

## Department of Anatomy (I)

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Yoshinori Kawai, *Professor*

### General Summary

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

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 recordings and immunoelectron microscopy. The cell-size distribution and numerical density of cNTS neurons were examined in subregions at the level 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. In total, small neurons ( $< 150 \mu\text{m}^2$ ) represented about 80% of the cell population in the cNTS. Predominant excitatory postsynaptic currents were observed in the adult small neurons, while 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. GABAergic synapses were more frequently associated with dendrites of large catecholaminergic cells (73%) than with those of small calbindin-containing cells (10%) in adults. These results indicate that differential synaptic input patterns were developmentally established in distinct small and large neurons.

#### *Local axonal arborization patterns of distinct neuronal types in the cNTS*

Neurons in the cNTS differ in size (50 to  $450 \mu\text{m}^2$  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 recordings. According to the patterns of axonal branching behaviors, cNTS cells could be classified into 2 groups: smaller cells ( $94.1 \mu\text{m}^2$  in mean somal area; range,  $62\text{--}120 \mu\text{m}^2$ ;  $n=22$ ) and larger cells ( $245 \mu\text{m}^2$  in mean somal area;

range, 142–411  $\mu\text{m}^2$ ;  $n=23$ ). Extensive axonal arborization with numerous possible synaptic boutons was specifically associated with smaller neurons, whereas larger cells possessed no or few 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 ( $P<0.05$ ). 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 results 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 NTS*

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 formation of the local neural network in the cNTS. In the present study, we focused on GABAergic axon terminals and investigated postnatal changes in GABAergic synaptic organization in the rat cNTS immunocytochemically at both the light and electron microscopic levels. Counting of synaptic and nonsynaptic GABAergic axon terminals revealed that the number of GABAergic axon terminal in the cNTS was constant until the second postnatal week and that GABAergic axon terminals were reorganized at approximately postnatal day (P)10. 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 the second postnatal week. Such morphologic changes in axon terminals could cause changes in electrophysiological activity and might contribute to the reorganization of the local network within the cNTS from the neonatal to the adult type. These postnatal changes in the cNTS local network might be required for the 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. *N*-methyl-D-aspartate receptor activation is critically implicated in this process, and blockade leads to disruption of normal circuit formation. This phenomenon has been thoroughly investigated in several neural systems, including the somatosensory system, but has not yet 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 antagonist of *N*-methyl-D-aspartate receptor,

when administered on 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 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.

### Publications

**Okada T, Yoshioka Y, Inoue K, Kawai Y.** Local axonal arborization patterns of distinct neuronal types in the caudal nucleus of the tractus solitarius. *Brain Res* 2006; **1083**: 134-44.

**Yoshioka M, Tashiro Y, Inoue K, Kawai Y.** Postnatal development of GABAergic axon terminals in the rat nucleus of tractus solitarius. *Brain Res*

2006; **1107**: 111-20.

**Yoshioka M, Okada T, Inoue K, Kawai Y.** Pattern differentiation of excitatory and inhibitory synaptic inputs on distinct neuronal types in the rat caudal nucleus of the tractus solitarius. *Neurosci Res* 2006; **55**: 300-15.