentration-dependent intake of saccharin and quinine (Ferssiw et al., 1987). The most profound influence of LH lesions was increased intake of normally aversion quinine and concentrated saccharin, and reduced intake of normally preferred dilute saccharin. CeA lesions, on the other hand, actually increased aversion to quinine and concentrated saccharin although preference for dilute saccharin also was reduced (Touzani et al., 1997). Interestingly, the normal preference for increasing concentrations of sucrose as well as decreased preference for increasing concentrations of quinine is eliminated by bilateral lesions of gustatory NST (Spector et al., 1993; Shimura et al., 1997b). Thus, forebrain excitation of taste processing, particularly in the NST, might serve to adjust ingestive behavior through increasing taste-elicited drive on specific pools of premotor neurons in the reticular formation. The contrasting effect of LH and CeA lesions on normally aversive stimuli is likely due to control of distinct populations of taste neurons. Previous research has shown that about half of forebrain responsive NST neurons were influenced by LH and CeA stimulation, while the other half were modulated by only one of these forebrain sites (Cho et al., 2003).

The LH, CeA, BNST, and GC also are implicated in learned and some forms of unlearned control of taste-guided behavior involving assignment of new hedonic value. For instance, transient inactivation of intracellular signaling or immediate early gene transcription in the CeA and GC or neural activity by tetrodotoxin in the LH impairs conditioned taste aversion learning (Lamprecht and Dudai, 1996; Caulliez et al., 1996; Lamprecht et al., 1997; Berman et al., 1998; Yasoshima et al., 2006), while BNST and CeA lesions impair sodium appetite (Zardetto-Smith et al., 1994). Altered palatability and intake induced by CTA and sodium appetite are critically dependent upon gustatory processing within the PBN (Reilly et al., 1993; Scalera et al., 1995; Grigson et al., 1998). Thus, centrifugal inhibition, particularly in PBN, might serve to adjust intake through decreasing taste-elicited drive on specific pools of premotor neurons in the reticular formation. Similar to the NST, some PBN taste cells receive input from multiple forebrain sites, while others do not (Cho et al., 2003; Lundy and Norgren, 2004; Li et al., 2005). Clearly, taste processing in the NST and PBN is a highly complex process involving forebrain modulation of overlapping and distinct populations of gustatory neurons and the engagement and disengagement of separate pools of forebrain neurons under different physiological circumstances.

4. Experimental procedures

4.1. Subjects

Six male Sprague–Dawley rats (Charles River), weighing 350–450 g, were housed in a temperature-controlled colony room on a 12-h light-dark cycle and maintained on *ad lib* access to normal rat pellets (Teklad 8604) and distilled water. All procedures complied with National Institutes of Health guidelines and were approved by the University of Louisville Institutional Animal Care and Use Committee.

4.2. Surgery

The rats were anesthetized with a 50-mg/kg injection (intraperitoneal, ip) of pentobarbital sodium (Nembutal). Atropine was administered to reduce bronchial secretions. Additional doses of Nembutal (0.1 ml) were administered as necessary to continue a deep level of anesthesia. The animals were placed on a feedback-controlled heating pad and body temperature maintained at 37 ± 1 °C. Animals were secured in a stereotaxic instrument and the skull was exposed with a midline incision then leveled with reference to bregma and lambda cranial sutures. A small hole was drilled through the bone overlying the cerebellum to allow access to the NST and the PBN.

4.3. Electrophysiological recording

Gustatory NST and PBN neurons were identified by recording multiunit activity through a glass-coated tungsten microelectrode (resistance: 1.5–3 M Ω) while stimulating the anterior tongue with 0.1 M NaCl. Only the anterior 2/3 of the tongue was stimulated because numerous prior studies have demonstrated that forebrain activation has a profound influence on brainstem taste cells that receive input via the chorda tympani nerve (Lundy and Norgren, 2001; Cho et al., 2002, 2003; Lundy and Norgren, 2004). Further, the concentration of NaCl used in the present study has been shown to produce a significant neural response in each “best-stimulus” class of PBN neurons (Lundy and Norgren, 2001, 2004). For access to the NST, the electrode was lowered at coordinates ranging from 12.0–12.8 mm posterior to bregma and 1.4–2.2 mm lateral to the midline according to the rat stereotaxic atlas (Paxinos and Watson, 1998). Typically, taste-evoked neural activity was encountered 6.0–7.0 mm ventral to the surface of the cerebellum. For the PBN, to avoid the transverse sinus, the electrode was oriented 20° off the vertical with the tip pointed rostral. The coordinates for PBN recordings were 11.8–12.4 mm posterior to bregma, 1.6–2.2 mm lateral to the midline and 5.0–6.0 mm below the brain surface. The surface of the cerebellum was kept moist throughout surgery with physiological saline.

4.4. Tracer injections

Once the gustatory region was identified, the tungsten electrode was replaced by a micropipette (ID 20–30 μ m) filled with a retrograde tracer. The recording side of this assembly was used to relocate the gustatory zone of the NST and the PBN. In two animals, fluorogold (FG) was injected iontophoretically (+ 2 μ A for 20 min; 2 min on and 1 min off) and fast blue (FB) by using pressure pulses (10 ms at 20 psi). In one of these animals, FG was injected into the NST and FB into the PBN, while in the other FB was injected into the NST and FG into the PBN. In four additional animals, green (GFB) and red (RFB) fluorescent latex microbeads (Lumafuor, Inc.) were pressure injected into the NST and PBN (10 ms at 20 psi). Again, half of the animals received injection of GFB into the NST and RFB into the PBN, the other half receiving the opposite.

4.5. Perfusion and histology

After a 7 day recovery period, the rats were euthanized with a lethal dose of Nembutal (100 mg/kg ip) and perfused through the ascending aorta, initially with 250 ml of 0.9% saline containing 5 ml of 100 units/ml heparin followed by 500 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brains were removed, blocked, frozen and then cut in the coronal plane at 20 μ m using a cryostat.

4.6. Immunohistochemistry

For the two animals that received injections of FG, brain sections mounted on gelatin-coated slides were incubated in 5% normal goat serum (NGS; Jackson Labs) mixed in 0.3% triton-x phosphate buffer saline (TPBS) for 1 h. Sections were then incubated overnight (4 °C) in rabbit FG antibody (Chemicon, AB153) diluted 1:2500 in TPBS. Sections were rinsed several times in PBS followed by 2 h incubation in FITC goat anti-rabbit diluted 1:100 in PBS with 5% NGS.

4.7. Analysis

Cell bodies positive for GFB (excitation filter: 490 nm; barrier filter: 550 nm) and RFB (excitation filter: 520–554 nm; barrier filter: 580 nm) immunoreactivity in the GC, CeA, BNST and LH were identified using an Olympus confocal microscope (sequential scanning). FG and FB positive neurons were identified using a Leica DM 2000 microscope equipped with UV and FITC filter cubes, Hamamatsu monochrome digital camera and Image-Pro Plus software. The number of positive cells per section (sum of cells divided by the number of sections) was calculated for statistical analyses using Image-Pro Plus software. The data sets were analyzed with 1 or 2-way ANOVAs and post hoc tests (Newman–Keuls) when appropriate. The results are presented as mean $\pm$ SE and a value of $P < 0.05$ was considered statistically significant.

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Fig. 1.

(A) The electrophysiological response to tongue application of 0.1 M NaCl recorded through the tracer filled injection pipette. The bottom panel shows the raw neural response and the top panel shows the peristimulus histogram. The stimulus sequence was water–NaCl–water. The scale bar at the bottom right equals 1 s. (B) Fluorescent images of tracer injection into the NST (panel 2, RFB) and PBN (panel 4, GFB) (montage taken at 20× magnification). The merged fluorescent and brightfield image of the same NST section (panel 1) and PBN section (panel 3) show the approximate area of the injections relative to subdivisions of the NST and PBN. The scale bar at the bottom right in panel 4 equals 0.3 mm. NST abbreviations: rc, rostral central; rl, rostral lateral; m, medial; v, ventral; Sp5n, spinal trigeminal nucleus. PBN abbreviations: cm, central medial; vm, ventral medial; vl, ventral lateral; Me5, mesencephalic nucleus; bc, brachium conjunctiva.

Fig. 2.

(A) Schematic representation of tracer injections concentrated in the rostral central (rc), rostral lateral (rl) and ventral (v) subdivision of the NST. (B) Schematic representation of tracer injections in the central medial (cm), dorsal medial (dm), ventral medial (vm), and ventral lateral (vl) subdivisions of the PBN. Sections are arranged from rostral (top) to caudal (bottom) and lateral is to the right. The approximate levels relative to bregma are indicated below each figure (Paxinos and Watson, 1998). Me5, mesencephalic trigeminal nucleus; bc, brachium conjunctiva.

Fig. 3.

Schematic representation showing the general location of retrogradely labeled neurons in each forebrain area. The gustatory cortex (GC) was identified as the area approximately 1 mm anterior and 0.8 mm posterior to bregma directly lateral to the claustrum (cl). The bed nucleus of the stria terminalis (BNST) was identified as the area approximately 0.2 mm anterior and 0.4 mm posterior to bregma directly medial to the internal capsule (ic). The central nucleus of the amygdala (CeA) was identified as the area approximately 1.8 to 3.3 mm posterior to bregma ventral to the striatum, medial to the basolateral nucleus of the amygdala (BLA), and lateral to the optic tract (ot). The lateral hypothalamus (LH) was identified as the area approximately 2.3 to 3.8 mm posterior to bregma sandwiched between the ic laterally and the fornix (fx)

medially. The approximate levels relative to bregma (A most rostral and H most caudal) are indicated at the top left corner in each section (Paxinos and Watson, 1998).

Fig. 4. The mean number of retrogradely labeled neurons in the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST), gustatory cortex (GC), and lateral hypothalamus (LH) following injections into the ipsilateral NST and PBN. **, significantly different from GC, BNST, and LH.

Fig. 5.

Representative photomicrographs of NST (GFB injection) and PBN (RFB injection) projection neurons in the bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), gustatory cortex (GC), and lateral hypothalamus (LH). Single-headed arrows in the panels on the left indicate GFB positive cells (green in each panel). Double-headed arrows in the middle panels indicate RFB positive cells (red in each panel). The merged images are shown on the far right and lines with a round head at the end indicate examples of double-labeled neurons. A scale bar is shown in lower right corner of LH (white, 50 μ m).

Fig. 6. High power (60 $\times$) photomicrographs of NST (GFB injection) and PBN (RFB injection) projection neurons in the bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), gustatory cortex (GC), and lateral hypothalamus (LH). Single-headed arrows in each panel indicates neurons positive for both tracers (yellow). A scale bar is shown in lower right corner of CeA (white, 50 μ m).