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Update article

Postnatal development and synapse elimination of climbing fiber to Purkinje cell projection in the cerebellum

Kouichi Hashimoto [a,b,*], Masanobu Kano [a,b]

[a] *Department of Cellular Neurophysiology, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8640, Japan*
[b] *Department of Cellular Neuroscience, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita 565-0871, Japan*

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Abstract

Cerebellar climbing fiber (CF) to Purkinje cell (PC) synapses in rodents provides a good model to study mechanisms underlying postnatal development of synaptic functions and elimination of redundant synapses in the central nervous system. At birth, each PC is innervated by multiple CFs. Then, single CF input is selected, matured and strengthened, while surplus CFs are eliminated. By the end of the third postnatal week, most PCs become innervated by single CFs. This up-date article aims to provide an overview of recent studies on the mechanisms of this process.

Keywords: Purkinje cell; Climbing fiber; Cerebellum; Parallel fiber; Postnatal development; Multiple innervation

1. Introduction

Proper functions of the nervous system rely on precise formation of neuronal circuitry during development. Immature neurons initially make synaptic connections not only to their final targets but also to other neurons. Then, functionally important synapses are strengthened, and less important synapses are weakened relative to the important ones. The weakened synapses are finally eliminated morphologically. Several previous studies suggest that neural activity during certain postnatal period (critical period) is essential for such refinement processes (Changeux and Danchin, 1976; Katz and Shatz, 1996; Lohof et al., 1996; Nguyen and Lichtman, 1996; Purves and Lichtman, 1980). This suggests the existence of activity-dependent process that works specifically in critical period. Mechanisms underlying such processes have been studied intensively in the neuromuscular junction (Sanes and Lichtman, 1999, 2001; Wyatt and Balice-Gordon, 2003). However, it is difficult to do detailed analyses in the central nervous system (CNS), because of the heterogeneity of inputs to individual neurons and the complexity of synaptic organization.

In this respect, the climbing fiber (CF) to Purkinje cell (PC) synapse in the cerebellum has provided a good model to study the cellular and molecular mechanisms of synapse elimination in the developing CNS. PCs in adult cerebellum receive two major excitatory inputs, namely parallel fibers (PFs) and CFs. PFs are axons of cerebellar granule cells (GCs) and form synapses on spines of PC dendrites. Each synaptic input is weak but 100,000–200,000 PFs form synaptic contacts on a single PC. In contrast, only one CF innervates each PC in the adult cerebellum (mono innervation) but each CF makes strong synaptic contacts on PC's proximal dendrites (Eccles et al., 1966; Palay and Chan-Palay, 1974). In early postnatal days, however, all PCs are innervated by multiple CFs (multiple innervation) (Crepel, 1982; Crepel et al., 1976; Lohof et al., 1996). These surplus CFs are eliminated eventually with the progress of postnatal development, and mono innervation

* Corresponding author. Tel.: +81 6 6879 3581; fax: +81 6 6879 3589.
E-mail address: hashik@cns.med.osaka-u.ac.jp (K. Hashimoto).

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is attained by the end of the third postnatal week in mice (Kano et al., 1995, 1997, 1998).

This up-date article aims to provide an overview of recent progress in studies how only one CF is selected and strengthened out of multiple CFs innervating a PC and how supernumerary CFs are eliminated subsequently.

2. Selection and strengthening of single CF input

2.1. Developmental change in the relative strengths of multiple CFs innervating the same PC

In the mature cerebellum, single CFs form synapses on proximal dendrites of PCs. Stimulation of such mono-innervating CFs elicits very large excitatory postsynaptic potentials (EPSPs) enough to activate voltage-gated Ca^{2+} channels over the whole dendrites (Hashimoto et al., 2001a; Konnerth et al., 1992; Miyakawa et al., 1992). In contrast, multiple CFs initially form synapses on the perisomatic processes of PCs in newborn mice (Chedotal and Sotelo, 1993; Morando et al., 2001). Amplitudes of excitatory postsynaptic currents (EPSCs) elicited by stimulating such multiply-innervating CFs are much smaller than those elicited by mature CFs (Hashimoto and Kano, 2003; Kano et al., 1995, 1997, 1998). These data indicate that CF inputs become stronger during postnatal development, while surplus CFs are eliminated during the same period. Mariani and Changeux showed that PCs were sometimes innervated by two CFs whose amplitudes were quite different around P10–P13 (Mariani and Changeux, 1981). This result suggests that only one CF is strengthened relative to other CFs before the completion of synapse elimination.

We have analyzed changes in the relative synaptic strengths of multiple CFs innervating the same PC (Hashimoto and Kano, 2003). Whole-cell patch clamp recording was conducted from the soma of a PC in a slice prepared from the vermis of mouse cerebellum. CFs were stimulated using a glass pipette positioned in the GC layer and CF-mediated EPSCs (CF-EPSCs) were recorded. To search multiple CFs innervating the recorded PC, the stimulation pipette was systematically moved and the stimulus strength was gradually increased from 0 V to around 50 V at each stimulation site. Fig. 1A and B illustrate the representative CF-EPSCs recorded at P3 (Fig. 1A) and P12 (Fig. 1B). At P3, the PC exemplified in Fig. 1A had five discrete CF-EPSC steps, indicating that this cell was innervated by at least five CFs. On the other hand, the PC recorded at P12 was judged to be innervated by two CFs (Fig. 1B). The decrease in the number of CF-EPSC steps at P12 reflects the progress of synapse elimination. Sizes of CF-EPSC steps were similar at P3, while those at P12 were very different between the larger CF and the smaller CF. The sizes of CF-EPSC steps reflect the strengths of individual CF inputs to a given PC. Therefore, these results suggest that synaptic strengths of multiply-innervating CFs are relatively

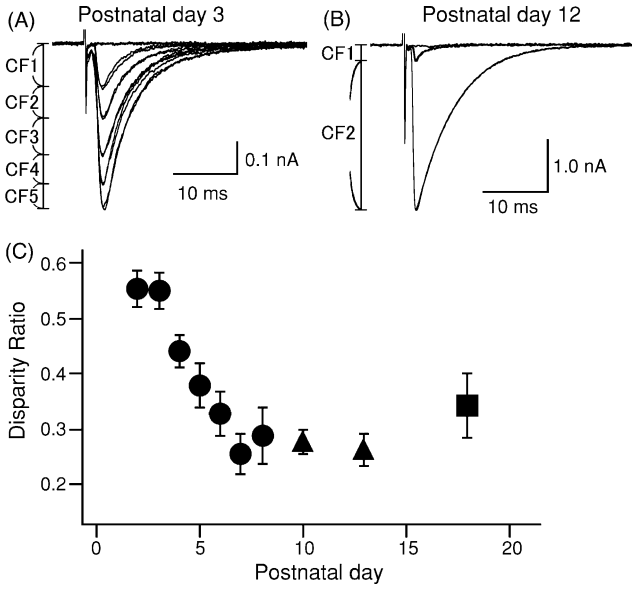

Fig. 1. Postnatal change in the disparity among multiple CF-EPSCs. (A, B) CF-EPSCs recorded in a PC at P3 (A) and in a different PC at P12 (B). CFs were stimulated in the granule cell layer at 0.2 Hz. Two to three traces are superimposed at each threshold stimulus intensity. Holding potential was −80 mV for A and −20 mV for B. The holding potential is corrected for junction potential. (C) To quantify the disparity in multiple CF-EPSCs recorded in a given PC, we calculated the Disparity Ratio for each PC. The amplitudes of individual CF-EPSCs in a given multiply-innervated PC were measured and they were numbered in the order of their amplitudes (A_1, A_2, …, A_i, …, A_N, $N \geq 2$, N is the number of CFs innervating a given PC. A_i is the EPSC amplitude for the CF_i recorded at the same holding potential. A_N represents the EPSC amplitude for the largest CF). Disparity Ratio $= \sum_{i=1}^{N-1}(A_i/A_N)/N - 1$, $(N \geq 2)$. If all CF-EPSCs in a given PC have the same amplitudes, the Disparity Ratio will be 1. Conversely, if the differences between A_N and other smaller CF-EPSCs are large, the Disparity Ratio will be small. The number of PCs for each data point is 18–92. Data for P9–P11 and those for P12–P14 are pooled and indicated with filled triangles. Data for P15–P21 are pooled and indicated with filled boxes (Reprinted from Neuron, 38, Hashimoto and Kano, 785–796 (2003), with permission from Elsevier).

similar at P3, and that one CF is strengthened during P3–P12. To examine the developmental course more in detail, we quantified the disparity among multiply-innervating CF-EPSC amplitudes by calculating a parameter, named Disparity Ratio. This value represents the predominance of the CF with the largest EPSC amplitude over the other weaker CFs innervating the same PC. As shown in Fig. 1C, the Disparity Ratio progressively decreased from P3 to P6 and reached a plateau after P7. This result indicates that one CF is strengthened among multiple CFs innervating the same PC during the first postnatal week (Hashimoto and Kano, 2003).

It has been reported very recently that the disparity among multiply-innervating CF-EPSC amplitudes becomes smaller from P4 to P7, and then progressively increases from P7 to P10 in CD1 strain mice (Scelfo and Strata, 2005). The reason for the apparent discrepancy between this report and our data is not clear. However, the average number of CFs innervating individual PCs at P4–P7 is around 3.3 in the