- 1P210 光捕捉による海馬神経シナプスの小胞ダイナミクス
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- Vesicle dynamics of hippocampal synapses in optical trapping
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Synaptic vesicle cycle in presynaptic terminals of nervous system is critical for information processing within the brain. Optical trapping has recently become a useful tool in handling nanomaterials. A single nanoparticle or molecule are difficult to be trapped stably because of small polarizability, however there are increasing evidences that polymers and nanoparticles can be trapped and assembled in the focal spot. We investigated the optical trapping effect on vesicle dynamics of hippocampal synapses by fluorescence spectroscopy. A cw-Nd:YVO₄ laser (λ = 1064 nm) was focused on the synapses in cultured hippocampal neurons labeled with the fluorescent endocytotic marker FM 1-43. When the trapping laser was focused on synapses, fluorescence was observed at the focal spot. The fluorescence spectrum was identified as fluorescence from FM 1-43 molecules around 605 nm, indicating that the dye molecules in synaptic vesicles emitted two-photon excited fluorescence by the trapping laser. From the temporal profiles of the fluorescence intensity at 605 nm of the spectra, the fluorescence intensity increased gradually within the irradiation time ~100 s of the trapping laser, suggesting that the synaptic vesicles are assembled in the focal spot. The present results can be applied to controlling synaptic activity of neurons with optical trapping.

- 1P212 加齢によるマウス聴性脳幹反応の変化
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- Auditory brainstem response in aged mice
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Auditory brainstem response (ABR) thresholds to the auditory stimuli in wide frequency range (1-32 kHz tone) were investigated in the aged wild-type mice (C57BL/6J). Common biological disorder (eg. auditory hearing loss, memory impairment) is known to occur in both humans and mice, and then, mice are recognized to be a good model to study aging process. In previous report, 100-week-old (at least more than 20-month-old) C57BL/6J mice actually show severe hearing loss to four auditory stimuli such as click and high frequency tone (8, 16, 32 kHz). Mice at 12-month (48-60 week)-old were also reported to reveal slight hearing loss to the four auditory stimuli. In our experiments, 30-week-old mice did not show hearing loss (for example, ABR thresholds in dB were 37 and 43 in click and 1 kH tone respectively). However, threshold level from the 60-week-old mice was higher compared with the 30-week-old one (in dB: 47 in click, 57 in 1 kHz tone). From above, it was shown for the first time that auditory response to low frequency tone stimuli in C57BL/6J mice also decreased with advancing age, and our results are correspondent with the previous study about other stimuli. In the poster presentation of annual meeting, we will also show result of ABR measurement using senescence-accelerated mice (SAM), which is a one of model mice for aging study.

- 1P211 瞬目反射条件付けの自家蛍光イメージング
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- Imaging of eyeblink conditioning using endogenous fluorescence
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During classical eyeblink conditioning, animals associate tone (conditioned stimulus) with stimulation of the eyelid (unconditioned stimulus). The cerebellum has been thought to be involved in the learning. In order to clarify which part of the cerebellum is activated during eyeblink conditioning, in vivo imaging was performed in mice using endogenous fluorescence of flavoproteins. Before the experiment, electrodes for eyelid stimulation and EMG recording were implanted in the eyelid of the mice. Tone (1 kHz, 352 ms) or electrical stimulus (100 Hz, 100 ms) was applied under anesthesia with urethane or in awake animals. Transcranial fluorescence images (excitation 470-490 nm, emission 515-550 nm) were acquired using a cooled CCD camera. Electrical stimulus of the eyelid evoked strong elevation in fluorescence almost in the entire cerebellum, and this reached its peak within 0.5 s from the onset of the stimulus. Tone stimulus evoked a weaker elevation in fluorescence, and the highest elevation was observed in the vermis. These results indicate that the cerebellum responds to both tone and electrical stimulus in eyeblink conditioning.

- 1P213 小脳抑制性シナプス伝達に対するエタノールの二重促進作用
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- Ethanol enhances both action potential-dependent and independent GABAergic synaptic transmission in the cerebellum via cyclic AMP pathways
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Ethanol (EtOH) modulates synaptic efficacy in various brain areas, including the cerebellum, which acts for the motor learning and memory. Previous studies have reported that EtOH exposure enhances tonic inhibition of cerebellar granule cells, which is thought to be a major reason for alcohol-induced impairment of motor behavior. However, the effects of EtOH on molecular layer interneurons (MLIs), such as basket and stellate cells in the mouse cerebellum have remained unknown. Here, we found that EtOH efficiently enhanced spontaneous activity of MLIs and increased their firing rate. We examined the underlying mechanism for the EtOH-induced facilitation of intrinsic firing of MLIs. The EtOH-mediated excitation was blocked by inhibitors for adenylyl cyclase and the hyperpolarization-activation cation current, *I*_h, suggesting that the HCN channels were activated by EtOH. Furthermore, high EtOH concentrations induced facilitation of miniature IPSCs, which was independent on *I*_h or PKA activation, but dependent on intracellular cyclic AMP concentration. Therefore, EtOH enhances GABAergic transmission onto Purkinje cells through *I*_h-dependent facilitation of MLI firing, and cyclic AMP-regulated and PKA-independent enhancement of GABA release at presynaptic terminals. This dual modulation of GABAergic synaptic transmission could influence spontaneous activity of cerebellar Purkinje cells, leading to regulation of the cerebellar neuronal circuit processing.