Department of Anatomy (Gross Anatomy and Neuroanatomy)

Yoshinori Kawai, *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 elucidate brain function and diseases by means of immunocytochemistry, electron microscopy, *in situ* hybridization histochemistry, single-cell tracer injection, and patch-clamp electrophysiology. Our primary interests are the architecture and dynamics of microcircuits. In gross anatomical research, the functional importance of variations of organ systems was studied using human 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

The region- and size-specific neuronal organization of the caudal nucleus tractus solitarii (cNTS) was investigated, after which excitatory and inhibitory synaptic input patterns onto specific cell types were analyzed with 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 all cells in this region. In total, small neurons ($<150\ \mu m^2$) represented about 80% of cells in the cNTS. Predominant excitatory postsynaptic currents were observed in 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 dendrites 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 cNTS

Neurons in the cNTS vary in cell size (50 to $450\ \mu m^2$ 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, 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 neurons possessed no or few axon collaterals; these findings suggest that smaller neurons serve as local-circuit neurons (or interneurons), whereas larger neurons are projection neurons. 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 number of dendritic branching points ($P<0.01$), than did smaller cells. These findings suggest that small cNTS neurons contribute specifically to an 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 function 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 local neural network in the cNTS. In the present study, we focused on 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 the second postnatal week and that GABAergic axon terminals were reorganized at about postnatal day 10. Electron microscopic observation revealed that most GABAergic axon terminals had formed axosomatic synapses on neurons with smaller somata (smaller neurons) by postnatal days 2 to 4 but that the number of axosomatic synapses decreased considerably after postnatal day 8. Orphan GABAergic boutons were present specifically near somata of smaller neurons at postnatal day 10, and the number axodendritic synapses on thicker dendrites gradually decreased during postnatal development. These results show that GABAergic axon terminals detach from somata of smaller neurons during the second postnatal week. 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 observed 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 is blocked by MK-801, an NMDA receptor antagonist, when administered on postnatal days 5 to 8, 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 by electron microscopy and electrophysiology into 2 cell groups: small and large cells under far stronger excitatory and inhibitory influences, 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 cNTS during postnatal development
Astrocytes are thought to be active participants in synaptic plasticity in the developing nervous system. Previous studies have suggested that the number of axosomatic synapses decrease on small cells of the rat cNTS toward the end of the first postnatal week. Astrocytes might be involved in this phenomenon. We examined the morphological development of astrocytic processes around small cell somata in the rat cNTS using light and electron microscopy. Structures positive for glial fibrillary acidic protein, glutamate-aspartate transporter (GLAST), and glutamate transporter 1 within the cNTS became more intensely stained as development proceeded. GLAST-positive structures encompassed calbindin-positive small cell somata after postnatal day 10. Electron microscopic observations indicated that astrocytic processes encompass the small cell somata, whereas the number of axosomatic synapses decreases as development proceeds. The timing for glial coverage of the small cell somata appears to be consistent with the decrease in axosomatic synapses on the small cells. These observations imply that astrocytes may participate actively in regulating the decrease in axosomatic synapses on small cells in the cNTS during postnatal development.

Publications

Tashiro Y, Kawai Y. Glial coverage of the small cell somata in the rat nucleus of tractus solitarius during postnatal development. *Glia* 2007; **55:** 1619–29.

Yoshioka M, Kawai Y. Activity-dependent reorganization of local circuitry in the developing visceral sensory system. *Neuroscience* 2007; **150:** 905–14.

Tashiro Y, Yanagawa Y, Obata K, Murakami F. Development and migration of GABAergic neurons in the mouse myelencephalon. *J Comp Neurol* 2007; **503**: 60–79.

Yasura T, Kawai Y. Activity-dependent glial coverage in the rat nucleus of tractus solitarius during postnatal development. *Neurosci Res* 2007; **58**(Suppl): 80.

Negishi Y, Kawai Y. Expression of neurocan in the rat nucleus of tractus solitarius during postnatal development. *Neurosci Res* 2007; **58**(Suppl): 206.