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Synaptic Connections in Developing and Adult Rat Taste Buds

John C. Kinnamon, Mary Dunlap and Ruibiao Yang

Department of Biological Sciences, University of Denver and the Rocky Mountain Taste and Smell Center, Denver, Colorado 80208, USA

Correspondence to be sent to: John C. Kinnamon, e-mail: jkinnamo@du.edu

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Synapses in adult rat circumvallate taste buds

Synaptic connections in rat taste buds closely resemble synapses found in the CNS. Taste bud synapses are characterized by parallel, apposed, thickened membranes that are separated by a cleft ranging from 16 to 30 nm. The putative synaptic vesicles are of two types: small, clear vesicles (40–70 nm) and large, dense-cored vesicles (90–120 nm). The small, clear vesicles predominate at most synapses. Most taste bud synapses fall into two structural types: small, macular synapses and 'finger-like' synapses (Kinnamon *et al.*, 1985). The finger-like synapses are characterized by a rod-like postsynaptic process that protrudes into an invagination of the presynaptic taste cell. Virtually all adult synapses are afferent, from the gustatory receptor cell onto a process of a cranial nerve. It has been proposed, however, that subsurface cisternae of smooth endoplasmic reticulum located at the close appositions between taste cells and nerve processes may participate in efferent modulation of the taste cell (Ide and Munger, 1980; Clapp *et al.*, 2004). Synapses in developing taste buds are more varied, including afferent, efferent, and neuro-neuronal synapses.

Most synapses in the CNS utilize a variety of proteins to translocate synaptic vesicles to the active zone of the presynaptic membrane, dock the vesicle, cause fusion of the vesicle membrane with the presynaptic membrane, and bring about exocytosis of the vesicle contents into the synaptic cleft. We are currently studying four synaptic proteins: SNAP-25, synaptobrevin, syntaxin and synaptotagmin. SNAP-25 and syntaxin are presynaptic membrane proteins, while synaptobrevin and synaptotagmin are synaptic vesicle proteins. SNAP-25, syntaxin and synaptobrevin (VAMP) are the three SNARE proteins (SNAP receptors) that form the core complex involved in synaptic vesicle docking and fusion (Söllner *et al.*, 1993; Jahn and Südhof, 1994). We have found that SNAP-25-like immuoreactivity (-LIR) is present in a small subset of taste cells (Yang *et al.*, 2000). Approximately 92% of the taste cells we observed with synapses displayed SNAP-25-LIR. Syntaxin, another presynaptic membrane protein, is also expressed in taste cells with synapses (Yang *et al.*, 2004a), as is synaptotagmin (unpublished observations). Using colloidal gold immunoelectron microscopy we have found that synaptobrevin-LIR is closely associated with vesicles at synapses from taste cells onto nerve processes. Based on their ultrastructural features we believe that most, if not all, of the taste cells with synapses in rat circumvallate taste buds are type III cells.

Synapses in developing taste buds

Postnatal day 0

Animals aged postnatal day 0 (P0) through P14 were examined with light and transmission electron microscopy. Profound ultrastructural changes occur in circumvallate taste buds during the first 2 weeks of postnatal life in the rat. At P0, developing taste buds contain undifferentiated cells that cannot be distinguished easily as to cell type. The gustatory epithelium is generally more electron-dense than the surrounding nongustatory epithelium. Only a few profiles of taste cells are present in any given section. We have not observed any mature taste buds (i.e. taste buds with a taste pore) at birth. Most of the undifferentiated taste cells are moderately electron-dense and contain many polyribosomes. Those cells often contain short chains of rough endoplasmic reticulum and a few mitochondria in close proximity to their nuclei. The nuclei of these undifferentiated taste cells are irregular in shape, with patchy heterochromatin adherent to the inner leaflets of the nuclear lamellae.

Postnatal day 1

During the first days of postnatal life a plexus of nerve processes is present in circumvallate taste buds of the rat. This nerve plexus is most prominent during postnatal days 1–4. At postnatal day 1, nerve processes take up much of the taste bud volume. In addition to conventional afferent synapses from taste cells onto nerve fibers, there are numerous neuro-neuronal synapses at P1. We have observed both asymmetrical and symmetrical neuro-neuronal synaptic contacts. Typically, both the presynaptic and postsynaptic elements contain numerous small clear vesicles and occasional large, dense-cored vesicles. Some, but not all of the nerve processes are possess several microtubules, as well as a few mitochondria.

Postnatal day 2

By postnatal day 2, taste buds within the circumvallate papillae have begun to assume a more adult appearance, yet the cells comprising the taste bud are still not well organized. Some of the taste cells have assumed a spindle-shape. Taste cells are replete with polyribosomes and individual ribosomes. Numerous mitochondria and lipid droplets are present in both the infranuclear and supranuclear cytoplasm. In addition, the ground substance of the developing type II cells has become electron-dense. Dense granules are present in the apical cytoplasm of the type I cells. Although the apical cytoplasm of the type II cell lacks the apical dense granules, numerous mitochondria and a few electron-lucent empty vesicles—presumably poorly preserved smooth endoplasmic reticulum are present in that region of the cell.

At postnatal day 2 most of the nerve plexus is restricted to the lower one-third of the taste bud. The nerve plexus is still present, but it is no longer the dominant feature of the taste bud as at postnatal day 1. The developing taste pore of a 2-day-old animal is often slender in appearance. Only a few layers of keratinized, stratified squamous epithelium separate the taste bud from the oral cavity. A few spindle-shaped cells have elongated and extended microvilli into the developing pore region. The microfilaments that comprise the roots of the microvilli can extend for considerable distances into the apical regions of the taste cells. A few tight junctions are present at the apices of the taste cells and desmosomes are present between taste cells in the upper one-third of the taste bud.

Postnatal day 4

The nerve plexus is present, but significantly smaller than at postnatal day 2. As differentiation of the taste cells proceed, it becomes progressively easier to distinguish type I from type II cells. More apical dense granules are present in type I cells, as well as several cisternae of rough endoplasmic reticulum. The type II cells have numerous supranuclear mitochondria. Most of the synapses are afferent, from taste cells onto nerve processes. Numerous mitochondria are usually present in the presynaptic region. Although most of the synaptic vesicles associated with these synapses are small, clear vesicles, a few large, dense-cored vesicles may be associated with the presynaptic region. Many small clear vesicles are often present in the postsynaptic nerve process, although only rarely are there any thickenings or other specializations from which one might infer the presence of an efferent synapse. Often aggregations of glycogen granules are present in the postsynaptic cytoplasm of the nerve fiber.

Postnatal day 6

Both the gustatory and nongustatory epithelium have increased in thickness, and the vertical extent of the developing taste bud has increased correspondingly. Most of the nerve processes are located near the base of the taste bud, as is characteristic of the adult condition. No significant nerve plexus is present. Many of the type I and type II cells are easily distinguished. In longitudinal section, several of the spindle-shaped taste cells in a taste bud from a 6-day-old animal span the entire length of the taste bud and terminate apically into the taste pore. The type II cells contain many mitochondria located above the centrally located nuclei. The nuclei are oval in profile with heterochromatin scattered throughout the nuclear matrix. At this stage in development, the taste pore has widened, and in many cases has access to the oral cavity. Many of the taste cells have assumed a spindle shape and project microvilli into the oral cavity. Accumulations of electron-dense pore substance are present in the taste pore region. Tight junctions are common between the taste cells. Typical afferent synapses are present. At this developmental stage we observed several examples of divergence, wherein a singe taste cell formed synapses onto two or more profiles of nerve fibers. Occasionally, glycogen deposits are still present in postsynaptic nerve processes. Postnatal day 6 is approximately the last day at which neuro-neuronal synapses are present.

Postnatal day 14

More taste cells have been added to the taste bud and it is essentially adult in appearance, albeit with fewer cells than an adult taste bud. Both type I and type II cells can easily be distinguished at postnatal day 14, as well as putative basal cells situated at the base of the taste bud. Numerous apical dense granules are located in the apical cytoplasm of the type I cells. Only a few nerve processes are present, and virtually all of the synapses are afferent. Moreover, by the end of the first two weeks of postnatal life, the taste pore has also taken on a mature appearance. Many taste cells project microvilli into the taste pore, and electron-dense pore substance fills most of the inter-microvillar spaces of the taste pore.

Conclusions

We speculate that the synaptic interactions between developing taste bud cells and the intragemmal neurons provide a basis for controlling the early developmental events associated with taste bud formation and development of the neurotrophic relationship between taste bud cells and sensory nerve fibers.

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