論文の内容の要旨

論文題目　**Desynchronization of cerebellar Purkinje cell population activity during postnatal development**

（生後発達期における小脳プルキンエ細胞集団活動の非同期化）

氏名　　グッド　ジャンマルク

INTRODUCTION

The inferior olivary nucleus constitutes one of the two major glutamatergic afferent systems to the cerebellum. In adult rodents, axons of olivary neurons divide into several climbing fibers (CFs) that innervate cerebellar Purkinje cells (PCs) in a one-to-one manner. The activation of CF-PC synapse generates bursts of action potentials known as complex spike (CS), which trigger a widespread influx of calcium into PCs. The presence of electrotonic coupling by gap junctions between olivary neurons is responsible for the synchronization of spontaneous CSs in nearby PCs. This synchrony is especially obvious between nearby PCs located within rostro-caudally oriented bands called microzones, and tends to fall off more sharply in the medio-lateral direction.

During the postnatal period, CF-PC synapses undergo several dynamic changes. At birth each PC soma is innervated by several CFs, and during the first postnatal week, only one CF on each PC soma is selectively strengthened relatively to the others in a process called functional differentiation. During the subsequent period, weak CFs are eliminated while the strong CF translocates to PC dendrite. In addition, morphological reconstructions of single olivary axons showed that soon after birth, each CF had collaterals making weak contacts with several PCs, a stage called “creeper” type terminal arbor, and that during the first postnatal week, CFs transformed into a “nest” type terminal arbor, characterized by a dense aggregation of terminals tightly surrounding single PC somata.

The spatio-temporal pattern of activity in populations of PCs has never been described during postnatal CF network refinement. In this study, I used *in vivo* two-photon calcium imaging to monitor spontaneous CF responses in populations of PCs from postnatal day (P) 3 to P24. I first examined wild-type (WT) mice and then, performed a similar analysis in PC-specific $Ca_v2.1$ knock out (PC-Cav2.1 KO) mice, in

which the functional differentiation of multiple CFs, dendritic translocation of a single CF, and elimination of weaker CFs are impaired in each PC.

RESULTS

The membrane permeable fluorescent calcium indicator Oregon Green 488 BAPTA-1/AM (OGB-1) was bulk loaded into the cerebellar cortex of anesthetized mice, and the activity in PCs was monitored by two-photon calcium imaging**.** Sudden increase in the fluorescence intensity corresponding to spontaneous calcium transients could be observed with a single-cell resolution. To gain insight into the nature of these events, cell-attached unit recordings were performed simultaneously to calcium imaging. In accordance with previous studies in adult mice, calcium transients exclusively reflected the occurrence of CSs in early postnatal mice. Then, I used an established temporal deconvolution algorithm to reconstruct CS firing rates based on fluorescent fluctuations. Simultaneous cell-attached recordings confirmed the reliability of the reconstructions.

I next examined the pattern of activity in populations of PCs. In particular, I evaluated the level of synchrony among CF responses by calculating pairwise correlation coefficients between several traces of reconstructed CSs firing rates from nearby PCs. Within the recorded field (~150-150 μm), population activity was highly correlated soon after birth and underwent prominent desynchronization as early as P5. 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.

To address whether the developmental desynchronization of cerebellar activity is related to the CF network refinement, I analyzed PC population activity in PC-Ca_v2.1 KO mice, in which the functional differentiation and subsequent CFs elimination are selectively impaired. At neonatal stage (P4-P5), 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 configuration of the CF network has a direct impact on the pattern of activity in populations of PCs and that the progressive desynchronization of CF responses during the development is attributed, at least in part, to the refinement of the CF network.

Finally, I explored the exact mechanism by which desychronization could be impaired in PC-Ca_v2.1 KO mice. 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 from “creeper” type to “nest” type. 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 in PC-Ca_v2.1 KO mice than in control mice. These results provide morphological basis for the impaired desynchronization of CF activity in PC populations in PC-Ca_v2.1 KO mice.

DISCUSSION

I found that a dramatic change occurred in the spatiotemporal pattern of activity in the cerebellar cortex during the postnatal development. CF responses, which were highly correlated among population of PCs soon after birth, underwent a progressive desynchronization as early as P5. The degree of synchrony declined to the adult level at around P8. In PC-Ca_v2.1 KO mouse, in which CF synapse refinement is impaired, the desynchronization observed in WT mice was incomplete. In particular, the activity in clusters of rostro-caudally oriented PCs remained highly correlated during the second postnatal week. Interestingly, the morphological analysis of this knockout mouse revealed the presence of abnormally long CF collaterals along the rostro-caudal axis in comparison with control littermates.

The results suggest that the desynchronization of PC population activity is a consequence of the CF network refinement. Two mechanisms might be involved. First, the contraction of the innervation territory of each CF during the remodeling of its terminal arbor might play a role. The presence of both abnormally long CF collaterals and an incomplete desynchronization in PC-Ca_v2.1 KO mouse is consistent with this interpretation. Second, the functional differentiation of CF-PC synapse might also be involved. The impairment of the functional differentiation in PC-Ca_v2.1 KO mouse could be the cause of the aberrant desynchronization in this mouse. A recent report showed that, while an input from the strong CF is sufficient to trigger a CS after the functional differentiation, the summation of several CF inputs is required to generate a CS before the functional differentiation. Based on this finding, it is conceivable that only the synchronized CF inputs can be transmitted to PCs in new born animals, while desynchronized CF inputs, which are not summated, cannot induce sufficient depolarization to trigger CSs.

The possibilities remain that the initial high synchrony and subsequent desynchronization of CF responses originates in the inferior olive. First, developmental regulation of the electrical coupling between olivary neurons might be involved. However, ultrastructural studies suggest that functional gap junctions might appear only around P10. Thus, there is currently no strong experimental evidence to support the contribution of the gap junction regulation in the inferior olive to the developmental desynchronization of CS activity. Second, afferents to inferior olive themselves could be synchronized. However, mature synapses onto olivary neurons are scarce during the first days of life. Taken together, these results suggest that CS activity patterns in PC populations are highly dependent on CF-PC synaptic wiring, and that the desynchronization of CS activity result, at least in part, from the CF network refinement.