Taste Representations in the Mouse Brain Revealed by Genetic Tracing

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The taste sensory system is responsible for detecting various compounds that are noxious or toxic, and that provide caloric energy, while playing a critical role in the life and nutritional status of organisms. Humans can detect and discriminate between sweet, bitter, sour, salty, and umami stimuli (Fig. 1). The sense of taste also evokes responses that range from innate behavioral actions such as aversion and attraction to food sources, to the pleasure of food consumption.

Taste perception in mammals is mediated by the specialized epithelial cells (taste receptor cells), which are arranged in taste buds on the tongue. Two families of mammalian taste receptors, the T1Rs and T2Rs, have been implicated in sweet, umami, and bitter detection (Scott, 2004) (Fig. 1). The 30 members of the T2R receptor family, most of which are co-expressed in the same subset of taste cells, appear to encode bitter receptors. The T1R receptor family generates at least two heteromeric receptors. T1R2 + T1R3, and T1R1 + T1R3 function as taste receptors for sweet and umami, respectively. Although subsets of T2Rs-positive cells and T1Rs-positive cells are localized in an identical taste bud, bitter and sweet receptors are not co-expressed in the same taste receptor cells (Scott, 2004). Recently, it was reported that the signal transduction molecules, phospholipase Cβ2 (PLCβ2) and the TRPM5 ion channel, is expressed in taste receptor cells (Zhang *et al.*, 2003). Mice lacking either PLCβ2 or TRPM5 abolish sweet, amino acid, and bitter taste reception, suggesting that both receptor families converge on a common signaling pathway in the cells (Zhang *et al.*, 2003). Furthermore, the PLCβ2 transgene expressed under the control of the T2R promoter rescued the response to multiple bitter compounds, but not to sweet or umami taste (Zhang *et al.*, 2003; Mueller *et al.*, 2005), supporting the finding that bitter and sweet receptors are not co-expressed in the same taste receptor cells, and the notion that the two modalities are recognized independently. Recent reports also show that activation of a single cell-type (sweet cells or bitter cells) is sufficient to generate a behavior (attractive or aversive), arguing against that taste receptor cells respond to multiple taste modalities (Zhao *et al.*, 2003; Mueller *et al.*, 2005).

We think it is a time to start examining how sweet and bitter taste information is processed in the central nervous system, when the discovery of bitter and sweet taste receptors indicates the coding of those tastes at the periphery, and importantly, when it is found that the coding qualities of those tastes are similar because of sharing intracellular signaling pathways in taste receptor cells (Zhang *et al.*, 2003; Zhao *et al.*, 2003; Mueller *et al.*,

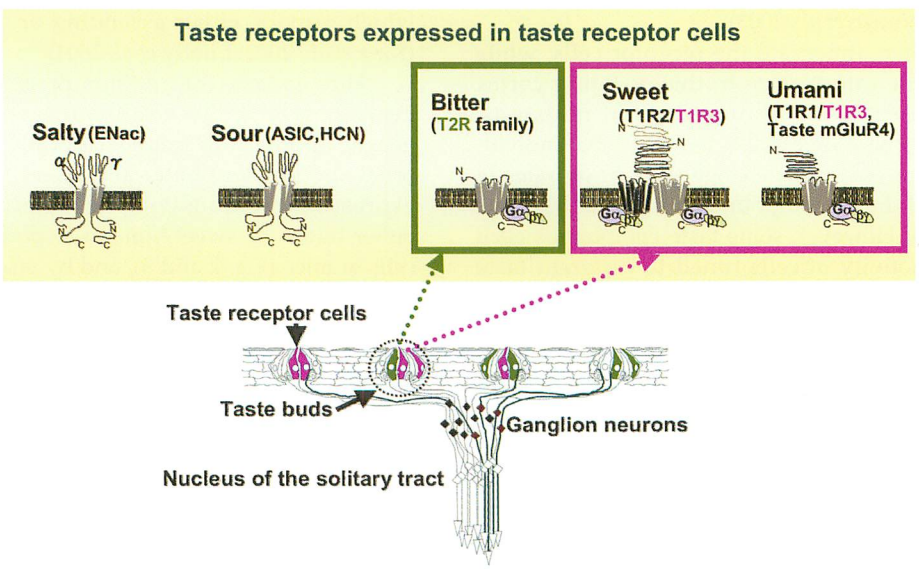

Fig. 1 Schematic representation of taste receptors expressed in taste receptor cells. The sensations of bitter and sweet/umami tastes are initiated by the interaction of sapid molecules with GPCRs such as the T2R family and T1R heterodimers in the apical membranes of taste receptor cells.

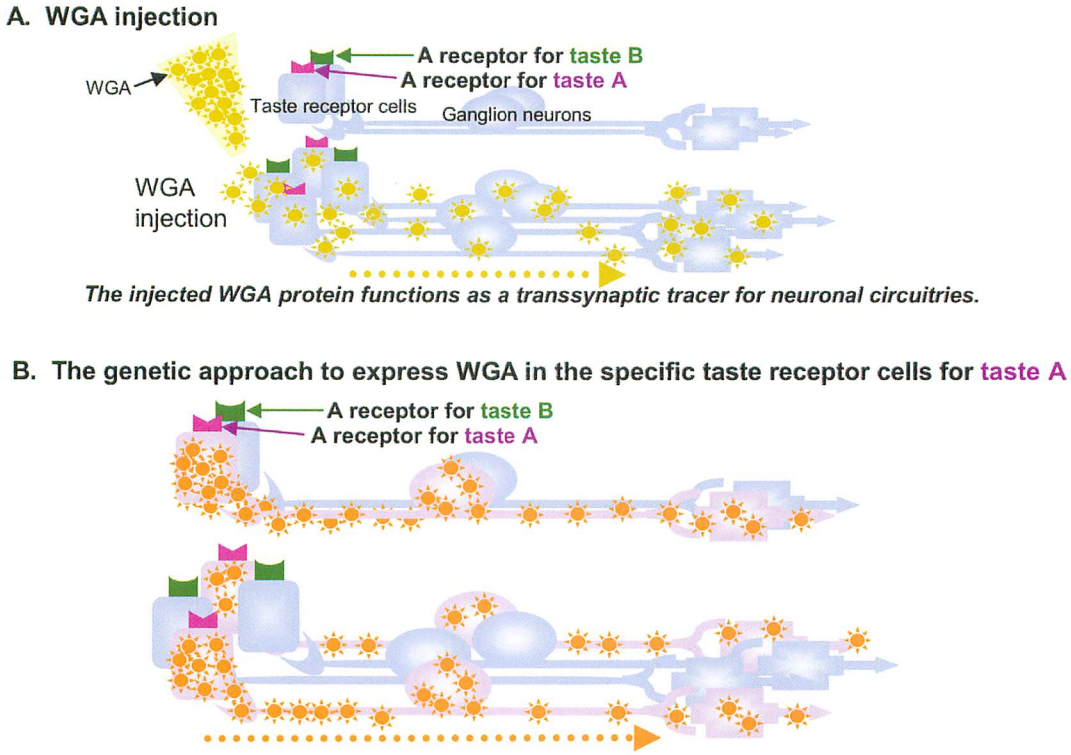

Fig. 2 The plant lectin, WGA, as a transsynaptic tracer. The previous reports indicated that the injected WGA protein functions as transsynaptic tracer for neuronal circuitries (A). However, the specific neuronal circuitries for taste A (sweet or bitter) in comparison with taste B were not clarified by using the WGA injection. How can we trace the specific neuronal circuitries for taste A? To address this issue, we expressed WGA in the specific taste receptor cells for taste A in transgenic mice (B).

2005). Animals lacking sweet and bitter reception and perception still respond sour and salty stimuli (Zhang *et al.*, 2003), importantly suggesting that sour and salty tastes are detected independently of bitter and sweet tastes although coding of sour and salty taste remains less clear (the labeled-line coding or the distinct quality of coding, such as temporal coding (Katz *et al.*, 2001; Katz *et al.*, 2002; Di Lorenzo *et al.*, 2003).

Afferent fibers innervate the receptor cells, while transmitting taste information to the gustatory cortex through synapses in the brain stem and thalamus. Previous electrophysiological data indicated that individual taste-responsive neurons change their firing rates in response to both bitter compounds and ones that taste sweet or umami. However, some taste compounds elicit the combined activity of cells tuned to different taste modalities. For example, quinine may activate not only T2Rs-expressing cells, but also the other taste receptor cells through the blockage of K^+ channels (Akabas *et al.*, 1990; Cummings *et al.*, 1992). Monosodium glutamate may evoke both salty and umami taste because of its Na^+ content (Zhao *et al.*, 2003). Many sweeteners exemplified by saccharine are likely to activate T2Rs-expressing cells. The potency of the side effects might depend on the experimental procedures. Thus, we need to carefully interpret and decode the previous reports. On the other hand, many data revealed that neurons in ganglions, the solitary tract nuclei and even in the gustatory cortex are differentially sensitive to bitter and sweet compounds (Frank, 1991; Danilova *et al.*, 1998; Lemon *et al.*, 2005; Yamamoto *et al.*, 1989), although neurons in the parabrachial nuclei and the gustatory cortex may also elicit the time-varying gustatory responses with more broadly tuned characteristics (Katz *et al.*, 2001; Nishijo *et al.*, 1997) because of interactions with other neurons within the same nucleus and with other gustatory nuclei, which provide either ascending or descending inputs (Katz *et al.*, 2002; Lundy *et al.* 2004).

How is taste information processed in the central nervous system? To address this issue, we applied a genetic approach to transsynaptically delineate the neuronal circuitries of bitter and sweet taste by selectively expressing the transsynaptic tracer, tWGA-DsRed, in either bitter- or sweet/umami-responsive taste receptor cells in mice (Fig. 2 and 3), and by visualizing the spatial distribution of tWGA-DsRed in the brain (Sugita *et al.*, 2005). The WGA proteins are well known to function as anterograde and retrograde tracers. Thus, tWGA-DsRed, which is originated from taste receptor cells and transferred to the neurons in the solitary tract nuclei, may be further transferred along their ascending pathways, or to the neurons which provide the descending inputs to the solitary tract nuclei. However, the labeling density is likely to be influenced by the efficiencies of transport and degradation of the tracer, governed by the molecular backbones which underlie anatomical and functional differences of the axonal and synaptic systems, and which may vary among neuronal types. Although the neurons,

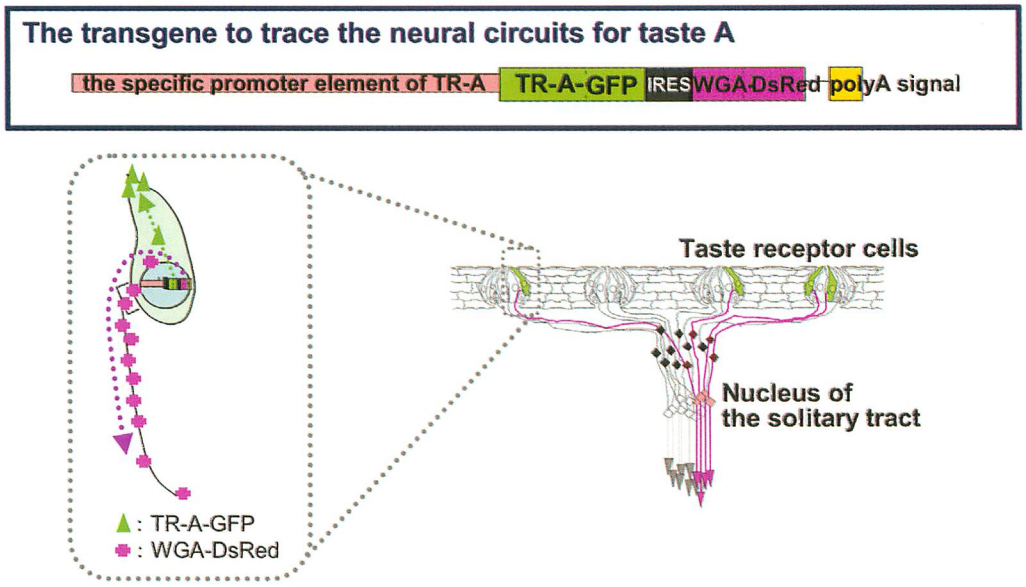

Fig. 3 The transgene to trace the specific taste neuronal circuitries. Specific taste neuronal circuitries were traced by the transgene employing the specific promoter element of a receptor for taste A (TR-A), the TR-A-GFP cDNA, the internal ribosome entry site (IRES), the WGA(truncated)-DsRed cDNA, and the polyadenylation signal. In the transgenic mice, the fusion proteins of TR-A-GFP and tWGA-DsRed were expressed in a subset of taste receptor cells, which expressed endogenous TR-A, and the WGA-DsRed fusion protein was transferred to the neurons with which they synapse, allowing us to visualize the specific transsynaptic neural pathways.

which contained only a small amount of tracer and were not detected, might also relay taste information, locations of the neurons containing a detectable amount of tWGA-DsRed revealed segregation of bitter- and sweet-inputs in the gustatory area in the brain (Sugita *et al.*, 2005), implying that the ascending input pathways are more effectively labeled than are the descending input pathways and the neuronal circuitries within the same nuclei. Comparison of the spatial distribution of tWGA-DsRed, transferred from either bitter- or sweet/umami-responsive taste receptor cells, revealed that the gustatory neurons, dispersed in the solitary tract nuclei, the parabrachial nuclei, the thalamic gustatory area, and the gustatory cortex, may be organized with sweet inputs rostral and with bitter inputs caudal, except for bitter inputs into the external lateral and external medial subdivisions of the parabrachial nuclei (Sugita *et al.*, 2005). In the amygdala and the gustatory cortex, the dispersed areas of tWGA-DsRed-labeled neurons in two strains appeared to partly overlap along the anterior-posterior axis (Sugita *et al.*, 2005). By mapping connections formed by small subsets of neurons, which process and integrate the information of bitter taste, separated from sweet taste, this genetic approach may be valuable for investigating the molecular aspects underlying the construction and refinement of taste neuronal circuitries to mediate taste discrimination, contrastive behavioral responses, emotional states and taste-associated learning. Electrophysiological characterization of tWGA-DsRed-labeled neurons under pharmacological and genetic manipulation of the descending inputs and the interactions with other neurons within the same nuclei is now of critical relevance for understanding the neuronal properties, which might be linked to taste discrimination, behavioral responses and emotional states.

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