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Reflex Topography in the Nucleus of the Solitary Tract

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Introduction and methods

The diverse influences that taste signals exert on organisms give rise to a complex anatomy. The first-order taste relay, the rostral nucleus of the solitary tract (rNST), is the origin for local reflex pathways, and ascending pathways important in motivational and cognitive functions. The intrinsic organization of the nucleus is characterized by topographic specialization. Cells with different morphologies, projections, receptive fields and responsiveness to taste versus oral tactile stimuli are preferentially distributed. Notably, however, a map of quality has not been evident.

Taste stimuli encompass not only food-related chemicals that promote ingestion, but also potentially toxic, often bitter, molecules that provide powerful signals to prevent intake. Based on Fos immunohistochemistry, we argue that there is a topographic representation of the bitter taste quality in rNST but one restricted to a subpopulation of neurons with a special function. In the Fos studies summarized below, taste stimuli (~7 ml/30 min) were infused through intraoral cannule into awake rats and their brains processed using immunohistochemical techniques to detect the Fos protein. Control groups were unstimulated and/or water-stimulated animals (see Harrer and Travers, 1996; Travers and Hu, 2000; Travers, 2002; Chan *et al.*, 2004).

Results and Discussion

The initial study compared two tastants with opposite effects on ingestion: sucrose (1.0 M) and QHCl (3 mM). There was a differential distribution of Fos-like immunoreactive (FLI) neurons. Sucrose elicited FLI more evenly distributed along the mediolateral axis, compared to QHCl, which elicited FLI more restricted medially (Harrer and Travers, 1996). Subsequent studies indicated that the QHCl pattern was maintained across concentration (Travers, 2002) and different bitter stimuli (Chan *et al.*, 2004). Additional data indicated that citric acid (30 and 100 mM) also evoked a different FLI distribution than QHCl (Travers, 2002; Chan *et al.*, 2004) but one similar to sucrose. With the NST divided into mediolateral thirds, there were two FLI patterns. Bitter stimuli evoked more FLI in the medial third of NST but sucrose, citric acid, water or no stimulation all were associated with maximal FLI in the middle subfield, with fewer Fos-positive neurons in the control groups. Thus the topographic organization does not extend to all qualities, since citric acid and sucrose evoke different sensations ('sour' and 'sweet') but similar FLI distributions. Neither does it represent a simple preferred-avoided axis, since both QHCl and citric acid are avoided in preference tests but elicit distinctive FLI.

Important insights were gained by studying another standard tastant. Surprisingly, 0.3 M NaCl, a potent stimulus, elicited FLI indistinguishable from water (Travers, 2002), demonstrating that not all activated taste neurons are revealed by Fos. This further implies that although QHCl, sucrose and citric acid are effective in eliciting Fos, the observed neurons may represent only a subset of the activated populations. As such, it is possible that these neurons have specialized functions. The clearest hypothesis derives from FLI neurons observed after bitter stimulation.

Decerebrate rats display FLI in the typical pattern following QHCl stimulation (Travers *et al.*, 1999), but IXth nerve transection greatly reduces the number of FLI neurons and obliterates their distinctive medial distribution (King *et al.*, 1999). Behaviorally, decerebration obliterates voluntary QHCl behaviors, but the reflex rejection response, described by Grill and Norgren (1978), are virtually untouched. In contrast, IXth nerve transection spares voluntary QHCl avoidance (Pfaffmann, 1952) and performance in discriminative tasks (St. John and Spector, 1998), but reflex rejection is severely compromised (Travers *et al.*, 1987). The persistence of QHCl-elicited FLI and oral rejection after decerebration, in contrast to the blunting of Fos and this behavior after IXth nerve section, is consistent with the hypothesis that neurons expressing Fos after QHCl stimulation are a subset of bitter-activated neurons with a critical role in reflex rejection (King *et al.*, 1999). Furthermore, since a particular NST region, the medial third, preferentially expresses Fos after bitter stimulation, we suggest the reflex has a topographic representation in NST. This hypothesis is also consistent with the relative effectiveness of various stimuli in eliciting reflex rejection, including one exception to the unique nature of the 'bitter' pattern. In two cases, an error resulted in infusion of a very high concentration of NaCl (~1.7 M), and revealed robust FLI with a medial, 'bitter-like' topography. Reliable behavioral observations on one case indicated clearly that rejection had occurred. This salt concentration constitutes a trigeminal, as well as a gustatory stimulus, but significantly, capsaicin (100 μM), a potent trigeminal non-gustatory stimulus, yielded sparse FLI in rNST without a preferential medial distribution and only minimal gaping (DiNardo, 1997). Thus, FLI in the medial NST is best associated with stimuli that evoke the gustatory rejection reflex.

Reflex rejection is not synonymous with gapes, but gapes are the most salient component of the rat rejection response and the most often quantified. Recent unpublished observations in the mouse demonstrate that intraoral QHCl evokes few gapes, and instead chin rubs and suppression of licking characterize rejection in this species. Nevertheless, QHCl evoked FLI in the mouse NST that closely resembled that in the rat in its cardinal feature, a pronounced increase in the medial third of NST. These results emphasize that the medial NST is not specifically associated with gapes, but the entire sequence of gustatory rejection.

Aside from the correlation between FLI and behavior, another approach to defining the function of these neurons is to establish their projections. Dual-retrograde tracing suggests that mostly separate populations of rNST cells project to the parabrachial nucleus (PBN) and the reticular formation (RF) subjacent to NST (Halsell *et al.*, 1996). Functional inactivation demonstrates that the RF is necessary for gustatory-elicited oromotor responses, including reflex rejection (Chen *et al.*, 2001). If cells expressing Fos after bitter stimulation contribute preferentially to oromotor rejection, a greater proportion might project to the RF than PBN. However, double-labeling with Fos immunohistochemistry combined with retrograde tracing revealed instead that approximately twice as many gustatory-

activated FLI cells projected to PBN. In fact, only about one-third of the Fos cells contacted either target, suggesting that a substantial proportion are interneurons.

These anatomical results suggest that Fos-activated neurons in the medial subfield trigger oromotor rejection via more complex circuits. A number of pathways could act in parallel. Although older data suggested that PBN lesions had little impact on gustatory oromotor reflexes (Flynn *et al.*, 1991), two recent studies (Matsuo *et al.*, 2001; King *et al.*, 2004) suggest that lesions or glutaminergic blockade in PBN suppress gapes. Thus an NST–PBN–RF projection is likely involved in oromotor rejection. In addition, a possible local pathway is for FLI cells in the medial subfield to make intranuclear connections to other NST cells that do not express FLI but that do project to the RF. The lack of Fos might mean that the higher-order NST neurons do not have the requisite intracellular signaling pathways, or that intranuclear connections are primarily inhibitory. Preliminary data ($n = 1$) with in-situ hybridization for GAD65 does suggest that some NST cells that express FLI after QHCl stimulation are inhibitory. A second possibility is that the connections between the NST and RF occur over very short distances, making them difficult to study precisely with extracellular tracing methods. Indeed a striking feature of the FLI elicited after bitter stimulation in awake animals is that Fos-positive cells extend in a continuous swath from the medial NST ventrally and laterally into the RF, suggestive of a multi-stage short connection pathway. Within this distribution, we have preliminary evidence for NST-RF communication via extension of RF dendrites into NST. With double-staining for NADPHd, a marker for nitric oxide synthase, Fos-activated nitrergic cells in the dorsal RF immediately subjacent to NST were observed to send dendrites toward and sometimes into the overlying nucleus (Travers, 2000).

In summary, these data provide evidence for a novel type of topography in the first-order gustatory nucleus. The critical variable underlying this topography appears related to a specific gustatory behavior– reflex rejection of potentially toxic foodstuffs. To the extent that these stimuli are most closely tied to bitter taste, this is a type of chemotopy. However, because reflex rejection is just one function of bitter tastants, it is more accurate to label this as a reflex topography. The suggested circuit by which these neurons exert their effects are speculative. However, the fact that we can functionally define neurons implicated in a specific behavior should help to resolve the critical connections and their mechanisms.

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