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Research Report

Terminal field specificity of forebrain efferent axons to the pontine parabrachial nucleus and medullary reticular formation

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ABSTRACT

The pontine parabrachial nucleus (PBN) and medullary reticular formation (RF) are hindbrain regions that, respectively, process sensory input and coordinate motor output related to ingestive behavior. Neural processing in each hindbrain site is subject to modulation originating from several forebrain structures including the insular gustatory cortex (IC), bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), and lateral hypothalamus (LH). The present study combined electrophysiology and retrograde tracing techniques to determine the extent of overlap between neurons within the IC, BNST, CeA and LH that target both the PBN and RF. One fluorescent retrograde tracer, red (RFB) or green (GFB) latex microbeads, was injected into the gustatory PBN under electrophysiological guidance and a different retrograde tracer, GFB or fluorogold (FG), into the ipsilateral RF using the location of gustatory NST as a point of reference. Brain tissue containing each forebrain region was sectioned, scanned using a confocal microscope, and scored for the number of single and double labeled neurons. Neurons innervating the RF only, the PBN only, or both the medullary RF and PBN were observed, largely intermingled, in each forebrain region. The CeA contained the largest number of cells retrogradely labeled after tracer injection into either hindbrain region. For each forebrain area except the IC, the origin of descending input to the RF and PBN was almost entirely ipsilateral. Axons from a small percentage of hindbrain projecting forebrain neurons targeted both the PBN and RF. Target specific and non-specific inputs from a variety of forebrain nuclei to the hindbrain likely reflect functional specialization in the control of ingestive behaviors.

1. Introduction

Several rostral forebrain areas like the insular gustatory cortex (IC), bed nucleus of stria terminalis (BNST), central nucleus of the amygdala (CeA), and lateral hypothalamus (LH) have widespread neural connections with hindbrain sites that process gustatory information and coordinate the control of oromotor nuclei (Veening et al., 1984; van der et al., 1984; Moga et al., 1990). One view is that these diverse centrifugal connections play a significant role in the establishment and elaboration of taste preference that can promote or discourage consumption.

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As the second-order relay in the rat central gustatory system, the parabrachial nucleus (PBN) of the pons transmits afferent taste information monosynaptically to the BNST, CeA and LH, and disynaptically via the thalamus to the IC, and, in turn, receives extensive projections from these same forebrain areas (Norgren, 1976; Saper and Loewy, 1980; Li and Cho, 2006; Nishijo et al., 2000; Yasoshima et al., 1995). Similar to decerebration, bilateral lesions of the classically defined caudomedial gustatory PBN eliminate learning and nutritional state dependent alteration of taste preference in rats (Reilly et al., 1993; Grigson et al., 1998; Scalera et al., 1995). In contrast, lesions of the gustatory area in the thalamus that only disrupts taste information along the disynaptic thalamocortical pathway have no obvious effects on these behavioral measures (Scalera et al., 1997). Together these findings support the notion that the neural mechanisms governing gustatory plasticity, at least in part, involve direct reciprocal communication between the PBN and ventral forebrain.

The medullary reticular formation (RF) is another hindbrain area critical for ingestive behavior, because it contains neurons that directly influence nuclei controlling muscles for licking and mastication (Travers et al., 1997; Travers and Norgren, 1983). Infusions of the $GABA_A$ agonist muscimol or glutamate antagonists into RF suppresses ingestion and rejection responses to sapid stimulation of the oral cavity in awake-behaving rats (Chen et al., 2001; Chen and Travers, 2003). Similar to the PBN, the medullary RF is targeted by descending axons of IC, BNST, CeA and LH origin (Notsu et al., 2008; Hopkins and Holstege, 1978; Yasui et al., 2004; Berk and Finkelstein, 1982; Notsu et al., 2008). The divergence of forebrain inputs to the PBN and RF provide an anatomical substrate for simultaneous control of sensory input and motor output related to ingestive behavior. However, clear evidence for the existence of individual forebrain neurons with divergent input to both hindbrain sites is lacking.

Thus, the present study investigated the extent to which individual neurons in the IC, BNST, CeA, and LH give rise to efferent axons that project both to the medullary RF and gustatory PBN. A different fluorescent retrograde tracer was injected into each hindbrain site and the number of single- and double-labeled neurons in each forebrain region was quantified.

2. Results

2.1. Injection sites

In three animals, retrograde tracer was concentrated in the intermediate zone of the medullary RF (IRt), while in the other three animals tracer material was mostly confined to the more lateral parvocellular region of RF (PCRt). In all six animals, the classically defined caudomedial taste responsive subnuclei of PBN were targeted including the central medial (cm), ventral medial (vm), and waist area between the cm and ventral lateral (vl) subdivisions. These electrophysiologically guided injections resulted in minimal spread into more rostral non gustatory responsive regions. Representative photomicrographs of cresyl violet stained sections revealing the location of tracer material are shown in Fig. 1A (IRt), B (PCRt), and C (PBN). Fig. 2 shows a summary of the six tracer injections in the RF and PBN.

2.2. Retrograde labeling

Figs. 3 and 4 show representative photomicrographs of single- and double-labeled neurons along with alternate cresyl violet stained sections depicting the general location within the IC and BNST (Fig. 3), and CeA and LH (Fig. 4). Neurons labeled in three different ways can be seen, green only cells project to the RF, red only cells project to the PBN, and green plus red cells project both to the RF and PBN. Relative to bregma, the approximate rostrocaudal extent of tissue examined for retrogradely labeled neurons was 1.8 to –0.4 for IC, 0.0 to –0.7 for BNST, –1.8 to –3.0 for CeA, and –2.4 to –4.0 for LH (Paxinos and Watson, 1982). All tracer labeled neurons in the LH area were counted without distinguishing whether they were nearer to the fornix (e.g. more medial) or internal capsule (e.g. more lateral). On the basis of prior tract tracing and anatomical studies, the portion of IC examined included the gustatory responsive region as well as a more posterior region that receives convergent inputs from baroreceptor, chemoreceptor, gustatory, and nociceptive organs (Cechetto and Saper, 1987; Kosar et al., 1986; Hanamori et al., 1998). The same is likely true for the other 3 forebrain areas and, thus, the present data cannot distinguish between the type(s) of sensory information processed by retrogradely labeled neurons. We can only say whether they project: (1) to an area that processes taste information (i.e. caudomedial PBN), (2) to one that directly influences oromotor neurons (i.e. RF), or (3) to both areas. Fig. 5 shows higher magnification images of single- and double-labeled neurons in each forebrain region.

Unlike the descending projections from other forebrain areas, which were almost entirely ipsilateral, the RF and PBN receive considerable input from both ipsilateral and contralateral IC. Given that in half of the animals' tracer material was placed within different subdivisions of the medullary RF, we first used analysis of variance to determine any differences in retrograde labeling between IRt and PCRt injections. Significant differences were not observed in terms of the number of single- and double-labeled neurons ($Fs_{4,30}<1.9$, $ps>0.10$; Table 1). Thus, the data from IRt and PCRt injections were combined for further analyses.

For each forebrain area and animal, the average number of neurons per section resulting from retrograde tracer injection into the RF and PBN was calculated and is graphically shown in Fig. 6. A two-way ANOVA varying injection and forebrain site revealed a significant interaction between factors ($F_{4,60}=5.05$, $p<0.01$). Irrespective of injection site, the major source of descending input originated in the CeA. Significantly fewer neurons in the ipsilateral IC projected to the RF, while the contralateral IC contained the fewest number of cells projecting to the PBN. A greater number of neurons in the CeA, BNST, and ipsilateral IC projected to the PBN compared to RF (all $ps\leq0.03$). Moreover, the distribution of cells within the BNST varied as a function of hindbrain target, ($F_{1,24}=11.3$, $p<0.01$). While a comparable number of cells in the caudal (60.8±7.6) and rostral (72.9±8.4) half of the BNST projected to the PBN ($p=0.26$), almost 5 fold more caudal BNST cells (46.5±8.4) targeted the RF compared to the rostral BNST (8.4±4.7). The photomicrographs of Fig. 7 show an example of the rostrocaudal distribution of RF and PBN projecting neurons in BNST.

Although the actual numbers of labeled neurons per section varied depending on injection size in the RF (ICi,