The organization of plasticity in the cerebellar cortex: from synapses to control

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Acknowledgments. We thank Leda Roggeri for technical assistance. This work was supported by European Union grants to ED [CEREBNET FP7-ITN238686, REALNET FP7-ICT270434, Human Brain Project (HBP-604102)].

Running Title: plasticity in the cerebellum
Key words: long-term synaptic plasticity, cerebellum, motor control

Abstract

The cerebellum is thought to play a critical role in procedural learning but the relationship between this function and the underlying cellular and synaptic mechanisms remains largely speculative. At present, at least 9 forms of long-term synaptic and non-synaptic plasticity (some of which bidirectional) have been reported in the cerebellar cortex and deep cerebellar nuclei. These include LTP and LTD at the mossy fiber - granule cell synapse, at the synapses formed by parallel fibers, climbing fibers and molecular layer interneurons on Purkinje cells, and at the synapses formed by mossy fibers and Purkinje cells on deep-cerebellar nuclear cells, as well as LTP of intrinsic excitability in granule cells, Purkinje cells and deep cerebellar nuclear cells. It is suggested that the complex properties of cerebellar learning would emerge from the distribution of plasticity in the network and from its dynamic remodeling during the different phases of learning. Intrinsic and extrinsic factors may hold the key to explain how the different forms of plasticity cooperate to select specific transmission channels and to regulate the signal-to-noise ratio through the cerebellar cortex. These factors include regulation of neuronal excitation by local inhibitory networks, engagement of specific molecular mechanisms by spike bursts and theta-frequency oscillations, and gating by external neuromodulators. Therefore, a new and more complex view of cerebellar plasticity is emerging with respect to that predicted by the original "Motor Learning Theory", opening issues that will require experimental and computational testing.

Introduction

Although the cerebellum is known to play a fundamental role in procedural learning, the underlying cellular and circuit mechanisms are still incompletely understood. The "Motor Learning Theory" proposed by Marr and Albus (Marr, 1969; Albus, 1971) predicted that learning had to occur at the parallel fiber - Purkinje cell synapse under climbing fiber control. The simplicity and elegance of
This prediction has attracted generations of scientists, who have tried to substantiate or invalidate the hypothesis. A fundamental breakthrough has been the discovery of parallel fiber-Purkinje cell LTD (Ito & Kano, 1982). The importance of this discovery followed that of LTP in the hippocampus (Bliss & Lomo, 1973), so that the two major forms of learning (declarative and non-declarative) had both their own form of plasticity. Conceptually, it was also relevant that synaptic transmission could be persistently changed in opposite directions, actually generating LTP and LTD at different synapses. However, the discovery of cerebellar LTD did not fully answer previous questions about cerebellar functioning but generated new questions instead. The first issue, very general indeed, is whether the cerebellum is a learning or a timing machine (Eccles et al., 1967; Eccles, 1973; Ivry et al., 2002; D'Angelo & De Zeeuw, 2009). The second issue is whether the cerebellum is the place where learning and memory take place or rather it is instrumental to cause learning in different brain regions (Diedrichsen et al., 2010; Shadmehr & Mussa-Ivaldi, 2012; Raymond et al., 1996). The third issue is whether parallel fiber-Purkinje cell LTD is needed and sufficient for cerebellar learning (Hansel et al., 2001b; Gao et al., 2012).

In order to answer these questions, various experimental approaches have been used and a constellation of evidences has been reported. Relevant results have been obtained performing accurate experimental investigations at the molecular, cellular and microcircuit level in experimental animals as well as using neuroimaging and neurostimulation in humans in vivo. Recently, the field has benefitted of various mathematical models, which helped assessing the consistency of functional hypotheses on the role of plasticity in cerebellar learning. In this review I will focus on how recent evidences help addressing the core of the issue: how does plasticity in the cerebellar circuit contribute to procedural learning and memory?

As noticed above, cerebellar long-term synaptic plasticity was initially thought to occur only at the parallel fiber-Purkinje cell synapse, but now synaptic plasticity is known to be distributed and to occur at several sites in the granular layer, molecular layer and deep-cerebellar nuclei (DCN) (Hansel et al., 2001b; Gao et al., 2012; Raman and Bean, 1999). The cerebellar circuit long-term modifications affect both excitatory and inhibitory synapses and involve regulation of both synaptic and non-synaptic mechanisms. Here, the salient properties of plasticity at different network locations are reviewed and discussed in terms of their potential contribution to learning and memory in the cerebellum.

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**FIG. 1**

**Plasticity in the granular layer**

Among all forms of cerebellar plasticity, that occurring in the granular layer is the hardest to understand since it has not been considered in previous theories. Actually, mossy fiber-granule cell plasticity was originally denied by stating that, since sooner or later it would saturate, it should not exist at all (Marr, 1969). Clearly, the concepts of LTP/LTD balance and of regulation of the inhibitory circuit were missing (see below). After its discovery, granular layer plasticity has been deeply investigated and has opened a view on how signal entering the cerebellum are processed and retransmitted.
**Mossy fiber - granule cell LTP and LTD.**

Long-term synaptic plasticity in the granular layer occurs between mossy fibers and granule cells and consists of a bidirectional change involving LTP and LTD (D'Angelo et al., 1999; Sola et al., 2004; Gall et al., 2005; D'Errico et al., 2009) as well as changes in granule cell intrinsic excitability (Armano et al., 2000; Nieus et al., 2006b). The induction depends on NMDA receptors and the subsequent calcium influx, which can be reinforced by activation of voltage-dependent calcium channels (VDCCs), metabotropic glutamate receptors (mGluRs) and calcium-induced calcium release (CICR) from intracellular stores: short low-frequency bursts and poor membrane depolarization favor LTD, while long high-frequency bursts and strong membrane depolarization favor LTP (Gall et al., 2005; D'Errico et al., 2009). The level of depolarization, in turn, depends on the excitatory/inhibitory balance and therefore on the number of active mossy fibers and on the intensity of Golgi cell inhibition (Mapelli & D'Angelo, 2007). The sensitivity to these factors, and therefore the effectiveness of induction, depends on neuromodulators. In particular, nicotine acting on alfa6 receptors can bias induction in favor of LTP by shifting the calcium/plasticity relationship, thereby causing LTP with short bursts otherwise ineffective or even causing LTD (Prestori et al., 2013). Therefore, the LTP/LTD balance depends on both the input pattern and neuromodulators (extrinsic factors) as well as on local inhibitory network activity (intrinsic factors).

Both LTP and LTD expression depend on changes in release probability, which increases with LTP and decreases with LTD. The communication from the postsynaptic induction site and the presynaptic expression site is not completely clarified but requires nitric oxide (NO) to operate (Maffei et al., 2002; Maffei et al., 2003).

Finally, LTP is accompanied by a change in intrinsic electroresponsiveness, which enhances granule cell firing (Armano et al., 2000). This change consists in a reduction of spike threshold possibly related to a change in the persistent Na current, and to changes in K currents (Nieus et al., 2006b).

**Plasticity in the granular layer inhibitory circuit.**

Given the strong dependence on GABAergic inhibition, the induction of LTP and LTD is expected to reflect activity in the inhibitory loop, which could in turn be regulated and be subject to plasticity. There is evidence that parallel fiber - Purkinje cell synapse can undergo LTD (Robberechts et al., 2010) and that membrane hyperpolarization can persistently change Golgi cell pacemaking (Hull et al., 2013). Moreover, several mechanisms may regulate inhibitory transmission, involving the tonic GABA level, metabotropic receptor systems, and various ionic channels in the pre-and post-synaptic elements of the granule cell - Golgi cell loops (Rossi et al., 2006; Mapelli et al., 2009; Brandalise et al., 2012). Although plasticity in the inhibitory Golgi cell loop has still to be investigated in detail, it may provide a powerful regulatory mechanism for mossy fiber-granule cell plasticity (Garrido et al., 2013) (Mapelli et al., submitted).

**Plasticity in the granular layer in vivo.**

A remarkable advancement in the understanding of granular layer plasticity has come from the demonstration that granular layer LTP and LTD occur in vivo (Roggeri et al., 2008). LTP and LTD
can be induced by patterned tactile stimulation and affect both the sensory component and the cerebro-
cortical component of granular layer response waves (Diwakar et al., 2011). Moreover, LTP in vivo can
be markedly enhanced by activation of nicotinic acetylcholine receptors (Prestori et al., 2013),
providing evidence for gating mechanisms relating plasticity to the behavioral state of attention and
learning (Schweighofer et al., 2001; Schweighofer et al., 2004).

The consequences of granular layer plasticity: geometry, timing and coding.

In situ experiments have revealed three remarkable properties of granular layer plasticity, which
is translated into timing, geometry and coding of output burst to be conveyed to Purkinje cells for
further elaboration.

LTP and LTD, thanks to their presynaptic expression mechanism, have a specific impact on the
short-term dynamics of facilitation and depression occurring during transmission of mossy fiber bursts
to granule cells (Nieus et al., 2006b). Actually, LTP accelerates release anticipating emission of the
first spike, while LTD does the opposite. Granule cell firing rate is controlled by plasticity of granule
cell intrinsic excitability. This effect has two remarkable consequences: to implement the time-window
control by regulating collision of the first spike with inhibition in the feed-forward loop (D'Angelo &
De Zeeuw, 2009b) and to regulate information transmission (see below).

LTP and LTD, by modifying the quantal properties of neurotransmission, can change the
input/output relationship of the granule cell. The impact of release probability has been quantified
using mutual information (MI) analysis showing that LTP is associated with an increase and LTD with
a decrease in MI. About half of MI is regulated by changes in release probability, the rest by changes in
intrinsic excitability (Arleo et al., 2010a).

LTP and LTD tend to be organized in center-surround exploiting the geometrical arrangement
of Golgi cell axons and dendrites with respect to mossy fibers and granule cells (Mapelli & D'Angelo,
2007). The reason of this is that the center is more excited making granule cells more depolarized, so
that the increased activation of NMDA receptors promotes LTP, while the surround is less excited and
the lower calcium influx through NMDA receptors causes LTD. Eventually, the center reinforces its
ability to convey spikes at higher frequency and with shorter delays than the surround, implementing
time-windowing and MI transfer in spatially organized manner (Solinas et al., 2010; D'Angelo et al.,
2013a).

Theoretical implications.

Mossy fiber - granule cell LTP and LTD are unsupervised and may serve to improve spatio-
temporal recoding of mossy fiber input patterns into new granule cell discharges (D'Angelo & De
Zeeuw, 2009). In the original Motor Learning Theory (Marr, 1969) and in the Adaptive Filter Model
theory (Dean & Porrill, 2010; Dean et al., 2010; Dean & Porrill, 2011), this operation has been
conceived as an "expansion recoding" subdividing the input bursts conveyed by mossy fibers into
multiple time-dependent spike sequences in granule cells. Given the properties explained above, it is
expected that recoding will in fact redesign the geometry, timing and information transfer through the
granular layer.

A first attempt at modeling the impact of granular layer plasticity was carried out using a firing
rate model in which synaptic weights were controlled by optimizing information transfer through the
granular layer (Schweighofer et al., 2001). The main observation was that the strength of mossy fiber synapses had to be counterbalanced by that of Golgi cell synapses and complemented by changes in intrinsic excitability. Moreover, mossy fiber - granule cells plasticity needed to be gated circumventing the absence of teaching lines in this part of the circuit. This model actually anticipated the existence of changes in intrinsic excitability (Armano et al., 2000) and gating (Prestori et al., 2013), which have been subsequently demonstrated, and predicted the role of inhibitory plasticity between Golgi cells and granule cells.

Recently, computational models have been used to simulate the impact of multiple distributed synaptic weights in the cerebellar granular layer network (Solinas et al., 2010; Garrido et al., 2013). In response to mossy fiber bursts, synaptic weights at multiple connections played a crucial role to regulate spike number and positioning in granule cells. The weight at mossy fiber to granule cell synapses regulated the delay of the first spike and the weight at mossy fiber and parallel fiber to Golgi cell synapses regulated the duration of the time-window during which the first-spike could be emitted. Moreover, the weights of synapses controlling Golgi cell activation regulated the intensity of granule cell inhibition and therefore the number of spikes that could be emitted. First-spike timing was regulated with millisecond precision and the number of emitted spikes ranged from 0 to 3. Interestingly, different combinations of synaptic weights optimized either first-spike timing precision or spike number, efficiently controlling transmission and filtering properties. These results predicted that distributed synaptic plasticity could regulate the emission of quasi-digital spike patterns on the millisecond time scale and allow the cerebellar granular layer to flexibly control burst transmission along the mossy fiber pathway.

**Plasticity in the molecular layer**

Long-term synaptic plasticity in the molecular layer has been predicted by the Motor Learning Theory based on the consideration that learning had to be supervised in order to be efficiently related to motor errors. Moreover, learning had to occur over the largest available set of information lines capable of carrying contextual information. Thus, the solution suggested by anatomy was to locate learning at the parallel fiber - Purkinje cell synapses (carrying contextual information) under supervision of climbing fibers (carrying teaching signals). The sign of the change was hypothesized to conform to either LTD (Marr, 1969) or LTP (Albus, 1971a). This logical solution has dominated the field of cerebellar plasticity suggesting that parallel fiber - Purkinje cell had to be the leading candidate to explain cerebellar learning (Ito, 1984). However, several additional aspects have emerged and the overall picture of cerebellar plasticity is now substantially changed (Gao et al., 2012).

The Purkinje cells and their afferent synapses provide a wide set of plastic mechanisms and different studies have supported the existence of multiple forms of plasticity, which can be summarized in five main groups differing for mechanisms, induction patterns and functional implications (Hansel et al., 2001a; Ito, 2001; Boyden et al., 2004; Coesmans et al., 2004):

1) postsynaptic parallel fiber LTP and LTD.
2) presynaptic parallel fiber LTP and LTD.
3) climbing fiber LTD
4) plasticity of Purkinje cell intrinsic excitability
5) plasticity at molecular layer inhibitory synapses

Mechanisms of postsynaptic parallel fiber LTP and LTD

Parallel fiber-Purkinje cell LTD induction requires a complex signal transduction pathway. parallel fiber stimulation causes glutamate release, which acts on two types of receptors: AMPA receptors and metabotropic receptors (mGluRs). The mGluRs bind the G-protein-GDP complex initiating a local signal transduction pathway activating phospholipase C (PLC) and the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into the diffusible second messenger molecules inositol 1,4,5-trisphosphate (IP3) and dyacylglycerol (DAG). The climbing fiber contribution to LTD induction consists of large, widespread calcium transients evoked by complex spikes (Konnerth et al., 1992). The release of glutamate by climbing fiber terminals activates AMPA receptors causing a strong Purkinje cell depolarization, with consequent Ca\(^{2+}\) increase caused by VDCCs and CICR. Moreover, climbing fibers also activate NMDA receptors further enhancing calcium influx (Piochon et al., 2010). The simultaneous increase of Ca\(^{2+}\) and DAG activates PKC, which acts as a coincidence detector of climbing fiber and parallel fiber activity. PKC phosphorylates the GluR2 subunit of AMPA receptors at the active parallel fiber synapses causing their desensitization and internalization through clathrin-mediated endocytosis, thus causing LTD parallel fiber -Purkinje cell contacts (Wang & Linden, 2000; Xia et al., 2000).

The effect of climbing fiber pairing can be substituted by postsynaptic depolarization suggesting that depolarization normally attributed to climbing fibers could be replaced by simultaneous activation of a few tens of parallel fibers. Indeed, simultaneous activation of several parallel fibers generates a postsynaptic calcium transient confined into the spines, whose amplitude increases with the number of active parallel fibers reaching levels similar to those produced in the same region by climbing fiber activation (Midtgaard et al., 1993; Denk et al., 1995). As result, 1-Hz parallel fiber stimulation at relatively high stimulus strength (either single pulses or brief 10-50 Hz bursts) produces a gradual summation of the calcium signal leading to LTD (Hartell 1996). In addition LTD can be induced by intense parallel fiber stimulation. Therefore, climbing fiber pairing may not be an essential requisite but rather a facilitatory and synchronizing factor (see below).

NO proved necessary for the induction of postsynaptic parallel fiber- Purkinje cell LTD evoked by brief parallel fiber bursts (Crepel & Jaillard, 1990; Shibuki & Okada, 1991; Daniel et al., 1993; Huang et al., 1993; Lev-Ram et al., 1997). In Purkinje cells, the activation of an NO-dependent form of guanylate cyclase (GC) triggers the cGMP/PKG pathway, whose effect is to prevent the dephosphorilation of AMPA receptors by blocking the PP2/PP1/PP2B cascade and therefore unblocking PKC. The NO synthase (NOS) required to produce NO is most likely located in the parallel fibers (Kimura et al., 1998; Southam et al., 1992). It has been proposed that NO production arises from the activation of NR2A-containing NMDARs located on parallel fibers (Casado et al., 2002; Shin & Linden 2005; Bidoret et al., 2009). The specific deactivation kinetics of these NMDAR could determine the high frequencies of activity required for NO-dependent LTD induction. Activation of NMDA receptors in molecular layer interneurons could be another source of NO (Carter & Regehr, 2000).
Postsynaptic parallel fiber-Purkinje cell LTD evoked by brief parallel fiber bursts is heterosynaptic and can spread tens of microns from the induction site (Hartell, 1996). This spread could involve NO (Reynolds & Hartell, 2000; Wang et al., 2000) and arachidonic acid (Reynolds & Hartell, 2001), both acting as potent activators of GC.

A form of parallel fiber-LTP can be induced by a single pulse of parallel fiber stimulation at 1 Hz for 5 minute (Lev-Ram et al., 2002; Coesmans et al., 2004; Belmeguenai & Hansel, 2005). Its induction is postsynaptic, involving the activation of PKA, PKC and - and -CaMKII (van Woerder et al., 2009) and the insertion of GluR2 subunit of AMPA receptors in the spine membrane (Kakegawa & Yuzaki, 2005). This molecular mechanism can be considered opposite to the AMPA receptors internalization that leads to parallel fiber-LTD, and indeed it has been shown that these forms of LTP and LTD are mutually reversible (Coesmans et al., 2004; Lev-Ram et al., 2003). The postsynaptic Ca2+ transient plays an important role in determining the direction of plasticity at parallel fiber-Purkinje cell synapse. Interestingly, the bidirectional plasticity at this synapse is the mirror image of the one previously unraveled in the hippocampus (Jornteil & Hansel, 2006). The model of different Ca2+ thresholds for bidirectional plasticity was proposed in 1982 (Bienenstock et al., 1982) and termed “BCM rule”. According to the BCM rule, lower and higher Ca2+ transients are associated with the induction of LTD and LTP respectively. In parallel fiber-Purkinje cell synapse there is a opposite scenario and lower and higher Ca2+ transients are associated with the induction of LTP and LTD respectively.

Therefore, classical parallel fiber-Purkinje cell LTD may be just a special case of a general mechanism of bidirectional parallel fiber-Purkinje cell plasticity expressed by regulation of AMPA receptor desensitization and membrane expression. These LTP and LTD are driven by two forces, the amount of intracellular calcium and the amount of NO. High calcium can be obtained by high-frequency coherent activation of parallel fibers and by activating the climbing fiber. High NO can be obtained by activation of parallel fiber presynaptic terminals and molecular layer interneurons. Various combinations of these factors would explain the variety of induction conditions for LTD and LTP.

Mechanisms of presynaptic parallel fiber LTP/LTD

In the last decade, it has become apparent that a climbing fiber-independent form of LTP with presynaptic expression can also occur. This form of LTP can be observed after low-frequency (2-8 Hz) parallel fiber stimulation (Sakurai et al., 1987; Hirano, 1991; Crepel et al., 1990; Shibuki & Okada, 1992). This LTP at the parallel fiber synapse is triggered by presynaptic calcium influx, which activates Ca-sensitive adenylyl cyclase (AC1), which rises cAMP and activates PKA increasing neurotransmitter release (Salin et al., 1996; Storm et al., 1998; Kimura et al., 1998; Linden & Ahn, 1999). PKA acts at the presynaptic site by phosphorylating vesicle-release related proteins, in particular the active zone protein Rac1 and vesicular proteins (Castillo et al., 1997; Powell et al., 2004). This LTP, like the one with postsynaptic expression, may also depend on NO production (Hartell, 2002; Qiu & Knopfel, 2007). NO regulates the probability of glutamate release at activated synapses and through transcellular diffusion at heterosynaptic synapses. This mechanism occurs via cGMP and PKG-dependent pathways. In addition endocannabinoids can regulate presynaptic LTP (Le Guen & De Zeeuw). Endocannabinoid release is evoked by high-frequency bursts, depends on the activation of postsynaptic mGluR1 and NMDA receptors in Purkinje cell or molecular layer interneuron. Activation of cannabinoid 1 (CB1)
receptors in parallel-fiber terminal suppresses adenylyl cyclase 1, and thereby attenuates cAMP-dependent PKA activity and induction of presynaptic LTP (van Beugen et al., 2006). Therefore, a retrograde regulation mechanisms takes part to presynaptic LTP control.

A presynaptically expressed parallel fiber LTD is observed when presynaptically expressed parallel fiber-LTP is prevented pharmacologically (Qiu & Knopfel, 2009). This type of plasticity is most effectively induced by 4 Hz parallel fiber stimulation, a protocol similar to that effective for presynaptic LTP, and requires activation of cannabinoid CB1 receptors. In this case, the endocannabinoids are released in an NMDA receptor-dependent, but not mGlu1 receptor dependent, fashion. Thus, in principle, bidirectional plasticity mechanisms exist for both postsynaptic and presynaptic plasticity at the parallel fiber-Purkinje cell synapse.

Mechanisms of climbing fiber LTD

For long time the climbing fiber to Purkinje cell synapse was considered to fire a large all-or-nothing action potential independent of strength of climbing fiber stimulation. However, the climbing fiber synapse can also undergo plastic changes (Hansel & Linden, 2000). Climbing fiber-LTD was induced by a short tetanization of climbing fibers causing a reduction of the slow component of the complex spike. The climbing fiber LTD also results in change in afterhyperpolarization (AHP), which can prolong the complex spike pause. Interestingly, the changes induced in calcium transients following climbing fiber-LTD can affect the induction of both postsynaptically expressed LTD and LTP at the parallel fiber to Purkinje cell synapse (Coesmans et al., 2004). Thus the climbing fiber-Purkinje cell synapse can exert a complex regulatory role on long-term synaptic plasticity and dendritic integration changing the spike output of the Purkinje cell (Othsuki, 2009).

Plasticity of Purkinje cell intrinsic excitability

Purkinje cell excitability can be enhanced by somatic current injections or parallel fiber stimulation protocols that also induce parallel fiber-LTP (Belmeguenai et al., 2010). Signal cascades involved in LTP are shared by intrinsic plasticity, which indeed requires postsynaptic Ca2+ signaling followed by activation of the PP1/PP2A/PP2B cascade. A complex interaction between these phosphatase and PKA and casein kinase 2 (CK2) ultimately leads to a downregulation of small conductance Ca2+-activate K channels. Intrinsic plasticity of Purkinje cells is promoted by parallel fiber-LTP, but inhibits the subsequent LTP induction. A possible explanation for this reduced LTP induction is that intrinsic plasticity is accompanied by enhanced spine calcium signaling (Belmeguenai et al., 2010) which could promote LTD rather than LTP (Coesmans et al., 2004). Thus, parallel fiber-LTP could be accompanied by intrinsic plasticity at activated parallel fiber synapses, while its induction could be reduced through intrinsic plasticity at weaker and neighboring non-potentiated synapses.

Plasticity at molecular layer inhibitory synapses

At low frequency, climbing fibers excite Purkinje cells but also suppress GABA release from inhibitory interneurons through AMPA receptor activation (Satake et al., 2000; 2006). However, repetitive climbing fiber stimulation can potentiate the amplitude of inhibitory postsynaptic current (IPSCs) in Purkinje cells (Kano et al., 1996; Kawaguchi & Hirano, 2002). This inhibitory LTP of
GABAergic interneuron-Purkinje cell synapse requires a postsynaptic Ca2+ transient in Purkinje cells due to activation of voltage-gate Ca2+ channels and IP3-mediated Ca2+ release from internal store (Hashimoto & Kano, 2001). This Ca2+ transient activates CaMKII, which in turn leads to a Ca2+-dependent upregulation of GABA-A receptors activity (Kano et al., 1992; Kano et al., 1996; Kawaguchi & Hirano, 2007).

The neurophysiological consequences of molecular layer plasticity

As a first step to understand the complex set of plasticity mechanisms in the molecular layer, the consequences of the classical form of parallel fiber – Purkinje Cell LTD (Ito et al., 1982) need to be revisited. This LTD, induced by low-frequency pairing of parallel fiber and climbing fiber activity, causes a decrease of simple-spike firing in the Purkinje cell and thus leads to reduced inhibitory input to DCN cells, increased output from the cerebellum and enhanced execution of movement (Ito, 1989; Mark et al., 1998; Ito 2001; Hansel et al., 2001). This LTD regulates the pattern of Purkinje cell discharge (Steuber et al., 2007) and it was recently observed at the parallel fiber-Purkinje cell synapse in alert animals (Márquez-Ruiz & Cheron, 2012).

According to the central statement of the Motor Learning Theory, classical parallel fiber – Purkinje Cell LTD is supervised and serves to store correlated granular layer patterns under the teaching signal generated by climbing fibers. A critical demonstration of this proposal is that the Purkinje cell may indeed operate as a "perceptron" capable of detecting the huge amount of combinations generated at its parallel fiber synapses, which can be "digitally" switched on or off by LTD or LTP (Brunel et al., 2004). However, in terms of mechanisms controlling the synaptic strength, the picture emerging from latest evidences is much more complex than previously thought. It is well established that the large majority of parallel fiber synapses are silent (Wang et al., 2000; Isope and Barbour, 2002; Ito, 2006) suggesting that LTD is the dominating plasticity process. If the default state of parallel fiber synapses during development is to be silent, LTP would then be the driving process required in order to obtain any kind of learning (Jortell & Hansel, 2006). This hypothesis is akin with the proposal that, by missing feed-back inhibition, the molecular layer may use LTD as a surrogate in order to enhance the signal-to-noise ratio in Purkinje cells (De Schutter, 1995).

Importantly, while postsynaptic LTD/LTP are at least partially supervised through climbing fiber activity, presynaptic LTP/LTD appear to depend on ongoing activity patterns in the cerebellar network instead. Presynaptic LTP may be driven by salient patterns selected in the granular layer, for example by high-frequency bursts or by low-frequency correlated activity. Therefore, learning would result from the interaction of several forms of synaptic plasticity and not just from classical LTD alone. But what is then the role of climbing fibers and complex spikes ? The climbing fibers may bias the learning process by regulating the LTP/LTD balance when learning becomes intense, like when attention is enhanced or errors in motor execution become large and frequent.

The behavioral consequences of molecular layer plasticity

A major proof that postsynaptic parallel fiber - Purkinje cell LTD could provide the critical cellular mechanism for cerebellar learning was that its analogy with the mechanisms of acquisition of associative eyelid conditioning (Medina and Mauk, 1999). The conditioned stimulus is conveyed to the cerebellar circuitry through the parallel fibers, while the unconditioned stimulus through the climbing
fibers. The association of these inputs would generate a depression of the parallel fiber-Purkinje cell synapses, producing a conditioned via disinhibition of the DCN. A new and more complex view on the impact of molecular layer plasticity on cerebellar learning and performance has been opened by blocking specific forms of plasticity and using cell-specific transgenic mice, which have recently shown that motor learning can occur normally in the absence of parallel fiber-Purkinje cell LTD (Schoenwille et al., 2011). In turn, investigations on transgenic mice have suggested that postsynaptic LTP at parallel fiber-Purkinje cell synapse may substantially contribute to cerebellar motor learning. CAMK2b−/− mice are ataxic and show deficit in the acquisition of new motor task (van Woerden et al., 2009), and mutant mice in which LTP induction is blocked show pronounced deficits in motor coordination (Schonewille et al., 2010). Moreover, the Purkinje cell-specific deletion of PP2B (L7-Pp2b), which leads to prominent impairments in motor performance and motor learning, affects not only LTP at parallel fiber-Purkinje cell, but also intrinsic excitability (Schonewille et al., 2010). Finally, plasticity in molecular layer interneuron-Purkinje cell synapses may also be relevant for cerebellar learning (Wulff et al., 2009). The selective deletion from Purkinje cells of the 2 subunit, and thereby GABAA receptors, (Purkinje cell-2 mice) affects Purkinje cell simple spike activity and motor behavior. Although these mutant mice are not ataxic they show a deficit in both phase reversal learning and gain and phase consolidation of the vestibular-ocular reflex (VOR). Thus, plasticity at molecular layer interneuron-Purkinje cell synapses might have a role in cerebellar signal coding and memory formation but may not be essential for normal motor performance.

**An integrated view of cerebellar cortical plasticity**

It is reasonable to believe that the numerous forms of plasticity of the cerebellar cortex need to be integrated into coherent patterns, although the corresponding regulation mechanisms remain poorly investigated. These forms of plasticity may be coordinated by **intrinsic** and **extrinsic** mechanisms. Intrinsic mechanisms include local biochemical cascades and inhibitory circuits, while extrinsic mechanisms include oscillation and resonance and neuromodulatory systems. (Figs 2, 3). This section proposes hypothetical theory-independent organizing schemes deserving experimental and modeling evaluation.

**Potentiation of transmission channels and signal-to-noise ratio in the mossy fiber pathway**

*The NO system of granule cells.* The granule cells are neurons producing NO both in the dendrites and in parallel fiber terminals following NMDA receptor activation. NO released in the granular layer is needed for mossy fiber-granule cell LTP (Maffei et al., 2003), while NO released in the molecular layer from parallel fibers causes presynaptic LTP and enhances postsynaptic LTD (Casado et al., 2002). NO is also responsible for heterosynaptic plasticity. Therefore, NO release may exert a coordinated regulatory action causing LTP both at the mossy fiber-granule cell and presynaptic parallel fiber-Purkinje cell synapse, potentiating selected transmission channels.

*The calcium control system in Purkinje cells.* Intracellular calcium in Purkinje cell spines depends on several regulatory mechanisms and controls several forms of plasticity. Basically all factors causing strong Purkinje cell excitation, including intense parallel fiber and climbing fiber activity, lead
to strong calcium elevations depressing AMPA receptors (postsynaptically expressed LTD) and enhancing GABA-A receptors (inhibitory LTP), therefore globally reducing Purkinje cell responsiveness. NO released by activity in parallel fibers and MLIs, favors postsynaptically expressed LTD. Conversely, weak calcium elevations enhance AMPA receptors (postsynaptically expressed LTP) and enhance Purkinje cell intrinsic excitability, therefore globally raising Purkinje cell responsiveness. As a whole, it appears that NO, by coordinating LTP, may potentiate transmission along selected mossy fiber channels, while the calcium control system in Purkinje cells may rescale Purkinje cell responsiveness enhancing the signal-to-noise ratio. It should also be considered that the presynaptic expression of NO-dependent LTP, both at the mossy fiber and parallel fiber synapses, effectively enhances short-term facilitation against short-term depression. This would improve transmission of single spikes or short spike bursts, further increasing the effectiveness of channels involved.

**Contrast enhancement and geometrical organization of plasticity**

The spatial organization of LTP and LTD in the granular layer, which depends on the geometrical arrangement of Golgi cell inhibition (Mapelli & D'Angelo, 2007), favors transmission of spikes at higher frequency and with shorter delays in the center than in the surround (Solinas et al., 2010; D'Angelo et al., 2013a)(Gandolfi et al., submitted). It would therefore be of interest to understand how long-term synaptic plasticity is organized in the molecular layer. It has been suggested that parallel fiber-Purkinje cell LTD occurs together with parallel fiber-MLI LTP and MLI-Purkinje cell iLTP, while parallel fiber-Purkinje cell LTP occur together with parallel fiber-MLI LTD and MLI-Purkinje cell iLTD (Gao et al., 2012). This coordinated chain of plastic events would not just concur to reinforce the changes occurring in Purkinje cells but also to regulate the spatial distribution of plasticity. Inhibition generated by stellate cells would preferentially spread along the parallel fiber beams, while that of basket cells orthogonal to it. Therefore, an active granule cell ascending axon bundle may generate spatially organized LTP and LTD also in the molecular layer, an issue that remains to be clarified.

**Coordination of plasticity during patterned circuit activity**

In response to the incoming mossy fiber bursts, the granule cells respond with new bursts which reach the parallel fiber terminals and are transmitted to Purkinje cells (Chadderton ...). Repetition of bursts is essential to generate LTP and LTD: long high-frequency bursts induce LTP while short low-frequency bursts induce LTD. Likewise, at the parallel fiber- Purkinje cell synapse, NO production requires short bursts in order to effectively regulate presynaptically and postsynaptically induced LTP and LTD. Although the impact of native granular layer patterns on Purkinje cell responses remains to be investigated, certain patterns of activity may facilitate coordinated plastic changes.

Particular attention has been given to theta-burst patterns that occur during certain behavioral states like active motion and cognition as well as during sleep. Bursts are transmitted from the thalamo-cortical system to the cerebellum through cortico-cerebellar projections (Ros et al., 2009). The granular layer is itself resonant at theta-frequency and actively amplifies the incoming theta-burst patterns generating coherent theta-frequency oscillations through a complex set of mechanisms (Hartmann and Bower, 1996; Gandolfi et al., 2013). The granule cell bursts reach the parallel fiber terminals and are transmitted to Purkinje cells, which are also activated by the climbing fibers conveying low-frequency
signals from the inferior olive. The climbing fibers have been shown to play the role of synchronizing subsets of Purkinje cells (Marshall and Lang, 2004) and coincidence of parallel fiber and climbing fiber low-frequency oscillations has been proposed to amplify specific Purkinje cell responses by resonance (D'Angelo, 2010). This mechanism may effectively select subsets of Purkinje cells at the intersection of climbing fiber and parallel fibers oscillating in phase and promote plasticity at their synapses.

Notably, presynaptically expressed LTP and LTD are generated by theta-patterns. Moreover, presynaptically expressed LTP and LTD are promoted by climbing fibers, whose activity needs to be paired with that of the parallel fibers within a time-window of about 200 ms (Wang et al., 2000), a duration coincident with a theta cycle. In this way, plasticity in Purkinje cell synapses could be coordinated by theta-burst cycles over large subsets of synapses and neurons.

**Gating of plasticity by neuromodulatory systems**

**Neuromodulatory mechanisms of gating.** Various neuromodulators (noradrenaline, serotonin, acetylcholine, dopamine) may play a critical role in gating cerebellar LTP and LTD. Given their wide distribution across the cerebellar cortex and nuclei, these systems have a remarkable potential to control when and how learning has to occur (Schweighofer et al., 2004). This mechanism is required to relate cerebellar learning to general functional state of the brain: serotonin would convey responsibility signals, noradrenaline would convey error-related signals, acetylcholine would convey success signals, dopamine would convey reward signals. Although it has been shown that acetylcholine can modify cerebellar plasticity (Prestori et al., 2013; Rinaldo and Hansel, 2013), the impact of other neuromodulators requires further investigation.

**Cerebellar cortical plasticity and timing**

The cerebellum has long been proposed to operate as a “timing machine” (Eccles, 1967) and a “learning machine” (Ito, 2006). The cerebellum controls motor behavior with millisecond precision (Timmann et al., 1999; Osborne et al., 2007), so it is expected that its computations are performed on a comparable time-scale. There are indications, mostly derived from cellular investigations in rat cerebellar slices, that the granular layer (see Fig. 1) is capable of exerting a close control on spike timing (D'Angelo & De Zeeuw, 2009a). The granule cells (GrCs) generate brief spike bursts in the axon initial segment, which are almost instantaneously (<0.3ms) transmitted to the dendrites and to synapses on the axon ascending branch (Diwakar et al., 2009; Diwakar et al., 2011). Moreover, the mossy fiber (MF) – GrC EPSCs have extremely fast kinetics [rise time <1ms (Silver et al., 1992)] and can therefore excite the GrCs with high temporal precision (Cathala et al., 2005). Finally, GrCs are endowed with specific ionic mechanisms capable of controlling the delay and persistence of spike emission (D'Angelo et al., 2001). Theoretical analysis has indeed revealed that half of the information carried by MF spike trains is retransmitted by GrCs as first-spike delay with millisecond precision and half as spike frequency (Arleo et al., 2010b). As noted above, long-term potentiation (LTP) and long-term depression (LTD) have been shown to regulate both first-spike delay and spike frequency through different mechanisms (Nieuws et al., 2006a), and the outstanding timing capabilities of this system have been summarized into the “time-window” hypothesis, which considers how these mechanisms compete.
with feed-forward synaptic inhibition mediated by Golgi cells (GoCs) in order to control spike emission from the GrCs during a period of a few milliseconds after MF burst discharge (D'Angelo & De Zeeuw, 2009a; D'Angelo et al., 2013b). A recent computational analysis has shown that appropriate tuning at the different synapses forming the granular layer excitatory and inhibitory loops can either optimize spike emission (maximum number of transmitted spikes / granule cell), signal to noise ratio (filtering of granule cell spikes), or firing precision (variability of granule cell spikes). Clearly, the granular layer appears to use distributed plasticity to generate temporal patterns, which are expected to have an impact on plasticity at the subsequent stage, the parallel fiber - Purkinje cell synapse. This latter would then learn and store the time correlations of the relevant granular layer patterns. In this sense, the learning and timing machines are indeed the same machine, in which plasticity is required to generate timing.

There is also an inverse relationship between timing and plasticity. Parallel fiber - Purkinje cell LTD requires a precise relationship between climbing fiber and parallel fiber discharge (Wang et al., 2000). Similarly, DCN plasticity (see below) requires appropriate phase relationship between mossy fiber and Purkinje cell discharge (Raman and Bean, 2007). It is however unclear whether spike-timing dependent plasticity (STDP) actually operates in granular layer synapses. This intriguing aspect, and the relationship of STDP with theta-cycles, requires experimental investigation.

**Integration of plasticity in the cerebellar cortex and nuclei**

In order to account for the complex properties of behavioral learning, which include fast adaptation, extinction, saving, generalization and rescaling, it is unlikely that granular and molecular layer plasticity are sufficient. Importantly, cerebellar learning may occur in two main stages, including fast acquisition of well timed correlations in the cerebellar cortex and transfer of memory into a stabilized form in the deep cerebellar nuclei (Shadmehr et al., 2010; Smith et al., 2006) followed by plastic changes in the cerebral cortex (Li Voti et al., 2013). Therefore, beyond the fact that plasticity is distributed, different nodes in the network may specifically take in charge different components of the learning process.

**Plasticity in the Deep Cerebellar Nuclei**

A further fundamental station for cerebellar learning is located outside the cerebellar cortex in the Deep Cerebellar Nuclei (DCN). The involvement of DCN in cerebellar learning was suggested experimentally at the single-cell level and supported by behavioral observations. Recent works (Masuda & Amari, 2008; Medina & Mauk, 1999; Medina & Mauk, 2000) have implied the importance for mossy fiber - DCN and parallel fiber-DCN plasticity in controlling cerebellar learning in eye-blink conditioning and vestibulo-ocular reflex (VOR). Actually, EBCC complex properties, including acquisition, extinction and saving, cannot be easily explained assuming only plasticity in the molecular layer. DCN plasticity would be especially useful for the slow phases of learning and consolidation, a view that has recently been expanded by means of closed-loop computational models (Garrido et l., 2013).
DCN plasticity is composed of multiple mechanisms generating glutamatergic mossy fiber - DCN (Bagnall et al., 2006; Pugh & Raman, 2006) and GABAergic Purkinje cell-DCN (Aizenman et al., 1998; Morishita & Sastry, 1996; Ouardouz & Sastry, 2000) LTP and LTD. Mossy fiber - DCN synaptic plasticity has been reported to depend on the intensity of DCN cell excitation (Racine et al., 1986; Medina & Mauk, 1999; Pugh & Raman, 2006; Zhang & Linden, 2006). Purkinje cell-DCN synaptic plasticity was reported to depend on the intensity of DCN cell and Purkinje cell excitation (Morishita & Sastry, 1996; Aizenman et al., 1998; Ouardouz & Sastry, 2000; Masuda & Amari, 2008). Moreover, as well as in granule cells and Purkinje cells, DCN cells can generate plasticity of intrinsic excitability (Aizenmann and Linden). These forms of plasticity can combine to generate an effective coincidence detector driven by intracellular calcium changes.

The effect of Integrated plasticity in the cerebellar cortex and nuclei.

Synaptic plasticity in the DCN is supervised and may serve to store correlated granular layer patterns under the teaching signal generated by Purkinje cells (Boyden et al., 2004; Gao et al., 2012; Hansel et al., 2001). In a recent work (Garrido et al., 2013), a theoretical model of the cerebellum was operated in the framework of a manipulation task, in which objects with different masses were moved along a desired trajectory. The main observation was that plastic mechanisms at DCN synapses effectively complemented the learning capabilities of parallel fiber-Purkinje cell synapses and contributed to the acquisition of the dynamic model of the arm/object plant. A proper synaptic weight adjustment at DCN synapses acted as a gain adaptation mechanism allowing the parallel fibers to work within their most effective operative range, thus making the plasticity mechanisms between parallel fibers and Purkinje cells more precise.

Cerebellar plasticity in learning and control

The cerebellum plays a critical role in the precise control of movements, as is evident when studying patients with cerebellar malfunctioning and diseases (Thach, 1996). The cerebellum receives proprioceptive signals (Sawtell, 2010) and copies of motor commands (Schweighofer et al., 1998a) together with haptic information (Ebner & Pasalar, 2008; Shadmehr & Krakauer, 2008; Weiss & Flanders, 2011) through MFs. By means of these signals and its own internal circuitry, the cerebellum is able to learn and process sensorimotor information, and thereby regulate the initiation, intensity and duration of motor acts in an anticipatory manner (Spencer et al., 2005; Manto et al., 2012). This gain control operation is a fundamental aspect of motor control in animals, as it allows not only the rapid regulation of motor acts according to contextual cues, but also, through learning, adaptation of these acts to bodily and environmental changes. This adaptable gain control requires closed-loop interactions between command centers and effectors and is thought to involve the cerebellum embedded in the so-called forward controller loop (Schweighofer et al., 1998a; Wolpert et al., 1998; Wolpert & Ghahramani, 2000). In fact, the abstraction of models (kinematics and dynamics) of objects under manipulation (Shadmehr & Mussa-Ivaldi, 2012b) is efficiently achieved thanks to close interaction between the cerebral and the cerebellar cortex (Middleton & Strick, 2000; Wang et al., 2008).
An effective understanding of cerebellar learning and control requires therefore a *closed loop* analysis, in which some specific issues have to be considered: first, the adaptable gain controller localized in the cerebellum should be able to optimize its performance in the face of broad and varying operative ranges, secondly, it should rapidly converge toward a stable solution, and thirdly it should be able to consolidate memory. In a recent work, an analog cerebellar model embedded into a control loop connected to a robotic simulator was implemented. In accordance with biological evidence, the cerebellum model was endowed with both LTD and LTP at the parallel fiber-Purkinje cell, MF-DCN and Purkinje cell-DCN synapses. This resulted in a network scheme whose effectiveness was extended considerably compared to one including just parallel fiber-Purkinje cell synaptic plasticity. Indeed, the system including distributed plasticity reliably *self-adapted* to manipulate different masses and to learn the arm-object dynamics over a time course that included fast learning and consolidation, along the lines of what has been observed in behavioral tests. In particular, parallel fiber-Purkinje cell plasticity operated as a *time correlator* between the actual input state and the system error, while MF-DCN and Purkinje cell-DCN plasticity played a key role in generating the *gain controller*.

This model, by incorporating distributed synaptic plasticity and by generating closed-loop simulations, allowed progressive error reduction based on feedback from the actual movement and accounted for three main theoretical aspects of cerebellar functioning. First, the model supported the principle that the cerebellum operates as a corrective inverse dynamic module (Schweighofer et al., 1996b, a; Schweighofer et al., 1998b; Spoelstra et al., 2000), in which the granular layer states are correlated with the error-based teaching signal received through the climbing fibers. As in an adaptive filter (Dean et al., 2009), granule cells set different delays responding to input stimuli along an arm trajectory trial [cf. (D’Angelo & De Zeeuw, 2009a)]. Then, parallel fiber-Purkinje cell plasticity temporally correlates the input state (represented in parallel fibers) and the error estimation obtained during execution of the manipulation task. Instead, MF-DCN and Purkinje cell-DCN plasticities store the excitatory and inhibitory gain of the neural network required to generate accurate correction of movement. Thus, the DCN afferent synapses infer the main properties of the object under manipulation, while the parallel fiber-Purkinje cell synapses store the temporal characteristics of the task. As a consequence of this, plasticity at DCN synapses provides a homeostatic mechanism capable of keeping Purkinje cell activity at its optimal range during learning. This effect can be observed in closed-loop simulations allowing progressive error reduction based on feedback from the actual movement. Thirdly, the model supports the existence of a learning consolidation process in the cerebellar nuclei. parallel fiber-Purkinje cell plasticity evolves rapidly, while DCN plasticity evolves more slowly, because it depends on the previous evolution of plasticity at the parallel fiber-Purkinje cell synapse itself, naturally implementing a double time-constant plasticity mechanism. Unfortunately, this model did not include granular layer plasticity, whose impact remains to be determined.

**Conclusions**

In conclusion, cerebellar learning is emerging as an integrated process involving numerous synaptic sites and forms of plasticity. The cerebellar cortex interacts with the cerebellar nuclei and different sites seem to be involved in elaborating different aspects of learning. Plasticity could be
organized in specific spatial patterns, with the emergence of plasticity channels operating against a background activity regulated itself by various forms of plasticity. Channeling begins in the granular layer and is concluded in the molecular layer, where Purkinje cells integrate signals coming from different cerebellar areas. Plasticity could be organized in specific temporal patterns, notably theta-burst oscillations, coordinating the activity of large neuronal fields in the granular and molecular layer. In this view, the predicted centrality of parallel fiber - Purkinje cells LTD for cerebellar learning is unclear, and this mechanism should be better viewed as part of a coordinated system of plasticities. Importantly, computational modeling has revealed that understanding the mechanisms of cerebellar learning requires that the whole system is analyzed in closed-loop, in order to allow feed-back and gating signals to dynamically modify plasticity at specific synaptic sites.
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Figure 1

Figure 1. Schematic drawing of the cerebellar circuit and its forms of plasticity. The principal elements of the cerebellar circuit and associate structures are indicated: mossy fiber (mf), parallel fiber (pf), climbing fiber (cf), granule cell (GrC), Golgi cell (GoC), Purkinje cell (PC), stellate cell/basket cell (SC/BC), deep cerebellar nuclei cell (DCN-C), inferior olive cell (IO-C). The cerebellar cortex is indicated by a shadowed area. The cerebellar circuit expresses at least 9 recognized forms of plasticity, some of which bidirectional, included into three main subcircuits.

1) Granular layer (green area): mossy fiber - granule cell LTP and LTD (mf-GrC LTP/LTD), granule cell LTP of intrinsic excitability (GrC IE-LTP).
2) Molecular layer circuit (yellow area): presynaptic parallel fiber - Purkinje cell LTP and LTD (presynaptic pf-PC LTP/LTD), postsynaptic parallel fiber - Purkinje cell LTP and LTD (postsynaptic pf-PC LTP/LTD), climbing fiber - Purkinje cell LTD (cf-PC LTD), stellate cell/basket cell inhibitory LTP (SC/BC iLTP), Purkinje cell LTD of intrinsic excitability (PC IE-LTP).
3) Deep cerebellar nuclei (red area): mossy fiber - DCN cell LTP and LTD (mf-DCNC LTP/LTD), Purkinje cell - DCN cell inhibitory LTP and LTD (PC-DCNC iLTP/LTD), and DCN cell LTP of intrinsic excitability (DCNC IE-LTP).
Figure 2. The major mechanisms of plasticity in cerebellar cortical circuit. The figure highlights two elements: the central position of granule cells in coordinating granular layer and molecular layer plasticity, and the PC pivotal role in coordinating molecular layer plasticity. The granule cells express NMDA receptors and release NO thus controlling plasticity both at the mossy fiber and parallel fiber synapses. The PC coordinates plasticity at the synapses formed with parallel fibers, climbing fibers and stellate cells / basket cells. mossy fiber (mf), parallel fiber (pf), climbing fiber (cf), granule cell (GrC), Golgi cell (GoC), Purkinje cell (PC), stellate cell/basket cell (SC/BC). Various membrane receptors and ionic channels are indicated in the figure. Intracellular elements have their usual meaning and are described in the text. Cyclic AMP, adenilate cyclase, protein kinase A (cAMP/AC/PKA), Cyclic GMP, guanyl cyclase, protein kinase G (cAMP/AC/PKA), diacylglycerol, IP3, protein kinase C (DAG, OP3, PKC), phospholipase 1, A1, A2 (PLA1/PL1/PLA2), calcium-calmodulin kinase II (CAMK-II) calcium (Ca$^{2+}$), nitric oxide (NO), nitric oxide synthase (NOS). Depolarization is indicate by yellow stars.