IDENTIFICATION OF PRE-NGI INTERNEURONS IN CENTRAL COMPENSATION OF EYESTALK POSTURE OF CRAYFISH

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When a statolith is removed unilaterally in crayfish, the eyestalk posture as well as the synaptic activity of nonspiking giant interneurons (NGIs) becomes bilaterally asymmetrical in the upright body position. The fact that this asymmetry disappears in several weeks in both posture and synaptic activity suggests that neurons that are presynaptic to NGIs regulate their outputs to recover the symmetrical eyestalk posture. Sustaining fibers that convey visual information from the eyestalk to the brain are known as presynaptic to NGIs. But spontaneous postsynaptic potentials were also observed in NGIs in the dark when sustaining fibers are generally supposed to be silent. In this study, we identified nerve cells involved in the presynaptic pathway to nonspiking giant interneurons using two glass microelectrodes to impale an NGI and its presynaptic cell simultaneously. We found 3 descending, 1 ascending and 1 local interneurons that connected to the NGI polysynaptically. We also identified physiologically one interneuron that suppressed the NGI activity monosynaptically. The result suggests that these interneurons are involved in the central compensation of eyestalk posture.

PHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERIZATION OF UROPOD MOTONEURONS IN CRAYFISH

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The uropods are paired appendages of the caudalmost (sixth) abdominal segment in crayfish. The movement of uropods in *Procambarus clarkii* Girard is controlled on each side by phasic and tonic muscles. The phasic adductor exopodite is innervated by two motoneurons, one of which travels the 2nd (R2add MN), and the other (R3add MN) the 3rd root of the terminal ganglion. The tonic reductor exopodite is innervated by at least three motoneurons which travel the 2nd root. In this study, we addressed a question of how the motoneurons differ in their passive membrane properties and dendritic structure. We characterized their functional structure using microelectrode techniques and three-dimensional morphometry. The input resistance and the membrane time constant of R2add MN were significantly smaller than those of reductor motoneurons ($P < 0.05$). The input resistance and the membrane time constant of R2add MN was significantly smaller than those of R3add MN ($P < 0.05$). Reductor motoneurons were classified into two types by their dendritic structure, but their passive membrane properties were not different each other.

ACTIVITIES OF CEREBELLAR NEURONS DURING CLASSICAL HEART RATE CONDITIONING IN GOLDFISH

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Activities of cerebellar neurons were monitored during classical heart rate conditioning in goldfish. Delay conditioning procedure was conducted on immobilized goldfish. The conditioned stimulus (CS) was a presentation of LED light and the unconditioned stimulus (US) was an electrical shock. Bradycardic response during the CS was quantified as a conditioned emotional response. We found that some neurons showed decreased firing rate during the CS as the conditioned response developed. We also found that the extracellularly recorded single units of those neurons consisted of two types of spike, one was simple spike and the other was complex spike. Therefore, the neurons which showed learning-dependent change of the response to the CS were classified as Purkinje cells. It has been shown that the intactness of the cerebellum is required for normal development of the classically conditioned bradycardic response in goldfish. Together with this, the present results show that the cerebellar circuit is closely involved in the classical emotional conditioning in goldfish.

SPECIFICITY OF OLFACTORY RECEPTOR NEURONS RESPONDING TO PHEROMONES IN *OSTRINIA FURNACALIS*

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We have recorded responses to female sex pheromone components, *(E)*- and *(Z)*-12-tetradecenyl acetates (E and Z12-14:Ac) and behavioral antagonists (Z9-14: Ac, E and Z11-14:Ac) in single receptor neurons of male *Ostrinia furnacalis* (Lepidoptera: Crambidae) using a cut-sensillum technique. According to the electrophysiological survey and electron microscopic observations the sensilla houses 1-3 neurons. Dose-response and cross-adaptation studies showed that type 1 neurons responded equally well to the two pheromone components. Neurons (type 2-4) responding selectively to one pheromone component and neurons (type 5-7) responding to behavioral antagonists were also observed. Co-localization between neurons responding to pheromone and antagonist compounds was found in some sensilla. *O. furnacalis* has a unique coding system for two major pheromone components built in neurons responding to both the two components. *O. nubilalis*, a close species of *O. furnacalis* has two types of neurons responding to each pheromone component specifically.

MOLECULAR BIOLOGICAL STUDIES ON OLFACTORY SIGNALING CASCADE IN JAPANESE COMMON NEWT, *CYNOPUS PYRRHOGASTER*

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The olfactory sensation in vertebrate starts from the interaction of odorant and receptor, and results in the activation of cascade of signal-transducing molecules. The G_s class G proteins, G_{olf} and G_s, are activated in response to reception of odorants and stimulate cAMP formation by an adenylyl cyclase. This leads to the opening of cyclic nucleotide-gated cation channels. However, various regulatory molecules were reported to be involved in this cascade, especially in the adaptation process. In order to clarify the relationship between the proteins in the olfactory cell and their functions, we investigated genes expressed in the olfactory cells. We chose the Japanese common newt, *Cynopus pyrrhogaster*, because it has been used for the electrophysiological studies. At first we made a cDNA library of 500 thousands independent clones from the newt olfactory epithelium. In the expression sequence tag (EST) data, the genes belong to the following categories are further investigated. 1) Channel proteins, 2) olfactory cell marker protein, 3) odorant receptor.

THE SECOND MESSENGER IN NEWT OLFACTORY TRANSDUCTION IS CYCLIC AMP

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It has been believed that olfactory transduction cascade uses two parallel pathways. One is a cAMP as a second messenger, and the other is an IP3. cAMP pathway was well known by many research of olfactory transduction and second messenger, on the other hands, IP3 pathway was still controversial. Because of lack of information regarding the single cell response induced by IP3 odorants.To make clear the possibility of second messenger in transduction system, we investigated electrophysiological properties by IP3 odorant. Our previous study reported that caged substances were useful tool for investigating the signal transduction system, which was able to control [cAMP]i. In this experiment, to deny involving IP3 in the olfactory transduction, we compared IP3 odorant responses to caged-cAMP responses. We could observe reversal potential and dose-dependence were very closely. Self- and cross-adaptation were observed between IP3 odorant responses and cAMP responses. Furthermore, summation was also observed at the peak of IP3 odorant responses, cAMP responses were added. These results showed that olfactory transduction used only one pathway with cAMP as a second messenger.

IDENTIFICATION OF TARGET PROTEINS OF P26OLF, A CALCIUM BINDING PROTEIN IN BULLFROG OLFACTORY AND BRONCHIAL CILIA

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A Ca^{2+}-binding protein, p26olf, was found in bullfrog (Rana catesbiana) olfactory and bronchial cilia. To search for the function of p26olf, we tried to identify its target proteins by using a p26olf-Sepharose affinity column. Several p26olf-binding proteins were isolated from solubilized membrane fractions of frog olfactory cilia and lung. Among them, we focused on a protein band of 35kDa on an SDS-PAGE because it was the most intensive band and was detected in both olfactory cilia and lung. Protein sequence analysis suggested that this band consisted of a mixture of annexin sub-types. In fact, we found many 35 kDa protein spots after 2D electrophoresis. The pI values of these 35kDa proteins were 5.5-8. Based on the partial amino acid sequences determined with the sequence analysis, we cloned annexin sub-types in a bullfrog. Mass spectroscopic analysis together with Western blot analysis revealed that the acidic proteins of approx. pI 8 are annexin II and those of approx. pI 5.5 are annexin V. The results suggested that p26olf functions in the olfactory and bronchial cilia through interaction with annexins in a Ca^{2+}-dependent manner.

FUNCTION OF Cl^- TRANSPORTER IN THE BULLFROG OLFACTORY RECEPTOR CELL

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Vertebrate olfactory receptor neurons (ORN) contain high concentration of Cl^- which flows out via Ca^{++}-activated Cl^- channels amplifying odor-induced inward receptor current. Concentration of Cl^- ($[Cl^-]_i$) in isolated bullfrog ORNs was monitored by analyzing fluorescence images of ORNs containing fluorescent chloride