[課題－2]

審査の結果の要旨

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The spatio-temporal pattern of activity in population of cerebellar Purkinje cells (PCs) has never been described during early postnatal period. It is of particular interest because well characterized plastic changes occur at climbing fiber (CF)-PC synapse in an activity dependent manner during this period. In this study, *in vivo* two-photon calcium imaging was used to monitor CF responses, known as complex spikes (CSs), in population of PCs during the early postnatal period in order to reveal the pattern of activity underlying the postnatal CF network refinement. First, developmental change in the pattern of CF activity was examined in wild type mice. Next a similar analysis was performed in PC-specific Ca_v 2.1 knockout (PC-Ca_v2.1 KO) mice, in which the postnatal CF network refinement is known to be impaired. The results are as follows.

1. The mean frequency of spontaneous calcium transients gradually increased during the first three postnatal weeks whereas the average half width was largest in newborn animals, and gradually decreased along with the postnatal development. To investigate the nature of the calcium signals, cell-attached unit recordings were performed simultaneously to calcium imaging and it confirmed that spontaneous calcium transients exclusively reflected the occurrence of CSs at all postnatal ages.
2. The pattern of CF activity in PC population was examined. In particular, the level of synchrony was evaluated by calculating pairwise correlation coefficients between CS activity from nearby PCs. The population activity was highly correlated soon after birth and underwent prominent desynchronization starting from postnatal day (P) 5. The degree of synchrony declined to the adult level at P8. To better characterize this phenomenon, mean correlation coefficients were plotted against the medio-lateral or rostro-caudal distance separating the pairs of PCs. In newborn animal, CF responses were highly correlated at all separations along both axis and the subsequent decrease of synchrony was relatively uniform.

3. To address whether the developmental desynchronization of cerebellar activity is related to the CF network refinement, PC population activity was analyzed in PC-Ca_v2.1 KO mice, in which this phenomenon is known to be impaired. At neonatal stage (P4-5), both PC-Ca_v2.1 KO and control littermates exhibited a highly correlated pattern of CF responses. In contrast, during the second postnatal week, while PC population activity underwent a marked desynchronization in control mice, it remained abnormally correlated in PC-Ca_v2.1 KO mice. In particular, the activity in rostro-caudally oriented clusters of cells failed to desynchronize, whereas more medio-laterally distant pairs desynchronized almost normally. This result suggests that the progressive desynchronization of CF responses during the development could be attributed, at least in part, to the refinement of the CF network.
4. Finally, the exact mechanism by which desychronization could be impaired in PC-Ca_v 2.1 KO mice was explored. One possibility is that, in the knockout mouse, the innervation territory of single CFs remains abnormally wide due to an incomplete remodeling of their terminal arbors. To test this hypothesis, a morphological analysis of the CF network by a triple fluorescent labeling for calbindin (PC marker), VGluT2 (CF terminal marker) and the anterograde tracer Alexa 594 (DA-594) was performed during the second postnatal week. This morphological analysis demonstrated that CF collaterals along the rostro-caudal axis were longer and had more terminals onto PC somata in PC-Ca_v 2.1 KO mice than in control mice. These results provide a morphological basis for the impaired desynchronization of CF activity in PC population in PC-Ca_v 2.1 KO mice.

In summary, these experiments showed that a dramatic desynchronization of CF responses occurs at the end of the first postnatal week. The results also strongly suggest that the refinement of the CF network might be involved in this developmental reshaping of the population activity. The immature pattern of CS activity in PC population remained so far widely unknown and hence, this research importantly contributes to a better understanding of the developmental processes taking place in the olivocerebellar system during the early postnatal period. It is hence sufficient to confer a PhD degree to the applicant.