UNIVERSITÀ DEGLI STUDI DI ROMA "TOR VERGATA"

FACOLTA' DI SCIENZE MM. FF. NN.

DOTTORATO DI RICERCA IN
BIOLOGIA CELLULARE E MOLECOLARE

CICLO XXII

Structural plasticity of adult cerebellar climbing fibres: an *in vivo* study on the role of the growth-associated protein 43

Giorgio Grasselli

A.A. 2009/2010

Docente Guida: Prof. Piergiorgio Strata
Tutor: Prof.ssa Maria Rosa Ciriolo

Controrelatore: Dott. Stefano Cannata
Coordinatore: Prof. Gianni Cesareni

E-mail: g [d o t] g r a s s e l l i [a t] h s a n t a l u c i a [d o t] i t

To my family and to my friends

Table of contents

List of figures and tables

Abbreviations

3-AP:	3-acetylpyridine
AMPA:	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
CaMKII:	Ca^{2+}/calmodulin-dependent protein kinases
CF:	climbing fibre
CKII:	casein kinase II
CNS:	central nervous system
CRF:	corticotropin-releasing factor
EPSC:	excitatory post-synaptic current
GAP-43:	growth associated protein 43
IO:	inferior olive
IPSC:	inhibitory post-synaptic current
LTD:	long-term depression
LTP:	long-term potentiation
mGluR:	metabotropic glutamate receptor
NMDA:	N-methyl-D-aspartic acid
PC:	Purkinje cell
PF:	parallel fibre
PKC:	protein kinase C
PLC:	phospholipase C
PPD:	paired-pulse depression
PPF:	paired-pulse facilitation
*sh*RNA:	short-hairpin RNA
siGAP:	slienced-GAP-43
TTX:	tetrodotoxin
VGLUT2:	vesicular glutamate transporter 2

Abstract

The growth-associated protein GAP-43 has a pivotal role in axonal growth and guidance during development. Towards the end of postnatal development its expression dramatically declines in most brain regions with few exceptions, such as cerebellar cortex and inferior olive (IO). When experimentally over-expressed in murine CNS *in vivo*, it is able to induce axonal sprouting, moreover it is up-regulated in conditions known to induce a structural remodelling of neuronal connectivity, such as injuries to the CNS and neurodegenerative disorders. However the reason why high expression is maintained through adulthood only in some regions of the brain has never been clarified.

Taking advantage of lentiviral vectors and *sh*RNAs to induce an efficient and stable gene silencing specifically in the IO, we chose to investigate GAP-43 role in axonal remodelling of the adult CFs, since they originate from one of the nuclei which retain GAP-43 high expression levels, they are conveniently arranged for morphological studies and they are endowed with profound plastic and regenerative potentials. Here we show *in vivo*, in adult rats, that silencing of GAP-43 causes an atrophy of olivocerebellar terminal axons and significant modification of its presynaptic *boutons*.

These data suggest that GAP-43 plays a pivotal role in the maintenance of axonal structure of CF and of the organization of presynaptic plasma membrane in physiological conditions and it plays a complex role in CF lesion-induced sprouting.

Finally, by means of two-photon microscopy and laser nanosurgery, we describe here for the first time *in vivo* lesion-induced sprouting in the mammalian CNS.

Introduction

1. The cerebellum

1. General organization and function

From a macroscopic point of view, the cerebellum is a very distinct region of the brain which occupies, in all vertebrates, the position immediately behind the tectal plate in the mesencephalon. It is the only region of the central nervous system (CNS) to span the midline without interruption, immediately over the fourth ventricle (Eccles, 1967; Herrup and Kuemerle, 1997; Shepherd, 2004).

The cerebellar cortex can be roughly divided into three longitudinal regions: the medial vermis and two lateral hemispheres. Each of them is connected to one of three groups of deep cerebellar nuclei (Ito, 1984). Two transverse fissures divide the cerebellum into three lobes: the postero-lateral fissure defines the most caudal flocculo-nodular lobe from the *corpus cerebelli* and the primary fissure lies in the *corpus cerebelli* between the posterior and the anterior lobe. The flocculo-nodular lobe receives mainly vestibular inputs and represents the evolutionary most ancient cerebellar region, referred to as *archicerebellum*. The region of the *corpus cerebelli*, which receives mainly spinal cord input is called the *paleocerebellum*. In primates, including humans, the largest component by far is the *neocerebellum* whose size is related to the development of the cerebral hemispheres, and it is dominated by cortical inputs. The cerebellum is connected to the rest of the CNS by three large fibre bundles, the *cerebellar pedunculi* (Kandel et al., 2003; Shepherd, 2004).

The *cerebellar cortex* (Fig. 1) generates a single efferent pathway consisting in the axons of the GABAergic Purkinje cells (PCs; Fig. 2), which regulate cerebellar nuclei activity by their inhibitory action. The large soma of PCs form a distinct monolayer in the cerebellar cortex, interposed between other two layers: the molecular layer, between the pial surface and the PC layer, containing the vast dendritic arbour of PCs, and the granular layer, between PC layer and the cerebellar white matter (Shepherd, 2004).

Neuronal input reaches the cerebellar cortex through two distinct glutamatergic pathways: the mossy fibres (MFs), originating from the spinal cord and a number of areas of the brainstem including the pontine nuclei, and the climbing fibres (CFs; Fig 3), identified as the terminal arborisation

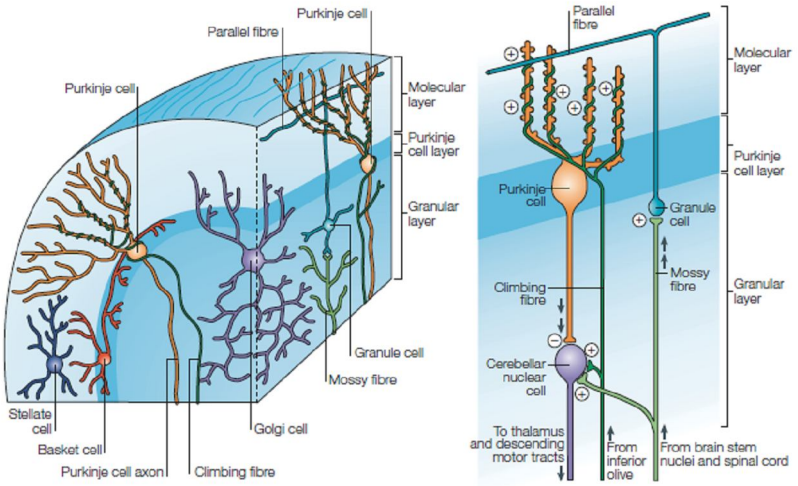

Fig. 1 - Basic structure of the cerebellar cortex.

There are two main afferents to the cerebellar cortex: climbing fibres (CF), which make direct excitatory contact with the Purkinje cells, and mossy fibres (MF), which terminate in the granular layer and make excitatory synaptic contacts mainly with granule cells, but also with Golgi cells. In some cases, the stem axons of CF and MF also provide collaterals to the cerebellar nuclei en route to the cerebellar cortex. The ascending axons of the granule cells branch in a T-shaped manner to form the parallel fibres, which, in turn, make excitatory synaptic contacts with Purkinje cells and molecular layer interneurons — that is, stellate cells and basket cells. Typically, parallel fibres extend for several millimetres along the length of individual cerebellar folia. With the exception of granule cells, all cerebellar cortical neurons, including the Purkinje cells, make inhibitory synaptic connections with their target neurons (Mod. from Purves, 2004).

of axons, whose cell bodies are located in the inferior olivary nucleus (IO, in the brain-stem). MFs, the major source of afferent input to the cerebellum, terminate in the molecular layer, where their bulbous terminals contact granule cells dendrites. Additionally, granule cells dendrites also receive the inhibitory input of Golgi cells axons; direct contacts between MF terminals and Golgi cells dendrites have been observed as well (Eccles, 1967). The whole synaptic complex here described is organized in a structure called cerebellar glomerulus (Eccles, 1967; Ramon y Cajal, 1911).

Both olivocerebellar axons and MFs give rise also to collaterals which innervate the deep cerebellar nuclei as well (Bloedel and Courville, 1981).

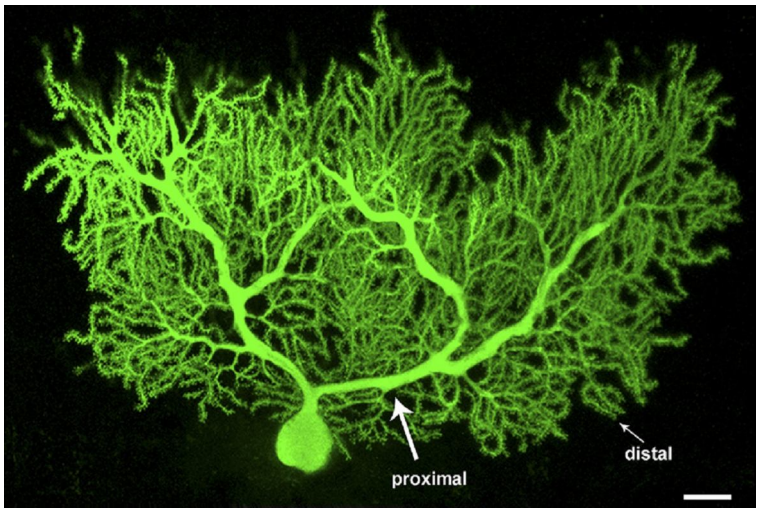

Fig. 2 – Purkinje cell morphology.
GFP-expressing PC from an adult rat visualized by confocal microscopy after lentiviral injection. The different calibre of the dendrites identifies two compartments, the proximal and the distal one. Note the difference in the density of the spines emerging from the two dendritic domains: the proximal one appears smooth since few spines are present; the distal one is covered with packed spines; scale bar 25 μm (Cesa & Strata 2009).

Granule cell axons form fibres ascending through the PC layer into the molecular layer, and, after a T-shaped branching, form parallel fibres (PFs). PFs run parallel to the pial surface and perpendicular to the fan-like PC dendritic arbours. PFs represent, together with CFs, the only excitatory input impinging on PCs dendrite. However their innervation territories on PC dendrites are different: PFs innervate distal dendritic branchlets while CFs innervate the thicker proximal dendritic branches (Fig. 2; Ito, 1984; Ito, 2006). A remarkable feature of these two types of synapses is that each PC is innervated in the rat by a single CF and by about 200,000 PFs (Carulli et al., 2004; Strata and Rossi, 1998).

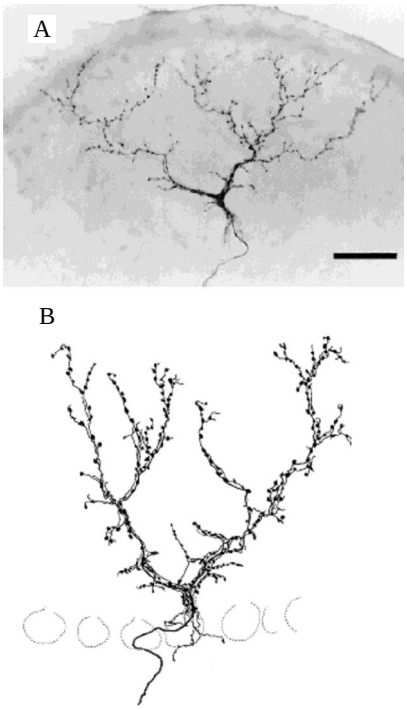

Fig. 3 - Terminal arborisations of labelled climbing fibres (CFs) in parasagittal sections.
A) Photomicrograph of a CF terminal arborisation. B) Reconstruction of a terminal arborisation. The organization in a main thick ramification (stalk) and thin lateral ramifications (tendrils) is clearly visible. Swellings along CF ramifications represent axonal *boutons* (varicosities). Scale bar 50 μm (Sugihara & Shinoda 1999).

PC activity is regulated by two types of inhibitory interneurons, the stellate and the basket cells, both receiving input from PFs. A third type of inhibitory interneuron is represented by Golgi cells, which form synapses on granule cell dendrite juxtaposed to the MF glomeruli. The first two inhibitory interneurons have their soma in the molecular layer while Golgi cells have their soma in the PC layer (Ito, 1984; Ito, 2006).

The cerebellar cortex also receives information by means of some additional diffuse projections from the brainstem. These consist in cholinergic fibres, originating from the pedunculo-pontine nucleus (Jaarsma et al., 1997), noradrenergic fibres from the *locus coeruleus* (Abbott and

Sotelo, 2000) and serotonergic fibres from raphe nuclei (Strahlendorf and Hubbard, 1983).

Lugaro cells and the recently discovered unipolar brush cells as additional elements of cerebellar neuronal circuitry and added to the classic diagram of cerebellar circuitry. Lugaro cells are inhibitory interneurons located in the granular layer which are activated by serotonergic fibres and, in turn, inhibit Golgi cells (Aoki et al., 1986; Sahin and Hockfield, 1990). Unipolar brush cells are located in the granular layer with a more extensive representation in the vestibule-cerebellum and receive excitatory synapses on their dendritic brush from a single MF terminal. The unipolar brush cell axon form branches within the granular layer, where they give rise to large terminals, similar to MF glomeruli, which synapse with both granule cells and unipolar brush cell dendrites. These cells receive inputs from glutamatergic primary vestibular fibres and choline-acetyltransferase-positive MFs (Dino et al., 1999; Ito, 2006).

The wide variety of sensory inputs processed and integrated by the cerebellum support an important computational rule resulting from such complex circuitry. The observation of an ordered disposition of neuronal elements led to the first hypothesis about the cerebellar computational function, known as the "Marr-Albus-Ito" theory of cerebellar motor learning, still largely accepted (Albus, 1971; Ito, 1984; Ito, 2006; Ito and Kano, 1982; Marr, 1969; Strata, 2009).

Until the 1960s, the cerebellum was thought to be involved exclusively in the control of movement; it was viewed as a comparator adjusting the motor output on the basis of sensory input. It was recently proposed that the cerebellum forms an internal model that reproduces the dynamics of the different parts of the body, helping the brain to perform precise movements, without the strict need of feedback information from the moving body part. (Ito, 2008; Wolpert et al., 1998). In the last twenty years, a growing body of anatomical, physiological, behavioural and clinical evidence has suggested that in addition to its role in motor control, the cerebellum is involved in cognitive and emotional functions, such as fear-associated memory, providing some sort of neuronal coordination not only with the motor cortex but also with many other cortical regions. This would explain, for instance, the great development of cerebellar hemispheres which has occurred in the evolution of primates in relation to the development of associative cerebral cortex (Sacchetti et al., 2009).

2. Physiology and synaptic plasticity

The cerebellum contains more neurons than the rest of the brain, due to the huge number of granule cells (Shepherd, 2004). Each PC receives a single CF axon which makes ~1,500 synapses (Strata and Rossi, 1998), and these synapses release glutamate with a very high probability, differently to PFs (Dittman and Regehr, 1998; Silver et al., 1998).

The stimulation of PFs causes a graded excitatory post-synaptic current (EPSC) in the PCs and triggers in a "simple spike" (i.e. conventional action potential), while CFs generate the largest depolarizing event in the CNS and a highly characteristic all-or-none burst of impulses known as "complex spike" (Fig. 4) composed of an initial fast action potential (due to a Na^+ spike), followed by two or three smaller spikelets superimposed on a sustained depolarization plateau (Eccles et al., 1966; Ito, 2006; Ohtsuki et al., 2009; Thach, 1967). The EPSCs at CF synapses are largely mediated by the activation of AMPA receptors (Konnerth et al., 1990; Llano et al., 1991; Perkel et al., 1990) that contain GluR2 subunits and are therefore not permeable to calcium (Ohtsuki et al., 2009). Only recently it was clarified that mature Purkinje cells express also functional NMDA receptors which contribute to the complex spike waveform, by influencing the number and timing of spikelets, as well as the after-depolarization plateau (Piochon et al., 2007; Renzi et al., 2007). The large number of synaptic contacts formed by a

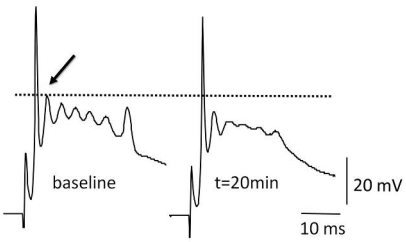

Fig. 4 – Climbing fibre complex spike and its long-term depression.
CF stimulation generates a large all-or-none depolarization of PC defined as complex spike and composed of an initial large and fast action potential, due to Na^+ conductance, followed by two or three smaller spikelets superimposed on a sustained depolarization plateau. A low frequency stimulation (5 Hz) of CF for 30s evokes long-term depression (LTD; shown in the right panel) of its excitatory post-synaptic potential (EPSP), consisting in a reduction in the amplitude of the first spikelet (arrow; Ohtsuki et al., 2009).

single CF input on PC and their high probability of glutamate release are responsible of a large, widespread calcium transient that accompanies complex spikes and plays a crucial role in cerebellar plasticity (Miyakawa et al., 1992; Ross and Werman, 1987). In addition, the CF activity exerts a tonic inhibition on the PC (Montarolo et al., 1982; Sugihara et al., 1999) due, at least partly, to a pure spillover connection to inhibitory interneurons in the molecular layer (Szapiro and Barbour, 2007). Direct synaptic contacts between CFs and inhibitory interneurons has been reported only morphologically by anterograde labelling of CF (Sugihara et al., 1999) but never confirmed functionally.

According to the classical model provided by the Marr-Albus-Ito theory of cerebellar motor learning, proposed in the late 60s, cerebellum could work as a learning machine, in which the synchronous activation of CF and PFs could lead to a long-lasting modification of the evoked synaptic potentials at PF-PC synapses, underlying learning and memory. It was shown that a protocol of low frequency stimulation of PF (1 Hz for 5 min) paired with CF stimulation can induce LTD at PF synapses (Ito, 1984; Ohtsuki et al., 2009). The induction of LTD at PFs depends on the activation of the mGluR1/PKC signalling cascade and activation of α-calcium/calmodulin-dependent kinase II (αCaMKII). CF signalling triggers dendritic calcium transients and contributes to PF LTD induction by activating these induction cascades. Co-activation of PF and CF inputs causes a supralinear calcium signalling in PF spines, thus providing a coincidence detection mechanism that might be required to reach a critical calcium threshold for LTD induction (Ito, 2001; Ohtsuki et al., 2009).

Recent findings suggest that there is no specific requirement for CF evoked calcium signals, but it has an important role in facilitating PF LTD induction by amplifying local calcium transients, since a strong PF stimulation alone is sufficient to induce PF LTD (Eilers et al., 1997; Hartell, 1996).

By applying the low-frequency/low-intensity PF stimulation protocol used for LTD induction but in the absence of any CF stimulation, PF develop a second and opposite form of synaptic plasticity, consisting in a long-term potentiation (LTP) of PF EPSC, expressed both pre- and postsynaptically (Lev-Ram et al., 2002; Salin et al., 1996). The induction of PF LTP depends on lower calcium transients than LTD induction (Coesmans et al., 2004), and requires the activation of protein phosphatases PP1, PP2A and PP2B (Belmeguenai and Hansel, 2005). It is interesting to observe that, concerning the level of calcium signalling and kinase/phosphatase activation

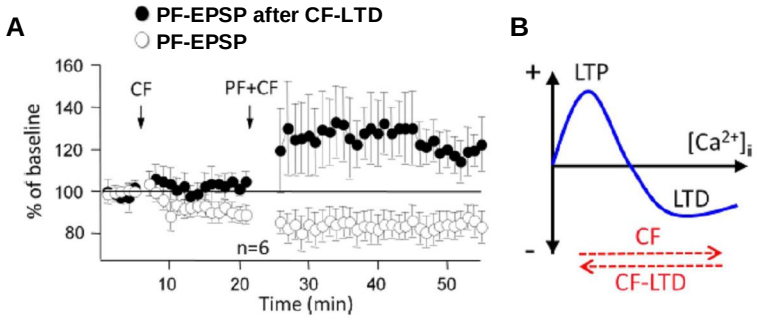

Fig. 5 – Bidirectional long-term plasticity of parallel fibre – Purkinje cell synapses regulated by CF plasticity.
A) CF function and plasticity plays a pivotal role in PF plasticity because it regulates the direction of the modification of PF-EPSP (LTP or LTD), resulting in a phenomenon of metaplastic modifications at PFs. Usually a long and low frequency stimulation (5 Hz, 30 s) of PF paired to CF induces a long-term depression (LTD) of PF-EPSP (open dots). Previous induction of CF-LTD inverts the sign of PF plasticity, leading to the induction of long-term potentiation (LTP) instead of LTD (closed dots). B) A high calcium threshold is needed at PF-PC synapses for the induction of LTD. CF stimulation is necessary for $[Ca^{2+}]_i$ to reach the threshold needed for PF-LTD. The reduction of PC calcium transients due to CF-LTD is sufficient to prevent the overcoming of the threshold, thus enabling the potentiation of PF synapses (modified from Ohtsuki et al., 2009).

requirements, PF bidirectional synaptic plasticity seems to be controlled by opposite mechanisms of those described in hippocampus and cortex (Jorntell and Hansel, 2006).

The idea of an invariant all-or-none response of the CF, has long prevailed. Nevertheless CF has been recently shown to undergo synaptic plasticity events itself: a low-frequency stimulation protocol (5 Hz for 30 s) is sufficient to induce LTD of CF EPSCs (Hansel and Linden, 2000), found to be expressed post-synaptically (Shen et al., 2002; Fig. 4). The biochemical cascade involved in the induction of CF LTD has several similarities with the LTD induction cascade at PF. At both types of synapses a postsynaptic calcium transient, the activation of mGluR1 receptors, and the activation of protein kinase C (PKC) are required for LTD induction (Hansel and Linden, 2000). Moreover CF LTD induction is also PKA-dependent (Schmolesky et al., 2007).

CF LTD plays a pivotal role in cerebellar plasticity because it was shown to control the direction of the modification of PF-EPSP (LTP or LTD), resulting in a phenomenon of metaplastic modifications at PFs. When CF LTD is induced before the application of the paired low-frequency stimulation protocol at PF and CF, this protocol induces LTP instead of LTD at PF synapses (Coesmans et al., 2004; Fig. 5). It was shown that CF LTD is accompanied by a reduction in calcium transients associated with the complex spike (Weber et al., 2003). The most likely explanation for this reversal of direction of PF plasticity is that CF LTD is sufficient to reduce the activity dependent calcium signal below the threshold for LTD induction at PF synapses (Coesmans et al., 2004; Jorntell and Hansel, 2006).

This is not the only way by which CF regulate PF plasticity. Indeed a post-synaptic release of endocannabinoids is triggered by CF and contributes to facilitate the induction of LTD at PFs by suppressing a form of pre-synaptic PKA dependent PF LTP (Brenowitz and Regehr, 2003; Safo and Regehr, 2005; van Beugen et al., 2006; Ohtsuki et al., 2009). CF activity can also induce the release of the neuropeptide corticotropin-releasing factor (CRF; Barmack and Young, 1990; Tian and Bishop, 2003), which has been shown to be involved in the induction of parallel PF LTD too (Miyata et al., 1999). CF input thus exerts a fine control over PF plasticity not only through the calcium transients associated with complex spike activity, but also by the activity-dependent production of endocannabinoids and the release of CRF.

To enhance the complexity of the system even further there are other synapses that were shown to undergo to various form of synaptic plasticity (Fig. 6; Hansel et al., 2001). The repetitive stimulation of CFs can induce an increase of inhibitory post-synaptic currents (IPSC) triggered by inhibitory interneurons on PC (IPSC-LTP; Kano et al., 1992). This IPSC-LTP requires a postsynaptic Ca^{2+} transient and the activation of αCaMKII and PKA (Hashimoto et al., 1996; Kano et al., 1996; Kawaguchi and Hirano, 2000; Khodakhah and Armstrong, 1997). The synapses between MFs and granule cells can undergo to LTP, too, as a consequence of high frequency stimulation (100 Hz for 100 ms repeated 8 times at 250 ms intervals) in a way that depends on activation of NMDA glutamate receptors, mGluR, and PKC, similarly to hippocampal LTP (Armano et al., 2000; D'Angelo et al., 1999; Rossi et al., 1996). Last, GABAergic synapses between PC and deep cerebellar nuclei neurons exhibit LTD or LTP themselves. This appears after repeated postsynaptic hyperpolarizing pulses paired or not with a postsynaptic hyperpolarization or application of an internal Na^{+} channel blocker (Aizenman et al., 1998).

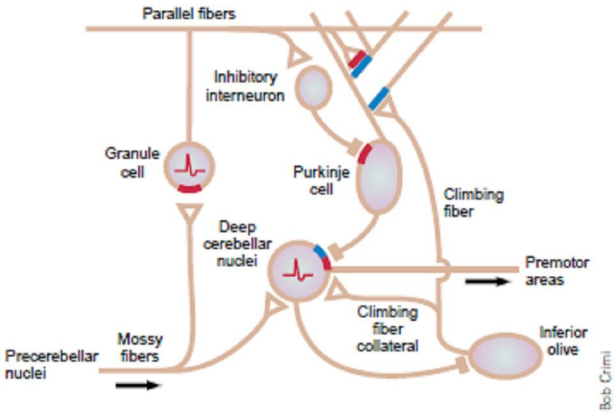

Fig. 6 - Summary of synaptic and intrinsic plasticity in the cerebellar circuit.
In this cartoon all known forms of functional plasticity occurring in the cerebellum are summarised: LTP is indicated by a red bar at synapses, LTD by a blue one. Changes in intrinsic excitability of a neuronal type are indicated by a red action potential in the soma (Hansel et al., 2001).

A further level of neuronal activity regulation in addition to classic plasticity at synaptic sites (LTP or LTD), is the modulation of intrinsic excitability of neurons, consisting in an increase or decrease in the voltage-sensitive conductances either locally at the level of single synapse or globally, affecting the whole neuron (Hansel et al., 2001; Turrigiano, 1999). This was shown to occur in PC, granule cells and in the neurons of the deep cerebellar nuclei (Aizenman and Linden, 2000; Armano et al., 2000; Ohtsuki et al., 2009; Schreurs et al., 1998).

3. Structural plasticity in the cerebellar cortex

Cerebellar cortex is not only able to modify its computational properties by a complex combination of functional modifications, but its well ordered circuitry is able to undergo several structural remodelling even during adulthood, increasing the levels of control of its network function. This was shown to occur either in relation to synaptic activity or as a reaction to experimental manipulations (Bosman and Konnerth, 2009; Carulli et al., 2004; Cesa and Strata, 2009; Rossi and Strata, 1995; Strata and Rossi, 1998). Several lines of evidence showed a relationship between synaptic plasticity

and structural modifications, mainly consisting in changes of the number of synapses. It was proposed that stimulation of PFs in a small dendritic area of a PC activates at the most 2.6 % (30/1140) of the PF–PC synapses located within the area, on the basis of the measurements of the area stimulated by an electrode, on the recorded EPSC and on the average number of synapses observed anatomically, (Wang et al., 2000). Moreover a burst stimulation of PFs alone or paired with CF is able to increase or decrease such receptive field (Jorntell and Ekerot, 2002). It was thus suggested that silent synapses are functionally produced by LTD, and that learning converts silent synapses to active synapses, or vice versa, by the induction of LTP or LTD (Jorntell and Ekerot, 2003; Ito, 2006). Pronounced structural plasticity, including a lasting change in synaptic number, was shown to occur in direct relation to learning processes: a decrease of the number of excitatory synapses was shown to occur in the cerebellar cortex following eye-blink conditioning, a behavioural learning protocol which is associated with PCs LTD (Connor et al., 2009).

In addition to the pivotal control on PF function and plasticity, CFs are characterized by a high degree of structural plasticity. They undergo a homosynaptic competition with other CFs during development and a heterosynaptic competition with PFs in the innervation of its PC target. Moreover they are able to expand their territory of innervation following an increase of the target space available, and, on the other hand, they undergo to regressive modifications after elimination of their target or block of electrical activity (Cesa and Strata, 2009; Hashimoto and Kano, 2005; Sacchetti et al., 2005; Strata and Rossi, 1998).

The first synaptic contact between PC and CF occurs around birth when the PC has not yet developed dendrites and the CF form synapses on its soma. The establishment of the first functional synapse evoking an EPSC occurs at P2 (Crepel, 1982). At P3, all PC respond to CF stimulation and in most of them in a way which is graded in steps, ranging from 2 to 5 (Crepel et al., 1981), supporting the notion of multiple innervation Surplus CFs are gradually eliminated during early postnatal development and most PCs in mice become innervated by single CFs within the third postnatal week (Hashimoto and Kano, 2003; Kano et al., 1995; Kano et al., 1997; Kano et al., 1998; Offermanns et al., 1997). The CF regression process is influenced also by the interactions of the target PCs with PFs. In three different mutant mice, in which loss of granule cells is generated by totally different mechanisms, multiple innervation of PCs by CFs persists into adulthood. This is indeed the case in the weaver (Crepel, 1976), staggerer (Crepel et al.,

1980; Mariani and Changeux, 1980; Steinmayr et al., 1998) and reeler mutant mice (Mariani et al., 1977). It was further shown in rats deprived of granule cells that the phase of CF regression starting from P9 needs PF innervation (Crepel, 1976; Mariani et al., 1990; Mariani et al., 1987).

Excitatory innervation on PC not only express a developmental homologous competition among CFs but also a heterologuos competition between CF and PFs. PFs and CF form synapses on distinct portion of the PC dendritic domain, the spiny distal and thin dendritic branchlets for PFs and the fairly smooth proximal and thick dendritic branches for CF (Cesa and Strata, 2009). Interestingly the orphan glutamate receptor delta-2 subunit (GluRδ2) is expressed selectively in cerebellum, and, there, only in PC distal spines (Lomeli et al., 1993). This protein plays a crucial role in the differential distribution and stabilization of PF and CF synapses (Guastavino et al., 1990; Ichikawa et al., 2002; Kurihara et al., 1997; Mandolesi et al., 2009a; Mandolesi et al., 2009b). Although no physiological ligand has been found yet, it was recently shown that it is involved in the stabilization and strengthening of synaptic connectivity between PFs and PCs, serving probably as an adhesion molecule (Mandolesi et al., 2009a; Yuzaki, 2003).

CF is able to expand their territory of innervation in response to enlarged target space, or to undergo to regressive modifications (Fig. 7; Cesa and Strata, 2009; Rossi and Strata, 1995; Strata and Rossi, 1998). Following a subtotal lesion of the inferior olive by means of 3-acetylpyridine (3-AP), most PCs remain deprived of their CFs. In these conditions, the surviving CF undergo sprouting and innervate the denervated PC (Benedetti et al., 1983; Rossi et al., 1991a; Rossi et al., 1991b) establishing new synapses (Benedetti et al., 1983). A few days after the lesion, a large number of new spines emerge from the PC proximal dendritic domain. The proliferation of spines that follows the CF terminal arbor deletion is accompanied by sprouting of adjacent PFs invading its territory of innervation (Rossi et al., 1991a; Sotelo et al., 1975). Moreover, GluRδ2 becomes expressed in the newly-formed spines innervated both by PFs and CFs (Cesa et al., 2003). After sprouting of CFs, the competitor PFs are confined to the distal dendritic domain and the GluRδ2 disappears from the proximal dendritic compartment (Cesa et al., 2003; Rossi et al., 1991a; Rossi et al., 1991b). This situation seems to recapitulate cerebellar cortex development, when, during an initial phase, the two inputs coexist on the same dendritic portions, a homogeneous distribution of spines occurs and the GluRδ2 is transiently expressed also in CF synapses (Zhao et al., 1998). The capacity of reactive synaptogenesis by the PF input has also been demonstrated following lesion of rat

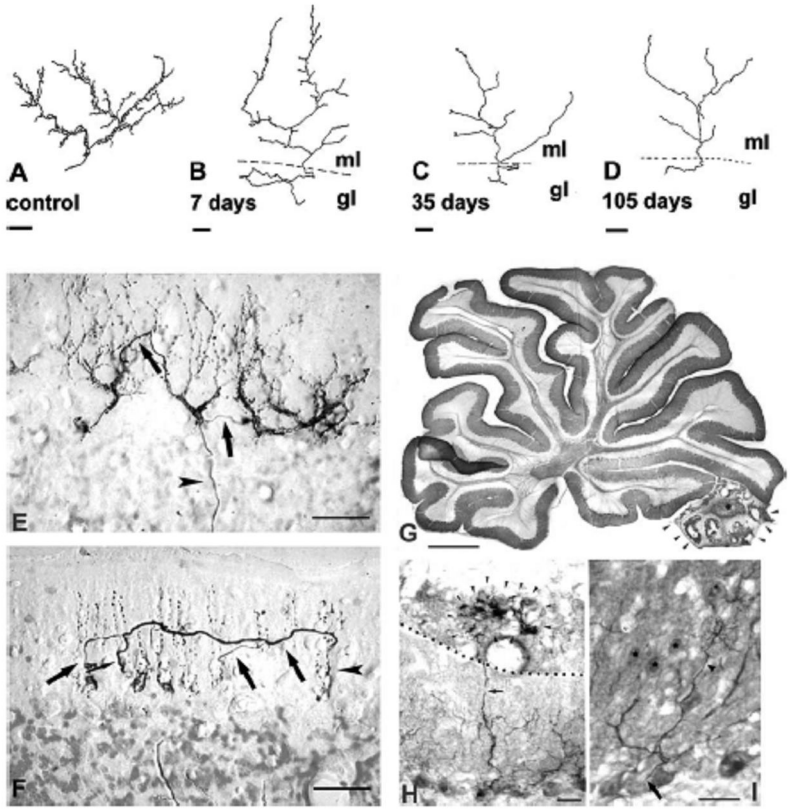

Fig. 7 - Structural plasticity of adult intact climbing fibres following target deletion or expansion.

(A–D) Camera lucida drawing of control and target-deprived CFs (mod. from Rossi et al., 1993). At 7 days after target deletion (B), a conspicuous loss of fine varicose branches already occurs comparing to control (A). The progressive retraction of distal varicose branches is enhanced at later survival times (C and D).

(E and F) Collateral sprouting of CFs after expansion of target territory. Following a subtotal lesion of the inferior olive by means of 3-acetylpyridine (3-AP), most PCs remain deprived of their CFs. Surviving CFs emit collateral sprouting (arrowheads) and reinnervate adjacent PCs, as shown in the sagittal (E) and coronal (F) planes (mod. from Rossi et al., 1991a).

(G–I) Innervation of grafted Purkinje cells by adult intact olivary axons. (G) The low-power micrograph shows the relationship between the graft (arrowheads) and the host cerebellum. (H) The arrow points to a thick CF collateral which runs straight to the host cerebellar surface to enter the transplant and give rise to an arborisation (arrowheads) impinging on Purkinje cells within the graft. (I) An olivary axon (arrow) forms a CF contacting an adult host Purkinje cells. This fibre emits a collateral (arrowheads) that extends on the dendrites of a grafted cell. Other grafted Purkinje cells (asterisks) have migrated in the host molecular layer. (Rossi et al., 1992). Scale bars: A, 30 μm; B, C, 20 μm; D, 25 μm; E and F, 30 μm; G, 1 mm; H 30 μm and I 50μm (Carulli et al., 2004).

peduncles). Also in this case, a few days after the lesion, new spines grow on deafferented proximal PC dendrites and become innervated by PFs (Cesa and Strata, 2005).

Adult olivary axons are able to expand their territory of innervation not only on vacant mature PCs following the loss of adjacent CFs, but also on additional sets of immature PCs (Fig. 7). When embryonic PCs taken from cerebellar primordia are transplanted onto the surface of the adult cerebellar cortex, collaterals of intact CF arbours grow through the molecular layer with moderate branching along the radial Bergmann glia, perforate *pia mater* and innervate the PCs inside the graft. Some grafted PCs which migrate inside the cerebellar cortex are similarly innervated by CF sprouting (Rossi et al., 1994). By electrophysiological recordings it has been shown that the new synapses are fully functional (Gardette et al., 1988; Tempia et al., 1996).

The opposite expression of CF structural plasticity is observed after the experimental deletion of its target neurons or, similarly, after the block of synaptic transmission. After PC degeneration induced by intracerebellar injections of kainic acid, CFs progressively undergo remarkable regressive modifications, consisting in the loss of most of the terminal arborisation and in the sparing of only its proximal thick branches. At about 1 month after the lesion, the total arbor length is 30% of control and only 10% of branches and varicosities are still present (Rossi et al., 1993). Similar results have been observed when PCs are deleted by propidium iodide (Rossi et al., 1993) or in mutant mice undergoing PC degeneration because of genetic mutations (Rossi and Strata, 1995). Interestingly, these regressive modifications are reversed whenever new PCs become available, for example when embryonic PCs are transplanted into the kainic acid lesion (Armengol et al., 1989) or into a mutant cerebellar cortex (Sotelo and Alvarado-Mallart, 1987). The infusion of the AMPA receptor inhibitor NBQX but not the infusion of the mGluR inhibitor MCPG causes a modification of CF boutons consisting of a reduction of their major and minor axes and their ratio (Cesa et al., 2007). These data suggest that CF has bidirectional target-dependent control of its morphology and that synaptic transmission through AMPA receptors (but not mGluR) may be involved, raising the possibility that this trophic relation with the target is dependent on the activity of some cerebellar neurons.

As an expression of the heterosynaptic competition between CFs and PFs, CF remodelling is usually accompanied by opposite PF modifications: after a subtotal lesion of the IO, PF sprout abundantly onto the proximal dendritic territory of the PC (Rossi et al., 1991a; Rossi et al., 1991b; Sotelo

et al., 1975); on the contrary, selectively weakening the PF synapses results in a larger CF innervation area on the PC dendritic tree and persistent multiple CF innervation (Hirai et al., 2005; Kano et al., 1998; Kurihara et al., 1997; Watanabe, 2008).

The block of synaptic transmission not only affects the morphology of CF but also that of PC, inducing spinogenesis on the PC proximal dendrite similarly to that observed after a lesion of IO or a peduncolotomy (Fig. 8). By infusion of tetrodotoxin (TTX), an inhibitor of voltage-gated Na^+ channels, or NBQX for 7 days in the cerebellar parenchyma *in vivo*, the CF terminal arbor loses a high number of synaptic contacts and a large number of new spines appear in the proximal dendritic domain limited to the regions surrounding their presynaptic varicosities (Bravin et al., 1999; Cesa et al., 2007). The CF synaptic loss is accompanied by expansion of the PFs which invade the proximal dendritic domain. Newly formed spines express GluRδ2. This is surprisingly expressed also on those innervated by GABAergic neurons and those still in contact with the CFs (Morando et al., 2001). Thus, in analogy with the acetylcholine receptor clustering during differentiation of the neuromuscular synapse (Yang et al., 2001), it was suggested that GluRδ2 expression is an intrinsic activity-independent property of all PC spines, and independent from the afferent input. After a recovery period of 135 days (4.5 months), GluRδ2s is down-regulated in the spines innervated by CF and the PFs are displaced by CF and confined to the distal dendritic territory. These findings led to the hypothesis that (1) an activity-independent, intrinsic mechanism (Sotelo, 1978; Sotelo, 1990) promotes spine growth over the whole dendritic territory and (2) an activity-dependent spine-pruning action is exerted by the CF in the territory of the target dendrite surrounding its synapses as a kind of lateral inhibition (Cesa et al., 2007). Therefore, active CFs exert a repressive action on the proximal dendritic domain through ionotropic AMPA receptors (Cesa et al., 2007). In addition it was shown that a delayed wallerian degeneration of CFs after the lesion of olivocerebellar axons in Wld^s mutant mice (described in the following sections) was sufficient to delay PC spinogenesis as well, suggesting that some adhesion molecules may also be involved in CF repression of PC spinogenesis (Cesa and Strata, 2005).

It was recently shown that the block of electrical activity affects not only glutamatergic innervation of PC but also inhibitory innervation (Fig. 8). After the block of electrical activity the density and the size of GABAergic terminals that derive from basket and stellate cells increase, expanding their territory of innervation but only to PC proximal dendritic domain.

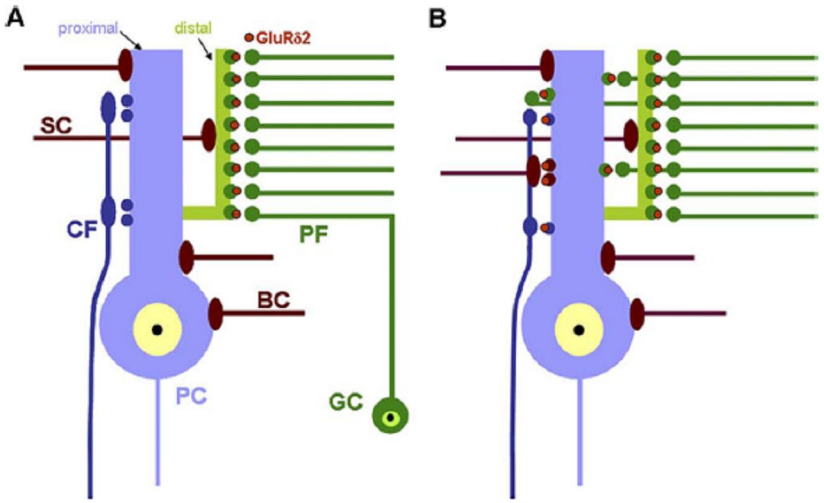

Fig. 8 – Summary of remodelling of the excitatory and inhibitory inputs to the mature PC in control condition and after block of neuronal activity by infusion of TTX.

Schematic illustration of the excitatory and inhibitory inputs to the mature PC in control condition and after TTX. (A) Normally, CF makes excitatory contacts on clusters of spines emerging from the proximal dendritic domain of the PC, whereas PFs form synapses with the spines of the distal compartment. GABAergic inputs from basket and stellate neurons are distributed along the PC somatodendritic region. (B) Block of electrical activity leads to a remarkable remodelling of the PC connections. New spines proliferate on the proximal dendritic domain and while the CF loses many of its synaptic contacts, PFs and GABAergic terminals expand they territory of innervation, GluRδ2 appears in all spines of the proximal dendritic domain, independently of the nature of their presynaptic partners. BC, basket cell; SC, stellate cell; GC, granule cell (Cesa & Strata, 2009).

GABAergic inputs, normally forming only symmetric synapses and only on the dendritic shaft, after TTX infusion innervate the dendritic spines forming not only symmetric synapses but also asymmetric synapses, typically excitatory. The postsynaptic densities of the spines innervated by the GABAergic input also express both excitatory (GluRδ2, AMPA GluR1 and GluR2/3 subunits) and inhibitory receptors ($GABA_A$ α1 and β2/3 subunits) during TTX infusion, coexisting on the same GABAergic-PC ectopic spine synapses (Cesa et al., 2008) The observation that under TTX the PF synapses are devoid of $GABA_A$ receptor labelling suggests that the

expression of these receptors, in contrast to the glutamate receptors, is induced by the GABAergic innervation even in the absence of activity (Cesa and Strata, 2009).

In conclusion, adult CFs, PFs and GABAergic terminals are endowed with a strong intrinsic plasticity This allows synaptic and structural modifications not only in response to lesions, but also to activity-dependent synaptic remodelling. Formation of a large number of new spines occurs after inferior olive lesion when the PC is hyperactive but also after the block of the cerebellar electrical activity, suggesting that this process is set up by neurons independently.

4. Climbing fibre plasticity regenerative properties and intrinsic factors

The plastic properties of mature IO neurons are associated with their basal expression of several growth-associated proteins: GAP-43 (Kruger et al., 1993), the remodelling-related foetal PSA-NCAM (Fernandez et al., 1999), the transcription factor EGR-1/KROX-24 (Herdegen et al., 1995) and the myristoylated alanine-rich C kinase substrate (MARCKS; McNamara and Lenox, 1997). Moreover they are able to respond with a cell body reaction to axonal injury, and can regenerate after axotomy in a permissive environment (Carulli et al., 2004). This does not happen in other neurons, such as PC, which do not undergo axonal regeneration after axotomy and are endowed by weak sprouting capabilities. Olivocerebellar axons undergo to regression after transection, being unable to grow into the surrounding mature cerebellar parenchyma (Bravin et al., 1997; Buffo et al., 1998; Rossi and Strata, 1995; Wehrle et al., 2001). However, the injured axons are able to elongate into transplants of embryonic cerebellar or neocortical tissue and innervate the grafted PCs, forming CF-like structures (Rossi and Strata, 1995). Similarly, injured olivocerebellar axons are able to growth through a transplant of neonatal Schwann cells and to elongate towards PCs (Bravin et al., 1997).

The high basal expression of growth markers was suggested to be a key factor for the low threshold for axon sprouting of olivary neurones. However, it was also suggested that the same genes may be involved in the vulnerability of IO neurons to axotomy, leading to their low survival rate (Carulli et al., 2004).

Transection of the olivocerebellar pathway at the inferior cerebellar pedunculi induces a vigorous cell body response (Buffo et al., 2003; Buffo et al., 1998; Florenzano et al., 2002), leading to a loss of 65% of olivary

neurones at 2 months after axotomy. Coincidentally, IO neurones upregulate several markers: the nitric-oxide synthase (NOS) related to cell injury, transcription factors (c-Jun and JunD) and the growth-associated protein GAP-43, associated with reparative processes. The upregulation of these proteins is sustained for at least 2 months after lesion and correlates with cell survival (Buffo et al., 2003; Buffo et al., 1998).

Although pedunculotomy transects the axons of all contralateral IO, the intensity of the cell body reaction is not uniform throughout the area. Two olivary cell types can be identified within precise sub-areas of the IO: responsive cells, able to intensely activate c-Jun, GAP-43 and NOS, mainly located in the medial accessory olive and in the principal olive, and unresponsive cells in the rest of the IO, which display weak c-Jun staining and no GAP-43 or NOS upregulation,. The pattern of reactive cells was shown to be independent to the distance of the lesion site from the IO cell bodies (Buffo et al., 2003). A similar reaction with the same pattern of gene up-regulation was obtained with a selective immunotoxin-mediated deletion of PC (Buffo et al., 2003). These data confirm the indication that the adult IO has an intrinsic heterogeneity in some cellular properties including the ability of activating growth-related genes.

The high plastic and regenerative properties of CFs are thus associated with a high (although heterogeneous) basal expression of growth-associated intrinsic neuronal factors (the growth-associated proteins GAP-43, MARCKS, the remodelling-related foetal PSA-NCAM, the transcription factors EGR-1/KROX-24) and to the ability to respond to axonal injury or neuronal target deletion by upregulating the expression of genes related to cell injury (NOS) or associated with reparative events (GAP-43, the transcription factors c-Jun and JunD).

2. Structural plasticity in the central nervous system: spontaneous and lesion-induced

1. Spontaneous pre-synaptic and post-synaptic structural plasticity during adulthood

During the last fifty years neuroscience has revealed that the nervous system has developed the ability to dynamically adapt the motor output to the changes of sensory stimuli, to keep memory of such adaptation and to re-establish pathways disrupted during injuries. Abnormalities of such plastic properties have been shown to be involved in many disorders, such as mental retardation (Bagni and Greenough, 2005; Ramakers, 2002). Many efforts have also been made to develop effective methods to enhance the functional recovery of patients with injuries in the CNS such as spinal cord injuries and those caused by stroke (Benowitz and Yin, 2007).

Neuronal plastic properties consist in the ability of the nervous system to modify the efficacy of synaptic transmission and their connectivity with other neurons. This properties can be respectively referred to as functional (or synaptic) plasticity and structural plasticity (Butz et al., 2009).

Functional plasticity comprises any change in the strength of a single synapse without changing the anatomical connectivity between neurons. Classic examples are provided by the well characterized long-term potentiation (LTP) and long-term depression (LTD) of the excitatory post-synaptic current occurring in hippocampus and cerebellum (Ito, 2001; Lynch, 2004). The changes in synaptic strength may occur by insertion or removal of postsynaptic receptors, by changing the presynaptic release of transmitters or the intrinsic excitability of the neurons (Butz et al., 2009; Hansel et al., 2001).

In contrast, any change of the anatomical connectivity between neurons is defined as structural plasticity. This comprises changes in synapse numbers, axonal and dendritic branching patterns, synaptic connectivity patterns, and even neuronal cell numbers (Butz et al., 2009).

Structural plasticity plays a major role during development, when continuously new connections are established and refined. This has been studied in detail in several brain areas and systems, such as in the case of ocular dominance columns of the visual system, activity-dependent pruning of motor fibres during establishment of single innervated motor fibres,

pruning of cerebellar CFs and development of single innervated PCs (Hashimoto and Kano, 2005; Butz et al., 2009)

Even when development is complete structural modifications occur spontaneously, and consist in the dynamic formation, modification and pruning of dendritic spines and axonal fibres (i.e. "spontaneous structural plasticity"; Butz et al., 2009). During the last decade a great attention has been paid to plasticity of dendritic spines, due to the high motility they can express (Holtmaat and Svoboda, 2009). For instance, in barrel cortex only one third of dendritic spines are stable for months, while the others can move within a few days or even in one day. However in other brain regions, such as the visual cortex, a lower rate of motile spines is observed, being 90% of the spines here stable in time, showing that spontaneous structural plasticity may be very variable in the different areas of the CNS (Butz et al., 2009).

Recent studies based on time-lapse imaging performed over time periods ranging from days to months have demonstrated that, during adulthood, also axon terminals can undergo spontaneous structural plasticity (Gogolla et al., 2007). For instance spontaneous synaptic rewiring by axonal turnover takes place in mature cortex within single columns or between one column and its direct neighbours (De Paola et al., 2006; Stettler et al., 2006; Butz et al., 2009). Recently it was shown in cerebellar CFs that spontaneous structural plasticity can vary even within the same axonal arbour, being higher in some types of branches and very subtle in others (Fig. 9; Nishiyama et al., 2007).

Spontaneous structural modifications in the axon can occur not only as motility of the axonal branches but also as changes in the position or shape of the presynaptic boutons. Similarly to what happens to dendritic spines, stable and dynamic boutons (persisting respectively for months or days) can be spatially intermingled along terminal arbours. Moreover their ratio can be different for different neuronal types (Holtmaat and Svoboda, 2009).

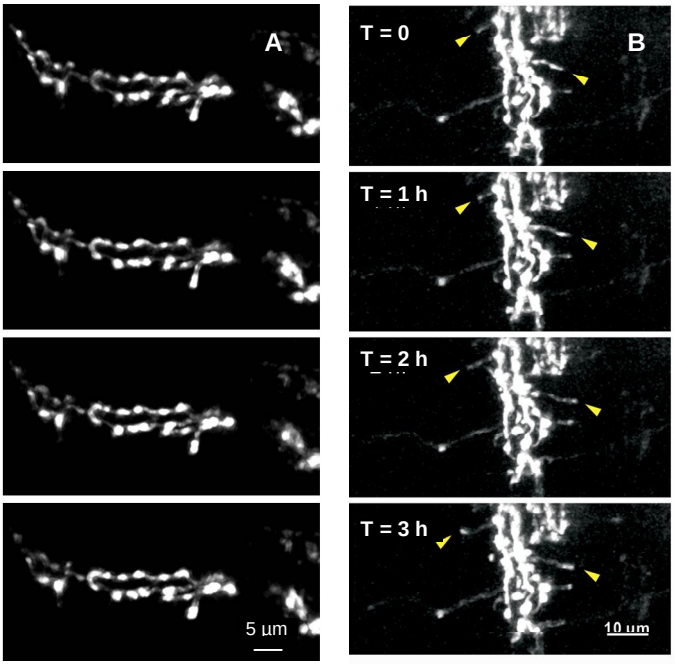

Fig. 9 - Axonal motility in CF specific for it transverse branches.
Frontal optical section of fluorescent mouse CF obtained by two-photon time-lapse microscopy in anesthetised living mice over a 3 h monitoring period. CFs were labelled by a fluorescent dye injected into the IO. A) CF ascending branches and their varicosities do not have any morphological change during the monitoring time. B) On the contrary, on the same monitoring period transverse branches have evident morphological changes. Yellow arrowheads indicate two different transverse branches that undergo elongation (mod. from Nishiyama et al., 2007)

2. Spontaneous structural plasticity, neuronal activity and behaviour

Structural plasticity can be influenced by electrical activity. Depolarization and synaptic transmission may increase the postsynaptic intracellular calcium concentration in dendrites and spines by activating ligand-gated calcium channels or voltage-dependent calcium channels. This may affect dendritic spinogenesis and spine morphology (Jourdain et al., 2003; Lipton and Kater, 1989).

Calcium can also act as a pre-synaptic second messenger, regulating growth cone motility and affecting in this way axonal outgrowth. Axonal

outgrowth is promoted by neuronal activity also by glutamatergic synaptic transmission (Butz et al., 2009).

Stabilization of immature axonal branches is further regulated by competition among growing axons for postsynaptic targets as well as competition between growing branches of the same axon (Huang and El-Husseini, 2005; Butz et al., 2009).

In the adult brain circuit changes mediated by structural plasticity and accompanied by synapse formation and elimination are thought to underlie aspects of long-term memory formation (Bailey and Kandel; Holtmaat and Svoboda, 2009)

A wealth of studies has addressed the problem of how experience may change cortical connectivity (Zuo et al., 2005; Holtmaat et al., 2006; Keck et al., 2008; Hofer et al., 2008). Experience-dependent cortical remapping is not only the result of a mere functional change in connectivity (i.e. by synaptic plasticity such as LTP and LTD) but is also associated with structural changes in terms of synapse formation and deletion (Butz et al., 2009).

Many studies showed for instance an increase in dendritic spines following synaptic activation or spatial learning. Experiments based on Golgi staining method have revealed that environmental enrichment (Volkmar and Greenough, 1972), extensive training (Kolb et al., 2008), stress levels (Magarinos et al., 1996) and abuse of drugs (Robinson et al., 2001; Robinson and Kolb, 1999) all might have profound influences on the complexity of dendritic arbours in some cortical areas (Holtmaat and Svoboda, 2009). In the adult brain spine and synapse density can change upon manipulation of sensory experience (Moser, 1999). Increased spine and synapse densities have been reported after rearing or training in enriched environments (Beaulieu and Colonnier, 1987; Greenough et al., 1985; Kolb et al., 2008; Moser, 1999; Moser et al., 1994), and also after long-term sensory stimulation (for example, Knott et al., 2002) and deprivation (for example, Zuo et al., 2005).

Furthermore in the hippocampus there are indications that axonal sprouting occurs during spatial learning (Holahan et al., 2009; Ramirez-Amaya et al., 2001) or in response to environmental enrichment (Galimberti et al., 2006; Holtmaat and Svoboda, 2009).

3. Lesion-induced structural plasticity

While the spontaneous structural plasticity underlying homeostatic and activity-dependent remodelling in the adult brain may be subtle and occurs

in a limited extension or in a limited portion of neuronal elements, structural plasticity following a lesion (i.e. “lesion-induced” or “reactive” plasticity) may be dramatic. For instance in mature primary sensory cortices the gross morphology of neurons and their circuitry does not change dramatically under normal conditions. However, following an injury, even areas that are regarded as stable such as primary sensory-motor areas can express profound reactive plasticity (Butz et al., 2009).

Reactive sprouting of intact fibre tracts can reoccupy postsynaptic targets left vacant by a lesion. The first example was provided by lesioning two afferent fibre systems of the septal nuclei complex: the hippocampal fimbrial fibres, which provide input to the septal nuclei, and the medial forebrain bundle, which provides hypothalamic input to the septal nuclei (Raisman, 1969; Butz et al., 2009). A clear example of reactive plasticity was provided by cerebellar CF, thanks to the simple planar organization of PC dendrite and to the fact that each PC is innervated by single CFs (Rossi et al., 1991b).

Reactive plasticity may even modify laminar distribution patterns of afferent fibres suggesting the possible competition between different afferent fibres impinging on different dendritic territories. In the hippocampus, for example, commissural and associative fibres (Frotscher et al., 1995; Lynch et al., 1973; Nadler and Cotman, 1978) terminate in the inner molecular layer of the dentate gyrus. After cutting the perforant path terminating in the outer molecular layer, also commissural and associative fibres sprout and make contact with targets in the outer molecular layer (Lynch, 1974; Nadler and Cotman, 1978). In the cerebellar cortex, either after pharmacological sub-total lesion of IO or by complete surgical deafferentation of cerebellar pedunculi, a similar expansion of PF innervation territory is observed, forming new synapses on PC proximal dendritic portion, normally innervated only by the CF (Carulli et al., 2004). Deafferented neurons within the lesion projection zone seem to change their receptive field properties (Giannikopoulos and Eysel, 2006), since they significantly increase their number of unstable spines and additionally form novel stable spines (Keck et al., 2008). In addition, neurons bordering the lesion projection zone show signs of axonal sprouting following the lesion, possibly due to disinhibition (Antonini et al., 1999; Antonini and Stryker, 1996). This can be similarly observed in the cerebellar cortex, where the loss of CF afferents leads to the formation of a high number of spines on PC proximal dendritic arbor (Sotelo et al., 1975).

4. Axonal retraction and degeneration

Structural plasticity involving presynaptic elements can in general involve the synaptic boutons, their turn-over, remodelling and movements (Gogolla et al., 2007; Holtmaat and Svoboda, 2009). More extensive modifications involve entire axonal portions.

There are two opposite mechanisms through which sections of axons are removed: retraction and degeneration (Low and Cheng, 2005; Luo and O'Leary, 2005). In retraction, the axonal process is gradually pulled backward, and the corresponding axonal material is transported back to more proximal sections of the axon. In contrast, degeneration involves rapid blebbing, and fragmentation of an entire axonal stretch into short segments, which are then removed by phagocytic cells such as locally activated glia or macrophages. Both processes are efficient and tightly regulated, and both can be followed by regrowth of the axon from its proximal end. However, although their ultimate outcome is similar, the two processes differ in that all cellular material, including intracellular signalling organelles (e.g. signalling endosomes), is conserved inside the neuron in retraction, whereas degeneration involves shedding of the axonal material, and its uptake by local phagocytes. Axon retraction has only been documented for modest local axon shortening events during axon outgrowth, whereas larger removal events all appear to involve axon degeneration (Saxena and Caroni, 2007).

When are axon segments eliminated by retraction and when by degeneration? As a simple rule, retraction seems to involve the elimination of shorter branches, whereas degeneration tends to affect longer axonal segments (Low and Cheng, 2005; Luo and O'Leary, 2005; Saxena and Caroni, 2007). During development axonal pruning has a major role to define the right nervous pathways. A large-scale axonal elimination occurs for instance in defining the right target of layer V cortical neuron axons, after having reached several distant target areas. On the contrary, a small-scale axonal pruning occurs to defining connections such as in the retino-geniculo-cortical pathways. A form of axonal elimination occurs also locally to define monoinnervated targets, by maintaining only one afferent axon among several competing for the same target. This happens in a similar way in the development of neuromuscular junctions and in the establishment of the CF-PC connectivity in the cerebellum (Crepel and Mariani, 1976; Hashimoto and Kano, 2005; Saxena and Caroni, 2007).

Most neurodegenerative diseases including Alzheimer’s, Parkinson’s, Huntington’s, motoneurons diseases (such as familiar amyotrophic lateral sclerosis), and prion diseases involve protracted gradual loss of distal

synapses and axons that can precede the death of neuronal cell bodies by months (Saxena and Caroni, 2007). In these pathologies an axonal "dying back" is observed, similar to that caused by toxic indults such as heavy metals, acrylamide, the anticancer drug vincristine, the antibiotic nitrofurantoin. It is not clear if it is due to a real axonal degeneration rather than axonal retraction.

Traumatic injuries cause the degeneration of the distal axonal portion referred to as Wallerian degeneration (Waller, 1850). The discovery of a slow Wallerian degeneration mouse mutant (Wld^s), in which the distal stump survives up to 3 weeks instead of few days, showed that this is an active process rather than just caused by nutrients deprivation (Lunn et al., 1989). The Wld^s dominant mutation consists of a fusion sequence encoding a chimeric protein composed by the N-terminal 70 aminoacids of the ubiquitin ligase of UFD2/E4 and the full-length NAD^+ synthesizing enzyme NMN adenylyltransferase 1 (Nmnat1), linked by a 18 amino acids region (Coleman et al., 1998; Conforti et al., 2000; Lyon et al., 1993; Mack et al., 2001). The protein has a predominant nuclear localization. The N-terminal domain of Wld^s influences the intranuclear location of the ubiquitin proteasome as well as its intrinsic NAD^+ synthesis activity (Wilbrey et al., 2008). In the Wld^s mutation, part of the protective effect could arise from the over-expression of Nmnat1 with a consequent increase in NAD^+ levels (Araki et al., 2004). It was also shown that SIRT1, a NAD^+-dependent histone deacetylase, is a downstream effector of Nmnat1 activation that leads to axonal protection. Differential proteomics analysis on isolated synaptic preparations from the striatum in mice has revealed 16 proteins with modified expression levels in Wld^s synapses (Wishart et al., 2007). Wld^s bind directly to vasolin-containing protein (VCP/p97), a protein with a key role in the ubiquitin proteasome system (Laser et al., 2006; Hilliard, 2009).

The molecular mechanism underlying axonal retraction and degeneration is poorly understood. However it was shown that, during axonal retraction, the microtubule cytoskeleton remains intact (Yamada et al., 1970) and the activation or disinhibition of RhoA pathway leads to an activation of myosin-based machinery of actin filament cytoskeleton retraction through the serine-threonine Rho kinase ROCK (Ahmad et al., 2000; Billuart et al., 2001; Eaton et al., 2002; Luo, 2002). Given the potential similarities between repulsive axon guidance and axon retraction, repulsive guidance molecules such as ephrins, Slits, or Semaphorins, all of which signal through RhoA in the context of axon guidance or inhibition of axon growth, could also signal axon retraction (Luo, 2002; Luo and O'Leary, 2005).

On the contrary, during degeneration the microtubule cytoskeleton is disrupted, axon undergoes blebbing, possibly due to the block of axonal transport, and axonal fragments are removed by phagocytes. In several models it was shown that the ubiquitin-proteasome system is involved in this process, as well as target derived neurotrophic factors. Axonal degeneration is thus a self-destruction process reminiscent in its logics and significance of apoptosis (Luo and O'Leary, 2005; Saxena and Caroni, 2007).

5. Axonal growth, regeneration and sprouting

Under specific intrinsic and environmental permissive conditions, which may be neuron type-specific, following a lesion an axon may regenerate the lost portion by axonal elongation or may generate new portions by sprouting.

After axotomy, while the distal segment of the severed axon undergoes Wallerian degeneration, the proximal fragment attached to the cell body may degenerate in a dying back fashion or attempt to reconnect with its target by regeneration. This can happen through regrowth of the axonal proximal fragment or through sprouting. However, following nerve injury, functional recovery can also occur through other mechanisms, for example by plasticity of surrounding neurons (Hilliard, 2009).

In humans and other mammals, axonal regeneration occurs in the PNS while it is reduced or absent in the CNS. Axonal regeneration fails mainly due to two causes: a non-permissive external environment and neuronal intrinsic factors. A large body of work in vertebrates has revealed the crucial inhibitory role that the glia play in the regenerative ability of a CNS axon following injury. Several myelin-associated inhibitors have been discovered, including Nogo, myelin-associated glycoprotein, oligodendrocyte myelin glycoprotein, ephrin B3 and the transmembrane semaphorin 4D/CD100. An additional important source of inhibition comes from the glial scar. Here, reactive astrocytes, recruited to the site of injury, secrete inhibitory extracellular matrix molecules such as chondroitin sulphate proteoglycans (Carulli et al., 2006). In addition to extracellular elements, numerous studies have revealed the crucial role that neuronal intrinsic factors play in determining the regenerative ability of a neuron. An example of this lies in the inability of some injured axons to regrow even if placed in a permissive environment. Important findings have shown that pre-conditioning nerve lesions generated days in advance can influence and enhance the regenerative potential of a subsequent lesion of the same nerve (McQuarrie and Grafstein, 1973). One mechanism underlying the increased axonal regrowth appears to be based on cAMP levels. In fact, increased axonal

regrowth can be induced with pre-treatment of the DRG neurons with a membrane-permeable cAMP analog (dibutyryl cAMP; db-cAMP), which elevates the intracellular concentration of cAMP in these cells (Benowitz and Yin, 2007; Hilliard, 2009).

6. Regeneration in the CNS

The failure of axotomised central neurons to undergo axon elongation is related to two main elements. The first is the poor intrinsic ability of the axon to regenerate and respond to growth-promoting cues. The second consists in several extracellular factors present in the environment of the damaged central nervous system that do not support regeneration or prevent it. What is clear, at present, is that the ability to regenerate is highly variable among different neuronal phenotypes (Carulli et al., 2004). At least some adult central nervous system neurones retain the ability to reactivate specific machineries necessary to elongate axons, to decode guiding cues and to form functional synapses when provided with a permissive environment.

However, regeneration can only occur in neurons able to respond to a lesion by activating a specific cell body response, which includes the activation of specific growth-associated genes (Caroni et al., 1997; Herdegen et al., 1997; Skene and Virag, 1989). Among the known growth-associated genes are found immediate early genes and transcription factors, such as c-Jun, JunD, KROX-24; growth-cone components, such as GAP-43 and CAP-23; cytoskeletal proteins, such as tubulin and actin isoforms; cell adhesion and other cell surface molecules, such as integrins, NCAM, L1 (Anderson et al., 1998; Caroni et al., 1997; Werner et al., 2000).

Furthermore, correlative evidence suggests that the molecular machinery leading to axon growth and cell death might be closely linked. Yet, the high propensity of a lesioned neurons to undergo cell death has to be considered as part of a vigorous cell body reaction. In fact, in several neuronal populations, severe neuronal degeneration following axotomy is also associated with strong gene upregulation and axon regeneration into permissive territories (Doster et al., 1991; Herdegen et al., 1997; Tetzlaff et al., 1991; Villegas-Perez et al., 1988). Both c-Jun and GAP-43 were not only associated with axonal regeneration but also with neuronal degeneration (Aigner et al., 1995; Buffo et al., 1997; Eilers et al., 1998; Gagliardini et al., 2000; Ham et al., 1995; Hull and Bahr, 1994; Wehrle et al., 2001).

The first evidence for the axon growth-inhibiting properties of the central nervous system environment comes from cell tissue culture experiments, which revealed strong inhibitory influences by central nervous system

myelin on neurite outgrowth, whereas peripheral nervous system myelin proved to be a good substrate for growth (Schwab and Thoenen, 1985). These experiments introduced the concept of myelin-associated neurite growth inhibitors. To date, three inhibitory components of myelin have been identified: myelin-associated glycoprotein (MAG; Filbin, 2003; McKerracher et al., 1994; Mukhopadhyay et al., 1994), Nogo (Chen et al., 2000; GrandPre et al., 2000; Prinjha et al., 2000; Savio and Schwab, 1989), and, most recently, oligodendrocyte-myelin glycoprotein (OMgp; Kottis et al., 2002; Wang et al., 2002). Although distinct in molecular structure, these proteins share a number of common attributes, such as their localisation in the myelin membrane directly adjacent to the axon. Moreover, they bind to a common receptor, the Nogo66 receptor (NgR), and may therefore act via a common signalling cascade (Filbin, 2003; McGee and Strittmatter, 2003; McKerracher and Winton, 2002; Schwab, 2002).

The injury response of the central nervous system is usually called reactive gliosis or glial scarring and involves several cell types, including astrocytes, microglia, oligodendrocyte precursors and meningeal cells. Central axons fail to regrow through regions of scar formation, which contains a number of different growth-inhibiting molecules, i.e. proteoglycans and tenascins (Fawcett and Asher, 1999; Morgenstern et al., 2002).

3. The growth-associated protein GAP-43

1. Expression in the CNS and relatioship with neuropathologies

The neuronal growth-associated protein GAP-43 (also known as neuromodulin, B-50, P-57, F1, F-57 and pp46) was initially found to be associated with nerve growth (Skene and Willard, 1981; Zwiers et al., 1976) and its gene was subsequently cloned (Basi et al., 1987; Oestreicher et al., 1997). It is rarely expressed in non-neuronal tissues and is therefore considered to be a nervous tissue-specific protein. (Mosevitsky, 2005; Oestreicher et al., 1997).

During development GAP-43 is abundantly expressed throughout the brain, but it decreases in most neurons when the axon has reached its target, with a few exceptions. Among these, a robust expression of GAP-43 mRNA is detectable in some neurons of the olfactory bulb, hypothalamus, hippocampus (particularly in the CA3 region), cerebellar cortex (only in granule cells) and in the IO nucleus (Fig. 10). In some neuronal types, such as hippocampal granule cells, PCs and inhibitory interneurons in the cerebellar cortex, GAP-43 seems to be completely absent (Kruger et al., 1993; McNamara & Lenox, 1997).

The functional relevance of GAP-43 is underlined by the low-survival rate observed during early postnatal period (< 5%) in homozygous knock-out mice (Maier et al., 1999; Strittmatter et al., 1995). In humans, heterozygote deletions of the locus comprising GAP-43 gene (3q13.10–3q13.21) were found to be associated with agenesis of the *corpus callosum*, together with severe mental retardation (Genuardi et al., 1994; Mackie Ogilvie et al., 1998).

The neuronal expression of GAP-43 is up-regulated *in vivo* in many conditions which are known to induce structural remodelling of neuronal connectivity: for instance, conditions which disrupt nervous system architecture such as nerve lesions, brain traumas or brain ischemia (Benowitz et al., 1990; Buffo et al., 2003; Oestreicher et al., 1997) or conditions which induce a robust neuronal activity, due, for example, to kainic acid administration, pilocarpine-induced seizure or electrical stimulation (Cantallops and Routtenberg, 1996; McNamara and Routtenberg, 1995; Miyake et al., 2002; Sharma et al., 2009). For this reasons the up-regulation of GAP-43 expression has been commonly used as a marker of structural plasticity and axonal remodelling (Oestreicher et al., 1997).

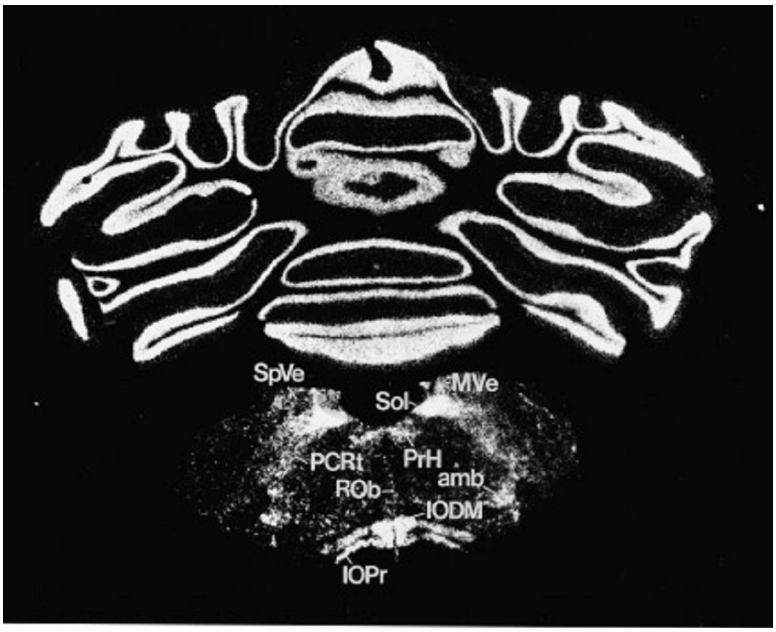

Fig. 10 – *In situ* hybridization of GAP-43 in cerebellum and brain stem.
Autoradiograph showing expression of GAP-43 mRNA in a coronal section of cerebellum and brain stem. Note the high expression level in the granular layer of cerebellar cortex and in the inferior olivary nucleus (IO) in the brain stem. IO, inferior olive nucleus; IODM, inferior olive dorso-medial nucleus; IOPr, inferior olive principle nucleus; MVe, medial vestibular nucleus; PCRt, parvocellular reticular nucleus; PrH, prepositus hypoglossal nucleus; ROb, raphe obscurus nucleus; SpVe, Spinal vestibular nucleus; Sol, solitary tract nucleus, Sp5I, spinal trigeminal nucleus, amb, ambiguous nucleus (McNamara & Lenox, 1997).

Alterations of GAP-43 expression pattern has been frequently observed also in human neuropathologies and their animal models, such as Alzheimer's disease, amyotrophic later sclerosis, epilepsy, diabetic neuropathy and schizophrenia (Oestreicher et al., 1997).

2. Role in axonal outgrowth and guidance

GAP-43 is localized at high concentrations at axonal growth cones in cultured neurons but virtually absent in dendrites after the end of

development period (Meiri et al., 1986; Nozumi et al., 2009; Oestreicher et al., 1997; Van Lookeren Campagne et al., 1990).

The involvement of GAP-43 in filopodia formation, axonal outgrowth and guidance was shown in many studies based on gene depletion or over-expression of its wild-type or mutated variants in cultured cells and transgenic mice (Mosevitsky, 2005; Oestreicher et al., 1997).

In primary dorsal root ganglion neurons plated on laminin silencing GAP-43 by anti-sense oligonucleotides leads to longer, less branched neurites with smaller growth cones. When these neurons are plated on poly-L-ornithine, no growth cone or neurite formation can be observed (Aigner and Caroni, 1993). In these conditions primary sensory neurons lose the adherence of their growth cones and the stability of their lamellar extensions. Moreover, NGF-induced spreading and IGF1-induced branching was also impaired (Aigner and Caroni, 1995).

Consistently with such evidence, the overexpression of GAP-43 *in vivo* leads to a dramatic increase of axonal sprouting. This was shown in several conditions. Motor nerves at the neuromuscular junction in transgenic mice overexpressing chicken GAP-43 under the control of Thy-1 promoter have a number of spontaneous terminal nerve sprouts in normal conditions and lesion-induced sprouting is enhanced if compared to wild-type. Moreover the same mice have a prominent spontaneous sprouting of mossy fibres in the dendate gyrus (Aigner et al., 1995). Similarly, enhancing GAP-43 expression in cultured cortical neurons accelerates formation of neurites (Anderson et al., 2001; Watterson et al., 2002). In transgenic mice, in which GAP-43 was overexpressed under the control of the PC-specific L7 promoter, PC axons profusely sprouted along the axon and at the axonal stump after lesion, even in portions covered by myelin (Buffo et al., 1997; Gianola and Rossi, 2004). Several studies reported that GAP-43 overexpression is sufficient to induce the formation of filopodia in several non-neuronal cell lines, although some conflicting results were obtained too. This was explained by the substantial difference in the cellular machinery expressed in non-neuronal cells, lacking, for instance, the molecules necessary for axonal guidance (Gauthier-Campbell et al., 2004; Mosevitsky, 2005; Oestreicher et al., 1997).

Several other works showed that, although GAP-43 overexpression is sufficient to induce neurite formation and axonal sprouting suggesting a promoting role on these phenomena, it is not necessary *per se* for axonal elongation. On the contrary, it was suggested to be necessary for a proper response of the growth cone to guiding cues and for their adhesion.

Embryonic dorsal root ganglia cells obtained from homozygous GAP-43 null mice extend similar axons and produce growth cones with similar morphological features to wild-type control. However the axons of their retinal ganglion cells fail to properly cross the optic chiasm (Strittmatter et al., 1995). These fibres follow abnormal trajectories within the chiasm and exit randomly into the ipsilateral or contralateral side (Sretavan and Kruger, 1998). A following study revealed that homozygous GAP-43 knock-out mice fail to form not only a normal optic chiasm but also the anterior commissure, the hippocampal commissure and *corpus callosum* (Shen et al., 2002). This is consistent with the agenesis of the *corpus callosum* observed in patients bearing a heterozygote deletions of GAP-43 locus (Genuardi et al., 1994; Mackie Ogilvie et al., 1998). Interestingly GAP-43 phosphorylation by PKC on Ser-41 in growth cones can be stimulated by Ig superfamily cell adhesion molecules, such as L1, that have been implicated in commissural axon guidance (Demyanenko et al., 1999; Kamiguchi et al., 1998).

From these observations it was proposed that GAP-43 is not essential for axonal growth, but it is necessary for a proper response of the growth cone to guiding cues. Accordingly to this idea, it was recently reported that in the hippocampus of transgenic mice overexpressing a mutant form of GAP-43, which can not be phosphorilated, mossy fibre growth ectopically to their normal target layer, innervating the distal stratum oriens (Holahan et al., 2009) in a way which is similar to that observed in mice with a deletion for the gene of NCAM or with its conditional ablation (Bukalo et al., 2004; Cremer et al., 1997).

3. Role in neurotransmitter release, long-term plasticity and memory

Several studies revealed that GAP-43 not only has a role in axonal growth and guidance but also in neurostransmitter release and long-term synaptic plasticity (Biewenga et al., 1996; Denny, 2006; Powell, 2006). The addition of anti-GAP-43 antibodies to permeabilized rat cortical synaptosomes blocked the phosphorylation of GAP-43 and the calcium-induced secretion of norepinephrine (Dekker et al., 1989). Inhibition of PKC, which phosphorilates GAP-43 at Ser-41, blocked dopamine release induced by amphetamine in rat striatal slices (Kantor and Gnegy, 1998), and LTP does not occur (Lovinger et al., 1986; Routtenberg et al., 2000). Interestingly at high concentration of Ca^{2+} (100-200μM), similar to those required for synaptic vesicle fusion, GAP-43 interacts directly with components of the synaptic machinery such as SNAP-25, sintaxin and

VAMP (Haruta et al., 1997). In addition, in conditions of high $[Ca^{2+}]_i$ or in the absence of the effector domain bound by calmodulin, GAP-43 interacts with rabaptin-5 (an effector of the GTPase Rab5), involved in membrane fusion during endocytosis, thus modulating endocytosis and synaptic vesicle recycling (Neve et al., 1998).

Several studies has revealed that LTD and LTP are associated respectively with a decrease and an increase of GAP-43 phosphorilation by PKC at Ser-41 in hippocampus indicating a possible role in learning and memory (Gianotti et al., 1992; Ramakers et al., 1995; Ramakers et al., 2000; Ramakers et al., 1999).

Furthermore heterozygous mice for GAP-43 deletion expressing a lower amount of protein have an impairment in learning and memory tasks (Rekart et al., 2005). Consistently, the overexpression of GAP-43 in transgenic mice leads to an enhancement of learning in several learning paradigms in a way which is dependent on the phosphorilation site of GAP-43. Moreover it was shown in transgenic mice overexpressing GAP-43 isoforms mutant at their PKC phosphorilation site, either mimicking phosphorilation or preventing it, that phosphorilation at this site regulates long-term forms of synaptic plasticity and the performance of memory-associated tasks (Holahan and Routtenberg, 2008; Holahan et al., 2009; Hulo et al., 2002; Routtenberg et al., 2000). For instance, transgenic mice overexpressing a GAP-43 sequence with its Ser-41 mutated to Asp (S41D), which mimics phosphorilated GAP-43, had an enhanced LTP when compared to wild type mice (Routtenberg et al., 2000). Such impairment in learning and memory has been recently attributed, at least partly, to the abnormal hippocampus architecture reported in these transgenic mice (Holahan et al., 2009).

4. Regulation of gene expression

GAP-43 gene in humans as well as in mice contains three exons, with the first exon coding for only the N-terminal 10 amino acids of GAP-43, while the second exon contains most of the coding region, and includes the residue phosphorylated by protein kinase C (Ser-41; Benowitz and Routtenberg, 1997). The expression of GAP-43 gene was found to be regulated by basic helix-loop-helix transcription factors, acting on a promoter containing several E-boxes (Chiaramello et al., 1996). A cis-acting element was found to contribute to GAP-43 neuron-specific gene expression, which is common also to SNAP-25 and nitric oxide synthase.

GAP-43 is regulated not only at the transcriptional level but also at the level of mRNA stability. The 3'-untranslated region of its mRNA is bound

by the protein HuD, belonging to the protein family ELAV (embryonic lethal abnormal vision; Mobarak et al., 2000) The binding of HuD to GAP-43 mRNA regulates its stability thus influencing GAP-43 protein level. HuD role on mRNA stability is dependent on PKCα and on the length of the mRNA poly(A) tail length (Denny, 2006). HuD, GAP-43 mRNA and ribosomes have been detected in axonal growth cones (Smith et al., 2004), suggesting that its local protein synthesis in axon may occur, contributing to provide locally growth-related proteins that sensitize the growth cone to guidance cues (Denny, 2006; Goldberg, 2003).

5. Protein structure and association with plasma membrane

Rat GAP-43 is a 226 amino acids protein (Basi et al., 1987). Its real molecular weight is of about 25 kDa, much lower than the apparent one of 43 kDa observed on SDS-polyacrylamide gels, probably because of the high number of charged residues present in the protein sequence, and the consequent lower amount of SDS bound by the protein (Benowitz and Routtenberg, 1997). It contains indeed 30 Lys, 5 Arg, 3 His, 35 Glu, and 22 Asp. Moreover the number of Pro is larger than average, contributing to the reduced organization in secondary structure elements (Hayashi et al., 1997). Consistently to the idea that GAP-43 is mainly unfolded, GAP-43 is cleaved by 20S proteasome, where folded proteins are too large to enter (Denny, 2004).

The charged residues and the post-translational modifications of GAP-43 protein are responsible for its physical interaction with the plasma membrane. The protein is palmitoylated at Cys-3 and Cys-4. Its N-terminal 10-11 amino acids are sufficient to target a report protein to the membrane. Palmitoylation is necessary for the initial step of targeting the protein to the plasma membrane. After that the basic residues in its N-terminal domain (Arg-6, Arg-7, and Lys- 9) are sufficient to maintain the association with the plasma membrane. It was suggested that palmitoylation facilitates the interaction of these basic residues with the plasma membrane bringing them into alignment with it. GAP-43 interaction with phospholipids is not only due to its N-terminal domain but also to the region called effector domain or IQ domain (Chapman et al., 1991; Laux et al., 2000). This region, rich in basic residues, interacts with the acidic phospholipid phosphatidylinositol 4,5-bisphosphate (PIP_2; Laux et al., 2000).

It was also suggested that palmitoylation may be regulated by extracellular signals, playing a role in the regulation of GAP-43 action,

similarly to what happens for PSD-95 (El-Husseini Ael et al., 2002; Milligan et al., 1995; Mumby, 1997; Skene and Virag, 1989).

6. *Localization at axon, growth cone and filopodia*

GAP-43 palmitoylation has also a role in targeting the protein to the axon, being sufficient to redirect to it dendritic proteins such as PSD-95 (El-Husseini Ael et al., 2001; Huang and El-Husseini, 2005; Kang et al., 2008).

It was proposed that GAP-43 palmitoylation and association with the membranes occur early in the secretory pathway, not later than at the trans-Golgi network (TGN; Bonatti et al., 1989). Prior to its sorting to the plasma membrane, GAP-43 becomes part of lipid rafts, rich in cholesterol and sphingolipids and detergent-resistant after the extraction of membranes (Arni et al., 1998; Brown and London, 2000; McCabe and Berthiaume, 2001). After association with membranes at trans-Golgi, GAP-43 travels along the axon with them by fast axonal transport, similarly to what happens for SNAP-25 (synaptosomes-associated protein of 25 kDa; Gonzalo and Linder, 1998).

It then reaches growth cone plasma membrane, where it accumulates at high concentration (estimated at 50-100 μM; He et al., 1997). Here it is associated with the formation of filopodia, which are thin projections that are considered to be the first phase in the process of neuronal branching (Gallo and Letourneau, 2004; Steketee and Tosney, 2002)

7. *Molecular interactions and regulation by calcium and protein kinase C*

GAP-43 has no enzymatic activity but it can interact with plasma membrane and several cytoskeletal and signaling proteins, influencing the conformation of bound proteins and modifying local composition of membranes (Fig. 11; Denny, 2006; Mosevitsky, 2005). In its domain called effector or IQ domain it interacts with calmodulin and it is phosphorilated by PKC at Ser-41 (Chapman et al., 1991; Spencer et al., 1992). In addition the binding with calmodulin has a negative effect on the association with vesicles in vitro (Gonzalo and Linder, 1998). Moreover, PKC-mediated phosphorylation introduces a negatively charged phosphate group that eliminates the binding to PIP_2 (Laux et al., 2000) and calmodulin (Chapman et al., 1991), and changes the interaction of GAP-43 with actin filaments (He et al., 1997). It was thus suggested that binding of GAP-43 to calmodulin occurs mainly in the resting cell. In support to this idea, crosslinking experiments showed that GAP-43 is bound to calmodulin under conditions

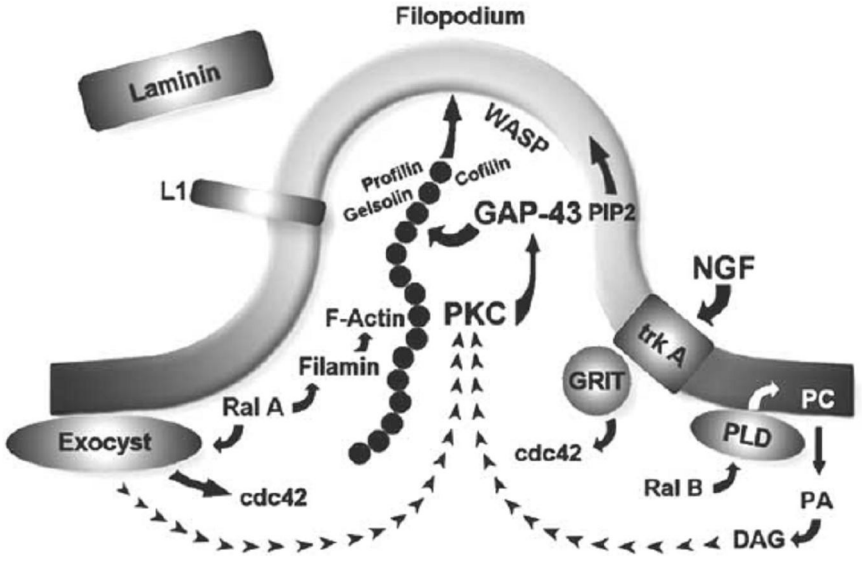

Fig. 11 - Summary of molecular pathways involved in GAP-43 promotion of filopodial formation.

The axonal growth cone plasma membrane is shown protruding as a filopodium begins to form. The three actin-binding proteins, gelsolin, profilin, and cofilin, shown near the F-actin filament, promote filament dynamics when they are not inactivated by being bound at the plasma membrane to phosphatidylinositol 4,5-bisphosphate (PIP2), because prior to local stimulation PIP2 is bound to GAP-43, as shown. Upon local stimulation, which in this case is the binding of nerve growth factor (NGF; on the right), a series of events are set into motion. RalB contributes indirectly to the activation of PKC by activating phospholipase D (PLD), which in turn cleaves the phospholipid phosphatidylcholine (PC) providing the substrate for the production of diacylglycerol (DAG).

PKC phosphorylates GAP-43 at Ser-41, releasing it from PIP2 and allowing it to potentially act as a lateral stabilizer of the F-actin. The released PIP2 diffuses in the membrane, promoting the adhesion of the F-actin with the membrane. Additionally it promotes actin polimarization via cdc42, the recruitment of the Arp2/3 complex and the activation of WASP (not shown). For simplicity, the binding of GAP-43 to calmodulin is not shown. Upon phosphorylation by PKC, GAP-43 would be released from calmodulin, as it is from PIP2. Actin polymerization is additionally induced by the activation of cdc42 via RalA and trkA pathway (Denny, 2006).

of low calcium but it is released when calcium concentration is increased or when GAP-43 is phosphorylated by protein kinase C (Gamby et al., 1996).

This suggests that GAP-43 role in promoting filopodia extension and association with the membrane may be inhibited by the binding with calmodulin in resting conditions or in subcellular domains with a low $[Ca^{2+}]_i$. The increase of $[Ca^{2+}]_i$ and/or the phosphorilation by PKC release the binding by calmodulin and enable the binding with membrane. In other words an inhibitory action is played by calmodulin in resting condition and an opposite role is played by phosphorilation and increase in $[Ca^{2+}]_i$ by removal of the inhibition on GAP-43.

GAP-43 was also proposed to bind directly to actin filaments (Hartwig et al,1992; He et al,1997). When phosphorylated at Ser-41, GAP-43 binds actin with a higher affinity and appears to act as a lateral stabilizer of actin filaments. This favours filopodial extension (He et al., 1997).

A crucial role in controlling GAP-43 action was proposed to be played by its binding to PIP_2 as well, occurring in its non-phosphorilated form. This binding was suggested to mask and inhibit PIP_2 microdomain under resting conditions. In response to PKC phosphorilation PIP_2 would be released by GAP-43 (Laux et al., 2000). Free PIP_2 would in turn sequester actin-binding proteins such as profilin, cofilin, and gelsolin, resulting in the promotion of growth and stabilization of peripheral actin. In addition, free PIP_2 could bind vinculin and WASP proteins, which promote focal contact formation, Arp2/3 function and actin recruitment (Laux et al., 2000).

A structural role of GAP-43 was also proposed, as it was shown to interact not only with actin but also with brain spectrin, another cytoskeletal protein (Riederer and Routtenberg, 1999). Brain spectrin (also known as fodrin, calspectin, or actin binding protein I) is one of the major cytoskeletal proteins associated with the plasma membrane, together with actin, tubulin and others, such as α-actinin and synapsin I (Goodman et al., 1995; Meiri and Gordon-Weeks, 1990). Since GAP-43 binds actin filaments (Hens et al., 1993) and brain spectrin binds the other cytoskeletal elements such as microtubules and neurofilaments (Hens et al., 1993; Riederer et al., 1986), the selective binding of GAP-43 to brain spectrin and filamentous actin could provide sites for the attachment of other proteins and promote the formation of molecular complexes essential for the organization of the plasma membrane (Riederer and Routtenberg, 1999).

Among the extracellular signals involved in GAP-43-dependent neuronal growth there are NGF (Perrone-Bizzozero et al., 1991), NCAM, L1, N-cadherin and FGF receptors (Dunican and Doherty, 2000; Zhang et al., 2005). It has been proposed that when the Ig-family adhesion molecule NCAM or L1 on the surface of a growth cone bind to an appropriate

substrate, FGF receptors are clustered and activated and this results in the phosphorylation of GAP-43 and the subsequent changes in membrane cytoskeleton, causing the growth cone to advance (Fig. 12; Ditlevsen et al., 2008; Dunican and Doherty, 2000; Zhang et al., 2005).

GAP-43 involvement in NCAM-mediated neurite outgrowth was first suggested by the observation that NCAM did not induce neurite outgrowth in cerebellar granule neurons from GAP-43 null mice (Meiri et al., 1998). GAP-43 phosphorylation at Ser41 by PKC and at Ser191/Ser192 by Casein Kinase II (CKII) has been shown to be related with NCAM-induced neurite outgrowth (Ditlevsen et al., 2008; Meiri et al., 1998). It was recently shown that GAP-43 regulates neurite outgrowth mediated specifically by the isoform NCAM-180 and that, for this function, spectrin and its phosphorylation by PKC and by CKII are relevant (Korshunova et al., 2007).

The cytoplasmic domains of both NCAM-140 and NCAM-180 interact with tubulin. Moreover spectrin interacts specifically with NCAM-180, but not with NCAM-140 or NCAM-120 (Pollerberg et al., 1987; Pollerberg et al., 1986). Consistently, in PC12 cells, dominant-negative mutant spectrin disrupts NCAM-180-mediated neurite outgrowth in conjunction with GAP-43, but not NCAM-140-mediated GAP-43-independent neurite outgrowth (Korshunova et al., 2007). The interaction between NCAM and spectrin has been shown to be important for the assembly of NCAM signaling complexes associated with lipidic rafts upon NCAM stimulation, by linking NCAM to signaling proteins such as PKCβ2 (Leshchyns'ka et al., 2003). It was thus suggested that GAP-43 role in NCAM-mediated neuritogenic signaling depends on the formation of NCAM-180/spectrin although their relation may be more complex than this, possibly implying additional mechanisms (Ditlevsen et al., 2008; Korshunova et al., 2007).

It has also been shown that the growth promoting effects exerted by the adhesion molecule L1 is dependent on GAP-43 (Meiri et al., 1998) and that they act synergistically to promote axon growth and regeneration *in vivo* in PC-specific double transgenic mice (Zhang et al., 2005). Interestingly L1 is clearly expressed at high levels in the IO (Horinouchi et al., 2005).

This is consistent with the findings on the impairment of crossing the optic chiasm by retinal ganglion cells axons in GAP-43 knock-out mice (Strittmatter et al., 1995) and the role reported to be played in commissural axon guidance (Demyanenko et al., 1999; Kamiguchi et al., 1998). It is also consistent with the aberrant innervation of the hippocampal distal stratum oriens by mossy fibres in mice which overexpress the non-phosphorilable GAP-43 (S41A) in the whole brain (Holahan et al., 2009), including

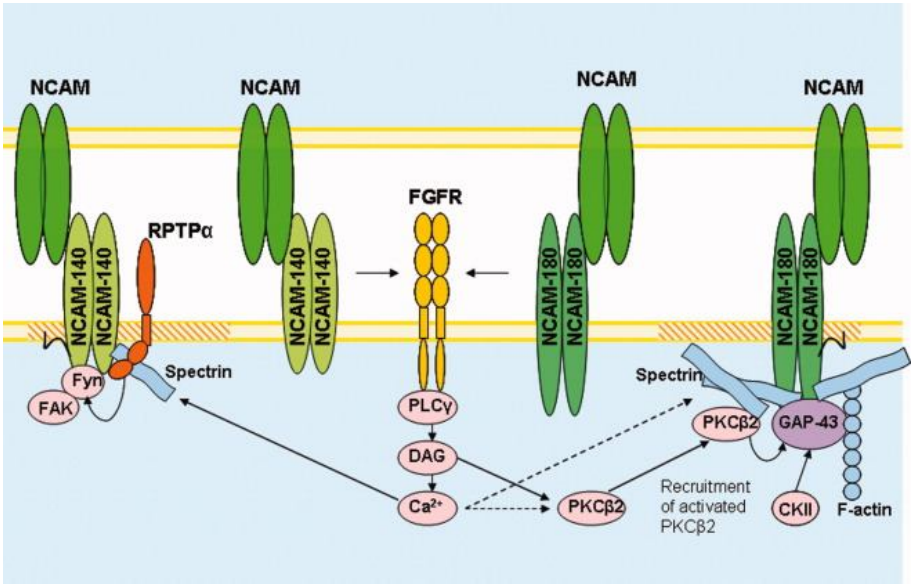

Fig. 12 - Schematic illustration of GAP-43 and NCAM interaction and signaling.
The illustration shows GAP-43 association with NCAM-180 in lipid rafts, which appears to favour NCAM-180/spectrin complex–mediated neuritogenic signaling in a way which is dependent on phosphorilation by PKC and casein kinase II. Upon NCAM trans-homophilic binding, it activates FGFR independently of localization of NCAM in lipid rafts. Furthermore, NCAM stimulation results in recruitment of NCAM into lipid rafts and assembly of raft-associated signaling complexes, the composition of which is at least partially specific for NCAM-140 and NCAM-180. NCAM-180 binds with high affinity to spectrin, which in turn links NCAM-180 to PKCβII. Upon NCAM stimulation and the resulting FGFR activation, activated PKCβII in conjunction with NCAM-180 and spectrin is recruited into lipid rafts, where PKCβII may function as an activator of GAP-43. For simplicity, RPTPa is depicted only in the NCAM-140-associated signaling complex, PKCbII is depicted as a component only of the NCAM-180-associated signaling complex and only cis-homodimers of NCAM isoforms are shown (CK, casein kinase; DAG, diacylglycerol; FAK, focal adhesion kinase; GAP-43, growth-associated protein 43; PKC, protein kinase C; PLC, phospholipase C; RPTP, receptor protein tyrosine phosphatase; Ditlevsen et al., 2008).

hippocampal granule cells normally devoid of GAP-43 expression (Kruger et al., 1993; McNamara and Lenox, 1997). This was reported to occur similarly to what observed in mice knock-out for NCAM or bearing its conditional ablation (Bukalo et al., 2004; Cremer et al., 1997).

Functional relations between GAP-43 and $G_{o/i}$ proteins have also been reported in several studies, showing that $G_{o/i}$ can be activated by N-terminal GAP-43 fragments introduced into the cells (Mosevitsky, 2005; Oestreicher et al., 1997). In particular it was shown by the use of isolated G_o, that complete GAP-43 molecules act similarly to the N-terminal fragments in activating G_o (Strittmatter et al., 1990).

Based on the findings that fragments of GAP-43 are naturally formed in neurons by m-calpain cleavage in the effector domain near Ser-41 (Mosevitsky, 2005; Zakharov and Mosevitsky, 2001) and that Ser-41 phosphorylation prevents this cleavage (Zakharov et al., 2005; Zakharov and Mosevitsky, 2007), GAP-43 was proposed to serve in growth cones as a switch for axonal guidance: PKC phosphorylation of Ser-41 preserves GAP-43 molecules from calpain action and leads them to bind to actin fibrils. This binding aids the growth of cytoskeleton fibrils and the protrusion of the growth cone (Mosevitsky, 2005).

Although a comprehensive picture of the dynamics of GAP-43 multiple interactions, its phosphorilation (PKC- and CKII-dependent) and calcium levels is not yet available, a short summary of the several models proposed can be attempted here (Denny, 2006; Ditlevsen et al., 2008; Mosevitsky, 2005; Oestreicher et al., 1997). GAP-43-dependent promotion of filopodia formation is inhibited by low $[Ca^{2+}]_i$ and stimulated by local increase of $[Ca^{2+}]_i$ and PKC-dependent phosphorilation with a mechanism which involves the release of calmodulin and PIP_2 and the interaction with actin and spectrin (which in turn binds to cytoskeleton and NCAM). In this way GAP-43 may contribute to the spatial organization of complexes of structural and signaling proteins associated with the membrane. Moreover GAP-43 may act as a lateral stabilizer of actin cytoskeleton and, following an increase of $[Ca^{2+}]_i$, it may release sequestered PIP_2, enabling its inhibition on acting-binding proteins thus promoting the growth and stabilization of actin filaments. GAP-43 role in the promotion of growth cone is triggered by stimuli converging on the production of diacylglycerol (DAG), the activation of PKC and the increase of $[Ca^{2+}]_i$ (Oestreicher et al., 1997). One of the signaling pathways in which GAP-43 plays a role is that controlled by NCAM/L1/FGFR which responds to homophilic interactions with similar adhesion molecules or to FGF (Ditlevsen et al., 2008). This pathway participates in a GAP-43 dependent manner in axonal guidance in regions such as optic chiasm (Sretavan et al., 1994; Sretavan and Kruger, 1998; Strittmatter et al., 1995) and hippocampus (Bukalo et al., 2004; Cremer et al., 1997; Holahan et al., 2009).

On the other hand, presumably in response to repulsive cues, GAP-43 free from the binding with calmodulin and from phosphorilation by PKC, can be cleaved by m-calpain, and its N-terminal fragment can interact with the G_o protein, promoting a collapse of the growth cone (Zakharov and Mosevitsky, 2007). By these mechanisms GAP-43 regulates axonal growth and guidance.

Finally, GAP-43 modulation of rabaptin-5 (Neve et al., 1998) and its interaction with SNAP-25, syntaxin and VAMP (Haruta et al., 1997), may play a role in the regulation of neurotransmitter release, possibly due to GAP-43 participation in the organization of protein complexes associated with the membrane (Riederer and Routtenberg, 1999).

Aims

GAP-43 represents a key intrinsic factor for the promotion of axonal growth and it is of crucial interest for the understanding of neuronal intrinsic regenerative potentials in the CNS.

A plethora of *in vitro* studies have investigated its role in promoting axonal growth on cultured cells, while knock-out mice helped to explore its function *in vivo* (Oestereicher et al., 1997; Mosevitsky, 2005; Denny, 2006). Despite its pivotal role during brain development and the dramatic alterations in neuronal connectivity which are present in knock-out mice, GAP-43 function in the adult brain is still largely speculative. A better understanding of the regulatory mechanisms which operate in the adult CNS will shed new light on axonal structural plasticity, neuronal regeneration and degeneration.

Our attention has been focused on the role of GAP-43 in axonal structure and remodelling in the adult brain of wild-type animals. Due to its high expression in the CFs, their peculiar morphological organization and their one-to-one relationship with PCs, we chose this structure as a convenient model for *in vivo* investigations. We took advantage of lentiviral vectors encoding both GFP and *sh*RNA. Moreover the IO nucleus in the brainstem, where CFs originate, is accessible to stereotaxic injection enabling their labelling and genetic manipulation without interfering with other neuronal components of the cerebellar cortex.

Preliminary results of this work have been previously presented (Grasselli et al., 2009; Allegra Mascaro et al., 2009).

Conclusions

In recent years axonal degeneration has been proposed to play a relevant role in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, multiple sclerosis, glaucoma, and Alzheimer's and Parkinson's diseases (Cifuentes-Diaz et al., 2002; Conforti et al., 2007; Fischer et al., 2004; Raff et al., 2002; Schlamp et al., 2006; Stokin et al., 2005; Trapp et al., 1998). We have shown that GAP-43 silencing determines a retraction of the CF. Finding effective pharmacological treatments for the promotion of GAP-43 expression could then contribute to sustain axonal structures, preventing dying back in models of neurodegenerative diseases.

We have here shown that during adulthood and in physiological conditions GAP-43 plays a pivotal role in the maintenance of CF axonal structure and organization of presynaptic plasma membrane. It may act together with cytoskeleton proteins, spectrin and adhesion molecules. The possibility that it is modulated via an activity-dependent pathway and its involvement in synaptic plasticity will however need further investigations.

Despite previous works showing that GAP-43 overexpression is sufficient to promote axonal sprouting (Aigner et al., 1995; Buffo et al., 1997; Gianola & Rossi, 2004).

We have also provided the first time-lapse description of lesion-induced sprouting in the mammalian CNS, and given new insight in the mechanisms underlying lesion induced sprouting of CF.

Acknowledgments

I am greatly thankful to: Prof. Piergiorgio Strata and Dr. Paolo Cesare for their careful supervision and training; Dr. Georgia Mandolesi, Dr. Eleonora Autuori, Dr. Valentina Vanni, Dr. Shin W. Kang, Dr. Theodoros Tsetsenis, Francesca Puglisi, Suresh Kumar Jetti and Nabila Haji for valuable scientific discussion and support; Vladimiro Batocchi for his skilled technical support in animal care and preparation of tissue samples.

Thanks to Anna Letizia Allegra-Mascaro, Dr. Leonardo Sacconi and Prof. Francesco Pavone (University of Florence, Italy) for their collaboration for two-photon microscopy and laser nanosurgery experiments.

Thanks also to Dr. Roberta Cesa, Dr. Bibiana Scelfo and Dr. Federica Premoselli (University of Turin) for their scientific support.

I am also greatful to Dr. Katsuhiko Mikoshiba, Dr. Chihiro Hisatsune and collegues (RIKEN Brain Science Institute, Wako-shi, Japan) for scientific and technical training provided during my visiting period in their laboratories.

References

Abbott, L. C., and Sotelo, C. (2000). Ultrastructural analysis of catecholaminergic innervation in weaver and normal mouse cerebellar cortices. J Comp Neurol *426*, 316-329.

Abramoff, M. D., Magelhaes, P. J., and Ram, S. J. (2004). Image Processing with ImageJ. Biophotonics International 11, 36-42.

Ahmad, F. J., Hughey, J., Wittmann, T., Hyman, A., Greaser, M., and Baas, P. W. (2000). Motor proteins regulate force interactions between microtubules and microfilaments in the axon. Nat Cell Biol *2*, 276-280.

Aigner, L., Arber, S., Kapfhammer, J. P., Laux, T., Schneider, C., Botteri, F., Brenner, H. R., and Caroni, P. (1995). Overexpression of the neural growth-associated protein GAP-43 induces nerve sprouting in the adult nervous system of transgenic mice. Cell 83, 269-278.

Aigner, L., and Caroni, P. (1995). Absence of persistent spreading, branching, and adhesion in GAP-43-depleted growth cones. J Cell Biol *128*, 647-660.

Aigner, L., and Caroni, P. (1993). Depletion of 43-kD growth-associated protein in primary sensory neurons leads to diminished formation and spreading of growth cones. J Cell Biol *123*, 417-429.

Aizenman, C. D., and Linden, D. J. (2000). Rapid, synaptically driven increases in the intrinsic excitability of cerebellar deep nuclear neurons. Nat Neurosci *3*, 109-111.

Aizenman, C. D., Manis, P. B., and Linden, D. J. (1998). Polarity of long-term synaptic gain change is related to postsynaptic spike firing at a cerebellar inhibitory synapse. Neuron *21*, 827-835.

Albus, J. S. (1971). A theory of cerebellar function. Mathematical Biosciences *10*, 25--61.

Allegra Mascaro, A. L., Cesare, P., Sacconi, L., Grasselli, G., Strata, P., and Pavone, F. S. (2009). Plasticity of climbing fibers after laser-axotomy, Paper presented at: Society for Neuroscience Annual Meeting (Chicago, IL, USA).

Alvarez, V. A., Ridenour, D. A., and Sabatini, B. L. (2006). Retraction of synapses and dendritic spines induced by off-target effects of RNA interference. J Neurosci 26, 7820-7825.

Anderson, K. D., Sengupta, J., Morin, M., Neve, R. L., Valenzuela, C. F., and Perrone-Bizzozero, N. I. (2001). Overexpression of HuD accelerates neurite outgrowth and increases GAP-43 mRNA expression in cortical neurons and retinoic acid-induced embryonic stem cells in vitro. Exp Neurol *168*, 250-258.

Anderson, P. N., Campbell, G., Zhang, Y., and Lieberman, A. R. (1998). Cellular and molecular correlates of the regeneration of adult mammalian CNS axons into peripheral nerve grafts. Prog Brain Res *117*, 211-232.

Anderson, W. A., and Flumerfelt, B. A. (1984). Sensitivity of rat inferior olivary neurons to 3-acetylpyridine. Brain Res *314*, 285-291.

Antonini, A., Fagiolini, M., and Stryker, M. P. (1999). Anatomical correlates of functional plasticity in mouse visual cortex. J Neurosci *19*, 4388-4406.

Antonini, A., and Stryker, M. P. (1996). Plasticity of geniculocortical afferents following brief or prolonged monocular occlusion in the cat. J Comp Neurol *369*, 64-82.

Aoki, E., Semba, R., and Kashiwamata, S. (1986). New candidates for GABAergic neurons in the rat cerebellum: an immunocytochemical study with anti-GABA antibody. Neurosci Lett *68*, 267-271.

Araki, T., Sasaki, Y., and Milbrandt, J. (2004). Increased nuclear NAD biosynthesis and SIRT1 activation prevent axonal degeneration. Science *305*, 1010-1013.

Armano, S., Rossi, P., Taglietti, V., and D'Angelo, E. (2000). Long-term potentiation of intrinsic excitability at the mossy fiber-granule cell synapse of rat cerebellum. J Neurosci *20*, 5208-5216.

Armengol, J. A., Sotelo, C., Angaut, P., and Alvarado-Mallart, R. M. (1989). Organization of Host Afferents to Cerebellar Grafts Implanted into Kainate Lesioned Cerebellum in Adult Rats. Eur J Neurosci *1*, 75-93.

Arni, S., Keilbaugh, S. A., Ostermeyer, A. G., and Brown, D. A. (1998). Association of GAP-43 with detergent-resistant membranes requires two palmitoylated cysteine residues. J Biol Chem *273*, 28478-28485.

Bagni, C., and Greenough, W. T. (2005). From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Nat Rev Neurosci *6*, 376-387.

Bailey, C. H., and Kandel, E. R. (1993). Structural changes accompanying memory storage. Annu Rev Physiol *55*, 397-426.

Barmack, N. H., and Young, W. S., 3rd (1990). Optokinetic stimulation increases corticotropin-releasing factor mRNA in inferior olivary neurons of rabbits. J Neurosci *10*, 631-640.

Basi, G. S., Jacobson, R. D., Virag, I., Schilling, J., and Skene, J. H. (1987). Primary structure and transcriptional regulation of GAP-43, a protein associated with nerve growth. Cell *49*, 785-791.

Bauer, P., Schols, L., and Riess, O. (2006). Spectrin mutations in spinocerebellar ataxia (SCA). Bioessays *28*, 785-787.

Beaulieu, C., and Colonnier, M. (1987). Effect of the richness of the environment on the cat visual cortex. J Comp Neurol *266*, 478-494.

Belmeguenai, A., and Hansel, C. (2005). A role for protein phosphatases 1, 2A, and 2B in cerebellar long-term potentiation. J Neurosci *25*, 10768-10772.

Benedetti, F., Montarolo, P. G., Strata, P., and Tempia, F. (1983). Inferior olive inactivation decreases the excitability of the intracerebellar and lateral vestibular nuclei in the rat. J Physiol *340*, 195-208.

Bennett, V., and Baines, A. J. (2001). Spectrin and ankyrin-based pathways: metazoan inventions for integrating cells into tissues. Physiol Rev *81*, 1353-1392.

Benowitz, L. I., and Yin, Y. (2007). Combinatorial treatments for promoting axon regeneration in the CNS: strategies for overcoming inhibitory signals and activating neurons' intrinsic growth state. Dev Neurobiol *67*, 1148-1165.

Benowitz, L. I., and Routtenberg, A. (1997). GAP-43: an intrinsic determinant of neuronal development and plasticity. Trends Neurosci *20*, 84-91.

Benowitz, L. I., Rodriguez, W. R., and Neve, R. L. (1990). The pattern of GAP-43 immunostaining changes in the rat hippocampal formation during reactive synaptogenesis. Brain Res Mol Brain Res *8*, 17-23.

Biewenga, J. E., Schrama, L. H., and Gispen, W. H. (1996). Presynaptic phosphoprotein B-50/GAP-43 in neuronal and synaptic plasticity. Acta Biochim Pol *43*, 327-338.

Billuart, P., Winter, C. G., Maresh, A., Zhao, X., and Luo, L. (2001). Regulating axon branch stability: the role of p190 RhoGAP in repressing a retraction signaling pathway. Cell *107*, 195-207.

Bloedel, J. R., and Courville, J. (1981). Cerebellar afferent systems In Handbook of Physiology, V. B. Brooks, ed. (Bethesda: American Physiological Society).

Bonatti, S., Migliaccio, G., and Simons, K. (1989). Palmitylation of viral membrane glycoproteins takes place after exit from the endoplasmic reticulum. J Biol Chem *264*, 12590-12595.

Bosman, L. W., and Konnerth, A. (2009). Activity-dependent plasticity of developing climbing fiber-Purkinje cell synapses. Neuroscience.

Bravin, M., Morando, L., Vercelli, A., Rossi, F., and Strata, P. (1999). Control of spine formation by electrical activity in the adult rat cerebellum. Proc Natl Acad Sci U S A *96*, 1704-1709.

Bravin, M., Savio, T., Strata, P., and Rossi, F. (1997). Olivocerebellar axon regeneration and target reinnervation following dissociated Schwann cell grafts in surgically injured cerebella of adult rats. Eur J Neurosci *9*, 2634-2649.

Brenowitz, S. D., and Regehr, W. G. (2003). Calcium dependence of retrograde inhibition by endocannabinoids at synapses onto Purkinje cells. J Neurosci *23*, 6373-6384.

Brevik, A., Leergaard, T. B., Svanevik, M., and Bjaalie, J. G. (2001). Three-dimensional computerised atlas of the rat brain stem precerebellar system: approaches for mapping, visualization, and comparison of spatial distribution data. Anat Embryol (Berl) *204*, 319-332.

Bridge, A. J., Pebernard, S., Ducraux, A., Nicoulaz, A. L., and Iggo, R. (2003). Induction of an interferon response by RNAi vectors in mammalian cells. Nat Genet 34, 263-264.

Brown, D. A., and London, E. (2000). Structure and function of sphingolipid- and cholesterol-rich membrane rafts. J Biol Chem *275*, 17221-17224.

Buffo, A., Carulli, D., Rossi, F., and Strata, P. (2003). Extrinsic regulation of injury/growth-related gene expression in the inferior olive of the adult rat. Eur J Neurosci *18*, 2146-2158.

Buffo, A., Fronte, M., Oestreicher, A. B., and Rossi, F. (1998). Degenerative phenomena and reactive modifications of the adult rat inferior olivary neurons following axotomy and disconnection from their targets. Neuroscience *85*, 587-604.

Buffo, A., Holtmaat, A. J., Savio, T., Verbeek, J. S., Oberdick, J., Oestreicher, A. B., Gispen, W. H., Verhaagen, J., Rossi, F., and Strata, P. (1997). Targeted

overexpression of the neurite growth-associated protein B-50/GAP-43 in cerebellar Purkinje cells induces sprouting after axotomy but not axon regeneration into growth-permissive transplants. J Neurosci 17, 8778-8791.

Bukalo, O., Fentrop, N., Lee, A. Y., Salmen, B., Law, J. W., Wotjak, C. T., Schweizer, M., Dityatev, A., and Schachner, M. (2004). Conditional ablation of the neural cell adhesion molecule reduces precision of spatial learning, long-term potentiation, and depression in the CA1 subfield of mouse hippocampus. J Neurosci *24*, 1565-1577.

Butz, M., Worgotter, F., and van Ooyen, A. (2009). Activity-dependent structural plasticity. Brain Res Rev *60*, 287-305.

Cantallops, I., and Routtenberg, A. (1996). Rapid induction by kainic acid of both axonal growth and F1/GAP-43 protein in the adult rat hippocampal granule cells. J Comp Neurol *366*, 303-319.

Caroni, P., Aigner, L., and Schneider, C. (1997). Intrinsic neuronal determinants locally regulate extrasynaptic and synaptic growth at the adult neuromuscular junction. J Cell Biol *136*, 679-692.

Carulli, D., Rhodes, K. E., Brown, D. J., Bonnert, T. P., Pollack, S. J., Oliver, K., Strata, P., and Fawcett, J. W. (2006). Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. J Comp Neurol *494*, 559-577.

Carulli, D., Buffo, A., and Strata, P. (2004). Reparative mechanisms in the cerebellar cortex. Prog Neurobiol *72*, 373-398.

Carulli, D., Cesa, R., Buffo, A., and Strata, P. (2002). Collateral sprouting of rat inferior olive neurons does not require GAP-43 up-regulation, Paper presented at: Society for Neuroscience Annual Meeting (Orlando, FL, USA).

Cesa, R., and Strata, P. (2009). Axonal competition in the synaptic wiring of the cerebellar cortex during development and in the mature cerebellum. Neuroscience *162*, 624-632.

Cesa, R., Morando, L., and Strata, P. (2008). Transmitter-receptor mismatch in GABAergic synapses in the absence of activity. Proc Natl Acad Sci U S A *105*, 18988-18993.

Cesa, R., Scelfo, B., and Strata, P. (2007). Activity-dependent presynaptic and postsynaptic structural plasticity in the mature cerebellum. J Neurosci *27*, 4603-4611.

Cesa, R., and Strata, P. (2005). Axonal and synaptic remodeling in the mature cerebellar cortex. Prog Brain Res 148, 45-56.

Cesa, R., Morando, L., and Strata, P. (2003). Glutamate receptor delta2 subunit in activity-dependent heterologous synaptic competition. J Neurosci *23*, 2363-2370.

Chapman, E. R., Au, D., Alexander, K. A., Nicolson, T. A., and Storm, D. R. (1991). Characterization of the calmodulin binding domain of neuromodulin. Functional significance of serine 41 and phenylalanine 42. J Biol Chem *266*, 207-213.

Chen, M. S., Huber, A. B., van der Haar, M. E., Frank, M., Schnell, L., Spillmann, A. A., Christ, F., and Schwab, M. E. (2000). Nogo-A is a myelin-

associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature *403*, 434-439.

Chiaramello, A., Neuman, T., Peavy, D. R., and Zuber, M. X. (1996). The GAP-43 gene is a direct downstream target of the basic helix-loop-helix transcription factors. J Biol Chem *271*, 22035-22043.

Chung, S. H., Clark, D. A., Gabel, C. V., Mazur, E., and Samuel, A. D. (2006). The role of the AFD neuron in C. elegans thermotaxis analyzed using femtosecond laser ablation. BMC Neurosci *7*, 30.

Coesmans, M., Weber, J. T., De Zeeuw, C. I., and Hansel, C. (2004). Bidirectional parallel fiber plasticity in the cerebellum under climbing fiber control. Neuron *44*, 691-700.

Coleman, M. P., Conforti, L., Buckmaster, E. A., Tarlton, A., Ewing, R. M., Brown, M. C., Lyon, M. F., and Perry, V. H. (1998). An 85-kb tandem triplication in the slow Wallerian degeneration (Wlds) mouse. Proc Natl Acad Sci U S A *95*, 9985-9990.

Conforti, L., Tarlton, A., Mack, T. G., Mi, W., Buckmaster, E. A., Wagner, D., Perry, V. H., and Coleman, M. P. (2000). A Ufd2/D4Cole1e chimeric protein and overexpression of Rbp7 in the slow Wallerian degeneration (WldS) mouse. Proc Natl Acad Sci U S A *97*, 11377-11382.

Connor, S., Bloomfield, J., LeBoutillier, J. C., Thompson, R. F., Petit, T. L., and Weeks, A. C. (2009). Eyeblink conditioning leads to fewer synapses in the rabbit cerebellar cortex. Behav Neurosci *123*, 856-862.

Cremer, H., Chazal, G., Goridis, C., and Represa, A. (1997). NCAM is essential for axonal growth and fasciculation in the hippocampus. Mol Cell Neurosci *8*, 323-335.

Crepel, F. (1982). Regression of functional synapses in the immature mammalian cerebellum. Trends Neurosci *5*, 266–269.

Crepel, F., Delhaye-Bouchaud, N., and Dupont, J. L. (1981). Fate of the multiple innervation of cerebellar Purkinje cells by climbing fibers in immature control, x-irradiated and hypothyroid rats. Brain Res *227*, 59-71.

Crepel, F., Delhaye-Bouchaud, N., Guastavino, J. M., and Sampaio, I. (1980). Multiple innervation of cerebellar Purkinje cells by climbing fibres in staggerer mutant mouse. Nature *283*, 483-484.

Crepel, F., and Mariani, J. (1976). Multiple innervation of Purkinje cells by climbing fibers in the cerebellum of the Weaver Mutant Mouse. J Neurobiol *7*, 579-582.

Crepel, F., Mariani, J., and Delhaye-Bouchaud, N. (1976). Evidence for a multiple innervation of Purkinje cells by climbing fibers in the immature rat cerebellum. J Neurobiol *7*, 567-578.

D'Angelo, E., Rossi, P., Armano, S., and Taglietti, V. (1999). Evidence for NMDA and mGlu receptor-dependent long-term potentiation of mossy fiber-granule cell transmission in rat cerebellum. J Neurophysiol *81*, 277-287.

Darian-Smith, C., and Gilbert, C. D. (1994). Axonal sprouting accompanies functional reorganization in adult cat striate cortex. Nature *368*, 737-740.

De Paola, V., Holtmaat, A., Knott, G., Song, S., Wilbrecht, L., Caroni, P., and Svoboda, K. (2006). Cell type-specific structural plasticity of axonal branches and boutons in the adult neocortex. Neuron *49*, 861-875.

Dekker, L. V., De Graan, P. N., Oestreicher, A. B., Versteeg, D. H., and Gispen, W. H. (1989). Inhibition of noradrenaline release by antibodies to B-50 (GAP-43). Nature *342*, 74-76.

Demyanenko, G. P., Tsai, A. Y., and Maness, P. F. (1999). Abnormalities in neuronal process extension, hippocampal development, and the ventricular system of L1 knockout mice. J Neurosci *19*, 4907-4920.

Denny, J. B. (2004). Growth-associated protein of 43 kDa (GAP-43) is cleaved nonprocessively by the 20S proteasome. Eur J Biochem *271*, 2480-2493.

Denny, J. B. (2006). Molecular Mechanisms, Biological Actions, and Neuropharmacology of the Growth-Associated Protein GAP-43. Curr Neuropharmacol *4*, 293-304.

Dino, M. R., Willard, F. H., and Mugnaini, E. (1999). Distribution of unipolar brush cells and other calretinin immunoreactive components in the mammalian cerebellar cortex. J Neurocytol *28*, 99-123.

Ditlevsen, D. K., Povlsen, G. K., Berezin, V., and Bock, E. (2008). NCAM-induced intracellular signaling revisited. J Neurosci Res *86*, 727-743.

Dittman, J. S., and Regehr, W. G. (1998). Calcium dependence and recovery kinetics of presynaptic depression at the climbing fiber to Purkinje cell synapse. J Neurosci *18*, 6147-6162.

Doster, S. K., Lozano, A. M., Aguayo, A. J., and Willard, M. B. (1991). Expression of the growth-associated protein GAP-43 in adult rat retinal ganglion cells following axon injury. Neuron *6*, 635-647.

Dunican, D. J., and Doherty, P. (2000). The generation of localized calcium rises mediated by cell adhesion molecules and their role in neuronal growth cone motility. Mol Cell Biol Res Commun *3*, 255-263.

Eaton, B. A., Fetter, R. D., and Davis, G. W. (2002). Dynactin is necessary for synapse stabilization. Neuron *34*, 729-741.

Eccles, J. C., Ito, M. & Szentágothai, J. (1967). The Cerebellum as a Neuronal Machine (Berlin: Springer-Verlag Press).

Eccles, J. C., Llinas, R., and Sasaki, K. (1966). The excitatory synaptic action of climbing fibres on the purinje cells of the cerebellum. J Physiol *182*, 268-296.

Eilers, A., Whitfield, J., Babij, C., Rubin, L. L., and Ham, J. (1998). Role of the Jun kinase pathway in the regulation of c-Jun expression and apoptosis in sympathetic neurons. J Neurosci *18*, 1713-1724.

Eilers, J., Takechi, H., Finch, E. A., Augustine, G. J., and Konnerth, A. (1997). Local dendritic Ca2+ signaling induces cerebellar long-term depression. Learn Mem *4*, 159-168.

El-Husseini Ael, D., Schnell, E., Dakoji, S., Sweeney, N., Zhou, Q., Prange, O., Gauthier-Campbell, C., Aguilera-Moreno, A., Nicoll, R. A., and Bredt, D. S. (2002). Synaptic strength regulated by palmitate cycling on PSD-95. Cell *108*, 849-863.

El-Husseini Ael, D., Craven, S. E., Brock, S. C., and Bredt, D. S. (2001). Polarized targeting of peripheral membrane proteins in neurons. J Biol Chem *276*, 44984-44992.

Fawcett, J. W., and Asher, R. A. (1999). The glial scar and central nervous system repair. Brain Res Bull *49*, 377-391.

Fernandez, A. M., Gonzalez de la Vega, A. G., Planas, B., and Torres-Aleman, I. (1999). Neuroprotective actions of peripherally administered insulin-like growth factor I in the injured olivo-cerebellar pathway. Eur J Neurosci *11*, 2019-2030.

Filbin, M. T. (2003). Myelin-associated inhibitors of axonal regeneration in the adult mammalian CNS. Nat Rev Neurosci *4*, 703-713.

Florenzano, F., Viscomi, M. T., Cavaliere, F., Volonte, C., and Molinari, M. (2002). Cerebellar lesion up-regulates P2X1 and P2X2 purinergic receptors in precerebellar nuclei. Neuroscience *115*, 425-434.

Frotscher, M., Heimrich, B., Deller, T., and Nitsch, R. (1995). Understanding the cortex through the hippocampus: lamina-specific connections of the rat hippocampal neurons. J Anat *187 (Pt 3)*, 539-545.

Gagliardini, V., Dusart, I., and Fankhauser, C. (2000). Absence of GAP-43 can protect neurons from death. Mol Cell Neurosci *16*, 27-33.

Galimberti, I., Gogolla, N., Alberi, S., Santos, A. F., Muller, D., and Caroni, P. (2006). Long-term rearrangements of hippocampal mossy fiber terminal connectivity in the adult regulated by experience. Neuron *50*, 749-763.

Gallo, G., and Letourneau, P. C. (2004). Regulation of growth cone actin filaments by guidance cues. J Neurobiol *58*, 92-102.

Gamby, C., Waage, M. C., Allen, R. G., and Baizer, L. (1996). Analysis of the role of calmodulin binding and sequestration in neuromodulin (GAP-43) function. J Biol Chem *271*, 26698-26705.

Gardette, R., Alvarado-Mallart, R. M., Crepel, F., and Sotelo, C. (1988). Electrophysiological demonstration of a synaptic integration of transplanted Purkinje cells into the cerebellum of the adult Purkinje cell degeneration mutant mouse. Neuroscience *24*, 777-789.

Gauthier-Campbell, C., Bredt, D. S., Murphy, T. H., and El-Husseini Ael, D. (2004). Regulation of dendritic branching and filopodia formation in hippocampal neurons by specific acylated protein motifs. Mol Biol Cell *15*, 2205-2217.

Genuardi, M., Calvieri, F., Tozzi, C., Coslovi, R., and Neri, G. (1994). A new case of interstitial deletion of chromosome 3q, del(3q)(q13.12q21.3), with agenesis of the corpus callosum. Clin Dysmorphol *3*, 292-296.

Giannikopoulos, D. V., and Eysel, U. T. (2006). Dynamics and specificity of cortical map reorganization after retinal lesions. Proc Natl Acad Sci U S A *103*, 10805-10810.

Gianola, S., and Rossi, F. (2004). GAP-43 overexpression in adult mouse Purkinje cells overrides myelin-derived inhibition of neurite growth. Eur J Neurosci *19*, 819-830.

Gianola, S., and Rossi, F. (2004). GAP-43 overexpression in adult mouse Purkinje cells overrides myelin-derived inhibition of neurite growth. Eur J Neurosci 19, 819-830.

Gianotti, C., Nunzi, M. G., Gispen, W. H., and Corradetti, R. (1992). Phosphorylation of the presynaptic protein B-50 (GAP-43) is increased during electrically induced long-term potentiation. Neuron *8*, 843-848.

Gogolla, N., Galimberti, I., and Caroni, P. (2007). Structural plasticity of axon terminals in the adult. Curr Opin Neurobiol *17*, 516-524.

Goldberg, J. L. (2003). How does an axon grow? Genes Dev *17*, 941-958.

Gonzalo, S., and Linder, M. E. (1998). SNAP-25 palmitoylation and plasma membrane targeting require a functional secretory pathway. Mol Biol Cell *9*, 585-597.

Goodman, S. R., Zimmer, W. E., Clark, M. B., Zagon, I. S., Barker, J. E., and Bloom, M. L. (1995). Brain spectrin: of mice and men. Brain Res Bull *36*, 593-606.

GrandPre, T., Nakamura, F., Vartanian, T., and Strittmatter, S. M. (2000). Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature *403*, 439-444.

Grasselli, G., Mandolesi, G., Batocchi, V., Vanni, V., Strata, P., and Cesare, P. (2009). Structural plasticity of adult cerebellar climbing fibres: An in vivo study on the role of growth-associated protein 43, Paper presented at: Society for Neuroscience Annual Meeting (Chicago, IL, USA).

Greenough, W. T., Hwang, H. M., and Gorman, C. (1985). Evidence for active synapse formation or altered postsynaptic metabolism in visual cortex of rats reared in complex environments. Proc Natl Acad Sci U S A *82*, 4549-4552.

Guastavino, J. M., Sotelo, C., and Damez-Kinselle, I. (1990). Hot-foot murine mutation: behavioral effects and neuroanatomical alterations. Brain Res *523*, 199-210.

Ham, J., Babij, C., Whitfield, J., Pfarr, C. M., Lallemand, D., Yaniv, M., and Rubin, L. L. (1995). A c-Jun dominant negative mutant protects sympathetic neurons against programmed cell death. Neuron *14*, 927-939.

Hansel, C., and Linden, D. J. (2000). Long-term depression of the cerebellar climbing fiber--Purkinje neuron synapse. Neuron *26*, 473-482.

Hansel, C., Linden, D. J., and D'Angelo, E. (2001). Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. Nat Neurosci *4*, 467-475.

Hartell, N. A. (1996). Strong activation of parallel fibers produces localized calcium transients and a form of LTD that spreads to distant synapses. Neuron *16*, 601-610.

Haruta, T., Takami, N., Ohmura, M., Misumi, Y., and Ikehara, Y. (1997). Ca2+-dependent interaction of the growth-associated protein GAP-43 with the synaptic core complex. Biochem J *325 (Pt 2)*, 455-463.

Hashimoto, K., and Kano, M. (2003). Functional differentiation of multiple climbing fiber inputs during synapse elimination in the developing cerebellum. Neuron *38*, 785-796.

Hashimoto, K., and Kano, M. (2005). Postnatal development and synapse elimination of climbing fiber to Purkinje cell projection in the cerebellum. Neurosci Res *53*, 221-228.

Hashimoto, T., Ishii, T., and Ohmori, H. (1996). Release of Ca2+ is the crucial step for the potentiation of IPSCs in the cultured cerebellar Purkinje cells of the rat. J Physiol *497 (Pt 3)*, 611-627.

Hayashi, N., Matsubara, M., Titani, K., and Taniguchi, H. (1997). Circular dichroism and 1H nuclear magnetic resonance studies on the solution and membrane structures of GAP-43 calmodulin-binding domain. J Biol Chem *272*, 7639-7645.

He, Q., Dent, E. W., and Meiri, K. F. (1997). Modulation of actin filament behavior by GAP-43 (neuromodulin) is dependent on the phosphorylation status of serine 41, the protein kinase C site. J Neurosci *17*, 3515-3524.

Hens, J. J., Benfenati, F., Nielander, H. B., Valtorta, F., Gispen, W. H., and De Graan, P. N. (1993). B-50/GAP-43 binds to actin filaments without affecting actin polymerization and filament organization. J Neurochem *61*, 1530-1533.

Herdegen, T., Blume, A., Buschmann, T., Georgakopoulos, E., Winter, C., Schmid, W., Hsieh, T. F., Zimmermann, M., and Gass, P. (1997). Expression of activating transcription factor-2, serum response factor and cAMP/Ca response element binding protein in the adult rat brain following generalized seizures, nerve fibre lesion and ultraviolet irradiation. Neuroscience *81*, 199-212.

Herdegen, T., Kovary, K., Buhl, A., Bravo, R., Zimmermann, M., and Gass, P. (1995). Basal expression of the inducible transcription factors c-Jun, JunB, JunD, c-Fos, FosB, and Krox-24 in the adult rat brain. J Comp Neurol *354*, 39-56.

Herrup, K., and Kuemerle, B. (1997). The compartmentalization of the cerebellum. Annu Rev Neurosci *20*, 61-90.

Hilliard, M. A. (2009). Axonal degeneration and regeneration: a mechanistic tug-of-war. J Neurochem *108*, 23-32.

Hirai, H., Miyazaki, T., Kakegawa, W., Matsuda, S., Mishina, M., Watanabe, M., and Yuzaki, M. (2005). Rescue of abnormal phenotypes of the delta2 glutamate receptor-null mice by mutant delta2 transgenes. EMBO Rep *6*, 90-95.

Hisano, S., Sawada, K., Kawano, M., Kanemoto, M., Xiong, G., Mogi, K., Sakata-Haga, H., Takeda, J., Fukui, Y., and Nogami, H. (2002). Expression of inorganic phosphate/vesicular glutamate transporters (BNPI/VGLUT1 and DNPI/VGLUT2) in the cerebellum and precerebellar nuclei of the rat. Brain Res Mol Brain Res 107, 23-31.

Holahan, M. R., Honegger, K. S., and Routtenberg, A. (2009). Ectopic growth of hippocampal mossy fibers in a mutated GAP-43 transgenic mouse with impaired spatial memory retention. Hippocampus.

Holahan, M., and Routtenberg, A. (2008). The protein kinase C phosphorylation site on GAP-43 differentially regulates information storage. Hippocampus *18*, 1099-1102.

Holtmaat, A., and Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev Neurosci *10*, 647-658.

Holtmaat, A., and Svoboda, K. (2009). Experience-dependent structural synaptic plasticity in the mammalian brain. Nat Rev Neurosci 10, 647-658.

Holtmaat, A., Bonhoeffer, T., Chow, D. K., Chuckowree, J., De Paola, V., Hofer, S. B., Hubener, M., Keck, T., Knott, G., Lee, W. C., et al. (2009). Long-term, high-resolution imaging in the mouse neocortex through a chronic cranial window. Nat Protoc 4, 1128-1144.

Horinouchi, K., Nakamura, Y., Yamanaka, H., Watabe, T., and Shiosaka, S. (2005). Distribution of L1cam mRNA in the adult mouse brain: In situ hybridization and Northern blot analyses. J Comp Neurol *482*, 386-404.

Huang, K., and El-Husseini, A. (2005). Modulation of neuronal protein trafficking and function by palmitoylation. Curr Opin Neurobiol *15*, 527-535.

Hull, M., and Bahr, M. (1994). Regulation of immediate-early gene expression in rat retinal ganglion cells after axotomy and during regeneration through a peripheral nerve graft. J Neurobiol *25*, 92-105.

Hulo, S., Alberi, S., Laux, T., Muller, D., and Caroni, P. (2002). A point mutant of GAP-43 induces enhanced short-term and long-term hippocampal plasticity. Eur J Neurosci *15*, 1976-1982.

Ichikawa, R., Miyazaki, T., Kano, M., Hashikawa, T., Tatsumi, H., Sakimura, K., Mishina, M., Inoue, Y., and Watanabe, M. (2002). Distal extension of climbing fiber territory and multiple innervation caused by aberrant wiring to adjacent spiny branchlets in cerebellar Purkinje cells lacking glutamate receptor delta 2. J Neurosci *22*, 8487-8503.

Ichikawa, R., Miyazaki, T., Kano, M., Hashikawa, T., Tatsumi, H., Sakimura, K., Mishina, M., Inoue, Y., and Watanabe, M. (2002). Distal extension of climbing fiber territory and multiple innervation caused by aberrant wiring to adjacent spiny branchlets in cerebellar Purkinje cells lacking glutamate receptor delta 2. J Neurosci 22, 8487-8503.

Ito, M. (1984). The cerebellum and neural control (New York: Raven Press).

Ito, M. (1984). The cerebellum and neural control (New York: Raven Press).

Ito, M. (2001). Cerebellar long-term depression: characterization, signal transduction, and functional roles. Physiol Rev *81*, 1143-1195.

Ito, M. (2006). Cerebellar circuitry as a neuronal machine. Prog Neurobiol *78*, 272-303.

Ito, M. (2008). Control of mental activities by internal models in the cerebellum. Nat Rev Neurosci *9*, 304-313.

Ito, M., and Kano, M. (1982). Long-lasting depression of parallel fiber-Purkinje cell transmission induced by conjunctive stimulation of parallel fibers and climbing fibers in the cerebellar cortex. Neurosci Lett *33*, 253-258.

Jaarsma, D., Ruigrok, T. J., Caffe, R., Cozzari, C., Levey, A. I., Mugnaini, E., and Voogd, J. (1997). Cholinergic innervation and receptors in the cerebellum. Prog Brain Res *114*, 67-96.

Jorntell, H., and Ekerot, C. F. (2002). Reciprocal bidirectional plasticity of parallel fiber receptive fields in cerebellar Purkinje cells and their afferent interneurons. Neuron *34*, 797-806.

Jorntell, H., and Ekerot, C. F. (2003). Receptive field plasticity profoundly alters the cutaneous parallel fiber synaptic input to cerebellar interneurons in vivo. J Neurosci *23*, 9620-9631.

Jorntell, H., and Hansel, C. (2006). Synaptic memories upside down: bidirectional plasticity at cerebellar parallel fiber-Purkinje cell synapses. Neuron *52*, 227-238.

Jourdain, P., Fukunaga, K., and Muller, D. (2003). Calcium/calmodulin-dependent protein kinase II contributes to activity-dependent filopodia growth and spine formation. J Neurosci *23*, 10645-10649.

Kamiguchi, H., Hlavin, M. L., Yamasaki, M., and Lemmon, V. (1998). Adhesion molecules and inherited diseases of the human nervous system. Annu Rev Neurosci *21*, 97-125.

Kandel, E. R., Schwartz, J. H., and Jessels, T. M. (2003). Principles of Neural Science: McGraw-Hill Education).

Kang, R., Wan, J., Arstikaitis, P., Takahashi, H., Huang, K., Bailey, A. O., Thompson, J. X., Roth, A. F., Drisdel, R. C., Mastro, R., *et al.* (2008). Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. Nature *456*, 904-909.

Kano, M., Hashimoto, K., Chen, C., Abeliovich, A., Aiba, A., Kurihara, H., Watanabe, M., Inoue, Y., and Tonegawa, S. (1995). Impaired synapse elimination during cerebellar development in PKC gamma mutant mice. Cell *83*, 1223-1231.

Kano, M., Hashimoto, K., Kurihara, H., Watanabe, M., Inoue, Y., Aiba, A., and Tonegawa, S. (1997). Persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking mGluR1. Neuron *18*, 71-79.

Kano, M., Hashimoto, K., Watanabe, M., Kurihara, H., Offermanns, S., Jiang, H., Wu, Y., Jun, K., Shin, H. S., Inoue, Y., *et al.* (1998). Phospholipase cbeta4 is specifically involved in climbing fiber synapse elimination in the developing cerebellum. Proc Natl Acad Sci U S A *95*, 15724-15729.

Kano, M., Kano, M., Fukunaga, K., and Konnerth, A. (1996). Ca(2+)-induced rebound potentiation of gamma-aminobutyric acid-mediated currents requires activation of Ca2+/calmodulin-dependent kinase II. Proc Natl Acad Sci U S A *93*, 13351-13356.

Kano, M., Rexhausen, U., Dreessen, J., and Konnerth, A. (1992). Synaptic excitation produces a long-lasting rebound potentiation of inhibitory synaptic signals in cerebellar Purkinje cells. Nature *356*, 601-604.

Kantor, L., and Gnegy, M. E. (1998). Protein kinase C inhibitors block amphetamine-mediated dopamine release in rat striatal slices. J Pharmacol Exp Ther *284*, 592-598.

Kawaguchi, S., and Hirano, T. (2000). Suppression of inhibitory synaptic potentiation by presynaptic activity through postsynaptic GABA(B) receptors in a Purkinje neuron. Neuron *27*, 339-347.

Keck, T., Mrsic-Flogel, T. D., Vaz Afonso, M., Eysel, U. T., Bonhoeffer, T., and Hubener, M. (2008). Massive restructuring of neuronal circuits during functional reorganization of adult visual cortex. Nat Neurosci *11*, 1162-1167.

Khodakhah, K., and Armstrong, C. M. (1997). Induction of long-term depression and rebound potentiation by inositol trisphosphate in cerebellar Purkinje neurons. Proc Natl Acad Sci U S A *94*, 14009-14014.

Knott, G. W., Quairiaux, C., Genoud, C., and Welker, E. (2002). Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice. Neuron *34*, 265-273.

Kolb, B., Cioe, J., and Comeau, W. (2008). Contrasting effects of motor and visual spatial learning tasks on dendritic arborization and spine density in rats. Neurobiol Learn Mem *90*, 295-300.

Konnerth, A., Llano, I., and Armstrong, C. M. (1990). Synaptic currents in cerebellar Purkinje cells. Proc Natl Acad Sci U S A *87*, 2662-2665.

Korshunova, I., Novitskaya, V., Kiryushko, D., Pedersen, N., Kolkova, K., Kropotova, E., Mosevitsky, M., Rayko, M., Morrow, J. S., Ginzburg, I., *et al.* (2007). GAP-43 regulates NCAM-180-mediated neurite outgrowth. J Neurochem *100*, 1599-1612.

Kottis, V., Thibault, P., Mikol, D., Xiao, Z. C., Zhang, R., Dergham, P., and Braun, P. E. (2002). Oligodendrocyte-myelin glycoprotein (OMgp) is an inhibitor of neurite outgrowth. J Neurochem *82*, 1566-1569.

Kruger, L., Bendotti, C., Rivolta, R., and Samanin, R. (1993). Distribution of GAP-43 mRNA in the adult rat brain. J Comp Neurol *333*, 417-434.

Kruger, L., Bendotti, C., Rivolta, R., and Samanin, R. (1993). Distribution of GAP-43 mRNA in the adult rat brain. J Comp Neurol 333, 417-434.

Kurihara, H., Hashimoto, K., Kano, M., Takayama, C., Sakimura, K., Mishina, M., Inoue, Y., and Watanabe, M. (1997). Impaired parallel fiber-->Purkinje cell synapse stabilization during cerebellar development of mutant mice lacking the glutamate receptor delta2 subunit. J Neurosci *17*, 9613-9623.

Laser, H., Conforti, L., Morreale, G., Mack, T. G., Heyer, M., Haley, J. E., Wishart, T. M., Beirowski, B., Walker, S. A., Haase, G., *et al.* (2006). The slow Wallerian degeneration protein, WldS, binds directly to VCP/p97 and partially redistributes it within the nucleus. Mol Biol Cell *17*, 1075-1084.

Laux, T., Fukami, K., Thelen, M., Golub, T., Frey, D., and Caroni, P. (2000). GAP43, MARCKS, and CAP23 modulate PI(4,5)P(2) at plasmalemmal rafts, and regulate cell cortex actin dynamics through a common mechanism. J Cell Biol *149*, 1455-1472.

Leshchyns'ka, I., Sytnyk, V., Morrow, J. S., and Schachner, M. (2003). Neural cell adhesion molecule (NCAM) association with PKCbeta2 via betaI spectrin is implicated in NCAM-mediated neurite outgrowth. J Cell Biol *161*, 625-639.

Lev-Ram, V., Wong, S. T., Storm, D. R., and Tsien, R. Y. (2002). A new form of cerebellar long-term potentiation is postsynaptic and depends on nitric oxide but not cAMP. Proc Natl Acad Sci U S A *99*, 8389-8393.

Lipton, S. A., and Kater, S. B. (1989). Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. Trends Neurosci *12*, 265-270.

Llano, I., Marty, A., Armstrong, C. M., and Konnerth, A. (1991). Synaptic- and agonist-induced excitatory currents of Purkinje cells in rat cerebellar slices. J Physiol *434*, 183-213.

Lomeli, H., Sprengel, R., Laurie, D. J., Kohr, G., Herb, A., Seeburg, P. H., and Wisden, W. (1993). The rat delta-1 and delta-2 subunits extend the excitatory amino acid receptor family. FEBS Lett *315*, 318-322.

Lovinger, D. M., Colley, P. A., Akers, R. F., Nelson, R. B., and Routtenberg, A. (1986). Direct relation of long-term synaptic potentiation to phosphorylation of membrane protein F1, a substrate for membrane protein kinase C. Brain Res *399*, 205-211.

Low, L. K., and Cheng, H. J. (2005). A little nip and tuck: axon refinement during development and axonal injury. Curr Opin Neurobiol *15*, 549-556.

Lunn, E. R., Perry, V. H., Brown, M. C., Rosen, H., and Gordon, S. (1989). Absence of Wallerian Degeneration does not Hinder Regeneration in Peripheral Nerve. Eur J Neurosci *1*, 27-33.

Luo, L. (2002). Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. Annu Rev Cell Dev Biol *18*, 601-635.

Luo, L., and O'Leary, D. D. (2005). Axon retraction and degeneration in development and disease. Annu Rev Neurosci *28*, 127-156.

Lynch, G. (1974). Functional recovery after lesions of the nervous system. 3. Developmental processes in neural plasticity. The formation of new synaptic connections after brain damage and their possible role in recovery of function. Neurosci Res Program Bull *12*, 228-233.

Lynch, G. S., Mosko, S., Parks, T., and Cotman, C. W. (1973). Relocation and hyperdevelopment of the dentate gyrus commissural system after entorhinal lesions in immature rats. Brain Res *50*, 174-178.

Lyon, M. F., Ogunkolade, B. W., Brown, M. C., Atherton, D. J., and Perry, V. H. (1993). A gene affecting Wallerian nerve degeneration maps distally on mouse chromosome 4. Proc Natl Acad Sci U S A *90*, 9717-9720.

Mack, T. G., Reiner, M., Beirowski, B., Mi, W., Emanuelli, M., Wagner, D., Thomson, D., Gillingwater, T., Court, F., Conforti, L., *et al.* (2001). Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/Nmnat chimeric gene. Nat Neurosci *4*, 1199-1206.

Mackie Ogilvie, C., Rooney, S. C., Hodgson, S. V., and Berry, A. C. (1998). Deletion of chromosome 3q proximal region gives rise to a variable phenotype. Clin Genet *53*, 220-222.

Magarinos, A. M., McEwen, B. S., Flugge, G., and Fuchs, E. (1996). Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci *16*, 3534-3540.

Maier, D. L., Mani, S., Donovan, S. L., Soppet, D., Tessarollo, L., McCasland, J. S., and Meiri, K. F. (1999). Disrupted cortical map and absence of cortical barrels in growth-associated protein (GAP)-43 knockout mice. Proc Natl Acad Sci U S A *96*, 9397-9402.

Mandolesi, G., Autuori, E., Cesa, R., Premoselli, F., Cesare, P., and Strata, P. (2009a). GluRdelta2 expression in the mature cerebellum of hotfoot mice promotes parallel fiber synaptogenesis and axonal competition. PLoS One *4*, e5243.

Mandolesi, G., Autuori, E., Cesa, R., Premoselli, F., Cesare, P., and Strata, P. (2009). GluRdelta2 expression in the mature cerebellum of hotfoot mice promotes parallel fiber synaptogenesis and axonal competition. PLoS One 4, e5243.

Mandolesi, G., Cesa, R., Autuori, E., and Strata, P. (2009b). An orphan ionotropic glutamate receptor: the delta2 subunit. Neuroscience *158*, 67-77.

Mariani, J., and Changeux, J. P. (1980). Multiple innervation of Purkinje cells by climbing fibers in the cerebellum of the adult staggerer mutant mouse. J Neurobiol *11*, 41-50.

Mariani, J., Benoit, P., Hoang, M. D., Thomson, M. A., and Delhaye-Bouchaud, N. (1990). Extent of multiple innervation of cerebellar Purkinje cells by climbing fibers in adult X-irradiated rats. Comparison of different schedules of irradiation during the first postnatal week. Brain Res Dev Brain Res *57*, 63-70.

Mariani, J., Crepel, F., Mikoshiba, K., Changeux, J. P., and Sotelo, C. (1977). Anatomical, physiological and biochemical studies of the cerebellum from Reeler mutant mouse. Philos Trans R Soc Lond B Biol Sci *281*, 1-28.

Mariani, J., Mulle, C., Geoffroy, B., and Delhaye-Bouchaud, N. (1987). Peripheral maps and synapse elimination in the cerebellum of the rat. II. Representation of peripheral inputs through the climbing fiber pathway in the posterior vermis of X-irradiated adult rats. Brain Res *421*, 211-225.

Marr, D. (1969). A theory of cerebellar cortex. J Physiol *202*, 437-470.

McCabe, J. B., and Berthiaume, L. G. (2001). N-terminal protein acylation confers localization to cholesterol, sphingolipid-enriched membranes but not to lipid rafts/caveolae. Mol Biol Cell *12*, 3601-3617.

McGee, A. W., and Strittmatter, S. M. (2003). The Nogo-66 receptor: focusing myelin inhibition of axon regeneration. Trends Neurosci *26*, 193-198.

McKerracher, L., and Winton, M. J. (2002). Nogo on the go. Neuron *36*, 345-348.

McKerracher, L., David, S., Jackson, D. L., Kottis, V., Dunn, R. J., and Braun, P. E. (1994). Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. Neuron *13*, 805-811.

McNamara, R. K., and Lenox, R. H. (1997). Comparative distribution of myristoylated alanine-rich C kinase substrate (MARCKS) and F1/GAP-43 gene expression in the adult rat brain. J Comp Neurol *379*, 48-71.

McNamara, R. K., and Lenox, R. H. (1997). Comparative distribution of myristoylated alanine-rich C kinase substrate (MARCKS) and F1/GAP-43 gene expression in the adult rat brain. J Comp Neurol 379, 48-71.

McNamara, R. K., and Routtenberg, A. (1995). NMDA receptor blockade prevents kainate induction of protein F1/GAP-43 mRNA in hippocampal granule cells and subsequent mossy fiber sprouting in the rat. Brain Res Mol Brain Res *33*, 22-28.

McQuarrie, I. G., and Grafstein, B. (1973). Axon outgrowth enhanced by a previous nerve injury. Arch Neurol *29*, 53-55.

Meijering, E., Jacob, M., Sarria, J. C., Steiner, P., Hirling, H., and Unser, M. (2004). Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. Cytometry A 58, 167-176.

Meiri, K. F., and Gordon-Weeks, P. R. (1990). GAP-43 in growth cones is associated with areas of membrane that are tightly bound to substrate and is a component of a membrane skeleton subcellular fraction. J Neurosci *10*, 256-266.

Meiri, K. F., Pfenninger, K. H., and Willard, M. B. (1986). Growth-associated protein, GAP-43, a polypeptide that is induced when neurons extend axons, is a component of growth cones and corresponds to pp46, a major polypeptide of a subcellular fraction enriched in growth cones. Proc Natl Acad Sci U S A *83*, 3537-3541.

Meiri, K. F., Saffell, J. L., Walsh, F. S., and Doherty, P. (1998). Neurite outgrowth stimulated by neural cell adhesion molecules requires growth-associated protein-43 (GAP-43) function and is associated with GAP-43 phosphorylation in growth cones. J Neurosci *18*, 10429-10437.

Milligan, G., Parenti, M., and Magee, A. I. (1995). The dynamic role of palmitoylation in signal transduction. Trends Biochem Sci *20*, 181-187.

Milosevic, N. T., and Ristanovic, D. (2007). The Sholl analysis of neuronal cell images: semi-log or log-log method? J Theor Biol 245, 130-140.

Miyakawa, H., Lev-Ram, V., Lasser-Ross, N., and Ross, W. N. (1992). Calcium transients evoked by climbing fiber and parallel fiber synaptic inputs in guinea pig cerebellar Purkinje neurons. J Neurophysiol *68*, 1178-1189.

Miyake, K., Yamamoto, W., Tadokoro, M., Takagi, N., Sasakawa, K., Nitta, A., Furukawa, S., and Takeo, S. (2002). Alterations in hippocampal GAP-43, BDNF, and L1 following sustained cerebral ischemia. Brain Res *935*, 24-31.

Miyata, M., Okada, D., Hashimoto, K., Kano, M., and Ito, M. (1999). Corticotropin-releasing factor plays a permissive role in cerebellar long-term depression. Neuron *22*, 763-775.

Mobarak, C. D., Anderson, K. D., Morin, M., Beckel-Mitchener, A., Rogers, S. L., Furneaux, H., King, P., and Perrone-Bizzozero, N. I. (2000). The RNA-binding protein HuD is required for GAP-43 mRNA stability, GAP-43 gene expression, and PKC-dependent neurite outgrowth in PC12 cells. Mol Biol Cell *11*, 3191-3203.

Montarolo, P. G., Palestini, M., and Strata, P. (1982). The inhibitory effect of the olivocerebellar input on the cerebellar Purkinje cells in the rat. J Physiol *332*, 187-202.

Morando, L., Cesa, R., Rasetti, R., Harvey, R., and Strata, P. (2001). Role of glutamate delta -2 receptors in activity-dependent competition between heterologous afferent fibers. Proc Natl Acad Sci U S A *98*, 9954-9959.

Morgenstern, D. A., Asher, R. A., and Fawcett, J. W. (2002). Chondroitin sulphate proteoglycans in the CNS injury response. Prog Brain Res *137*, 313-332.

Moser, M. B. (1999). Making more synapses: a way to store information? Cell Mol Life Sci *55*, 593-600.

Moser, M. B., Trommald, M., and Andersen, P. (1994). An increase in dendritic spine density on hippocampal CA1 pyramidal cells following spatial learning in adult rats suggests the formation of new synapses. Proc Natl Acad Sci U S A *91*, 12673-12675.

Mosevitsky, M. I. (2005). Nerve ending "signal" proteins GAP-43, MARCKS, and BASP1. Int Rev Cytol *245*, 245-325.

Mukhopadhyay, G., Doherty, P., Walsh, F. S., Crocker, P. R., and Filbin, M. T. (1994). A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron *13*, 757-767.

Mumby, S. M. (1997). Reversible palmitoylation of signaling proteins. Curr Opin Cell Biol *9*, 148-154.

Nadler, J. V., and Cotman, C. W. (1978). Interactions between afferents to the dentate gyrus after entorhinal lesion during development: long-term regulation of choline acetyl-transferase activity. Brain Res *142*, 174-181.

Neve, R. L., Coopersmith, R., McPhie, D. L., Santeufemio, C., Pratt, K. G., Murphy, C. J., and Lynn, S. D. (1998). The neuronal growth-associated protein GAP-43 interacts with rabaptin-5 and participates in endocytosis. J Neurosci *18*, 7757-7767.

Nishiyama, H., Fukaya, M., Watanabe, M., and Linden, D. J. (2007). Axonal motility and its modulation by activity are branch-type specific in the intact adult cerebellum. Neuron *56*, 472-487.

Nishiyama, H., Fukaya, M., Watanabe, M., and Linden, D. J. (2007). Axonal motility and its modulation by activity are branch-type specific in the intact adult cerebellum. Neuron 56, 472-487.

Nolan, T., Hands, R. E., and Bustin, S. A. (2006). Quantification of mRNA using real-time RT-PCR. Nat Protoc 1, 1559-1582.

Nozumi, M., Togano, T., Takahashi-Niki, K., Lu, J., Honda, A., Taoka, M., Shinkawa, T., Koga, H., Takeuchi, K., Isobe, T., and Igarashi, M. (2009). Identification of functional marker proteins in the mammalian growth cone. Proc Natl Acad Sci U S A *106*, 17211-17216.

Oestreicher, A. B., De Graan, P. N., Gispen, W. H., Verhaagen, J., and Schrama, L. H. (1997). B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system. Prog Neurobiol *53*, 627-686.

Oestreicher, A. B., De Graan, P. N., Gispen, W. H., Verhaagen, J., and Schrama, L. H. (1997). B-50, the growth associated protein-43: modulation of cell morphology and communication in the nervous system. Prog Neurobiol 53, 627-686.

Offermanns, S., Hashimoto, K., Watanabe, M., Sun, W., Kurihara, H., Thompson, R. F., Inoue, Y., Kano, M., and Simon, M. I. (1997). Impaired motor coordination and persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking Galphaq. Proc Natl Acad Sci U S A *94*, 14089-14094.

Ohtsuki, G., Piochon, C., and Hansel, C. (2009). Climbing fiber signaling and cerebellar gain control. Front Cell Neurosci *3*, 4.

Palay, S. L., Chan-Palay, V. (1974). Cerebellar Cortex: Cytology and Organisation (Berlin: Springer-Verlag).

Paves, H., Neuman, T., Metsis, M., and Saarma, M. (1990). Nerve growth factor-induced rapid reorganization of microfilaments in PC12 cells: possible roles of different second messenger systems. Exp Cell Res 186, 218-226.

Perkel, D. J., Hestrin, S., Sah, P., and Nicoll, R. A. (1990). Excitatory synaptic currents in Purkinje cells. Proc Biol Sci *241*, 116-121.

Perrone-Bizzozero, N. I., Neve, R. L., Irwin, N., Lewis, S., Fischer, I., and Benowitz, L. I. (1991). Post-transcriptional regulation of GAP-43 rnRNA levels during neuronal differentiation and nerve regeneration. Mol Cell Neurosci *2*, 402-409.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29, e45.

Piochon, C., Irinopoulou, T., Brusciano, D., Bailly, Y., Mariani, J., and Levenes, C. (2007). NMDA receptor contribution to the climbing fiber response in the adult mouse Purkinje cell. J Neurosci *27*, 10797-10809.

Pollerberg, G. E., Burridge, K., Krebs, K. E., Goodman, S. R., and Schachner, M. (1987). The 180-kD component of the neural cell adhesion molecule N-CAM is involved in cell-cell contacts and cytoskeleton-membrane interactions. Cell Tissue Res *250*, 227-236.

Pollerberg, G. E., Schachner, M., and Davoust, J. (1986). Differentiation state-dependent surface mobilities of two forms of the neural cell adhesion molecule. Nature *324*, 462-465.

Powell, C. M. (2006). Gene targeting of presynaptic proteins in synaptic plasticity and memory: across the great divide. Neurobiol Learn Mem *85*, 2-15.

Prinjha, R., Moore, S. E., Vinson, M., Blake, S., Morrow, R., Christie, G., Michalovich, D., Simmons, D. L., and Walsh, F. S. (2000). Inhibitor of neurite outgrowth in humans. Nature *403*, 383-384.

Qiu, Z., and Ghosh, A. (2008). A calcium-dependent switch in a CREST-BRG1 complex regulates activity-dependent gene expression. Neuron 60, 775-787.

Raisman, G. (1969). Neuronal plasticity in the septal nuclei of the adult rat. Brain Res *14*, 25-48.

Ramakers, G. J. (2002). Rho proteins, mental retardation and the cellular basis of cognition. Trends Neurosci *25*, 191-199.

Ramakers, G. M., De Graan, P. N., Urban, I. J., Kraay, D., Tang, T., Pasinelli, P., Oestreicher, A. B., and Gispen, W. H. (1995). Temporal differences in the phosphorylation state of pre- and postsynaptic protein kinase C substrates B-50/GAP-43 and neurogranin during long-term potentiation. J Biol Chem *270*, 13892-13898.

Ramakers, G. M., Heinen, K., Gispen, W. H., and de Graan, P. N. (2000). Long term depression in the CA1 field is associated with a transient decrease in pre- and postsynaptic PKC substrate phosphorylation. J Biol Chem *275*, 28682-28687.

Ramakers, G. M., McNamara, R. K., Lenox, R. H., and De Graan, P. N. (1999). Differential changes in the phosphorylation of the protein kinase C substrates

myristoylated alanine-rich C kinase substrate and growth-associated protein-43/B-50 following Schaffer collateral long-term potentiation and long-term depression. J Neurochem *73*, 2175-2183.

Ramirez-Amaya, V., Balderas, I., Sandoval, J., Escobar, M. L., and Bermudez-Rattoni, F. (2001). Spatial long-term memory is related to mossy fiber synaptogenesis. J Neurosci *21*, 7340-7348.

Ramon y Cajal, S. (1911). Histologie du Système Nerveux de l'Homme et des Vertébrés, Vol II (Paris: Maloine).

Rekart, J. L., Meiri, K., and Routtenberg, A. (2005). Hippocampal-dependent memory is impaired in heterozygous GAP-43 knockout mice. Hippocampus *15*, 1-7.

Renzi, M., Farrant, M., and Cull-Candy, S. G. (2007). Climbing-fibre activation of NMDA receptors in Purkinje cells of adult mice. J Physiol *585*, 91-101.

Reynolds, A., Leake, D., Boese, Q., Scaringe, S., Marshall, W. S., and Khvorova, A. (2004). Rational siRNA design for RNA interference. Nat Biotechnol 22, 326-330.

Riederer, B. M., and Routtenberg, A. (1999). Can GAP-43 interact with brain spectrin? Brain Res Mol Brain Res *71*, 345-348.

Riederer, B. M., and Routtenberg, A. (1999). Can GAP-43 interact with brain spectrin? Brain Res Mol Brain Res 71, 345-348.

Riederer, B. M., Zagon, I. S., and Goodman, S. R. (1986). Brain spectrin(240/235) and brain spectrin(240/235E): two distinct spectrin subtypes with different locations within mammalian neural cells. J Cell Biol *102*, 2088-2097.

Robinson, T. E., and Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. Eur J Neurosci *11*, 1598-1604.

Robinson, T. E., Gorny, G., Mitton, E., and Kolb, B. (2001). Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse *39*, 257-266.

Ross, W. N., and Werman, R. (1987). Mapping calcium transients in the dendrites of Purkinje cells from the guinea-pig cerebellum in vitro. J Physiol *389*, 319-336.

Rossi, F., and Strata, P. (1995). Reciprocal trophic interactions in the adult climbing fibre-Purkinje cell system. Prog Neurobiol *47*, 341-369.

Rossi, F., Borsello, T., and Strata, P. (1994). Embryonic Purkinje cells grafted on the surface of the adult uninjured rat cerebellum migrate in the host parenchyma and induce sprouting of intact climbing fibres. Eur J Neurosci *6*, 121-136.

Rossi, F., Borsello, T., Vaudano, E., and Strata, P. (1993). Regressive modifications of climbing fibres following Purkinje cell degeneration in the cerebellar cortex of the adult rat. Neuroscience *53*, 759-778.

Rossi, F., Borsello, T., Vaudano, E., and Strata, P. (1993). Regressive modifications of climbing fibres following Purkinje cell degeneration in the cerebellar cortex of the adult rat. Neuroscience 53, 759-778.

Rossi, F., van der Want, J. J., Wiklund, L., and Strata, P. (1991a). Reinnervation of cerebellar Purkinje cells by climbing fibres surviving a subtotal lesion of the

inferior olive in the adult rat. II. Synaptic organization on reinnervated Purkinje cells. J Comp Neurol *308*, 536-554.

Rossi, F., Wiklund, L., van der Want, J. J., and Strata, P. (1991b). Reinnervation of cerebellar Purkinje cells by climbing fibres surviving a subtotal lesion of the inferior olive in the adult rat. I. Development of new collateral branches and terminal plexuses. J Comp Neurol *308*, 513-535.

Rossi, F., Wiklund, L., van der Want, J. J., and Strata, P. (1991). Reinnervation of cerebellar Purkinje cells by climbing fibres surviving a subtotal lesion of the inferior olive in the adult rat. I. Development of new collateral branches and terminal plexuses. J Comp Neurol 308, 513-535.

rossi, P., D'Angelo, E., and Taglietti, V. (1996). Differential long-lasting potentiation of the NMDA and non-NMDA synaptic currents induced by metabotropic and NMDA receptor coactivation in cerebellar granule cells. Eur J Neurosci *8*, 1182-1189.

Routtenberg, A., Cantallops, I., Zaffuto, S., Serrano, P., and Namgung, U. (2000). Enhanced learning after genetic overexpression of a brain growth protein. Proc Natl Acad Sci U S A *97*, 7657-7662.

Sacchetti, B., Scelfo, B., and Strata, P. (2005). The cerebellum: synaptic changes and fear conditioning. Neuroscientist *11*, 217-227.

Sacchetti, B., Scelfo, B., and Strata, P. (2009). Cerebellum and emotional behavior. Neuroscience *162*, 756-762.

Sacconi, L., O'Connor, R. P., Jasaitis, A., Masi, A., Buffelli, M., and Pavone, F. S. (2007). In vivo multiphoton nanosurgery on cortical neurons. J Biomed Opt 12, 050502.

Safo, P. K., and Regehr, W. G. (2005). Endocannabinoids control the induction of cerebellar LTD. Neuron *48*, 647-659.

Sahin, M., and Hockfield, S. (1990). Molecular identification of the Lugaro cell in the cat cerebellar cortex. J Comp Neurol *301*, 575-584.

Salin, P. A., Malenka, R. C., and Nicoll, R. A. (1996). Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. Neuron *16*, 797-803.

Savio, T., and Schwab, M. E. (1989). Rat CNS white matter, but not gray matter, is nonpermissive for neuronal cell adhesion and fiber outgrowth. J Neurosci *9*, 1126-1133.

Saxena, S., and Caroni, P. (2007). Mechanisms of axon degeneration: from development to disease. Prog Neurobiol *83*, 174-191.

Saxena, S., and Caroni, P. (2007). Mechanisms of axon degeneration: from development to disease. Prog Neurobiol 83, 174-191.

Schmolesky, M. T., De Ruiter, M. M., De Zeeuw, C. I., and Hansel, C. (2007). The neuropeptide corticotropin-releasing factor regulates excitatory transmission and plasticity at the climbing fibre-Purkinje cell synapse. Eur J Neurosci *25*, 1460-1466.

Schreurs, B. G., Gusev, P. A., Tomsic, D., Alkon, D. L., and Shi, T. (1998). Intracellular correlates of acquisition and long-term memory of classical

conditioning in Purkinje cell dendrites in slices of rabbit cerebellar lobule HVI. J Neurosci *18*, 5498-5507.

Schwab, M. E. (2002). Increasing plasticity and functional recovery of the lesioned spinal cord. Prog Brain Res *137*, 351-359.

Schwab, M. E., and Thoenen, H. (1985). Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. J Neurosci *5*, 2415-2423.

Sharma, N., Marzo, S. J., Jones, K. J., and Foecking, E. M. (2009). Electrical stimulation and testosterone differentially enhance expression of regeneration-associated genes. Exp Neurol.

Shen, Y., Mani, S., Donovan, S. L., Schwob, J. E., and Meiri, K. F. (2002). Growth-associated protein-43 is required for commissural axon guidance in the developing vertebrate nervous system. J Neurosci *22*, 239-247.

Shepherd, G. M. (2004). The synaptic organization of the brain, Vol The synaptic organization of the brain, 5 ed. edn (New York: Oxford Univ. Press).

Sholl, D. A. (1953). Dendritic organization in the neurons of the visual and motor cortices of the cat. J Anat 87, 387-406.

Silver, R. A., Momiyama, A., and Cull-Candy, S. G. (1998). Locus of frequency-dependent depression identified with multiple-probability fluctuation analysis at rat climbing fibre-Purkinje cell synapses. J Physiol *510 (Pt 3)*, 881-902.

Skene, J. H., and Virag, I. (1989). Posttranslational membrane attachment and dynamic fatty acylation of a neuronal growth cone protein, GAP-43. J Cell Biol *108*, 613-624.

Skene, J. H., and Willard, M. (1981). Characteristics of growth-associated polypeptides in regenerating toad retinal ganglion cell axons. J Neurosci *1*, 419-426.

Skene, J. H., and Willard, M. (1981). Characteristics of growth-associated polypeptides in regenerating toad retinal ganglion cell axons. J Neurosci 1, 419-426.

Smith, C. L., Afroz, R., Bassell, G. J., Furneaux, H. M., Perrone-Bizzozero, N. I., and Burry, R. W. (2004). GAP-43 mRNA in growth cones is associated with HuD and ribosomes. J Neurobiol *61*, 222-235.

Sotelo, C. (1978). Purkinje cell ontogeny: formation and maintenance of spines. Prog Brain Res *48*, 149-170.

Sotelo, C. (1990). Axonal abnormalities in cerebellar Purkinje cells of the 'hyperspiny Purkinje cell' mutant mouse. J Neurocytol *19*, 737-755.

Sotelo, C., and Alvarado-Mallart, R. M. (1987). Embryonic and adult neurons interact to allow Purkinje cell replacement in mutant cerebellum. Nature *327*, 421-423.

Sotelo, C., Hillman, D. E., Zamora, A. J., and Llinas, R. (1975). Climbing fiber deafferentation: its action on Purkinje cell dendritic spines. Brain Res *98*, 574-581.

Spencer, S. A., Schuh, S. M., Liu, W. S., and Willard, M. B. (1992). GAP-43, a protein associated with axon growth, is phosphorylated at three sites in cultured neurons and rat brain. J Biol Chem *267*, 9059-9064.

Sretavan, D. W., and Kruger, K. (1998). Randomized retinal ganglion cell axon routing at the optic chiasm of GAP-43-deficient mice: association with midline recrossing and lack of normal ipsilateral axon turning. J Neurosci *18*, 10502-10513.

Sretavan, D. W., Feng, L., Pure, E., and Reichardt, L. F. (1994). Embryonic neurons of the developing optic chiasm express L1 and CD44, cell surface molecules with opposing effects on retinal axon growth. Neuron *12*, 957-975.

Steinmayr, M., Andre, E., Conquet, F., Rondi-Reig, L., Delhaye-Bouchaud, N., Auclair, N., Daniel, H., Crepel, F., Mariani, J., Sotelo, C., and Becker-Andre, M. (1998). staggerer phenotype in retinoid-related orphan receptor alpha-deficient mice. Proc Natl Acad Sci U S A *95*, 3960-3965.

Steketee, M. B., and Tosney, K. W. (2002). Three functionally distinct adhesions in filopodia: shaft adhesions control lamellar extension. J Neurosci *22*, 8071-8083.

Stettler, D. D., Yamahachi, H., Li, W., Denk, W., and Gilbert, C. D. (2006). Axons and synaptic boutons are highly dynamic in adult visual cortex. Neuron *49*, 877-887.

Strahlendorf, J. C., and Hubbard, G. D. (1983). Serotonergic interactions with rat cerebellar Purkinje cells. Brain Res Bull *11*, 265-269.

Strata, P. (2009). David Marr's theory of cerebellar learning: 40 years later. J Physiol *587*, 5519-5520.

Strata, P., and Rossi, F. (1998). Plasticity of the olivocerebellar pathway. Trends Neurosci *21*, 407-413.

Strittmatter, S. M., Fankhauser, C., Huang, P. L., Mashimo, H., and Fishman, M. C. (1995). Neuronal pathfinding is abnormal in mice lacking the neuronal growth cone protein GAP-43. Cell *80*, 445-452.

Strittmatter, S. M., Valenzuela, D., Kennedy, T. E., Neer, E. J., and Fishman, M. C. (1990). G0 is a major growth cone protein subject to regulation by GAP-43. Nature *344*, 836-841.

Sugihara, I., Wu, H., and Shinoda, Y. (1999). Morphology of single olivocerebellar axons labeled with biotinylated dextran amine in the rat. J Comp Neurol *414*, 131-148.

Sugihara, I., Wu, H., and Shinoda, Y. (1999). Morphology of single olivocerebellar axons labeled with biotinylated dextran amine in the rat. J Comp Neurol 414, 131-148.

Svoboda, K., and Yasuda, R. (2006). Principles of two-photon excitation microscopy and its applications to neuroscience. Neuron 50, 823-839.

Szapiro, G., and Barbour, B. (2007). Multiple climbing fibers signal to molecular layer interneurons exclusively via glutamate spillover. Nat Neurosci *10*, 735-742.

Szulc, J., Wiznerowicz, M., Sauvain, M. O., Trono, D., and Aebischer, P. (2006). A versatile tool for conditional gene expression and knockdown. Nat Methods 3, 109-116.

Tempia, F., Bravin, M., and Strata, P. (1996). Postsynaptic currents and short-term synaptic plasticity in Purkinje cells grafted onto an uninjured adult cerebellar cortex. Eur J Neurosci *8*, 2690-2701.

Tetzlaff, W., Alexander, S. W., Miller, F. D., and Bisby, M. A. (1991). Response of facial and rubrospinal neurons to axotomy: changes in mRNA expression for cytoskeletal proteins and GAP-43. J Neurosci *11*, 2528-2544.

Thach, W. T., Jr. (1967). Somatosensory receptive fields of single units in cat cerebellar cortex. J Neurophysiol *30*, 675-696.

Tian, J. B., and Bishop, G. A. (2003). Frequency-dependent expression of corticotropin releasing factor in the rat's cerebellum. Neuroscience *121*, 363-377.

Tiscornia, G., Singer, O., Ikawa, M., and Verma, I. M. (2003). A general method for gene knockdown in mice by using lentiviral vectors expressing small interfering RNA. Proc Natl Acad Sci U S A 100, 1844-1848.

Tiscornia, G., Tergaonkar, V., Galimi, F., and Verma, I. M. (2004). CRE recombinase-inducible RNA interference mediated by lentiviral vectors. Proc Natl Acad Sci U S A 101, 7347-7351.

Turrigiano, G. G. (1999). Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. Trends Neurosci *22*, 221-227.

van Beugen, B. J., Nagaraja, R. Y., and Hansel, C. (2006). Climbing fiber-evoked endocannabinoid signaling heterosynaptically suppresses presynaptic cerebellar long-term potentiation. J Neurosci *26*, 8289-8294.

Van Hooff, C. O., De Graan, P. N., Boonstra, J., Oestreicher, A. B., Schmidt-Michels, M. H., and Gispen, W. H. (1986). Nerve growth factor enhances the level of the protein kinase C substrate B-50 in pheochromocytoma PC12 cells. Biochem Biophys Res Commun 139, 644-651.

Van Lookeren Campagne, M., Oestreicher, A. B., Van Bergen en Henegouwen, P. M., and Gispen, W. H. (1990). Ultrastructural double localization of B-50/GAP43 and synaptophysin (p38) in the neonatal and adult rat hippocampus. J Neurocytol *19*, 948-961.

Villegas-Perez, M. P., Vidal-Sanz, M., Bray, G. M., and Aguayo, A. J. (1988). Influences of peripheral nerve grafts on the survival and regrowth of axotomized retinal ganglion cells in adult rats. J Neurosci *8*, 265-280.

Vogel, A., Noack, J., Hüttman, G., and G., P. (2005). Mechanisms of femtosecond laser nanosurgery of cells and tissues. Appl Phys B 81, 1015–1047.

Volkmar, F. R., and Greenough, W. T. (1972). Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science *176*, 1445-1447.

Waller, A. (1850). Experiments on the section of glossopharyngeal and hypoglossal nerves of the frog and observations of the alternatives produced thereby in the structure of their primitive fibers. Phil Trans R Soc London *140*, 423–429.

Wang, K. C., Koprivica, V., Kim, J. A., Sivasankaran, R., Guo, Y., Neve, R. L., and He, Z. (2002). Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. Nature *417*, 941-944.

Wang, S. S., Khiroug, L., and Augustine, G. J. (2000). Quantification of spread of cerebellar long-term depression with chemical two-photon uncaging of glutamate. Proc Natl Acad Sci U S A *97*, 8635-8640.

Watanabe, M. (2008). Molecular mechanisms governing competitive synaptic wiring in cerebellar Purkinje cells. Tohoku J Exp Med *214*, 175-190.

Watterson, J. M., Watson, D. G., Meyer, E. M., and Lenox, R. H. (2002). A role for protein kinase C and its substrates in the action of valproic acid in the brain: implications for neural plasticity. Brain Res *934*, 69-80.

Weber, J. T., De Zeeuw, C. I., Linden, D. J., and Hansel, C. (2003). Long-term depression of climbing fiber-evoked calcium transients in Purkinje cell dendrites. Proc Natl Acad Sci U S A *100*, 2878-2883.

Wehrle, R., Caroni, P., Sotelo, C., and Dusart, I. (2001). Role of GAP-43 in mediating the responsiveness of cerebellar and precerebellar neurons to axotomy. Eur J Neurosci *13*, 857-870.

Werner, A., Willem, M., Jones, L. L., Kreutzberg, G. W., Mayer, U., and Raivich, G. (2000). Impaired axonal regeneration in alpha7 integrin-deficient mice. J Neurosci *20*, 1822-1830.

Wilbrey, A. L., Haley, J. E., Wishart, T. M., Conforti, L., Morreale, G., Beirowski, B., Babetto, E., Adalbert, R., Gillingwater, T. H., Smith, T., *et al.* (2008). VCP binding influences intracellular distribution of the slow Wallerian degeneration protein, Wld(S). Mol Cell Neurosci *38*, 325-340.

Wishart, T. M., Paterson, J. M., Short, D. M., Meredith, S., Robertson, K. A., Sutherland, C., Cousin, M. A., Dutia, M. B., and Gillingwater, T. H. (2007). Differential proteomics analysis of synaptic proteins identifies potential cellular targets and protein mediators of synaptic neuroprotection conferred by the slow Wallerian degeneration (Wlds) gene. Mol Cell Proteomics *6*, 1318-1330.

Wolpert, D. M., Miall, R. C., and Kawato, M. (1998). Internal models in the cerebellum. Trends in Cognitive Sciences *2*, 338-347.

Yamada, K. M., Spooner, B. S., and Wessells, N. K. (1970). Axon growth: roles of microfilaments and microtubules. Proc Natl Acad Sci U S A *66*, 1206-1212.

Yang, J. F., Cao, G., Koirala, S., Reddy, L. V., and Ko, C. P. (2001). Schwann cells express active agrin and enhance aggregation of acetylcholine receptors on muscle fibers. J Neurosci *21*, 9572-9584.

Yanik, M. F., Cinar, H., Cinar, H. N., Chisholm, A. D., Jin, Y., and Ben-Yakar, A. (2004). Neurosurgery: functional regeneration after laser axotomy. Nature 432, 822.

Yuzaki, M. (2003). The delta2 glutamate receptor: 10 years later. Neurosci Res *46*, 11-22.

Zakharov, V. V., and Mosevitsky, M. I. (2001). Site-specific calcium-dependent proteolysis of neuronal protein GAP-43. Neurosci Res *39*, 447-453.

Zakharov, V. V., and Mosevitsky, M. I. (2007). M-calpain-mediated cleavage of GAP-43 near Ser41 is negatively regulated by protein kinase C, calmodulin and calpain-inhibiting fragment GAP-43-3. J Neurochem *101*, 1539-1551.

Zakharov, V. V., Bogdanova, M. N., and Mosevitsky, M. I. (2005). Specific proteolysis of neuronal protein GAP-43 by calpain: characterization, regulation, and physiological role. Biochemistry (Mosc) *70*, 897-907.

Zhang, Y., Bo, X., Schoepfer, R., Holtmaat, A. J., Verhaagen, J., Emson, P. C., Lieberman, A. R., and Anderson, P. N. (2005). Growth-associated protein GAP-43

and L1 act synergistically to promote regenerative growth of Purkinje cell axons in vivo. Proc Natl Acad Sci U S A *102*, 14883-14888.

Zhang, Y., Bo, X., Schoepfer, R., Holtmaat, A. J., Verhaagen, J., Emson, P. C., Lieberman, A. R., and Anderson, P. N. (2005). Growth-associated protein GAP-43 and L1 act synergistically to promote regenerative growth of Purkinje cell axons in vivo. Proc Natl Acad Sci U S A 102, 14883-14888.

Zhao, H. M., Wenthold, R. J., and Petralia, R. S. (1998). Glutamate receptor targeting to synaptic populations on Purkinje cells is developmentally regulated. J Neurosci *18*, 5517-5528.

Zuo, Y., Yang, G., Kwon, E., and Gan, W. B. (2005). Long-term sensory deprivation prevents dendritic spine loss in primary somatosensory cortex. Nature *436*, 261-265.

Zwiers, H., Veldhuis, H. D., Schotman, P., and Gispen, W. H. (1976). ACTH, cyclic nucleotides, and brain protein phosphorylation in vitro. Neurochem Res *1*, 669-677.