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Synapses need coordination to learn motor skills

Abstract: Experience-dependent remodeling of synaptic structure and function underlies information storage in the mammalian central nervous system. Although accumulating evidence suggests synergistic roles of long-term depression (LTD) and long-term potentiation (LTP) in cerebellar motor learning, their structural correlates and operational mechanisms have not been clearly addressed. A recent three-dimensional electron microscopic study provides insight for a potential complementary interplay between LTP and LTD in local dendritic segments of Purkinje cells of motor skill-trained animals. Complex motor skill training induced strengthening of a subset of parallel fiber synapses onto Purkinje cells by forming multiple-synapse boutons (MSBs) contacting spine pairs arising from the same dendrite, whereas MSB-neighboring synapses were weakened by reducing the size of the postsynaptic density. Here, we discuss these orchestrated structural modifications of neighboring synapses that may sharpen synaptic weight contrast in local dendritic segments, leading to enhanced signal-to-noise ratio for optimal motor skill retention.

Keywords: dendritic spine; motor learning; parallel fiber; plasticity; Purkinje cell; synapse.

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Introduction: motor learning and synapse plasticity in the cerebellar cortex

Experience changes synaptic structure, number, and function by which neural information is thought to be stored in the mammalian central nervous system (Rosenzweig and Bennett, 1996; Yuste and Bonhoeffer, 2001; Holtmaat and Svoboda, 2009). For many years, neuroscientists have focused on deciphering the fundamental physiological

mechanisms of synaptic plasticity that underlie learning and memory, as well as understanding the pathological synaptic alterations that occur in memory-related disorders such as Alzheimer's disease. Hebbian, or associative, type of synaptic plasticity includes long-term potentiation (LTP) and long-term depression (LTD), which are the most popular and intensively studied cellular correlates of memory formation (Bliss and Lomo, 1973; Ito, 1986). Although numerous factors play contributory roles, LTP- and LTD-induced changes in postsynaptic receptor composition and morphology of synapses have been widely accepted as major mechanisms of activity-dependent synaptic plasticity (Wheal et al., 1998; Luscher et al., 2000; Bosch and Hayashi, 2012).

More recently, it has been shown that neurons also exploit multiple modes of compensatory homeostatic mechanisms to constrain neuronal output within an optimal working range (Turrigiano and Nelson, 2004; Pozo and Goda, 2010; Lee et al., 2011, 2013b; Queenan et al., 2012; Vitureira and Goda, 2013). However, it is still unclear how multiple synaptic plasticity models complement each other to fine-tune the structural and functional differences between neighboring synapses and encode neural information at the synaptic circuit level.

Motor skill learning is critical to maintain our daily life activities ranging from handwriting to riding a bicycle and playing online games. Complex motor skills are acquired in the long-term by repetition and, once acquired, are persistent without further reinforcement (Kleim et al., 1997). The cerebellum is a crucial area for motor skill learning (Thach et al., 1992; Thach, 1998; Ito, 2000). Although it takes up only 10% of the total brain volume of individuals, more than 3/4 of the brain's neurons reside in this area, forming complicated yet highly organized patterns of neuronal circuits (Llinas et al., 2004). Most excitatory synapses in the brain are formed between presynaptic axonal boutons and postsynaptic dendritic spines, small dendritic protrusions that exhibit activity-dependent dynamic structural and functional plasticity (Harris and Stevens, 1988; Luscher et al., 2000; Hering and Sheng, 2001; Yuste and Bonhoeffer, 2001). Purkinje cells, a major projection neuron in the cerebellar cortex, receive two main excitatory synaptic inputs. Climbing fibers arising from inferior olive mainly innervate primary dendrites of Purkinje neurons, whereas parallel fibers, bifurcated axons of cerebellar granule cells, make numerous synaptic

contacts with higher order dendritic branches of Purkinje cells (Figure 1). Decades ago, electrophysiologists found that repetitive and synchronous activation of both inputs onto Purkinje cells induces LTD at parallel fiber synapses (Ito, 1986; Linden and Connor, 1995). Since then, LTD has been considered a cellular substrate for error-driven motor learning in the cerebellar cortex, in which inappropriate signals delivered by parallel fibers are selectively weakened by temporal association with climbing fiber firing. A number of subsequent studies with cultured Purkinje neurons and genetically engineered animal models have supported the idea that LTD and its molecular signaling components are crucial for motor learning (Linden and Connor, 1991, 1993, 1995; Shibuki and Okada, 1991; Aiba et al., 1994; Linden, 1994; Boxall et al., 1996; Crepel, 1998; Levenes et al., 1998; Eto et al., 2002; Feil et al., 2003; Hansel et al., 2006), although there have been some conflicting or opposing findings (Shibuki et al., 1996; Welsh et al., 2005; Schonewille et al., 2011).

More recently, the LTD-centric model has become somewhat muddled by the observations that LTP is required for proper motor skill acquisition (Lev-Ram et al., 2002, 2003; Schonewille et al., 2010). Moreover, several lines of knock-in animal models with impaired

LTD exhibited normal learning behavior in some forms of motor skill tasks such as vestibulo-ocular reflex (VOR) adaptation, eyeblink conditioning, and locomotion learning (Schonewille et al., 2011). Thus, the relative contribution of individual plasticity models during different types of motor skill learning is still under active debate in the field. New evidence suggests that neighboring synapses must be finely regulated to enhance signal-to-noise ratio, allowing appropriate information storage in the brain. Here, we review motor learning-induced synaptic plasticity in the cerebellar cortex and explore the emerging concept that synergistic strengthening and weakening of neighboring synapses in a local dendritic segment is essential for optimal encoding of complex motor skills (Lee et al., 2013a). This line of investigation also led us to propose that coordinated remodeling between nearby synapses is important to homeostatically maintain overall activity levels within local dendritic segments relatively stable.

Multiple-synapse boutons: learning-induced local synaptic strengthening

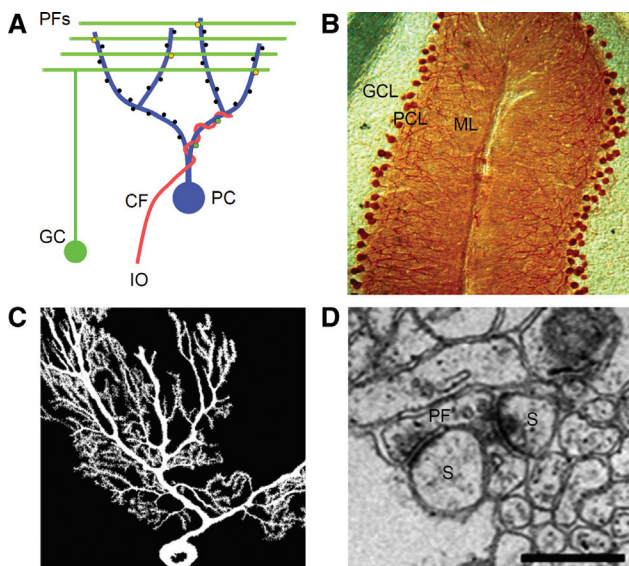


Figure 1 Synaptic connectivity of PCs in the cerebellar cortex. (A) Schematic diagram of excitatory synaptic inputs onto PCs. (B) Immunohistochemical image of rat cerebellar cortex using anti-Calbindin-28K antibody, a marker for Purkinje cells. (C) High-voltage electron microscopic image of a Golgi-impregnated Purkinje neuron. (D) Transmission electron microscopic image of an MSB. Scale, 0.5 μ m. PF, parallel fiber; GC, granule cell; CF, climbing fiber; PC, Purkinje cell; IO, inferior olive; GCL, granule cell layer; PCL, Purkinje cell layer; ML, molecular layer; S, dendritic spine.

A number of studies have consistently demonstrated that motor skill training induces synaptogenesis in the cerebellar cortex selectively between parallel fiber boutons and dendritic spines of Purkinje cells (Black et al., 1990; Anderson et al., 1996; Kleim et al., 1998; Federmeier et al., 2002; Kim et al., 2002). However, simple motor activities including running or locomotion failed to induce the formation of new synapses, indicating that learning, not merely the activity component of a motor skill task, is required for the synaptogenesis observed at parallel fiber-to-Purkinje cell synapses. In addition, astrocytic glial hypertrophy has been observed in the cerebellar cortex following complex motor skill training (Anderson et al., 1994; Kleim et al., 2007), likely involved in supporting the motor learning-induced synaptogenesis.

At the ultrastructural level, parallel fiber-to-Purkinje cell synapses can be categorized into three types by their distinct synaptic contact features (Kim et al., 2002; Lee et al., 2013a) (Figure 2). Single-synapse boutons (SSBs), contacting one postsynaptic dendritic spine, are most commonly observed, although multiple-synapse boutons (MSBs), contacting spine pairs of Purkinje cells, are also frequently detected. In contrast, multiple-synapse spines (MSSs), which make synaptic contacts with two parallel

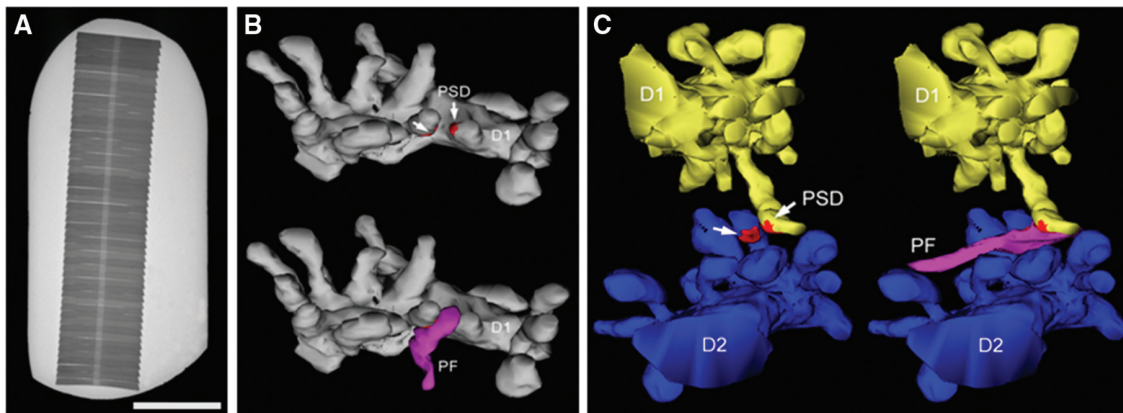


Figure 2 Serial section electron microscopy.

(A) Serial ultrathin sections mounted on the Formvar-coated one-hole slot grid in preparation for 3D reconstruction. Scale, 0.5 mm. (B, C) Three-dimensional reconstruction of MSBs. MSB contacting spine pairs originating from the same dendrite (B) and MSB contacting two spines arising from different neighboring dendrites (C). D, Dendrite. PF, parallel fiber.

fiber boutons, are much more rarely observed. Quantitative analysis of electron micrographs revealed that motor skill training significantly increased the number of MSBs by approximately twofold and SSBs by 34%–40% compared with both active (simple locomotor or running) and inactive (sedentary) control animals (Federmeier et al., 2002; Kim et al., 2002; Lee et al., 2013a). There were no differences observed in the number of MSSs among groups. These results clearly suggest that SSB and MSB formation may have critical functional roles in encoding motor skills.

Importantly, an increase in the number of MSBs has been found in multiple brain regions such as cerebellum, hippocampus, motor cortex, and visual cortex following different behavioral training paradigms (Jones et al., 1997, 1999; Geinisman et al., 2001; Federmeier et al., 2002; Kim et al., 2002; Lee et al., 2013a), estrogen treatment (Woolley et al., 1996; Yankova et al., 2001), and LTP-inducing stimulation (Toni et al., 1999; Fiala et al., 2002; Medvedev et al., 2012). These results suggest that MSB formation may be widely associated with various forms of learning and memory, although the molecular mechanisms of MSB formation and its functional role in information processing are not yet clearly understood.

One caveat of conventional electron microscopic studies is that we can glimpse only fragmented, two-dimensional profiles of MSBs because of the ultrathin section thickness (typically in the tens of nanometers). Depending on the dendritic origin of spine pairs on MSBs, we would predict two completely different functional roles of MSBs in neural information processing (Lee et al., 2013a): if two spines on MSBs arise adjacent to one another on the same parent dendrite, local synaptic strengthening would be expected from activation of both spines in

response to an action potential delivered to the contacting parallel fiber bouton. On the other hand, if spines on MSBs originate from different neighboring dendrites, then preexisting SSBs have presumably recruited an additional spine from a nearby dendrite during motor skill training, indicating extensive remodeling of neuronal circuits. The only approach to identify which case is true would be to three-dimensionally reconstruct synaptic connectivity using serial section electron microscopy (3D-EM) (Harris and Stevens, 1988; Xu-Friedman et al., 2001; Lee et al., 2013a).

In this context, we recently investigated this issue using 3D-EM reconstruction and demonstrated that acrobatic motor skill training selectively induced MSBs contacting spine pairs arising from the same dendrite, indicating enhancement of synaptic efficacy in local dendritic segments (Lee et al., 2013a). Most MSBs observed in the cerebellar cortex after motor skill learning contacted completely separate neighboring spines rather than branched-type spines sharing a common neck, consistent with a previous report focused on the hippocampal CA1 area (Fiala et al., 2002). These findings suggest that MSBs are formed by new spine outgrowth rather than splitting of preexisting spines. Same-dendrite MSBs are formed in both rat hippocampal slices and dentate gyrus of awake adult rats after LTP stimulation (Toni et al., 1999; Fiala et al., 2002; Medvedev et al., 2012). Because an action potential-driven, simultaneous activation of two adjacent spines on the same dendrite would produce a larger net response when compared with activation of single postsynaptic spines, we proposed that acrobatic motor skill training strengthens a subset of synapses between parallel fibers and Purkinje cells via LTP-generated same-dendrite

MSBs. Indeed, accumulating evidence suggests that LTP is associated with acquiring motor skills (Asanuma and Keller, 1991; Rioult-Pedotti et al., 2000; Kargo and Nitz, 2004; Schonewille et al., 2010; Carrillo et al., 2013).

Is MSB formation induced in other types of cerebellum-mediated learning such as eyeblink conditioning? To our knowledge, this question has not been addressed in the cerebellar cortex after delay eyeblink conditioning. However, trace eyeblink conditioning (requiring both cerebellum and hippocampus) has been found to increase the number of MSBs in the stratum radiatum of hippocampal subfield CA1 (Geinisman et al., 2001), although the dendritic origin of such MSBs has yet to be determined. This finding may suggest the contribution of MSBs to multiple forms of motor learning. It would be interesting to test whether other cerebellum-mediated paradigms including VOR adaptation and eyeblink conditioning can lead to formation of same-dendrite MSBs (Lee et al., 2013a).

In addition to the cerebellar cortex, acrobatic motor skill training also produces MSBs in the forelimb sensorimotor cortex (Jones et al., 1999), indicating the concurrent involvement of other parts of the brain in such learning. Acrobatic tasks, requiring coordination of both gross and fine motor skills, are distinguished from other cerebellum-mediated learning tasks involving refinement of very specific behaviors such as eyeblink conditioning and VOR adaptation (Federmeier et al., 2002). Moreover, acrobatic training engages potential non-motor learning factors such as familiarity and motivation (Lee et al., 2013a). These points hint that widespread circuit remodeling in multiple brain areas is recruited to encode complex motor skills.

Why does motor skill training induce same-dendrite MSBs with two separate spines to enhance synaptic efficacy, instead of simply expanding the size of single-contact synapses? One possible reason is that each synapse may have a physical limit of synaptic strengthening achievable via increasing the amount of their surface receptors and the size of the postsynaptic density (PSD). This view implies that expansion and shrinkage of individual synapses should be tightly constrained to a certain range to avoid a winner-take-all effect that can irreversibly destabilize neuronal circuits. Thus, when it is necessary to persistently enhance efficacy at a specific synapse beyond the level of a single synaptic contact, the formation of new adjacent synapses sharing a common presynaptic bouton presents an attractive solution. The formation of same-dendrite MSBs supports the requirement for LTP in motor skill retention (Lev-Ram et al., 2002, 2003; Schonewille et al., 2010) but is seemingly in conflict with the well-documented role for LTD in motor learning. To address this discrepancy, we examined potential alterations to

MSB-neighboring synapses in animals trained with a complex motor skill task.

Weakening of MSB-neighboring synapses

Recent computational, electrophysiological, and imaging work suggests that coordinated regulation of dendritic spines along a single dendritic segment might act as the basic functional unit of plasticity (Poirazi and Mel, 2001; Polsky et al., 2004; Gray et al., 2006; Losonczy and Magee, 2006; Harvey and Svoboda, 2007; Harvey et al., 2008; Losonczy et al., 2008; Makino and Malinow, 2011). In accordance with this idea, our recent 3D-EM study demonstrated that excitatory synapses near same-dendrite MSBs (i.e., on the same dendritic segment) were smaller compared with both active and inactive controls (Lee et al., 2013a). Calculation of PSD areas on individual synapses (residing $<5\ \mu\text{m}$ linear distance from same-dendrite MSBs) by measuring serial PSD profiles revealed a close correlation between the size of the PSD and the distance from same-dendrite MSBs following motor learning; specifically, the PSD areas of excitatory synapses located $<2\ \mu\text{m}$ from same-dendrite MSBs were significantly smaller compared with excitatory synapses located within $2\text{--}5\ \mu\text{m}$ from same-dendrite MSBs. Considering the proportional relationship between PSD size and both postsynaptic AMPA receptor density and presynaptic docked vesicle number at excitatory synapses (Hirai, 2001; Nusser et al., 1998; Schikorski and Stevens, 1999; Takumi et al., 1999), these results suggest a potential compensatory depression of MSB-neighbor synapses. Indeed, reduced PSD size of MSB-neighbor synapses is consistent with a previous observation that motor skill-trained animals displayed more thin-type spines on tertiary dendritic branchlets of Golgi-impregnated Purkinje cells (Lee et al., 2007). Although the precise mechanism underlying this putative weakening of MSB-nearby synapses has not been addressed, it is plausible that local dendritic redistribution of synaptic components to MSBs from neighboring synapses could be one candidate mechanism to explain such a phenomenon.

Together with synaptic strengthening via formation of same-dendrite MSBs, these findings led us to propose that concerted strengthening and weakening of adjacent synapses occur with motor learning, which may sharpen synaptic weight contrast for enhanced signal-to-noise ratio while maintaining stable overall activity levels within local dendritic segments.

Synergistic interplay between multiple types of synaptic plasticity

LTD at parallel fiber synapses onto Purkinje cells has long been associated with cerebellar motor learning. Indeed, numerous studies have shown that diverse mutant mice lacking LTD-related intracellular signaling molecules have impairment in cerebellar motor learning tasks such as eyeblink conditioning and VOR adaptation (Aiba et al., 1994; Boxall et al., 1996; Shibuki et al., 1996; Feil et al., 2003; Hansel et al., 2006). However, there have been arguments that LTD is not essential for cerebellar motor learning. For example, blockade of LTD in the brains of behaving animals using a pharmacological reagent failed to induce impairment in motor learning and coordination (Welsh et al., 2005), and mutant mice lacking glial fibrillary acidic protein required for LTD induction and maintenance exhibited no detectable deficits in motor coordination tasks (Shibuki et al., 1996). Furthermore, several mutant mice strains lacking LTD by virtue of blockade of AMPA receptor internalization showed normal motor learning capacity when tested by eyeblink conditioning, locomotion learning on the Erasmus ladder, and VOR adaptation (Schonewille et al., 2011). Finally, Purkinje cell-specific deletion of protein phosphatase 2B impaired both postsynaptic LTP and acquisition of motor learning without affecting LTD (Schonewille et al., 2010), suggesting that LTP in Purkinje cells is required for proper motor skill retention and LTD is not the sole mechanism of cerebellar motor learning. Thus, it seems evident that multiple types of synaptic plasticity cooperate to obtain complex motor skills in the cerebellar cortex (Gao et al., 2012).

Motor learning-induced formation of same-dendrite MSBs and decreased PSD size of MSB-neighbor synapses indicate that LTP and LTD synergistically act on neighboring synapses in local dendritic segments of Purkinje cells. In this case, both plasticity mechanisms complement each

other for optimal information processing under physiological conditions. In fact, motor learning-induced recruitment of parallel fiber LTD and LTP has been implicated previously (McCormick and Thompson, 1984; Ojakangas and Ebner, 1992; Hartell, 2002). In addition to computational benefits, we envision that synaptic weakening through LTD may serve as a local negative feedback mechanism preventing overstimulation of Purkinje cells in a homeostatic manner, as was first suggested almost two decades ago (De Schutter, 1995), although more experimental data are needed to validate the hypothesis.

It would be of interest to examine whether this interplay between opposing plasticity mechanisms operates to integrate activity within specific local dendritic branches or at the whole-cell level to regulate overall neuronal firing. In line with previous work on coordinated structural plasticity between excitatory and inhibitory synapses along hippocampal CA1 dendrites during LTP (Bourne and Harris, 2011), another major open question is how motor learning affects inhibitory synapses onto Purkinje cells. Although it was reported that motor skill training did not increase the number of synapses between parallel fibers and interneurons (Kleim et al., 1998), large-scale ultrastructural reconstruction of both excitatory and inhibitory synaptic circuits would be informative to definitively address this issue in the near future. Further studies are warranted in order to better understand the coordinated relationship between multiple types of synaptic plasticity and the structural basis for neural encoding of motor memory in the brain.

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