movements to form the adult body. Apoptosis is the process that eliminates cells which are no longer biologically necessary. During the apoptotic process, the dying cell is squeezed out from the tissue by mechanical forces produced within the cell and by forces produced within the neighboring cells. We experimentally demonstrated that the forces that squeezed out the apoptotic cell also contributed to tissue movements during development in *Drosophila*. This active mechanical role is not classically attributed to apoptosis.

1D1510 A model of cell-division stop for the limb regeneration

Hiroshi Yoshida (*Faculty of Math. Kyushu Univ.*)

Cell-division stop is essential for the limb regeneration. The steepness hypothesis was submitted as a model that explains regulation of the cell-division stop under an assumption of a gradient across cells. The *Dachsous/Fat* heterodimers are now considered to substantiate the steepness hypothesis and to be involved in regeneration in insect legs. Little is, however, known about the way *Dachsous* and *Fat* molecules are distributed during cell division. Extending the steepness hypothesis, we here show that some condition of the distribution ratio provides the cell with cell-division stop, enabling regeneration. We found that the obtained condition provides a molecular-based explanation for a variety of limb regeneration such as distal outgrowth and intercalary regeneration. It has been revealed that the condition can explain several results of experiments using regeneration-dependent RNAi in cricket legs. We anticipate our result to provide a unified view of regeneration based on molecules within the cell.

1D1522 ラット培養神経回路網の長期発達過程における同期活動に関わる機能的分子の探索

Analysis of functional molecules underlying synchronous activity during long-term development of rat cultured neuronal networks

Daisuke Ito¹, Keiko Yokoyama², Kazutoshi Gohara² (¹*Fac. Adv. Life Sci., Hokkaido Univ.*, ²*Fac. Eng., Hokkaido Univ.*)

Synchronous activity is a remarkable phenomenon in the electrical activity of neuronal networks and is thought to be involved in the higher function of the nervous system, such as memory and learning. Studies using multi-electrode arrays (MEAs) have revealed that cultured neuronal networks reconstructed in vitro show synchronized bursts (SBs). However, the molecular mechanisms underlying the generation of SBs during long-term development remain unclear. In order to clarify the mechanisms of this phenomenon at a molecular level, we analyzed the gene expression involved in the changes in network activity. We cultured rat cortical neurons for 1 month, and the spontaneous electrical activity was recorded using MEAs. SBs were observed starting at approximately 2 weeks and the rate increased gradually during the culture periods. Reverse transcription PCR analysis revealed that the housekeeping gene and transcription factor gene *creb* were found to be consistently expressed during the culture period. However, the immediate early gene *arc* was not identified at 1-7 days in vitro (DIV), but the expression level increased up to 28 DIV. This increase in the expression of *arc* resembled the increase in the SB rate. These results suggest that the generation of SBs is correlated with the increase in immediate early gene expression during the culture periods. Based on these preliminary results, we have begun to quantify the expression level of *arc* and investigate the expression of other genes and proteins involved in network activity.

1D1534 モノアラガイの長期記憶におけるインスリンとグルコースの役割 Insulin and glucose for memory in a snail

Etsuro Ito¹, Ryuichi Okada¹, Mika Morikawa¹, Satoshi Takigami², Akiko Okuta³, Manabu Sakakibara² (¹*Kagawa School of Pharmaceutical Sciences, Tokushima Bunri University*, ²*School of High-Technology for Human Welfare, Tokai University*, ³*Graduate School of Science, Kyoto University*)

The pond snail *Lymnaea stagnalis* is capable of learning taste aversion and consolidating this learning into long-term memory (LTM) that is called conditioned taste aversion (CTA). First, we examined the role of insulin in the LTM formation of CTA. When we applied insulin to the brain, we observed a long-term change in synaptic efficacy (i.e., enhancement) of the synaptic connection between some neurons. This synaptic enhancement was blocked by application of an insulin receptor antibody to the brain. Injection of the insulin receptor antibody into the snail before CTA training, while not blocking the acquisition of taste aversion learning, blocked the memory consolidation process, thus LTM was not observed. Second, we examined the relationship

among learning ability, starvation period and hemolymph glucose concentration, and tested the effect of insulin on the learning score. One-day mild starvation improves the learning score and keeps good LTM. When we injected glucose into the abdominal cavity of mild starved snails for a short period, the snails showed worse scores than the case of no injection. Further, when insulin was injected into snails with a complete bellyful of food, the glucose concentration decreased and the learning score and LTM were improved. These results showed that learning ability depends on the starvation period and the glucose concentration and that learning ability must be controlled by manipulation of glucose concentration and injection of insulin in CTA learning of *Lymnaea*.

1D1546 光捕捉された神経細胞内シナプス小胞群の集合ダイナミクス

Assembling dynamics of optically trapped synaptic vesicles in neuronal cell

Yusuke Ueda^{1,2}, Suguru N. Kudoh², Takahisa Taguchi¹, Chie Hosokawa^{1,2} (¹*Health Res. Inst., AIST*, ²*Grad. Sci. Eng., Kwansei Gakuin Univ.*)

The turnover of synaptic vesicles in presynaptic terminals of neurons is essential for information processing within the brain. For aiming artificial control toward modulating the synaptic transmission in a neuronal network with optical tweezers, we investigated assembling dynamics of optically trapped synaptic vesicles in neuronal cells by fluorescence analysis. When a 1064-nm trapping laser beam is focused on synapses of a neuronal cell labeled with a fluorescent endocytic marker, the fluorescence intensity increased gradually with the laser irradiation time, suggesting that optical trapping force causes vesicles assembly at the focus. The synaptic vesicle dynamics in an optical trap was evaluated by fluorescence correlation spectroscopy. The decay time of fluorescence autocorrelation curves increased with the trapping laser power and the laser irradiation time, indicating that vesicle motion was restrained at the focus due to optical trapping force. Moreover, the decay time of autocorrelation curves was decreased after applying the phosphatase inhibitor okadaic acid causes vesicles to diffuse freely and the degree of decrement was related to the trapping laser power, which will be presented and discussed.

1D1558 膜電位感受性色素・カルシウムイメージングに使える新しい超高速共焦点顕微鏡の開発

A new class of confocal microscope for a fast voltage-sensitive dye (VSD) and Ca²⁺ imaging

Takashi Tominaga, Yoko Tominaga (*Dept. Neurophysiol., Kagawa Sch Pharm Sci, Tokushima Bunri Univ*)

Monitoring neuronal activities using imaging devices is central to our understanding of brain microcircuit functions. Recent progresses in imaging devices together with the rapidness of VSD response have allowed us to follow the sub-millisecond timescale of neuronal activities by VSD imaging. Confocal microscopy provides a more efficient S/N ratio in terms of spatial resolution and thus could be used to obtain better S/N ratios from the VSD signal. However, sampling rates have been difficult to achieve in millisecond timescales. Here we describe the basic configuration of a new no-scanning type of confocal microscopy designed for VSD imaging. The confocal optics was designed to fit a standard camera adaptor of an epi-fluorescent microscope (e.g., BX51WI, Olympus). The images of the subject placed under the objective lens (20x/0.95, Olympus) were projected onto a 100 × 100 pinhole array placed at a conjugation point. An excitation light reflected by a standard mirror unit was projected onto the pinhole array and illuminated onto the subject. Fluorescence emitted from the subject was then converted into an image using the MOS-type 100 × 100 pixel imager (MiCAM Ultima, Brainvision). The pinhole array and pixel imager correspond with each other in terms of pixels. Since the pinhole array is at a fixed position, there are no mechanical disturbances. We tested the microscope by imaging a rat hippocampal slice preparation by bulk and single cell VSD staining of the slices, and bulk staining with a Ca²⁺ indicator.

1D1610 De novo アセンブルによる軟体動物脳的全トランスクリプトーム解析 De novo sequencing and transcriptome analysis of the molluscan brain by deep RNA sequencing

Hisayo Sadamoto¹, Hironobu Takahashi², Taketo Okada¹, Hiromichi Kenmoku², Yoshinori Asakawa² (¹*Facul. Pharmaceut. Sci. Kagawa Camp, Univ. Tokushima Bunri*, ²*Inst. Pharmacognosy, Univ. Tokushima Bunri*)

The pond snail *Lymnaea stagnalis* is among several mollusc species that have been well investigated due to the simplicity of their nervous systems and large