The presynaptic active zone protein Bassoon as a marker for synapses between Type III cells and afferent nerve fibers in taste buds

公立大学法人
福岡女子大学
FUKUOKA WOMEN'S UNIVERSITY

Rio Ikuta & Shun Hamada

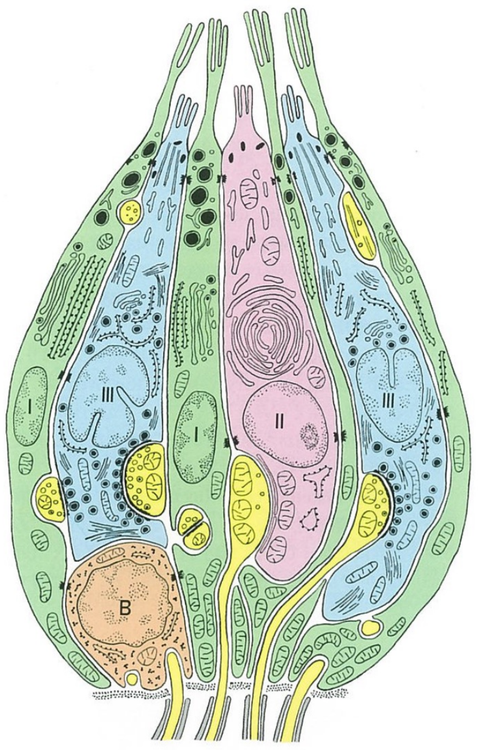
Background & Purpose

Taste buds are receptor organs for gustation. Each taste bud consists of 50-100 cells that are classified into four types (Type I, II, III and basal cell, ***Right***). Type II and Type III cells are directly involved in the transduction of different taste modalities and activate afferent nerve fibers.

The structures of synapses in Type II and III cells are markedly different from each other. The synapses of Type III cells are conventional synapses that show the accumulation of synaptic vesicles. In contrast, the synapses of Type II cells lack synaptic vesicles and release a neurotransmitter through channels.

Reliable markers for synapses at the light microscopic level greatly contribute to the study of synapse in various nervous systems. Although a synaptic marker for the synapses of Type II cells has been recently identified, there is currently no useful marker for conventional synapses of Type III cells.

In the present study, we examined the distribution pattern of Bassoon, a scaffolding protein of the cytomatrix at the active zones of conventional synapses, to determine whether it could be a synaptic marker for Type III cell synapses.



I: Type I cell, II: Type II cell, III: Type III cell, B: basal cell. Nerve fibers are shown in yellow.
From Yoshie et al .(1990)
Arch. Histol. Cytol., 53: 103-119

Result

We examined the localization of Bassoon in the taste buds of the circumvallate papillae of mice using confocal laser microscopy with immunofluorescence (**Figures 1 and 2**) and immunoelectron microscopy (**Figure 3**).

Bassoon was detected in Type III cells but not in Type II cells

At the light microscopic level, Bassoon-immunoreactivity (IR) was detected as round or elongated puncta in longitudinal sections of the taste buds (Figure 1a,b). Most Bassoon-positive puncta were observed in Type III cells that were detected by immunostaining for carbonic anhydrase (CA4) (Figure 1c-h). In contrast, few Bassoon-positive puncta were observed in Type II cells that were detected by immunostaining for phospholipase C β2 (PLCβ2) (Figure 2a-e).

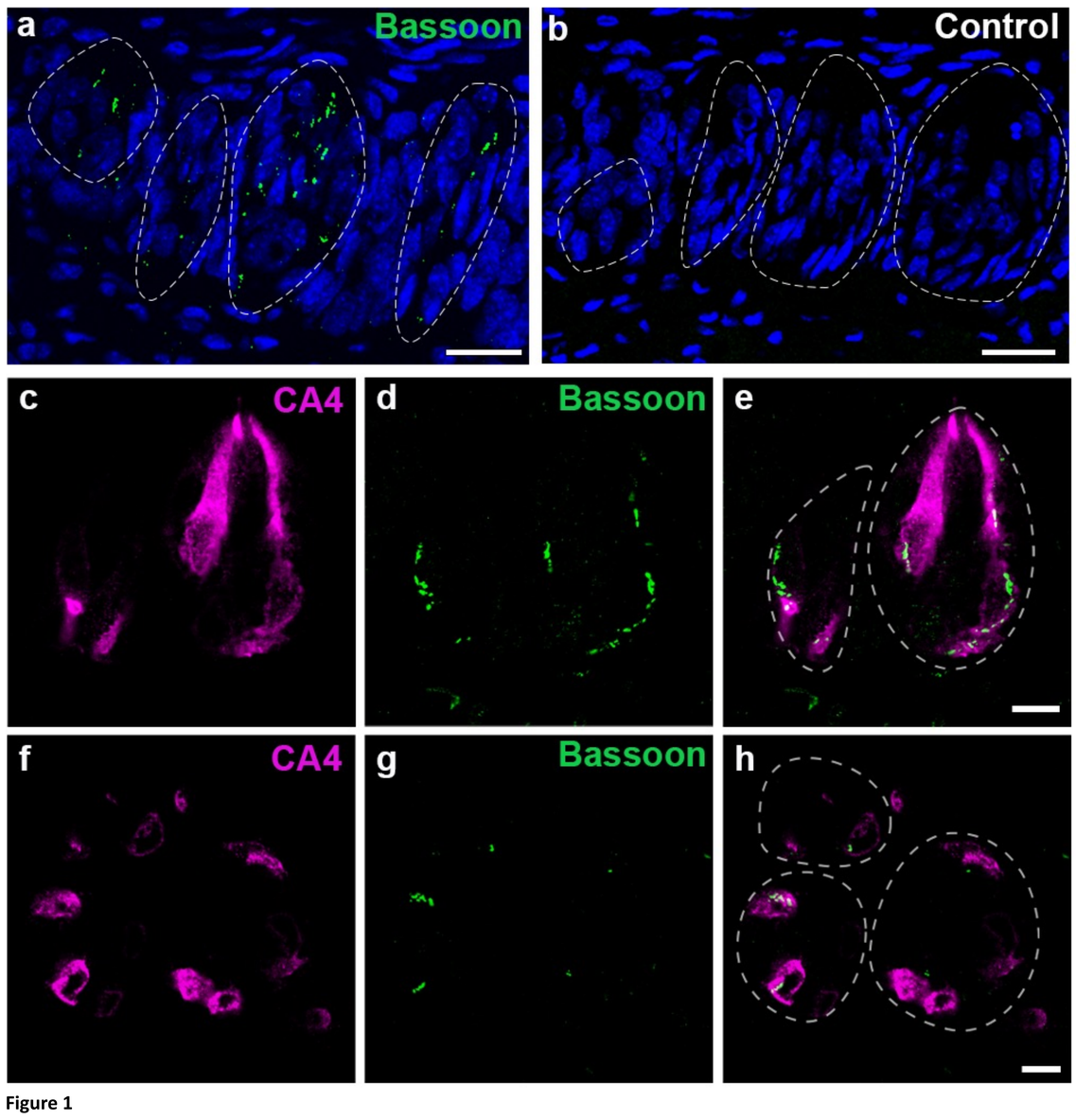


Figure 1
Representative confocal images (individual Z sections) of single (a) or double immunolabeling for Bassoon and the Type III cell marker, CA4 (c-h).
Bassoon was detected as round or elongated puncta in taste buds (a, d and g). No immunolabeling was observed in the negative control (b). Nuclei were stained with DAPI (a, b). Double immunolabeling for Bassoon and CA4 showed that most Bassoon-positive puncta were observed in CA4-positive cellular profiles (c-h). Longitudinal (c-e) and transverse (f-h) images to the long axis of taste buds. Scale bars = 20 µm (a, b); 10 µm (e, h).

Bassoon was detected in proximity to or partially overlapping with nerve fibers

We then examined the spatial relationship between Bassoon-IR and intragemmal nerve fibers. We used GAP-43 as a marker for intragemmal nerve. Triple immunolabeling for GAP-43, CA4, and Bassoon showed that the majority of Bassoon-IR was in proximity to or partially overlapping with both GAP43-positive fibers and CA4-positive cellular profiles (Figure 2f-j).

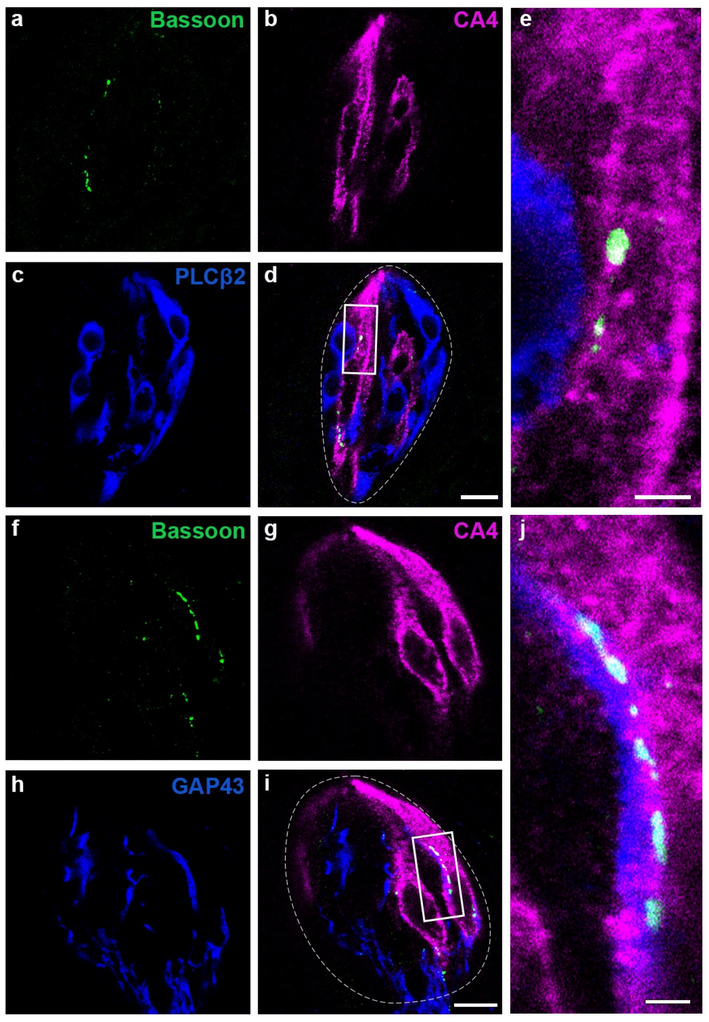


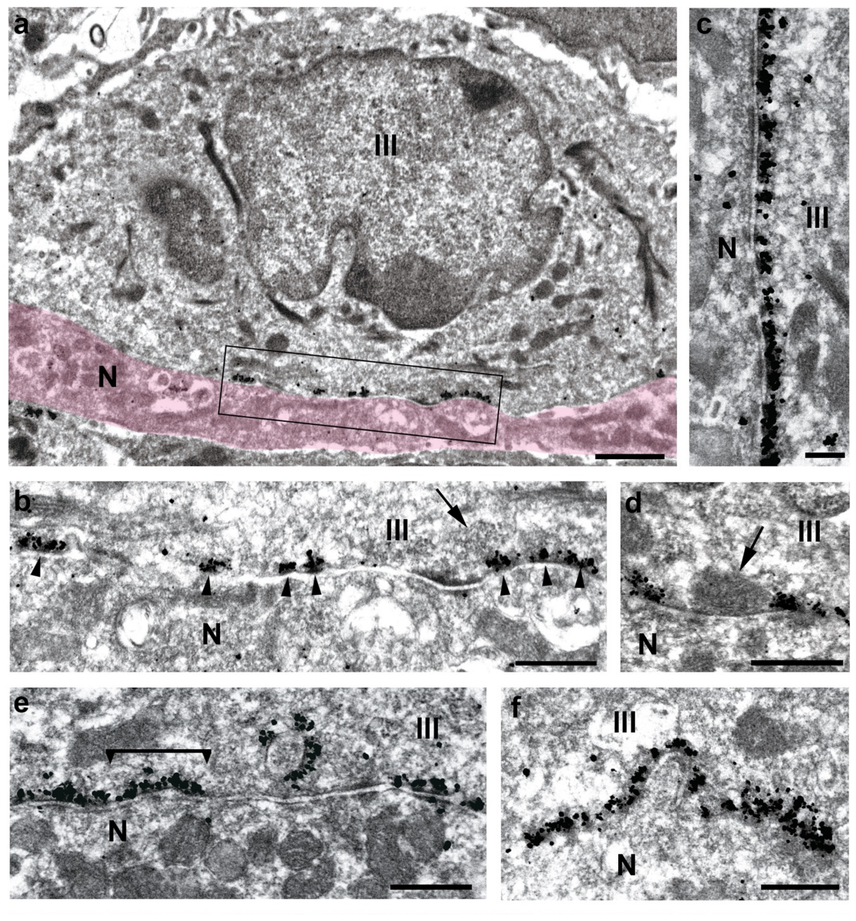
Figure 2
Representative confocal images (individual Z sections) of triple immunolabeling for Bassoon, CA4 and the Type II cell marker, PLCb2 (a-e), or Bassoon, CA4 and the nerve fiber marker, GAP-43 (f-j).
Triple labeling for Bassoon (a), CA4 (b), and PLCβ2 (c) showed that Bassoon-positive puncta were detected in CA4-positive cells but rarely in PLCβ2-positive cells (d, e). Panel e corresponds to the rectangle area in d.
Triple labeling for Bassoon (f), CA4 (g) and GAP-43 (h) showed that Bassoon-positive puncta were observed in the regions of CA4-positive cells contact with nerve fibers (i, j). Panel j corresponds to the rectangle area in i. Scale bars = 10 µm (d, i); 2 µm (e, j).

Immunoelectron microscopy confirmed Bassoon localization at the synapses of Type III cells

We used a pre-embedding immunogold method with silver enhancement to examine the localization of Bassoon in taste buds at the ultrastructural level (**Figure 3**). Bassoon-IR was observed along the inner side of the membranes of Type III cells apposed to the nerve fibers. Silver deposits for Bassoon-IR appeared to cluster in an intermittent manner at the membrane regions at which Bassoon-IR localized (**Figure 3b, c**). Some of the clusters of silver deposits were accompanied by clusters of synaptic vesicles (**Figure 3b, e**). These silver deposit clusters were often observed in groups along the plasma membrane of Type III cells in contact with nerve fibers. The groups of clusters of Bassoon-IR sometimes extended to several micrometers (**Figure 3c**). This distribution pattern of Bassoon-IR is consistent with that of the active zones of conventional synapses in taste buds.

We also detected Bassoon-IR around mitochondria that tightly adhered to the plasma membranes apposed to nerve fibers (**Figure 3d**). This Bassoon-IR around mitochondria was considered to correspond to the active zones of mixed synapses, which are unique to Type III cells. We occasionally observed circular membrane structures with Bassoon-IR in Type III cells (**Figure 3e**). These structures with Bassoon-IR were likely to be "fingerlike", which are characterized by a protrusion of the nerve fiber into an invagination of Type III cells. The profiles of the active zones of fingerlike synapses vary in shape from invaginations to circles depending on the planes of ultrathin sections. Figure 3f shows the distribution pattern of Bassoon-IR in a fingerlike synapse shown as a simple invagination.

Figure 3
Representative immunoelectron micrographs showing the localization of Bassoon-IR in taste buds. Silver deposits for Bassoon-IR were observed along the plasma membranes of Type III cells apposed to nerve fibers. The region labeled in red is a profile of an afferent nerve fiber (a). A higher magnification of the boxed area in a is shown in b. Arrowheads indicate clusters of silver deposits. Some of the clusters of Bassoon-IR were accompanied by clusters of synaptic vesicles (b, arrow; e, bar). The membrane thickening of nerve fibers was frequently observed at the regions apposed to the membranes of Type III cells bearing Bassoon-IR (c, e). Bassoon-IR was also observed around mitochondria (d, arrow) that adhered to the plasma membranes apposed to nerve fibers (d). Bassoon-IR was observed at the active zones of "fingerlike" synapses (e, f). Type III cells and afferent nerve fibers are labeled with "III" and "N," respectively. Scale bars = 1 µm (a); 500 nm (b, d–g); 250 nm (c).



Conclusion

Bassoon is a reliable marker for the synapses between Type III cells and afferent nerve fibers in taste buds. Immunohistochemistry for Bassoon will contribute to investigations on the formation of and impairments in conventional synapses in taste buds.

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