

SYNAPTIC PLASTICITY AT THE CEREBELLUM INPUT STAGE: MECHANISMS AND FUNCTIONAL IMPLICATIONS

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INTRODUCTION

Long-term synaptic plasticity is a well-established biological process consisting in persistent changes in synaptic strength, which follow specific activity patterns in synapses and neurons (Bliss and Collingridge, 1993; Hawkins *et al.*, 1993; Malenka and Nicoll, 1999). Long-term synaptic plasticity is considered the cellular basis for learning and memory (Bliss and Collingridge, 1993; Bliss, Collingridge and Morris, 2003) and typically takes the form of potentiation, LTP, or depression, LTD. In fact, LTD is probably a separated process from depotentiation, the reverse of LTP (Lisman, 2003). LTP and LTD have been reported at several central synapses in the neocortex, hippocampus, cerebellum and other brain structures. Despite an amazing complexity and variety of mechanisms, LTP and LTD respect some general features which are reviewed below. Then, I'll consider the case of LTP at the mossy fiber – granule cell synapse of cerebellum, which is opening interesting perspectives on the relationship between synaptic structure, function and plasticity. The analysis of this new form of plasticity, together with others recently discovered in the cerebellum (Hansel *et al.*, 2001), provides new cues to explain cerebellar information processing and sensori-motor control.

GENERAL PROPERTIES OF LONG-TERM SYNAPTIC PLASTICITY

The existence of long-term synaptic plasticity was predicted by the theoretical work of Donald Hebb in 1947. Then, LTP was first demonstrated in the rabbit hippocampus *in vivo* by Bliss and Lomo in 1973. A parallel story developed in the cerebellum, where LTD at the parallel fiber – Purkinje cell synapse was predicted by David Marr in 1969 and subsequently demonstrated by Ito in 1982. These forms of LTP and LTD represent to date the most intensely investigated paradigms of synaptic learning in the mammalian brain.

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LTP and LTD are called Hebbian when they arise from contingency of pre- and postsynaptic activity, anti-Hebbian in the opposite case, non-Hebbian if activity in either pre- or postsynaptic element is sufficient to induce plasticity. These definitions descend from the Hebb's (1949) postulate stating that "When an axon of cell A is near enough to excite cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A's efficiency, as one of the cells firing B, is increased".

After an *induction* phase, which is typically very brief (e.g. 1 sec at 100 Hz for LTP), the synaptic change lasts for a long time, possibly for the entire life of the animal. This persistent phase during which the plastic change is maintained is called *expression*. Typically, according to Hebb's rule, the pre- and postsynaptic neurons have to discharge together, intensely and repeatedly to have LTP. LTD arises in the opposite case of weak, asynchronous, sporadic activity in the two elements. In hippocampal neurons, which present a favorable input organization, experimental manipulation of the pre- and postsynaptic activity patterns allowed to identify three fundamental LTP properties called *cooperativity* (multiple fibers need to cooperate to cross a certain intensity threshold), *associativity* (different inputs can be associated so that a strong can help a weak input to develop LTP), and *specificity* (LTP develops only in inputs that were tetanized).

Learning rules

An important issue is the definition of patterns generating LTP and LTD. The basic learning rules descend from Hebb's postulate and relate LTP/LTD to postsynaptic activity, were it expressed as spike frequency, membrane depolarization, or intracellular Ca^{2+} concentration (this seems indeed the ultimate most important factor, see below). In general, strong postsynaptic activity determines LTP, weak activity LTD. The simple covariance rule (Stanton and Sejnowsky, 1989) is unbound, allowing for unlimited changes in both LTP and LTD directions. A more realistic rule is the BCM (Bienenstok, Cooper and Munro, 1982), which shows saturation of LTP at high postsynaptic activities, and extinction of LTD at low postsynaptic activity. A more recent rule, which is based on precise spike coincidence with postsynaptic depolarization, is called spike-timing-dependent plasticity or STDP (reviewed in Sjöström and Nelson, 2002). Clearly, covariance and BCM address the cases of rate-coding while STDP that of time-coding. It is not always clear which of the rules applies to specific synapses.

Synaptic mechanisms

The mechanisms of LTP and LTD are also debated. Research on mechanisms has produced an amazing amount of results, unveiling unexpected complexity in the metabolic chains involved in regulating synaptic transmission. Nonetheless, some general statements can be done.

Induction usually requires a Ca^{2+} increase in the post-synaptic terminal, although cases of presynaptic induction are known. The most common molecular coincidence detector is the NMDA receptor, which needs glutamate for activation and depolarization for channel unblock. In addition, forms of long-term synaptic plasticity based on Ca^{2+} -permeable AMPA receptors and VDCCs have been reported. Ca^{2+} ele-

vation and activation of other second messenger systems involved in LTP/LTD can occur through activation of mGlu receptors.

Downstream of Ca^{2+} , several Ca^{2+} -sensitive enzymes (like CaMK-II, PKA, PKC, NOS) start the expression phase. CaMK-II plays a central role at least in hippocampal LTP, and has been proposed as a key element for channel phosphorylation and insertion in the postsynaptic membrane (Lisman, 1993). NOS is supposed to be the key element for trans-synaptic expression mechanisms. A noticeable example is LTD at the parallel fiber – Purkinje cell synapse of cerebellum, where NO (the gaseous diffusible neurotransmitter produced by NOS) released from granule cells determines postsynaptic changes. In the hippocampus, NO released from postsynaptic neurons could explain presynaptic increase in neurotransmitter release (Arancio *et al.*, 1996), although the issue is debated.

Several expression mechanisms have been reported and proved to vary from synapse to synapse and even during different stages of LTP. Two main categories of expression mechanisms can be recognized, the pre- and postsynaptic. In the presynaptic hypothesis, vesicular release is increased. This could occur through the action of a retrograde messenger (e.g. NO, see below) either because the number of releasing sites or their probability of release is increased. It should be noted that a site with zero release probability is presynaptically silent. In the postsynaptic hypothesis, synaptic channels can be modulated by phosphorylation or inserted into the synapse. Insertion may occur in discrete quantal units uncovering a postsynaptically silent site. Clearly, the potential existence of pre- or postsynaptically silent sites confuses quantal analysis (e.g. see discussion in Lisman, 2003; Kullman *et al.*, 1996), as considered below.

After an initial phase of about 30-60 minutes, synaptic strength is determined by new protein synthesis and gene expression probably affecting both the pre- and postsynaptic terminals. An important related issue is that of tagging (reviewed in Martin and Kosik, 2002; Sajikumar and Frey, 2004), so that protein trafficking can be properly directed and proteins addressed to the synapses which need to express LTP or LTD.

Non-synaptic mechanisms

In their original discovery of LTP, Bliss and Lømo (1973) reported the existence of E-S potentiation, a process increasing EPSP-spike coupling without intervention of a synaptic modification. Recently, the existence of non-synaptic plasticity has been documented in an increasing number of neurons, which change their intrinsic excitability in response to specific stimulus patterns (Zhang and Linden, 2003; Daoudal and Debanne, 2004). This form of plasticity is intrinsically different from classical neuromodulation (e.g. by acetylcholine or noradrenaline) since it persists after the presentation of the stimulus and occurs at glutamatergic synapses. An important form of plasticity that could be confused with E-S potentiation is that occurring at inhibitory synapses. Since these eventually control GABA-A receptors and cell input resistance, they will eventually regulate neuronal intrinsic excitability.

From mechanisms to function

The concept of long-term synaptic modifications developed from theoretical considerations showing that neuronal networks need learning and plasticity in synapses in order to perform high-level computations and mimic cognitive functions (e.g. see Churchland and Sejnowsky, 1990). A basic question that remains to be answered is whether LTP is necessary to form memories or is memory itself (Bliss *et al.*, 2003). As far as computational issues are concerned, three additional aspects need to be addressed.

First, in complex networks, synaptic weights cannot show unlimited growth or decrease without causing a severe impairment of global network performance. So changes in one direction at some synapses must be balanced by changes in the opposite direction at others. Changes in intrinsic excitability could also provide a global compensation for generalized increases (or decrease) in cell synaptic excitation. This is the concept of homeostatic plasticity, evidence for which is increasing (Turrigiano and Nelson 2000, 2004).

Secondly, several forms of synaptic and non-synaptic plasticity can coexist at the same synaptic relay or in the same neuron. Moreover, in a network, plasticity is synapse- and neuron-specific. Thus, understanding plasticity means understanding how the different forms interact in space and time to optimize certain network parameters and eventually network computation and performance in controlling specific tasks. So, the variety and spatial distribution of synaptic plasticity needs to be understood.

Thirdly, the mechanisms of LTP and LTD expression are tightly bound to those of synaptic transmission. For example, if postsynaptic receptors are saturated, increasing their number would increase the response while increasing release would have no effect. However, wakening a silent site, either pre- or postsynaptically, would enhance the response irrespective of receptor saturation at individual PSDs. At multi-site synapses, increasing release probability would be unaffected by saturation at single sites, and the response should reflect the number of quanta released at each impulse. The complexity of these interactions requires that classical quantal analysis developed at the NMJ is revisited and extended. Importantly, if receptors are not saturated, spillover should be able to affect the response in proportion to average neurotransmitter release. Moreover, regulating neurotransmitter release or postsynaptic receptor properties (like desensitization or gating) is expected to have different effects on temporal dynamics during repetitive neurotransmission.

LTP AT THE CEREBELLAR MOSSY FIBER - GRANULE CELL RELAY

The cerebellum is a brain structure of primary importance for the coordination of movement, and is also probably involved in processing higher brain functions (Eccles *et al.*, 1967; Ito, 1984; Ghez and Thach, 2003). The most famous form of cerebellar plasticity is certainly pf-PC LTD (Ito *et al.*, 2001). Its history is emblematic: it was predicted by the famous Marr's theory (1969) and subsequently disco-

vered by Ito (Ito *et al.*, 1982). However, most influential cerebellar theories neglect the existence of plasticity at the mf-GrC relay. For instance, Marr (1969) explicitly negated the possibility that mf-GrC synaptic weights could be modified by activity. He noted that “sooner or later all weights would be saturated” so that plasticity would be inefficient. Thus, the Marr’s model did not include any mf-GrC synaptic plasticity, although the subsequent extension due to Albus (1971) was more permissive. However, the observation that mfs discharge consists of high-frequency bursts (Kase *et al.*, 1984; Chadderton *et al.*, 2004)) and that GrCs express NMDA receptors, which trigger the induction of LTP/LTD at other central synapses (see Bliss and Collingridge, 1993; Hawkins *et al.*, 1993; Malenka and Nicoll, 1999), led us to revisit the question. In fact, once theta-burst stimulation or prolonged (1 sec) high-frequency (100 Hz) stimuli were applied to mfs in cerebellar slices, GrC synaptic exci-

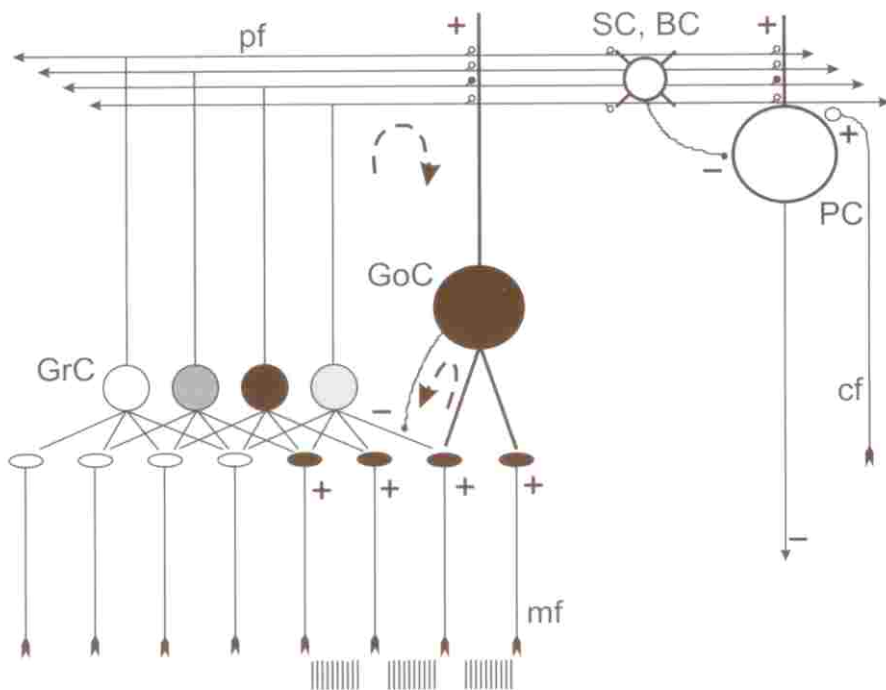


Fig. 1. - Schematic representation of the neuronal circuit of the cerebellar cortex: mf, mossy fiber; pf, parallel fiber; cf, climbing fiber; GrC, granule cell; GoC, Golgi cell; PC, Purkinje cell; SC, stellate cell; BC, basket cell.

The granular layer is primarily composed of GrCs and GoCs. Note divergence and convergence at the mf-GrC relay and the double feed-back and feed-forward inhibition of GrC through GoC (dashed arrows). SC, BC, PC are outside the granular layer. Inhibitory neurons are in black and arrows indicate the direction of information flow. Excitation of mossy fibers with bursts of action potential (e.g. a theta-burst stimulation, TBS, consisting of 100-Hz 100-ms bursts repeated every 250 ms) activates a certain set of granule cells, some of which are inhibited by Golgi cells. The cell depicted in red is strongly excited by 2 mfs, the one in yellow is weakly excited by 1 mf, the one in blue (although also receiving mf excitation) is inhibited by the Golgi cell. According to our investigations (Armano *et al.*, 2000) the cell in red will generate LTP and the one in yellow will generate potentiation of intrinsic excitability, while the one in blue could generate LTD.

tation was persistently strengthened indicating the occurrence of long-term potentiation (LTP; Fig. 1).

Mf-GrC LTP has been investigated using extracellular recordings (Maffei *et al.*, 2002, 2003), whole-cell recordings (D'Angelo *et al.*, 1999; Rossi *et al.*, 2002), and perforated-patch recordings (Armano *et al.*, 2000). Thus LTP can be induced without altering the cytoplasm and can be revealed both in neuronal ensembles and in single cells. In all these cases potentiation of synaptic currents is between 30% and 50% and affects both the AMPA and NMDA receptor-mediated component of the response. At present, several aspects of the induction and expression mechanism of mf-GrC LTP have been clarified. It should also be noted that other forms of plasticity have been discovered in the cerebellum, which is emerging as a highly adaptable network (Hansel *et al.*, 2001).

mf-GrC LTP induction

Repetitive high-frequency stimulation activates a cascade of events leading to mf-GrC LTP. AMPA receptor activation causes membrane depolarization and NMDA receptor unblock, which, in addition to reinforce depolarization, determines a remarkable cytoplasmic Ca^{2+} increase in the dendrites (Gall, Prestori, Sola, Rossi and D'Angelo, unpublished observation). The Ca^{2+} increase is reinforced by mGlu receptor activation, probably through a PLC-mediated pathway initiated by mGlu receptor stimulation and leading to DAG and IP_3 production (Monti *et al.*, 2002). These events are critical for LTP, since either preventing membrane depolarization, blocking NMDA or mGlu receptors, or buffering intracellular Ca^{2+} prevented LTP (Rossi *et al.*, 1996; D'Angelo *et al.*, 1999; Armano *et al.*, 2000). LTP was also reduced by intra- or extracellular blockage of PKC (D'Angelo *et al.*, 1999; Maffei *et al.*, 2002), a kinase requiring DAG and Ca^{2+} for activation. Mf-GrC LTP induction resembles the general scheme in which high frequency stimulation activates ionotropic and metabotropic glutamate receptors initiating a Ca^{2+} -dependent signaling cascade (Bliss and Collingridge, 1993). Mf-GrC LTP is therefore a classical form of NMDA receptor-dependent LTP with Hebbian properties.

Another process that is likely to occur during induction is NO production. The NO producing enzyme, nitric oxide synthase (NOS), is expressed in GrCs (Bredt *et al.*, 1990; Garthwaite *et al.*, 1988). During LTP induction, NO raises in the nanomolar range, reaching concentrations sufficient to fully activate soluble guanylyl cyclase, the major effector of the NO pathway (Maffei *et al.*, 2003). Thus, NO is a potential candidate for signal back-propagation from post- to presynaptic site (see below).

Mf-GrC LTP expression: synaptic conductance changes

The origin of synaptic conductance changes during long-term synaptic plasticity is the object of an open debate focused on hippocampal LTP (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). At the mf-GrC synapse, the fact that both the AMPA and NMDA currents are potentiated could indicate either a common presynaptic mechanism or a simultaneous postsynaptic change in both receptor types

(D'Angelo *et al.*, 1999; Fig. 3A). A presynaptic mechanism of expression during the first 30 minutes of LTP has been supported by a recent investigation (Sola *et al.*, 2004). The demonstration is based on several points. First, during LTP, EPSC coefficient of variation (CV), failures and paired-pulse ratio (PPR) decreased. Similar changes were observed by raising neurotransmitter release (high $\text{Ca}^{2+}/\text{Mg}^{2+}$), while the opposite occurred by decreasing release (low $\text{Ca}^{2+}/\text{Mg}^{2+}$, Cl-adenosine). No changes followed postsynaptic modifications (different holding potential), while only CV and failures decreased by raising the number of active synapses. LTP was occluded by raising release probability and was observed in the spillover-dependent component of AMPA EPSCs and in NMDA EPSCs. Finally, during LTP, minis did not change their amplitude or variability but increased their frequency. Binomial analysis explained EPSC changes through an increased release probability. It should be noted that these observations may not be sufficient due to the several complications depending on synaptic organization. However, we recall that neither AMPA nor NMDA receptors are saturated at this synapse allowing quantal analysis to be applied in its classical terms. Moreover, spillover increased both the AMPA and NMDA EPSC, as expected from increased release in the glomerulus. Finally, short-term plasticity changes during LTP are consistent with a presynaptic modification (Nieus, Sola, Mapelli, Saftenku, Rossi and D'Angelo, submitted). Thus, mf-GrC LTP makes a particularly well documented case of presynaptic LTP expression.

As well as in mf-GrC LTP, an increased neurotransmitter release was proposed to occur at other central synapses *in situ* (Malinow and Tsien, 1990; Schultz *et al.*, 1994; Kullmann *et al.*, 1996; Gasparini *et al.*, 2000) and in neuronal cell cultures (Bekkers and Stevens, 1990; Malgaroli *et al.*, 1995). Coupling between postsynaptic NMDA receptor-dependent induction and presynaptic expression may be provided by NO (Arancio *et al.*, 1996). Indeed, blocking NOS, scavenging NO, or blocking sGC prevented mf-GrC LTP, while NO donors induced it (Maffei *et al.*, 2003). The present observation does not exclude that, in different functional or developmental conditions, LTP expression might change. For instance, silent synapse awakening characterizes the developmental process of the cerebellar mf-GrC relay, leading from purely NMDA to mixed AMPA-NMDA EPSCs (see above: D'Angelo *et al.*, 1993; Losi *et al.*, 2002). Moreover, NMDA receptor stimulation leads to CREB activation in GrCs (Monti *et al.*, 2002), and may therefore prime postsynaptic gene expression and protein synthesis in later LTP phases.

Mf-GrC LTP expression: changes in GrC intrinsic excitability

In addition to showing an increased synaptic conductance, during LTP GrCs show an increased intrinsic excitability (Armano *et al.*, 2000; Fig. 3A). GrC E-S potentiation can be identified in the presence of blockers of inhibitory transmission and reflects therefore changes in intrinsic excitability. GrC E-S potentiation consists of an increased input resistance in the subthreshold region and of a spike threshold decrease. E-S potentiation is induced by NMDA receptor activation but is less sensitive to membrane depolarization than potentiation of synaptic conductance. Thus, a protracted weak stimulation may be able to enhance GrC excitability, and E-S potentiation would assume an homeostatic effect. Clearly, E-S potentiation is all

postsynaptic, although the channels involved remain speculative. A reduction in voltage-dependent K^+ currents and an increase in persistent Na^+ currents may be involved. Further forms of E-S potentiation related to Golgi cell synaptic inhibition are currently under investigation (Rossi, Roggeri, Gall, de Kerkov d'Exaerde, Shiffmann, Taglietti, and D'Angelo, *in preparation*).

Mf-GrC LTP expression: changes in mf intrinsic excitability

During mf-GrC LTP, modifications can be revealed in the presynaptic terminal current measured by using loose-patch recording techniques (Maffei *et al.*, 2002; Fig. 3B). During LTP, the presynaptic current increases with the same time course of LTP. The presynaptic current increase is prevented by the same factors preventing LTP, including block of NMDA receptors, mGlu receptors, or PKC, as well as by post-synaptic inhibition through Golgi cells. Moreover, the presynaptic current increase is prevented by NOS inhibitors, NO scavengers, and sGC inhibitors, and is mimicked by the NO donor DEA-NO (Maffei *et al.*, 2003). Thus, it seems that postsynaptic induction can cause presynaptic expression through NO production.

The role of presynaptic current changes is unknown at present. The Ca^{2+} -dependent component of the presynaptic terminal current increased during LTP, and LTP as well as the presynaptic current change could be occluded by application of the K^+ channel blocker TEA (Maffei *et al.*, 2002). Thus, presynaptic current changes may reflect regulation of excitation dynamics during repetitive stimulation and may be linked to the release process through a regulation of Ca^{2+} influx. It is currently unknown whether there is a mechanistic relationship between the increased neurotransmitter release revealed by EPSC quantal analysis and the presynaptic terminal current change. Moreover, it is unknown whether NO could determine LTP by raising presynaptic terminal excitability or by regulating the release machinery.

Mf-GrC LTP impairment in mutant mice

If LTP is of functional significance, then it should be possible to impair LTP and motor learning in mice bearing mutation in molecules linked to the LTP mechanism. To this aim we have used mice with a mutation in the NMDA receptor, in particular a C-terminal truncation of the NR2A and NR2C C-terminal chain (Rossi *et al.*, 2002). Due to the late expression of these subunits, the ontogenetic process is unaltered. Moreover, NR2A and NR2C show a selective co-expression in the cerebellar GrCs. Thus, NR2A- $C^{\Delta C/\Delta C}$ mice provide a rather target- and time-specific mutation. Interestingly, mutated NMDA channels show a reduced open probability but their density in the plasma membrane appears normal. In NR2A- $C^{\Delta C/\Delta C}$ mice LTP is impaired, and mice show impaired motor learning in the thin-rod test (Sprengel *et al.*, 1998; Georg Kohr, unpublished observation). It seems therefore that mf-GrC LTP plays a role in motor coordination and learning. Investigation of other mutants is currently being performed in order to extend this observation.

Functional consequences of mf-GrC LTP

The cerebellum is thought to operate in feed-forward mode anticipating the corrections needed to regulate complex sequences of movements (Ghez and Thach, 2003).

As every feed-forward device, the cerebellum needs to store information to be used in a predictive manner. No surprise therefore that the cerebellar circuitry expresses mechanisms for learning and memory. As proposed by Marr (1969), a major form of plasticity occurs at the pf-PC synapse, allowing heterosynaptic depression when a motor error is detected (pf-PC LTD). Instruction or error signals are conveyed by cfs, so that pf-PC synapses relaying relevant sensori-motor signals are persistently depressed. The experimental observation of pf-PC LTD has obviously a huge impact on present understanding of cerebellar functions (reviewed by Ito, 2001). Nonetheless, the discovery of mf-GrC LTP, together with various forms of plasticity at other cerebellar synapses, suggests that the classical concept of cerebellar learning needs to be extended (Hansel *et al.*, 2001; see also Llinas *et al.*, 1997; DeSchutter 1997).

By being induced by homosynaptic activity, mf-GrC LTP implements a modality of unsupervised learning. By being dependent on high-frequency mf activity and postsynaptic depolarization, LTP is associative, implementing a process of coincidence detection. The functional consequences of mf-GrC LTP depend on several factors including the molecular and cellular mechanisms involved, the spatial distribution of plasticity, local network activity (primarily related to endogenous rhythms and synaptic inhibition), and long-range modulation (primarily related to cholinergic, serotonergic and noradrenergic innervation of the cerebellum). Although understanding of these mechanisms is far from being complete, some hypothesis can be advanced.

- 1) The enhanced release probability during LTP is associated with complex changes in short-term neurotransmission dynamics. During LTP, short-term synaptic depression is enhanced and the spillover current is increased. This remarkably anticipates the first-spike latency and raises the frequency of output bursts (Nieus, Sola, Mapelli, Rossi and D'Angelo, *in preparation*). Thus, mf-GrC LTP may play an important role in regulating temporal recoding in the cerebellum (DeSchutter and Bjjale, 2001) providing the substrate for adaptable delay lines envisioned by theoretical models of the cerebellum (Braitenberg, 1967; Medina and Mauk, 2000).
- 2) Since EPSC variability decreased (Sola *et al.*, 2004), LTP should improve the reliability of neurotransmission. The effect could be an increase in mutual information transfer (MI), a subject currently under investigation (Bezzi, Nieus, Coenen, and D'angelo, unpublished observations). Optimization of MI is indeed at the core of two recent theories on granular layer function. In the first (Schweighofer *et al.*, 2000), LTP enhances MI while the postsynaptic increase in intrinsic excitability (Armano *et al.*, 2000) is suited for determining the number of active GrCs minimizing redundancy and optimizing sparse representation of mf activity. In the second (Philippona and Coenen, 2003), LTP follows to an unsupervised learning rule intrinsic in the glomerular structure requiring a global feedback signal that could correspond to NO.
- 3) A critical step in understanding LTP functional implications is that of defining the *learning rules* (Sjöström and Nelson, 2002). What we know is that a single 100-

ms train is not usually sufficient for inducing LTP, while the train has to be repeated at least 4 times to be effective. LTD can be observed when the induction is weakened by pharmacological manipulations (such as intracellular Ca^{2+} buffering, NMDA receptor blockage, synaptic inhibition; D'Angelo *et al.*, 1999; Armano *et al.*, 2000; Maffei *et al.*, 2002, 2003), and when mossy fiber bursts are short (Gall *et al.*, 2005).

- 4) NO diffusion may determine spatial spread of LTP. By diffusing transcellularly, NO released from neighboring GrCs may summate exerting a collective control on the mf terminal. In turn, membrane depolarization needed to unblock NMDA receptors and release NO should follow synchronous discharge in several mfs (see Armano *et al.*, 2000). Thus, the NO signal may be generated depending on the effective number and location of active GrCs, influencing temporo-spatial processing of mf discharge and sensori-motor control by the cerebellum.
- 5) Finally, it should be noted that LTP would enhance coincidence detection of mf impulses thereby reducing the codon size (i.e. the number of mf synapses that need to be simultaneously active to fire a GrC: Marr, 1969). So, LTP would impair spatial pattern separation.

SUMMARY

The mf-GrC relay provides the case of a synapse at which elementary neurotransmission mechanisms are particularly well understood allowing a precise investigation of synaptic plasticity. An interesting consequence is that a presynaptic mechanism of LTP could be precisely documented on the basis of quantal analysis. By being presynaptically expressed, LTP becomes instrumental to regulation of short-term synaptic dynamics thereby controlling time-dependent transformations of the incoming mossy fiber input. It is unknown to what extent these considerations could be generalized, but early observations were provided for comparable concepts and mechanisms in neocortical synapses (Tsodyks and Markram, 1997). Although several aspects remain to be investigated, mf-GrC LTP provides a wide substrate for information storage in the cerebellum. In the rat cerebellum, there are 10^{11} GrCs and 4 times as many mf-GrC synapses. Mathematical models have suggested that mf-GrC LTP improves mutual information transfer, and that the combination of synaptic and non-synaptic changes improves sparse representation of the mf input (Schweighofer *et al.*, 2000; Philipona *et al.*, 2003). Moreover, mf-GrC LTP could play a key role in regulating neurotransmission dynamics, implementing adaptability in delay lines early envisioned by Breitenberg (1967) and then revisited by Medina and Mauk (2002). These observations challenge the simple view of spatial pattern separation proposed by Marr (1969). The potential consequences of mf-GrC LTP need to be further investigated and confronted with computational models of the cerebellar network.

APPENDIX

Synaptic transmission at the mf-GrC relay

Mossy fibers (mfs) form the largest cerebellar afferent system and originate from various regions in the spinal cord, brain-stem, and cerebral cortex. Mfs activate GrCs and Golgi cells (GoCs), the main inhibitory interneurons of the granular layer. Golgi cells are also excited by parallel fibers (pf), the GrC axons. Thus, GoCs inhibit GrCs through a double feed-forward and feed-back loop. The excitatory nature of the mf-GrC synapse and its inhibitory control by Golgi cells were early recognized (Eccles *et al.*, 1967). Mfs diverge onto numerous GrCs (about 28 in the rat), which in turn receive just 4 different mf inputs on as many independent dendrites. GrCs dendrites terminate with 3-4 bulbs endowed with postsynaptic densities aligned with releasing sites in the mf terminal (Hamory and Somogy, 1983; Jakab and Hamory, 1988). Miniature synaptic currents are unquantal (Chatala *et al.*, 2003) and quantal analysis indicates that EPSCs are determined by release of 1 to 3 quanta (Sola *et al.*, 2004), as expected from ultrastructural investigation. The probability of release estimated with binomial models ranges from 0.2 to 0.7. Mf are glutamatergic and activate AMPA, NMDA, and mGlu receptors in GrCs. AMPA receptors are located in clusters facing the releasing sites (DiGregorio *et al.*, 2003), while NMDA receptors are in part synaptic and in part extrasynaptic (Rossi *et al.*, 2002; Petralia *et al.*, 2002; Chatala *et al.*, 2003). Glutamate spillover in the glomerulus can activate both AMPA receptors located at different postsynaptic sites and NMDA receptors. Non-stationary fluctuation analysis (Silver *et al.*, 1996) and analysis of the spillover current (DiGregorio *et al.*, 2003; Sola *et al.*, 2004) indicate that neither AMPA nor NMDA receptors are saturated by synaptically released glutamate. Spillover is also thought to generate a remarkable AMPA receptor desensitization contributing to shape EPSC dynamics during repetitive neurotransmission (Xu-Friedmann and Regher, 2003). EPSPs generated by a single mf are usually not sufficient to activate an action potential from rest, and the synchronous activation of 2-3 synapses is needed (D'Angelo *et al.*, 1995). During repetitive stimulation, the ongoing depression in AMPA receptor-mediated responses is compensated by a large increase in spillover currents (AMPA and NMDA) enhancing temporal summation. Glutamate also activates class-I mGlu receptors coupled to the PIP₂ pathway. Both NMDA and mGlu receptors play an important role in regulating GrC intracellular Ca²⁺ increase (Gall *et al.*, 2004; Gall, Prestori, Sola, Rossi and D'Angelo, unpublished observation).

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