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Functional Reciprocal Connections between Olfactory and Gustatory Pathways

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Introduction

The gustatory and olfactory chemosensory systems rely on receptors in the oral and nasal cavities that interact with the relevant molecules and generate receptor and action potentials, thus transmitting the effects of chemical stimuli to appropriate regions of central nervous system. The primary gustatory cortex (GC) is defined as the cortical region that receives direct fiber projection from the parvicellular part of the ventral posteromedial nucleus in the thalamus and the terminal labeling area is situated rostrally in the granular division (GI) and caudally in the dysgranular division (DI) of the insular cortex (IC) (Nakashima *et al.*, 2000). In fact, unit recordings found gustatory responses of neurons from both the GI and DI (Ogawa *et al.*, 1992), suggesting the location of the GC in both IC divisions. On the other hand, the primary olfactory cortex is defined as the region that receives direct fiber projections from the olfactory bulb (OB) (Price, 1987). The piriform cortex (PC) is the largest structure in the olfactory cortex and further the piriform region includes a nucleus localized in between the PC proper and the caudate-putamen, endopiriform nucleus (EPN). The PC is an important center before delivery of olfactory information to the higher system via the EPN (Haberly, 1998). In the primate, the orbitofrontal cortex is the highest chemosensory center where the olfactory and gustatory information might be integrated (Rolls, 1989), but in other mammalian species, no taste-sensitive neuron has been found in the orbitofrontal cortex. We report the olfactory and gustatory integrative region in the central nervous system from mapping data obtained from electrical and optical recordings.

Methods

Animal protocols used in this study complied with all pertinent institutional and Japanese Government regulations and every attempt was made to minimize the number of animals utilized. Young Wistar rats ($n = 25$) were used. The frontal slices including PC and GC were prepared at a plane ~10° off the frontal plane. After recovery, stimulating electrodes were inserted into the layer I/II of the PC and, layer II/III of the GC and/or the EPN. Single square pulses were delivered at 0.05–0.07 Hz. Glass microelectrodes containing 2% Brilliant Blue in 0.85% NaCl were used for field potentials and unit recordings. For optical imaging, a slice was stained with a voltage-sensitive dye NK2761, transferred to the recording chamber and perfused with artificial cerebrospinal fluid (ACSF). The camera unit of the optical imaging system (Fujix HR-Deltaron 1700) contains a 128 × 128 photodiode array. Sixteen responses were averaged to form a run. The details have been described elsewhere (Sugai *et al.*, 1997). After electrical or optical recordings, stimulating, recording and appropriate sites in the pathway of signal propagation following stimulation of the PC/GC and/or EPN were marked. The slice was then fixed and resectioned. Sections were processed by an immunohistochemical technique. After elimination of endogenous peroxidase activity, alternative sections were incubated with an antibody against a B-type receptor of γ-amino butyric acid ($GABA_B$-R) at a dilution of 1:10 000 or with an antibody against calcitonin gene-related peptide (CGRP) at a dilution of 1:4000 and then incubated in ABC solution. The location of the camera field in the slice was also histologically reconstructed.

Results

Mapping studies of field potentials

Electrical stimulation of layer II in the PC evoked a field potential with a latency of 35 ms in the central region of the EPN. A field potential with a latency of 66 ms was elicited by a single shock of layer II/III of the GC in the EPN in ACSF containing Mg^{2+} (normal solution). The latencies of the PC- and GC-evoked field responses became longer gradually in normal solution and then the field responses often disappeared after multiple stimulation. Disappearance of the GC- or PV-evoked field potentials was blocked in Mg^{2+}-free solution. These electrophysiological results suggest that both the PC and GC are connected with the EPN. On the other hand, electrical stimulation of the EPN provoked field potentials with their latency of 24 and 114 ms in the PC and GC, respectively, in Mg^{2+}-free solution. These results suggest that the PC and GC are connected reciprocally with the EPN.

Results of signal propagation by optical imaging

Optical recordings were carried out in normal or Mg^{2+}-free solution. PC stimulation provoked excitation propagation to the EPN. The patterns of signal propagation and propagation velocity (51.3 ± 10.5 mm/s, mean ± SE) were similar in six slices. The GC shock provoked excitation propagation to the EPN via deep layers in the agranular division (AI) of the IC. The pattern of signal propagation and propagation velocity (42.5 ± 7.6 mm/s) were similar in all slices ($n = 4$). The GC shock produced stronger and longer optical responses in Mg^{2+}-free solution than in normal solution. The propagation velocity was 33.9 ± 3.0 mm/s ($n = 10$) in Mg^{2+}-free solution. These results suggest that the PC and GC are connected with the EPN. On the other hand, EPN stimulation evoked signal propagation toward PC or GC via AI in Mg^{2+}-free solution. The propagation velocity to the PC and GC were 40.4 ± 5.9 mm/s ($n = 4$) and 19.7 ± 2.4 mm/s ($n = 5$), respectively. Thus, the above electrophysiological findings were confirmed visually by these excitation propagations in optical imaging. Both GC–EPN and EPN–GC propagations were found to pass through the AI.

Immunohistochemical results and histological reconstruction

CGRP-positive fibers were observed in the AI. A marking site, which was presumed to be the deep layer in the AI was found in the region containing CGRP-positive fibers of the ventral part of the AI (Yasui *et al.*, 1989). $GABA_B$-R-positive cells were observed in an adjacent section. This marking site was found under the layer containing

$GABA_B$-R-positive cells, which were located in layer V of the AI. Thus, the marking site appeared to be in layer VI of the AI (Jasmin *et al.*, 2003). A distinct layer of cells can be seen in the GI, suggesting layer IV. As the stimulation site for GC was located in the region without layer IV and just dorsal to the AI, it appeared to be in layer III of the DI, which is the GC.

Unit recording study

PC stimulation provoked a long burst of spike discharges in the EPN units in Mg^{2+}-free solution. The same EPN unit responded to GC shocks. Unit responses to PC and GC shocks showed similar long bursting patterns. Of the 30 EPN units we recorded, 25 units responded to both PC and GC shocks. These results suggest that olfactory and gustatory connections converge onto a single EPN neuron. Stimulation of EPN evoked spike discharges in layer II of the PC and layer II/III of the GC. Out of 24, 19 PC units responded to EPN shocks, whereas out of 31, 28 GC units did. Thus, PC–EPN and GC–EPN connections appeared to be reciprocal.

Discussion

A tracer injection study into the OB demonstrated that the OB of the mouse had a direct terminal projection to a sector of the IC. However, a direct OB–IC projection has not been found in other mammalian species. In all species for which data is available, at least two additional routes from the OB to the IC are suggested: (i) one way is from the OB to the PC and from the PC to the IC; (ii) the second route is from the PC by way of the EPN to the mediodorsal thalamic nucleus (MD) and from the MD to the IC. Therefore, the PC–IC projection by way of the EPN observed in this study is considered as another route.

Our present optical results demonstrated EPN–GC propagation of signals via the AI and vice versa, although polysynaptic connections were responsible for slow propagation speed of optical signals. The remarkably slow propagation times may be due to bath temperature, smaller diameter axons in the EPN, inhibitory synaptic action, or wave-like propagation.

The AI is associated with visceral sensory and autonomic functions rather than taste. An immunohistochemical study revealed that the AI and DI were innervated by CGRP-immunoreactive fibers and the density of CGRP-positive innervation was richer in the ventral AI (Yasui *et al.*, 1989). Our results indicate that the AI is an important region for propagation to the GC from the EPN. A recent immunohistochemical study has shown, further, that $GABA_B$-Rs are concentrated on pyramidal neurons of layer V in the AI and $GABA_B$ neuron activity could change the pain threshold and then might cause hyperalgesia (Jasmin *et al.*, 2003). From results obtained from unit recordings, furthermore, olfactory and gustatory activity converged onto a single EPN neuron. The EPN–PC and EPN–GC connections were also observed. Our results led us to conclude reciprocal connections between olfactory and gustatory pathways. Together with the descending connections from the EPN and AI to the amygdala (Price, 1987; Haberly, 1998; Nakashima *et al.*, 2000), it is possible that the cortical integration of olfactory, gustatory, visceral and nociceptive information could modulate mechanisms involved in food selection and emotional reactions relating to the chemical and pain senses.

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