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Involvement of Muscarinic Acetylcholine Receptors and Hippocampal Theta Oscillation in Eyeblink Serial Feature-Positive Discrimination Task in Mice

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Abstract

Classical eyeblink conditioning is considered as one of the best models of learning for studying the interaction between higher and lower levels of the nervous system. Mounting evidence has been collected on the underlying neural substrates responsible for standard delay eyeblink conditioning, which essentially depends on the cerebellum and brainstem, the role of higher brain regions, such as the hippocampus and medial prefrontal cortex have also been under intense experimental scrutiny. Although the effect of the hippocampal lesion in serial feature-positive discrimination has not been investigated, the pivotal role of the hippocampus in the simultaneous feature-positive discrimination and the impairment of conditional discrimination in amnesic patients, and, suggested an important role of the hippocampus in the top-down modulation of the CR in eyeblink serial feature-positive discrimination task. Hippocampal theta rhythm is considered as one of the candidates for neural activities that contribute to the top-down modulation during the conditional discrimination which showed a strong correlation with the learning rate during eyeblink conditioning. Recently, the dynamics of hippocampal local field potential (LFP) was investigated in rats and showed that the relative power of theta oscillation (5–8 Hz) increased after the light cue, which revealed a

significant correlation with the expression of CR on a trial-to-trial basis.

I investigated the role of muscarinic acetylcholine receptors (mAChRs) in eyeblink serial feature-positive discrimination learning in mice using the mAChR antagonist. Behavioral data revealed that saline-injected control mice were successfully discriminating the identical tone presented in cued and non-cued trials within a few days of conditioning. Analysis of CR dynamics revealed that mice exhibited differentially timed CR depending on the presence/absence of the preceding cue: the CR onset latency was shorter in cued trials than in non-cued trials although CR peak amplitude values were not significantly different between trial types. These results suggest that the top-down modulation mainly occurred before the execution of the CR, leading to the difference in its frequency of occurrence, but not during the CR itself. In contrast, scopolamine influenced CR temporal pattern as scopolamine-injected mice showed the equivalent CR onset latency in cued and non-cued trials, suggesting an involvement of mAChRs in refining CR timing in serial feature-positive discrimination learning. I found that scopolamine-injected mice developed an equivalent number of CRs irrespective of the presence of the cue during the acquisitions, indicating in a failure to acquire conditional discrimination. Post-training administration of scopolamine to the control mice did not impair the conditional discrimination and expression of pre-acquired CR. These results suggest that mAChRs may play a pivotal role in memory formation in the conditional brain state associated with the feature cue, however, they are unlikely to be

involved in the development of discrimination after conditional memory had formed in the serial feature-positive discrimination task during eyeblink conditioning.

Also, I investigated the hippocampal LFP during eyeblink conditional discrimination task in mice. I found the existence of differential sub-bands by analyzing the hippocampal theta oscillation in cued trials: the higher (7–9 Hz) but not the lower (4–6 Hz) sub-band increased after the conditional cue, suggesting the higher sub-band reflects the cognition of the conditional cue, which might lead to the acquisition of discriminative responses. In addition, the cue-evoked increase in the higher sub-band correlated significantly with the earlier onset of the subsequent conditioned response, suggesting a precise role of the higher sub-band in preparation for the coming CS-US presentation. Also, I found that during the course of training both the lower (4–6Hz) and the higher type 2 oscillation (7–9 Hz) were enhanced, suggesting an increase in general attention. Administration of scopolamine after sufficient learning suppressed both the sub-bands during the conditioning but not the pre-acquired discrimination, indicating both the sub-bands are type 2 theta oscillation and not involved in memory expression for conditional discrimination. These findings are consistent with the behavioral result, where post-training administration of scopolamine did not weaken the pre-acquired discrimination, despite impaired the acquisition of discrimination in eyeblink serial feature-positive discrimination task. Collectively, the behavioral as well as hippocampal LFP data revealed that mAChRs play an important role in acquiring the discrimination

between cued and non-cued trials through activation of sub-bands of type 2 theta oscillation but not after robust learning, where the association of other structures other than the hippocampus might be involved. Thus, mAChRs and its dependent sub-bands might play an important role in the formation, but not the expression, of the memory for top-down modulation associated with the feature cue in the serial feature-positive discrimination task in mouse eyeblink conditioning.

【論文審査の結果の要旨】

本博士論文は、学習モデルの１つである瞬目反射条件付けの逐次型特徴陽性弁別課題を用いて、記憶獲得過程および記憶発現過程におけるムスカリン性アセチルコリン受容体と海馬の集団的同期活動の１つであるシータ波の役割を調べた研究の結果をまとめた論文である。

本研究では、ムスカリン性受容体がこの弁別課題の記憶獲得過程において重要な役割を果たすことを初めて明らかにした。また、ムスカリン性受容体は条件記憶が形成された後は弁別の発現にはあまり関与しないことも示した。さらに、本課題学習中のマウスの海馬局所場電位を調べて、ムスカリン性受容体依存的な type 2 シータ波には高周波数帯 (7-9 Hz)と低周波数帯(4-6 Hz)の 2 種類があることを初めて明らかにし、そしてそれらの記憶獲得過程における応答性の違いから、弁別課題における役割の違いを示した。特に、高周波数帯が条件手がかりの認識を反映することを示唆する結果は重要である。総合的に考えて、行動実験および海馬局所場電位の結果は、ムスカリン性アセチルコリン受容体が type 2 シータ振動の活性化を通じて、弁別記憶の獲得において重要な役割を果たすことを示唆している。

これらの研究成果および論文内容はとても優れており、本学大学院生命融合科学教育部における博士（工学）としてふさわしいと判断される。