Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *Professor* Mitsuyo Maeda, *Assistant Professor*

General Summary

Our department's research activities have focused on neuroanatomy and gross anatomy. In neuroanatomical research, the development and organization of neuronal networks were investigated to clarify brain function and diseases by means immunocytochemistry, electron microscopy, *in situ* hybridization histochemistry, single-cell tracer injection, and patch-clamp electrophysiology. Our primary interests are the architecture and dynamics of the microcircuitry and their relationships. In gross anatomical research, the functional importance of variations in organ systems was explored in cadavers and animals.

Research Activities

Pattern differentiation of excitatory and inhibitory synaptic inputs on distinct neuronal types in the rat caudal nucleus of the tractus solitarius

Region- and size-specific neuronal organizations of the caudal nucleus of the tractus solitarius (cNTS) were investigated, followed by analyses of excitatory and inhibitory synaptic input patterns onto specific cell types by patch-clamp recording and immunoelectron microscopy. The cell-size distribution and numerical density of cNTS neurons were examined in subregions at levels of the area postrema. In the subpostremal and dorsomedial subnuclei, characterized by the presence of dense glutamatergic and sparse GABAergic somata, small calbindin neurons constituted 42% of all cells. The medial subnucleus contained large numbers of glutamatergic, GABAergic, and catecholaminergic somata, and large tyrosine hydroxylase—containing cells constituted 13% of cells in this region. Overall, small neurons ($<150\ \mu m^2$) represented about 80% of the cell population in the cNTS. Predominant excitatory postsynaptic currents were observed in the adult small neurons, whereas inhibitory postsynaptic currents were more evident in larger neurons, regardless of subnuclear location. This distinct differentiation of postsynaptic current patterns was not evident in neonates. In adults GABAergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%). These results indicate that differential synaptic input patterns are developmentally established in distinct small and large neurons.

Local axonal arborization patterns of distinct neuronal types in the cNTS

Neurons in the cNTS are heterogeneous in cell size (50 to 450 μm^2 in somal area) and other morphologic characteristics. For a more objective classification of cNTS neurons, their morphologic features were analyzed quantitatively on the basis of reconstructed biocytin-filled cells after whole-cell patch-clamp recording. According to the patterns

of axonal branching behaviors, cNTS cells could be classified into 2 groups: smaller cells (94.1 μm^2 in mean somal area; range, 62–120 μm^2; n=22) and larger cells (245 μm^2 in mean somal area; range, 142–411 μm^2; n=23). Extensive axonal arborization with numerous possible synaptic boutons was specifically associated with smaller neurons, whereas larger cells possessed few or no axon collaterals, suggesting their distinct roles as local-circuit neurons (or interneurons) and projection neurons, respectively. With regard to somatodendritic characteristics, the following correlations with cell size were found: smaller cells had larger form factors than did larger cells ($P<0.05$), and larger neurons had more extensive dendritic arborization, expressed by total dendritic length ($P<0.01$) and the number of dendritic branching points ($P<0.01$), than did smaller cells. These findings suggest that small cNTS neurons contribute specifically to the integration of input information generated in local circuits, whereas large neurons convey the integrated information to other autonomic brain regions.

Postnatal development of GABAergic axon terminals in the rat cNTS

The proper functioning of the brain depends on a precise arrangement of excitatory and inhibitory synapses. Although the cNTS plays a pivotal role in cardiorespiratory reflexes, we know little about the formation of the local neural networks in the cNTS. In the present study, we examined GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organizations in the rat cNTS with immunocytochemical methods at both the light and electron microscopic levels. The counting of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminals in the cNTS was constant until postnatal week 2 and that GABAergic axon terminals were reorganized at about postnatal day 10 (P10). Electron microscopic observation revealed that most GABAergic axon terminals formed axosomatic synapses on neurons with smaller soma (smaller neurons) at P2 to P4 but that the number of axosomatic synapses decreased considerably after P8. Orphan GABAergic boutons were present specifically near somata of smaller neurons at P10, and the number of axodendritic synapses on thicker dendrites decreased gradually during postnatal development. These results show that GABAergic axon terminals detach from somata of smaller neurons during postnatal week 2. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to reorganization of the local network within the cNTS from the neonatal type to the adult type. These postnatal changes in the cNTS local network might be required for cardiorespiratory reflexes of the adult type.

Activity-dependent reorganization of local circuitry in the developing visceral sensory system

Neural activity during critical periods could fine-tune functional synaptic connections. Activation of *N*-methyl-D-aspartate (NMDA) receptors is implicated in this process, and blockade leads to disruption of normal circuit formation. This phenomenon has been investigated in several neural systems, including the somatosensory system, but has not been evidenced in the visceral sensory system. Ultrastructural analysis of GABAergic synapses and electrophysiological analysis of inhibitory and excitatory postsynaptic

currents of cNTS cells revealed that developmental changes in the synaptic organizations were blocked by MK-801, an NMDA-receptor antagonist, when administered from P5 to P8, a presumed critical period for the visceral sensory system. Normal synapse reorganization during postnatal development dictates undifferentiated neonatal cNTS neurons in terms of synaptic input patterns measured with electron microscopy and electrophysiology into 2 cell groups: small cells and large cells under far stronger excitatory and inhibitory influence, respectively. Blockade by MK-801 during the critical period might leave adult neurons wired in the undifferentiated synaptic networks, possibly preventing synapse elimination and subsequent stabilization of the proper wiring.

Glial coverage of the small cell somata in the rat nucleus of tractus solitarius during postnatal development

Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Studies have suggested that the number of axosomatic synapses on the small cells of the rat cNTS decreases toward the end of postnatal week 1. Astrocytes might be involved in this phenomenon. We examined the morphological development of astrocytic processes around the small cell soma in the rat cNTS by means of light and electron microscopy. Structures positive for glial fibrillary acidic protein, glutamate-aspartate transporter, and glutamate transporter-1 within the cNTS became more intensely stained as development proceeded. Structures positive for glutamate-aspartate transporter encompassed calbindin-positive small cell somata after P10. Electron microscopic observations indicated that astrocytic processes encompass the small cell soma, whereas the number of axosomatic synapses decreases as development proceeds. The timing for glial coverage of the small cell soma appears to be consistent with the decrease in axosomatic synapses on the small cells. These observations suggest that astrocytes participate actively in regulating the decrease of axosomatic synapses on small cells in the cNTS during postnatal development.

Quantitative and immunohistochemical analysis of neuronal types in the mouse cNTS: Focus on GABAergic neurons

GABAergic neurons are major inhibitory interneurons that are widely distributed in the central nervous system. The cNTS, which plays a key role in respiratory, cardiovascular, and gastrointestinal function, contains GABAergic neurons regulate neuronal firing. In the present study, GABAergic neuronal organization was analyzed in relation to the location of subnuclei in the mouse cNTS. According to the differential expression of glutamate decarboxylase 67, vesicular glutamate transporter 2, calbindin, and tyrosine hydroxylase (TH) messenger RNAs, the cNTS was divided into 4 subnuclei: the subpostrema, dorsomedial, commissural, and medial subnuclei. The numerical density and size of soma in the four subnuclei were then quantified with an unbiased dissector analysis. Calbindin-positive cells constituted subpopulations of small non-GABAergic neurons preferentially localized in the subpostrema subnucleus. TH-positive cells constituted large neurons preferentially localized in the medial subnucleus. GABAergic neurons constituted a subpopulation of small neurons, preferentially localized in the

commissural and medial subnuclei, which represented at least 50% of small cells in these subnuclei. Thus, small GABAergic neurons were located around TH-positive large cells in the ventrolateral portion of the cNTS. This finding, in combination with the results of previous studies in the rat cNTS showing that large cells originate efferents from the cNTS, suggests that GABAergic small neurons in the commissural and medial subnuclei might regulate output from the cNTS.

Publications

Okada T, Tashiro Y, Kato F, Yanagawa Y[1], Obata K[1], Kawai Y ([1]RIKEN). Quantitative and immunohistochemical analysis of neuronal types in the mouse caudal nucleus tractus solitarius: focus on GABAergic neurons. *J Chem Neuroanat* 2008; **35:** 275–84.

Tashiro Y, Okada T, Kawai Y. Postnatal development of axosomatic synapses in the rat caudal nucleus tractus solitarus. *Neurosci Res* 2008; **61(Suppl):** S160.

Negishi Y, Kawai Y. Spontaneous synaptic input patterns in the rat caudal nucleus tractus solitarius. *Neurosci Res* 2008; **61(Suppl):** S216.