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examinined whether the inositol 1,4,5-trisphosphate (IP3)-induced Ca2+ release (ICR) is involved in the regulation of the synaptic clustering of GABA receptors. In hippocampal neurons from mice lacking intracellular Ca2+ releasing channel (IP3) receptor type 1 (IP3R1), clusters of GABA receptors and those of its scaffold protein gephyrin were significantly smaller than in wild type neurons. In wild type neurons, the inhibition of ICR by specific inhibitors resulted in the decrease in GABAR and gephyrin cluster size, without reducing the amount of GABAR on the cell surface. Single particle tracking with quantum dot (QD-SPT) revealed that the loss of IP3R1 and inhibition of ICR enhanced the lateral diffusion of GABAR. The increase of GABAR lateral mobility resulting from the loss of ICR was completely prevented by an inhibitor of calcineurin. Our results suggest that IP3/ Ca2+ signaling contributes to the stabilization of synaptic GABAR clusters through the regulation of GABAR lateral diffusion, possibly through phosphorylation.

3PT207 海馬でのアクチンによるシナプス可塑性の急性制御

Acute Modulation of Synaptic Plasticity of Pyramidal Neurons by Activin in Adult Hippocampus

Yoshitaka Hasegawa, Hideo Mukai, Makoto Asahima, Yuki Ooishi, Suguru Kawato

Activin is known as a sex-hormone in mammalian. We attempt to reveal the role of activin as a neuromodulator in the adult hippocampus. Activin is a homodimer of inhibin β, and activin belong to the superfamily of transforming growth factor-β (TGF-β). We showed endogenous/basic activation of activin in the hippocampal neurons. Localization of activin receptors in spines was demonstrated by immunoelectron microscopy. The incubation of hippocampal acute slices with activin A altered the density and morphology of spines in CA1 pyramidal neurons. The total spine density was increased by activin treatments. Activin increased the head diameter of spines. Blocking of MAPK, PKA or PKC prevented the activin-induced spineogenesis by reducing the density of spines. We also demonstrated that activin induced the long term potentiation (LTP) of the hippocampal neurons.

3PT208 カルシウム依存型ホスファトゲンキナーゼ1変異体を含むシナプス可塑性制御の機序解析

Binomial distribution analysis of increase of transmitter release depending on [Ca2+]c at the frog neuromuscular junction

Taisuke Matsuda, Naoya Suzuki (Dept. Phys., Sch. Sci., Univ. Nagoya)

To investigate the action of Ca2+ to induce the transmitter release, we analyzed the dependency of quantal release parameters on extracellular Ca2+ concentration ([Ca2+]o). Binomial distribution analysis with two parameters, release probability (p) and number of releasable synaptic vesicles (n) having that release probability was applied to the release induced by single stimulations at frog neuromuscular junction. Endplate potentials (EPFs) and miniature endplate potentials (MEPPs) were electrophoretically recorded with an intracellular glass microelectrode under five different [Ca2+]oc from 0.55 mM to 0.95 mM at intervals of 10.00 mM. The averaged size of EPFs was proportional to the about 3.0-3.5 power of [Ca2+]o. The binominal analysis of EPFs distribution with using MEPPs distribution as single unit event gave values of p about 0.1-0.5 and n about 10-50. Detail analysis of the change of two parameters, p and n, showed p was approximately proportional to [Ca2+]o and n was proportional to the about square of that. It suggests that increase of n contributed largely and increase of p contributed slightly to increase of transmitter release with change of [Ca2+]o.

3PT209 アクロトイド系に特有で神経活動に影響を与える膜キナーゼによる保護効果の検討

Thymooquinone, the Nigella sativa Bioactive Compound, Prevents β amyloid neurotoxicity in cultured rat primary neurons

Amani Alhibsh, Ikuro Suzuki, Masato Gotoh (Tokyo University of Technology)

Alzheimer disease (AD) is a neurodegenerative disease characterized by extracellular abnormal accumulation and extensive deposition of amyloid beta peptide (A β). This accumulation is associated with oxidative damage, inflammatory reactions, synaptic function impairment, synaptic loss and finally leads to neuronal death, and the use of antioxidants and anti-inflammations could reduce this risk. Thymooquinone (TQ), the abundant essential oil compound of Nigella sativa L. seeds, known to be the active principle responsible for many of the seed's antioxidant and anti-inflammatory effects, was used in this study. In every experience, rat cultured embryonic hippocampal and cortical neurons were treated simultaneously with Aβ1-42 and TQ for 72 h. The results showed that co-treatment with TQ efficiently attenuated Aβ1-42-induced neurotoxicity, as evidenced by the improved cell viability. In addition, TQ inhibited the mitochondrial membrane potential depolarization and reactive oxygen species generation caused by Aβ1-42. TQ also restored synaptic vesicle recycling inhibition, partially reversed the loss of spontaneous activity, and inhibited Aβ1-42 aggregation. These beneficial effects may contribute to the protection against Aβ-induced neurotoxicity. Together, our results suggest that the natural antioxidant TQ has potential for neuroprotection and therefore, may be a promising candidate for AD treatment.

3PT210 インスリンを介した神経シナプスによって制御される線虫 C. elegans の温度適応の解析

Insulin-mediated neural signals negatively regulate temperature tolerance in C. elegans

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Temperature is one of the most critical environmental stimuli and cause biochemical change in the body. Therefore, animals have adaptation mechanisms against environmental temperature changes, however, its molecular mechanisms are still poorly understood. Here we show that C. elegans has a cultivation temperature-dependent cold resistance, which is regulated by insulin-mediated pathway. 25°C -grown wild-type animals isolated from UK. were destroyed by cold stimuli, 2°C for 4hr. By contrast, most of 15°C -grown wild-type animals can survive for 48 hours at 2°C. These facts suggest that C. elegans has cold resistance with changes in environmental temperature. To reveal the molecular mechanisms underlying the cold resistance, we measured cold-resistance in the various mutant animals defective in temperature sensation and temperature-controlled hormonal signaling. We found that the mutants defective in insulin-like molecule DAF-28, insulin receptor DAF-2 and its downstream molecules showed enhancement in cold resistance. These results suggest that insulin-mediated neural signals negatively regulate cold resistance. In another approach, we focused on the natural variation of wild-type strains isolated from different areas. We so far found that British strain showed weaker phenotypes in cold resistance than California or Vancouver strains, respectively. We are also using artificial evolution approaches and forward genetic screening to isolate the mutation in cold resistance.

3PT211 海馬での年齢依存的なホルモンホスファチドリンスフィンガーターに含まれる2種の変異解析

Age-related changes in the expression of mRNAs encoding for sex steroidogenic enzymes and sex hormone receptors in the hippocampus

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Although sex steroids play a crucial role in the development/maintenance of brain function, the age-related changes in the hippocampal sex steroidogenesis remain largely unknown. We examined the mRNA expression levels of sex steroidogenic enzymes and sex steroid receptors in the hippocampus of aged (24 month-old) male rats compared with those of young adult (3 month-old) male rats by means of semi-quantitative RT-PCR. The levels of mRNAs for cytochrome P45017a, 17β-HSD3 and 5α-reductase2 reduced in the hippocampus of aged rats to approx. 45%, 76% and 75% of young adult rats, respectively. On the other hand, the levels of 17β-HSD1, 5α-reductase1 and P450aromatase were almost the same between aged and young adult hippocampus. The levels of estrogen receptor β and androgen receptor reduced in aged hippocampus to approx. 84% and 55% of young adult hippocampus, respectively, while almost no change was observed in the mRNA expression of estrogen receptor β between aged and young adult hippocampus. These results indicate that the hippocampal sex steroidogenic properties are substantially altered between aged and young animals.

3PT212 NMDA 受容体と相互作用することにより CaMKII は分子メカニズムとして機能する in vitro 実験系による実証

CaMKII functions as a molecular memory through interaction with NMDA receptors - validation in in vitro experiments -
In the process of long-term potentiation (LTP) at synapses, calcium-calmodulin-dependent protein kinase II (CaMKII) is interacted with NMDA receptors, and the interaction leads to Ca^2+ /CaM-induced, but CaM-independent CaMKII activity (Bayer 2001, Nature 411, pp. 801-805). This sustained activity is considered as a part of mechanisms of LTP maintenance; however, additional mechanisms are needed to explain reversibility of LTP, and stable basal CaMKII activity even with spontaneous Ca^2+ increase at synapses. Here, in an in vitro experimental system, we found that CaMKII autophosphorylation at T286 shows hysteresis through interaction of a NMDA receptors-derived peptide. This indicates that CaMKII activity can work as a molecular memory for LTP maintenance. The hysteresis was regulated by protein phosphatase 1 (PP1), and cancelled by high PP1 application, correspondingly decrease of CaMKII activity. Ca^2+ concentration dependence of the hysteresis showed that the hysteresis is robust against spontaneous Ca^2+ increase. This can provide a mechanism of reversibly LTP and stable basal CaMKII activity at synapses.

3PT213 新機能神経スパイラル解析プログラム
Novel program and its applications for spines in neurons
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Dendritic spines in the brain are substrates of synaptic contacts that may be involved in neuronal computational processes. Visualization and analysis of dendritic spines is of critical importance to elucidate physiological changes of morphological plasticity, as well as effects of various hormones and pharmacological agents. Advent of laser-scanning confocal microscopy / 2-photon microscopy and fluorescent dye allowed us to collect large body of neuronal image data, namely tens of dendrites and several tens of thousands of spines residing on each dendrite of single neurons. Conventional methods using manual tracing software need time-consuming efforts by researchers and not suitable to handle large data. Therefore introduction of protocol for a new automatic analysis of neuronal structure including spines will greatly enhance progress of research in the area. Here we developed a new software, Spiso-3D, to extract spines as well as dendrites and in neuronal image based on their geometrical features. Our “geometric method” utilizes scale-free, shape-dependent analysis, making less dependent on brightness in the image. Because Spiso-3D is a Java-based program, it will readily work on a PC with a relatively limited resource.

Using the Spiso, we analyzed the effect of activin, an endogenous sex hormone in the mammalian brain, on spines of hippocampal neurons. Ref. Mukai et al., Cerebral Cortex, 21, (12): 2704-2711. (2011)

3PT214 高速ビデオカメラ法および高電圧法によるサル専門反射系構造解析システムの開発
Evaluation of eyelink classical conditioning in monkey by using high-speed video sensing and electromyogram signal
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Classical eyeblink conditioning is one of the best-characterized behavioral models of associative learning in mammals. Especially, standard delay paradigm has been used for assessing the motor learning or cognitive performance in a variety of mammalian species including, rabbit, rat, human and mouse. In the present study, we first developed the system for evaluating the delay eyeblink conditioning in monkey (Macaca mulatta). The eye blinking of a monkey was measured and evaluated by eyelid electromyogram (EMG) method and a 1kHz high-speed image processing (Intelligent Vision Sensor, C8201; Hamamatsu Photonics K. K.), simultaneously. Within the conditioning procedure, a 1kHz tone served as the CS while an air-puff was used as the US. The EMG-based analysis indicated that monkeys exhibited over 60 % of conditioned response (CR) frequency during 5-days acquisition session (100 trials per day), and rapid extinction of CRs during 2-days extinction session. Also in the high-speed image processing analysis, the monkeys exhibited similar results of both in acquisition and extinction of CRs. Hence, we have concluded that both methods of the EMG-based and video-based analysis are effective in measuring eyelink conditioning in monkey. Our results indicated that the conventional delay conditioning procedure can also effective in conditioning monkeys. The novel multi-measuring system of eyeblink CRs in monkeys will provide useful tools for elucidating the neural mechanism underlying motor learning in higher mammals.

3PT215 新機能神経スパイラル解析プログラム
Histamine H1 receptor-expressing neurons in the anterior part of the hypothalamic paraventricular nucleus inhibit food intake
Shuhei Horio (Institute of Health Biosciences, The University of Tokushima Graduate School)

Food intake in mammals is controlled by neurons in the hypothalamus. To suppress food intake, the information from the periphery is conveyed to the hypothalamus via two distinct pathways; one is a humoral pathway that transmit satiety information to hypothalamic paraventricular nucleus (PVH) via arcuate nucleus (ARC) that lies adjacent to blood circulation, and the other is a neuronal pathway that send information to PVH via nucleus solitarius (NTS). Thus satiety information converges in PVH, which is now considered as a center for suppressing food intake. Thus far, several neurons in PVH including CRH (corticotropin-releasing hormone) neurons, oxytocin neurons, and melanocortin neurons are found to suppress food intake. We studied the role of histamine H1 receptor (H1R)-expressing PVH neurons in food intake. H1R is highly expressed in murine PVH in both the anterior and posterior parts. We developed gene-targeted mice that expressed human IL-2Ra selectively in H1R-expressing neurons. Injection of immunotoxin to PVH selectively ablated these neurons (the toxin binds to IL-2Ra and disrupts the cell). The ablation of these neurons caused a 30% increase in food intake. The body weight was also increased approximately 30% compared to control. The ablation of the posterior part of H1R-expressing PVH neurons had no effect on food intake.

These results indicate that H1R-expressing neurons in the anterior PVH have a crucial role in the regulation of food intake. It is probable that these neurons constantly suppress food intake.

3PT216 眼 Blink hormone不感帯領域の役割を高まる神経に与える影響
Sex difference in profile of hippocampal hormones generates sex difference in hippocampal function
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Sex difference in the brain is a very attractive problem. For example, the hypothalamus, the brain region responsible for reproductive behavior, exhibits a clear sex difference in the size of nerve nucleus. In contrast, the hippocampus, a center for learning and memory, does not have sex difference at the anatomical level including the volume and the number of neurons. Nevertheless, the significant sex difference in the performance of hippocampus-dependent task such as spatial memory using Morris water maze or radial arm maze task.

What is the cause of sex difference in the performance of learning and memory depending on hippocampus? So far, sex difference in the hippocampus had been attributed to the level of sex hormones in the blood. However, we revealed that the hippocampal level of sex hormones is much higher than the blood level. Surprisingly, hippocampal estradiol (E2), the most potent female hormone, in the male is 7-fold higher than female whereas female E2 in the blood is much higher than male. Moreover, the density of spines (postsynapses) as well as the level of E2 in female hippocampal fluctuates with a period of 4 days (estrus cycle), whereas those in male are retained at a constant level.

This clear sex difference in hormonal profile in hippocampus may generate the sex difference in the hippocampal structure at more subtle level, that is, synaptic level, resulting in the sex difference in the performance of hippocampus-dependent task.

3PT217 新機能神経スパイラル解析プログラム
Age-related changes in spine density and morphology of hippocampal neurons in relation to memory impairment
Suguru Kawato, Koren Li, Yasushi Hojo (Grad Sch of Arts & Sci, Univ Tokyo)

We have investigated the spine density and morphology of neurons in...