2P256 コレラ菌走化性受容体のアミノ酸リガンド認識機構 Recognition of amino acid ligands by *Vibrio cholerae* chemoreceptors

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Vibrio cholerae, an etiological agent of cholera, shows chemotaxis, which is implicated in pathogenicity as well as survival in various environments. A classical biotype strain of *V. cholerae* O395N1 appear to possess 44 chemoreceptor homologs, which we name methyl-accepting chemotaxis protein-like proteins (MLPs). Among them, Mlp24 and Mlp37, which are highly homologous to each other, mediate attractant responses to serine and other various amino acids. Our recent ITC results using purified periplasmic fragments of Mlp24 and Mlp37 strongly suggest direct binding of various amino acids to the fragments. Recently, the structure of the periplasmic domain of Mlp37 from an El Tor biotype strain of *V. cholerae* was determined by X-ray crystallography. The structure, mainly composed of two homologous domains with two putative binding pockets in tandem, is not related to those of amino acid chemoreceptors of *E. coli*. To understand the ligand-recognition mechanism, we mutated residues of putative binding pockets of Mlp24: alanine was substituted for Y120, R125 (pocket I), Y192, H205 (pocket II). Both the pocket I mutants showed defects in mediating taxis toward serine, arginine, asparagine and proline, although they were expressed at the wild-type level, implying the involvement of pocket I in amino acid recognition. On the other hand, defects of the pocket II mutants were milder. Construction and characterization of other mutants of Mlp24 and Mlp37 are in progress to further understand their ligand recognition mechanisms.

2P257 コレラ菌走化性応答能の環境温度変化による制御 Temperature-control of chemotactic properties of *Vibrio cholerae*

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Vibrio cholerae, the causative agent of cholera, senses and responds to various stimuli to survive in host animals and other environments. Chemotaxis is one of these critical environmental responses. Chemotaxis has been best studied in *Escherichia coli*. Stimuli are sensed by five methyl-accepting chemotaxis proteins (MCPs), which regulate directions of flagellar rotation via the control of phosphorylation levels of Che proteins. *V. cholerae* has 44 MCP-like proteins (MLPs) and 3 sets of Che proteins. We previously showed that only system II is directly responsible for chemotaxis and that Mlp24 and Mlp37 are the major chemoreceptors for mediating taxis toward amino acids. Mlp24, also known as McpX, has been implicated in pathogenicity. In this study, we asked whether *V. cholerae* modulates the MLP expression pattern in response to environmental changes. We found that culture temperature influences taxis for amino acids and the expression pattern of MLPs. When cultured at 37C, *V. cholerae* showed stronger responses toward serine than when cultured at 23C or 30C. Immunoblotting revealed that the cellular amounts of Mlp10 and Mlp37 decreased and increased with increasing temperature, respectively. CheA2, which belongs to Che system II, was constitutively expressed at different temperatures. These results suggest that *V. cholerae* can control its chemotactic ability by changing the MLP repertoire in response to temperature.

2P258 音がもたらす不快情動と自律神経応答の関係 Correlation between sound-induced aversiveness and autonomic response

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An ample body of fMRI and PET studies have shown that sound-induced aversiveness has a strong correlation with the activation of the amygdala, the center of stress-related memory, such as fear or anxiety. In addition, it is well known that the activation of the amygdala enhances the sympathetic response, one of the stress-related responses. However, to date little is known about the relationship between aversiveness and stress-related responses. The goal of the present study is therefore to reveal the correlation between sound-induced aversiveness and the sympathetic response. Healthy males aged 25-35 years were exposed to three types of sound at several sound pressure levels 40-80 dB (A). One of these sounds was the chalkboard scratching sound recorded as an aversive stimulus and the others were synthesized from this with the aversiveness reduced by signal processing. A finger photoplethysmograph

(PPG) was measured and the degree of the decrease in PPG amplitude was used to derive information about the sympathetic response mediated by adrenergic receptor $\alpha 1$. The results showed that the degree of the decrease in PPG amplitude varied in accordance with not only the sound pressure levels but also with the type of sound. The sound that was assessed as the most aversive by participants was the most effective stimulus for inducing the sympathetic response at 70 dB (A), suggesting that the sympathetic response mediated by adrenergic receptor $\alpha 1$ can be a good indicator of aversiveness aroused when people listen to unpleasant sounds.

2P259 培養神経回路網の長期発達過程における興奮性・抑制性シナプス分布の免疫蛍光計測 Immunofluorescence investigation for the distribution of excitatory and inhibitory synapses in the development of cultured neural networks

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Dissociated neurons cultured in vitro spontaneously construct the networks and show electrical activity. It has been reported that the activity was correlated with the density of whole synapses in the development of the network. However, the network activity is regulated by the balance of excitatory and inhibitory synaptic inputs. Thus, it is required to reveal the development and distribution of both excitatory and inhibitory synapses with the network activity.

In the present study, the spontaneous electrical activity of cultured cortical neurons was recorded for more than one month using Multi-electrode arrays (MEAs). The network firing rate of cultured networks was found to increase at from 2 to 3 weeks. This activity did not increase over 5 weeks but become saturated within 1 month.

To clarify the developmental change and distribution of excitatory and inhibitory synapses, we performed immunocytochemistry of the both synapses during long-term culture of rat cortical networks using antibodies against vesicular glutamate transporter 1 (VGLUT1) and vesicular transporter of γ -amino-butyric acid (VGAT). The number of both VGLUT1 and VGAT-labeled terminals increased gradually along the culture ages from 7 to 21 days in vitro (DIV), whereas the number of the puncta became saturated and did not increase at 1 month. The VGLUT1-labeled terminals predominated on neuronal dendrites compared to VGAT terminals. Based on these results, further investigation of spatiotemporal dynamics of neuronal networks will be discussed.

2P260 雄海馬で女性ホルモンは Sub-threshold 刺激でも LTP を成立させる Estradiol induces the long term potentiation even under sub-threshold stimulation in male hippocampus

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We revealed that the estradiol (female hormone) perfusion induced the long-term potentiation (LTP) of male hippocampal neurons under sub-threshold stimulation. Without estradiol, LTP was not induced under sub-threshold stimulation. On the other hand, LTP induced by strong tetanic stimulation was not further enhanced by the estradiol perfusion. It is deduced that the neurotrophic estradiol enhances weak LTP to become complete LTP, but does not further enhance the already established LTP. It should be noted that the male brain has more estradiol than the female brain.

2P261 電極法によるマウス脳組織における一酸化窒素 (NO) 測定 Direct measurement of nitric oxide (NO) in mice brain

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Nitric oxide (NO) is known to play important roles in a number of physiological processes, such as vasodilation, neurotransmission, immune response, over various animal species of vertebrate and invertebrate. Especially, relationship between NO and memory learning has been received a lot of attention in recent years. From previous reports, NO-related reagents influenced memory formation by different learning tasks in various animals. Our previous study showed that NO synthase inhibitor impaired eyeblink conditioning with delay paradigm in mice. It is also reported that NO metabolite levels increased after Y-maze task in rat brain. However NO production in learning process is suggested to be important for memory formation from above, physiological role of NO and its dynamics in memory learning process have not been studied in detail. For investigating detailed NO dynamics in brain, NO production in mice brain was measured using NO-specific electrode. In our preliminary experiment, we successfully measured enzymatic NO production in sub-molar concentration in isolated mice brain tissue. In the presentation of annual