TRANSCRIPTION REPRESSOR CREB2 REGULATES LONG-TERM MEMORY IN *LYMNAEA STAGNALIS*

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The transcription factor, cAMP response element binding protein (CREB), is believed to play an important role in formation of long-term memory (LTM) in many animals. Using a quantitative real-time PCR method, we measured the mRNA copy numbers of CREB1 (activator) and CREB2 (repressor) in both the central nervous system (CNS) and the single key neuron in the pond snail *Lymnaea stagnalis*, where LTM for conditioned taste aversion is formed. The copy number of CREB2 mRNA was larger than that of CREB1 mRNA in the CNS. In the single key neuron, the similar results were obtained and the copy number of CREB1 mRNA was less than 10 copies. These results suggest that CREB2, but not CREB1, regulates the transcriptional activity in LTM formation. To confirm this hypothesis we examined the change in the copy number of CREB2 mRNA during LTM formation. After LTM consolidation, the copy number of CREB2 mRNA decreased significantly in the single key neuron. Thus, the present results showed that the decrease in the transcription repressor CREB2 in the single key neuron is critical for LTM formation in *L. stagnalis*.
Supported by JSPS and Narishige Scientific Instrument Laboratory.

DISTRIBUTION OF OLFACTORY AND GUSTATORY INPUT AND DESCENDING OUTPUT REGIONS IN THE BRAIN OF THE TERRESTRIAL SLUG *LIMAX VALENTIANUS*

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Terrestrial slugs have the ability of odor-taste associative learning, but the underlying neural pathway has not been elucidated. We examined the distribution of olfactory and gustatory input and descending output regions in the brain by backfilling with Co^{2+} ions from the nerves and connectives of the brain and by labeling of sensory neurons with horseradish peroxidase. Backfill staining revealed that the tentacular nerve and the internal, medial, and external lip nerves project to the cerebral ganglion and the subesophageal ganglion. Backfill staining of the cerebro-pleural and cerebro-pedal connectives revealed that the somata and dendrites of descending neurons are mainly located in the ventral side of the cerebral ganglion. Horseradish peroxidase staining revealed that some of the sensory neurons in the olfactory epithelium project to several regions in the cerebral ganglion, including the procerebral lobe.

SPATIOTEMPORAL NEURAL DYNAMICS OF THE OLFACTORY INFORMATION PROCESSING SYSTEM IN THE CEREBRAL GANGLION OF THE TERRESTRIAL SLUG, *INCILARIA FRUHSTORFERI*

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The cerebral ganglion of the terrestrial slug consists of three regions, the procerebrum, the mesocerebrum and the metacerebrum. Anatomically afferent fibers from the olfactory organs, the superior and the inferior tentacles (STs and ITs), project to all these regions. The spatiotemporal neural dynamics among these regions seems to be important to the olfactory information processing, therefore we optically recorded the response of the whole cerebral ganglion evoked by electrical stimulation of the ST or IT nerve.

By the ST stimulation, the metacerebral posteromedial area responded and the procerebral response followed, and by the IT stimulation, in addition to these two responses, the metacerebral posterolateral area responded. The response latencies of the metacerebral two areas were about 50 msec shorter than that of the procerebrum. In the metacerebrum, the response duration of the posteromedial area was longer than that of the posterolateral area. In the procerebrum, the response duration was as long as that of the metacerebral posteromedial area regardless of the stimulated nerve, but the response patterns evoked by each stimulation was different in fine structure.

CENTRAL CONTROL ON THE PERIPHERAL NEURON-EVOKED MOTILITY OF THE POSTERIOR GIZZARD IN *APLYSIA*

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We examined the role of intrinsic peripheral neurons and interactions between the central and the peripheral nervous system in the control of the digestive organs of *Aplysia kurodai* and *A. juliana*. A large number of peripheral neuronal somata are located in neural plexus on the outer surface of the posterior gizzard; some these are clustered together to form small groups. These neurons showed synchronous bursting activities periodically, which were followed by constrictions of the posterior gizzard. This suggests that some of the clustered neurons on the posterior gizzard may function as motor neurons for the circular muscle in the posterior gizzard. Electrical stimuli at the distal cut-stump of the esophageal nerve augmented and/or inhibited the bursting activities of the neurons, which resulted in, respectively, an increase and/or a decrease in activities in posterior gizzard constriction. Central nervous control on the motility of the digestive tract may be exerted, at least in part, by changing activities of the peripheral neurons in the neural plexus.

PROJECTION MAP OF PHEROMONE AND GENERAL ODOR PROCESSING PATHWAYS IN THE PROTOCEREBRUM OF THE MALE SILKWORM MOTH

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In the insect antennal lobe (AL), odor information is represented topographically as a glomerular map. How this topographic map in the AL is represented in the protocerebrum (PC), which is the projection site of the AL projection neurons (PNs), is not well known. In the male silkworm moth *Bombyx mori*, we found that NO-induced cGMP immunohistochemistry revealed specific immunoreactivity of the AL PNs that respond to the major pheromone component (bombykol). Thus, their projection area in the PC clearly represents the pheromone processing pathway of the major pheromone component. Furthermore, we performed double-labeling of major pheromone component PNs and non-pheromone or minor pheromone component PNs, combined with cGMP immunohistochemistry. We found that each type of PNs had projection to the different area in the PC.

IMMUNO-DOUBLE STAINING ANALYSIS OF LATERAL ACCESSORY LOBE (LAL) RELATED NEURONS IN THE BRAIN OF THE MALE SILKWORM MOTH *BOMBYX MORI*

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Male silkworm moths, Bombyx mori , exhibit a characteristic zigzagging pattern as they walk upwind toward pheromones released by conspecific females. We are clarifing the neural pathways responsible for the generation of the zigzag behavior. Bilateral neurons which connect both lateral accessory lobes (LALs) maybe involved generation of the zigzag behavior.

The LAL neural circuit was clarified by immonocytochemical and intracellular recording methods. Applying immonocytochemical methods, we identified characteristic serotonin-immunoreactive LAL bilateral neurons (SI LAL-BLs). Using intracellular recording, we found many LAL related neurons. For example, 1)LAL bilateral neurons and LAL local interneurons 2)Group-1 and Group-2 descending neurons. All of the neurons were registered on NeuronDB (Database of neurons on Bombyx mori). In this study we applied serotonin-immuno-double staining in order to examine possible connection between the SI LAL-BLs and other types of LAL neurons. So far, we have found connection between the SI LAL-BLs and some LAL related neurons.

THREE-DIMENSIONAL RECONSTRUCTION AND IDENTIFICATION OF THE ANTENNAL LOBE GLOMERULAR STRUCTURES IN *BOMBYX MORI*

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We present three-dimensional reconstruction of the antennal lobe (AL) of *Bombyx mori* based on confocal microscopic analysis of glomeruli stained with 0.1% Lucifer Yellow in DW, followed by image processing. The three-dimensional reconstruction demonstrates the spatial distribution of ordinary glomerular structures. Moreover we identified 33 glomeruli according to the following criteria: shape, size, relative positioning and fiber bundles in the AL. We classified all glomeruli into six classes by using identified glomeruli and characteristic fiber bundles in the AL as landmarks. These six classes allowed us to analyze the number of glomeruli and their variation in detail. The number of glomeruli was 58 ± 1 ($N = 5$). There were individual differences. However, we could determine which glomeruli were different by using our six classes classification system. On the other hand, there was little variability between right and left ALs in the same individual. Furthermore, we applied this method of glomerular identification and three-dimensional reconstruction to localize the dendritic arborizations of AL interneurons. We analyzed which glomeruli were innervated by these interneurons.