The Neuroscientist

http://nro.sagepub.com

Book Review: Serotonergic Neuromodulation in the Cerebellar Cortex: Cellular, Synaptic, and Molecular Basis

Stéphane Dieudonné

Neuroscientist 2001; 7; 207
DOI: 10.1177/107385840100700306

The online version of this article can be found at:
http://nro.sagepub.com/cgi/content/abstract/7/3/207

Published by:

http://www.sagepublications.com

Additional services and information for *The Neuroscientist* can be found at:

Email Alerts: http://nro.sagepub.com/cgi/alerts

Subscriptions: http://nro.sagepub.com/subscriptions

Reprints: http://www.sagepub.com/journalsReprints.nav

Permissions: http://www.sagepub.com/journalsPermissions.nav

Citations http://nro.sagepub.com/cgi/content/refs/7/3/207

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

Serotonergic Neuromodulation in the Cerebellar Cortex: Cellular, Synaptic, and Molecular Basis

STÉPHANE DIEUDONNÉ
Department of Biology
Ecole Normale Superieure
Paris, France

The cerebellum, like most sensorimotor areas of the brain, receives a serotonergic innervation from neurons of the reticular formation. It is well established that local application of serotonin modulates the firing rate of cerebellar Purkinje cells in vivo and in vitro, but the mechanisms by which serotonin affects the cerebellar function are still poorly understood. Whereas interactions between serotonin, glutamate, and GABA have been reported to increase or decrease the firing frequency of Purkinje cells, there is little evidence for a modulation of excitatory and inhibitory synapses by serotonin in the cerebellar cortex. Changes in the intrinsic electrical properties of Purkinje cells upon application of serotonin have also been reported, but their impact on Purkinje cell firing is unclear. The recent finding that serotonin specifically modulates the activity of Lugaro cells, a class of inhibitory interneurons of the cerebellar cortex, offers new insights on the action of this neuromodulator. The peculiar axonal projection and specific interneuronal targets of the Lugaro cells suggest that the action of serotonin might occur upstream of Purkinje cells through a resetting of the computational properties of the cerebellar cortex. Understanding the mechanisms of the serotonergic modulation of the cerebellar cortex is of clinical relevance, as abnormal serotonin metabolism has been observed in animal models and pathological cases of motor disorders involving the cerebellum, and as chronic intravenous administration of L-5-hydroxytryptophan (5-HTP), a precursor of serotonin, was the first treatment shown to improve significantly cerebellar symptoms. NEUROSCIENTIST 7(3):207–219, 2001

KEY WORDS *Serotonin, Cerebellum, Cerebellar cortex, Interneurons, Neuromodulation, Motor control*

The serotonergic innervation of the cerebellar cortex in the form of thin varicose fibers was first described using fluorescent histochemical methods (Höckfelt and Fuxe 1969), and serotonergic afferents arrive early in the immature cerebellum of both the rat (Lidow and Molliver 1982) and the opossum (Bishop and others 1988). Serotonergic modulation of the mature cerebellum has been a subject of clinical interest for 10 years (Trouillas and Fuxe 1993). Daily long-term administration of L-5-hydroxytryptophan (5-HTP), a precursor of serotonin, improves the dysfunctions associated with some cerebellar disorders and was the first treatment for cerebellar ataxia. The design of more efficient treatments could benefit from a better knowledge of the cellular and synaptic mechanisms of the action of serotonin and of the serotonin receptor subtypes involved. The published data are reviewed here, in an attempt to extrapolate from cellular-level to network-level neuromodulatory effects. Such extrapolation is based on our detailed knowledge of the cellular and synaptic organization of the cerebellar cortex and its afferents.

The cerebellar cortex receives two classes of glutamatergic afferent fibers: the mossy fibers, originating from many sensorimotor areas, and the climbing fibers, originating exclusively from the inferior olivary nuclei. The Purkinje cell axonal projection to the deep cerebellar nuclei constitutes in turn the only output of the cerebellar cortex. The climbing fibers make a powerful synapse on Purkinje cells, which produces an all-or-none response called a complex spike, a distinctive burst of several action potentials at 300 to 500 Hz. Although complex spikes constitute a qualitatively important aspect of the cerebellar cortex output, the modulation of complex spikes firing by serotonin, because it depends entirely on the action of serotonin on inferior olivary neurons, is beyond the scope of this article. The input of mossy fibers is relayed to Purkinje cells by glutamatergic granule cells, through their axons the parallel fibers. This disynaptic pathway is controlled by several inhibitory interneurons: the Golgi cells, at the level of granule cells, and the molecular layer interneurons (basket cells and stellate cells), at the level of Purkinje cells. The treatment of mossy fibers input therefore depends on all the cellular components of the cortex and is ultimately coded in the pattern of Purkinje cell single spike firing. The cellular and molecular

This work was supported by a postdoctoral fellowship from the Foundation Fyssen, France. I thank Boris Barbour for careful reading of the manuscript.

Address correspondence to: Stéphane Dieudonné, Ph.D., Laboratoire de Neurobiologie, CNRS UMR 8544, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France (e-mail: sdieudon@wotan.ens.fr).

Copyright © 2001 Sage Publications
ISSN 1073-8584

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

substrates for the serotonergic modulation of Purkinje cell single spike firing rate are discussed in relation with data concerning serotonin innervation of the cerebellar cortex and serotonin receptor expression patterns. The physiological and biochemical data concerning neuromodulatory actions of serotonin on the different cellular components of the cerebellar cortex are presented. An emphasis is put on the recently described excitatory effect of serotonin on Lugaro cells, a cerebellar interneuron with original synaptic projections (Dieudonné and Dumoulin 2000). It is proposed that the Lugaro cell constitutes the main target and effector of serotonergic neuromodulation in the cerebellar cortex. By making long-distance connections with the other cerebellar inhibitory interneurons, the Lugaro cell may control the pattern of activity in granule and Purkinje cells, allowing for a serotonin-operated intracortical switch.

Serotonergic Afferents to the Cerebellum

Serotonin-Containing Fibers in the Cerebellum

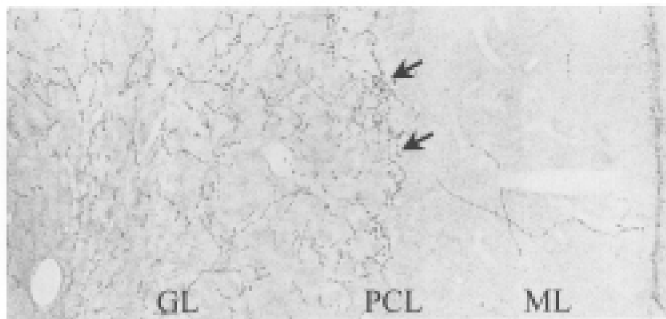
Biochemical measurements of 5-HT content in various parts of the rat brain have shown that the adult cerebellum is among the CNS structures containing the least 5-HT (Palkovits and others 1974). This is due to the low overall density of 5-HT-containing varicosities, which number 240,000 per mm^3 of the rat cerebellar cortex (Beaudet and Sotelo 1981). The first detailed studies of the serotonergic innervation of the cerebellar cortex, including electron microscopic observations, were performed using autoradiographic tracing techniques following intraventricular infusion of tritiated 5-HT in rats and Rhesus monkeys (Chan-Palay 1975) or superfusion of the rat cerebellar vermis with tritiated 5-HT (Beaudet and Sotelo 1981). In both studies, two putative serotonergic afferents were described: large terminals typical of mossy fibers (about 1% of all mossy fibers) and fine beaded axons distributed in all layers of the cerebellar cortex. Only the thin varicose axons were found in all subsequent studies, which used immunohistochemical methods to detect serotonin directly in the rat (Takeuchi and others 1982; Bishop and Ho 1985), cat (Takeuchi and others 1982; Bishop and Ho 1985; Kerr and Bishop 1991), and opossum (Bishop and others 1985). Immunocytochemical detection of the serotonin transporter in the cerebellum also revealed only fine beaded fibers similar to the serotonin-immunoreactive fibers (Sur and others 1996). The transporter is expressed at high density in the varicosities of these axons, which can be found both in the granular and molecular layers (Sur and others 1996). An estimated 10% of the serotonergic varicosities display a classical axosomatic or axodendritic synaptic junction (Beaudet and Sotelo 1981). In the cerebellum, most of the serotonergic transmission is therefore nonjunctional and serotonin exerts its effects through volume transmission, by acting on high affinity receptors.

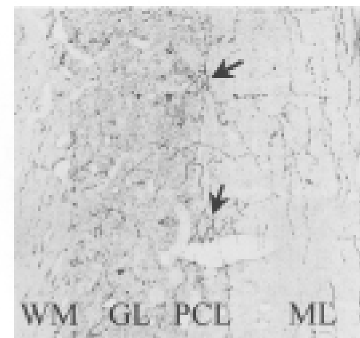
Although some mossy fibers display a nonspecific uptake of [^{3}H]serotonin when it is present at high concentrations in the extracellular space (10^{-4} M in the presence of nialamide, an MAO inhibitor), serotonin is not found at high enough concentrations in these mossy fibers to be a potential neurotransmitter. The local origin of labeled mossy fibers is supported by the fact that these fibers (but not the thin varicose serotonin-immunopositive ones or the classical serotonin-immunonegative mossy fibers) disappear when the cerebellum is X-irradiated to produce an agranular cortex. This [^{3}H]serotonin uptake might therefore reveal the population of mossy fibers originating from the deep cerebellar nuclei. Indeed, when the concentration of serotonin is artificially raised by pretreating cats with L-tryptophan and pargyline, some lightly serotonin-immunoreactive cell bodies are found in the deep cerebellar nuclei (Kerr and Bishop 1991). These cells are similar in their morphology to deep nuclear neurons sending mossy fiber–like projections to the cerebellar cortex (Tolbert and others 1976; Tolbert and others 1978) and may therefore account for the labeling of some mossy fibers by tritiated serotonin.

Organization of Serotonergic Fibers in the Cerebellum

In the rat (Takeuchi and others 1982), the cat (Takeuchi and others 1982; Kerr and Bishop 1991), and the opossum (Bishop and others 1985), the deep cerebellar nuclei receive a rather dense and homogeneous plexus of fine (0.25 µm diameter) varicose (average 1.3 µm diameter every 4 µm) axons (Bishop and others 1985) with a preferred medio-lateral orientation. In contrast, in the cerebellar cortex, the density of the plexus of serotonergic fibers, their preferred orientation, and their partition between the three cortical layers differs between species and even regions of the cerebellar cortex in a given species (see Fig. 1). This complex organization may indicate variations in the cellular targets of serotonin (see the section on Lugaro cells). In the cat, the density of serotonergic fibers is very homogeneous across lobules (Takeuchi and others 1982; Kerr and Bishop 1991), whereas in the rat the density is higher in vermal lobules VII to X, paramedian lobule and crusII (Bishop and Ho 1985). In the opossum, the highest density is found in lobules VIII and IX and the lowest density is found in lobules II to V, paramedian lobule and flocculus (Bishop and others 1985). In the cat, there is a very small number of fibers in the molecular layer and a diffuse plexus in the whole depth of the granular layer (Takeuchi and others 1982; Kerr and Bishop 1991) (Fig. 1*A*). In the opossum, serotonergic fibers are also virtually absent from the molecular layer but the plexus concentrates almost exclusively in the upper part of the granular layer and in the adjacent Purkinje cell layer (Bishop and others 1985). When observed in a plane of section tangential to the Purkinje cell layer, the fibers orient mainly in the mediolateral and rostrocaudal directions, forming a grid-like network (Bishop and others

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009





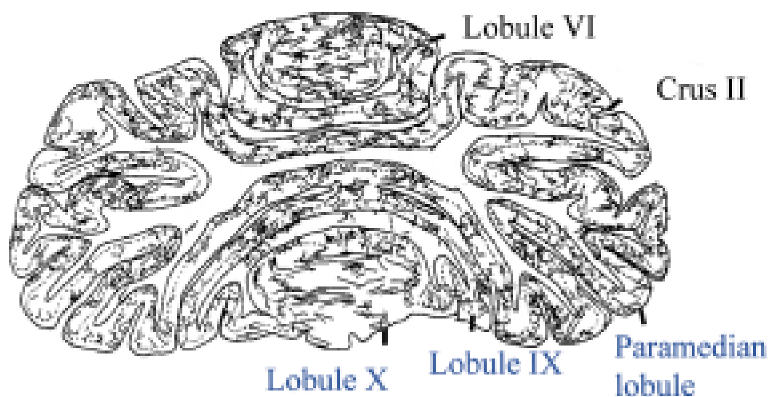


Fig. 1. Serotonergic fibers in the cerebellar cortex, as detected by immunohistochemistry against serotonin. *A*. In the cat, the plexus of thin varicose serotonin-immunoreactive fibers is nearly exclusively restricted to the granular and Purkinje cell layers. In the rat, serotonergic fibers ascend to the molecular layer where they extend in the longitudinal direction. Note the higher density of fibers in and around the Purkinje cell layer (PCL), arrows. Frontal sections, adapted from Takeuchi and others (1982) (© Springer-Verlag 1982). *B*. Reconstruction of the serotonergic plexus in a frontal section of the whole rat cerebellar cortex. In the detail of the lobus simplex, note again the dense plexus around the Purkinje cell layer. Adapted from Bishop and Ho (1985) (© Elsevier 1985). GL = granule cell layer; ML = molecular layer; PCL = Purkinje cell layer; WM = white matter.

1985). In the rat (Chan-Palay 1975; Takeuchi and others 1982; Bishop and Ho 1985) and mouse (Triarhou and Ghetti 1991), the same distribution as in the opossum is found in the upper granule cell layer and in the Purkinje cell layer, but there is a substantial plexus of fibers in the molecular layer (Fig. 1). In most lobules, this molecular layer plexus is composed of radial fibers ascending toward the pia and bifurcating into tangential fibers parallel to the parallel fibers (Chan-Palay 1975; Takeuchi and others 1982; Bishop and Ho 1985) (Fig. 1). However, in the flocculus, paraflocculus, and some regions of the hemispheres, tangential and oblique fibers, but not parallel fibers, are found (Takeuchi and others 1982; Bishop and Ho 1985). Therefore, depending on the region, small populations of afferents can modulate wide parasagittal modules through their parallel fiber-like axons or more restricted stripes through oblique and radial axons.

Origin of the Serotonergic Afferents to the Cerebellum

Serotonergic neurons are mostly found in brainstem areas belonging to the reticular formation. Most of them are clustered around the raphe (raphe nuclei), but others are found in lateral nuclei. The distribution of the serotonergic neurons projecting to the cerebellar cortex has been studied by several authors in the rat, the cat, and the opossum, by combining retrograde transport of peroxidase with serotonin immunohistochemistry. Retrogradely labeled neurons are found in all the known precerebellar nuclei, which give rise to the glutamatergic projections to the cerebellum in the form of mossy fibers and climbing fibers. In the ventral precerebellar regions belonging to the reticular formation, a proportion (0.7% to 14%) of the retrogradely labeled neurons were also immunoreactive for serotonin (Kerr and Bishop

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

1991). In the dorsal precerebellar nuclei (vestibular and cuneate nuclei), the same authors found no serotonin-immunoreactive cell bodies, but Batini (1993) found that a substantial portion of the neurons contained serotonin. In the region of the raphe, some cell bodies are retrogradely labeled, but as in the other reticular nuclei, many of them (70%) are not immunoreactive for serotonin (Kerr and Bishop 1991; Batini 1993). The small number of double-labeled neurons in these studies, compared to the dense plexus of serotonergic fibers in the cerebellum, suggests that each serotonergic neuron ramifies extensively in the cerebellar cortex. This ramification is not, however, accompanied by a complete loss of topographical organization (Kerr and Bishop 1991; Batini 1993; Bishop and others 1993). In the anterior vermis, serotonergic fibers arise from neurons of the paramedian reticular nucleus, the lateral reticular nucleus, and the lateral tegmental field. In the posterior vermis and paramedian lobule, serotonergic afferents come from the lateral reticular nucleus. Finally, the hemisphere receives serotonergic afferents from the lateral tegmental field, the periolivary reticular formation, and the paramedian reticular nucleus. These data suggest that a large fraction of the glutamatergic cerebellar afferents are accompanied by serotonergic projections to the same region of the cerebellar cortex.

Kitzman and Bishop (1994) showed that the loci of origin of the serotonergic projections to the deep cerebellar nuclei are essentially distinct from the loci of origin for the cerebellar cortex. They include the medullary raphe nuclei, the pontine superior central raphe nucleus, the nucleus locus coeruleus, the dorsal tegmental nucleus, and the periolivary reticular formation. These results suggest that serotonergic neuromodulation may have different roles in the cerebellar nuclei and cerebellar cortex. Controversial studies on the action of serotonin in the deep cerebellar nuclei (Gardette and others 1987; Cumming-Hood and others 1993; Kitzman and Bishop 1997) will therefore not be reviewed in the next sections, to concentrate on the mode of action of serotonin in the cerebellar cortex.

Serotonin Receptors in the Cerebellar Cortex

In the cerebellar cortex, serotonin mostly acts through volume transmission, because most varicosities lack a morphologically differentiated synapse. Modulation by serotonin must therefore rely on the activation of high affinity serotonin receptors. Many of the physiological data concerning the modulation of the cerebellar cortical neurons by serotonin were obtained at a time when the molecular and pharmacological characterization of the serotonin receptors was just beginning. In addition to the 5-HT$_1$ and 5-HT$_2$ families of metabotropic receptors for serotonin, then defined according to crude pharmacological criteria, five other genes have now been cloned that code for receptors with distinct pharmacological and transductional properties (5-HT$_4$ to 5-HT$_7$). It is therefore useful to review the pattern of expression of the metabotropic serotonin receptors (Table 1) to guide the interpretation of the physiological data. The distribution of many of these receptors in the cerebellar cortex has been quantified by in situ hybridization at the tissue level or by radiolabeling studies. With these techniques, the signals reflect the average level of expression of the receptors. Therefore, the receptors expressed by a small population of interneurons would not be detected, and negative results can only be taken as an indication that the receptors are not expressed by granule cells or Purkinje cells, the main cell populations of the cortex.

The pattern of expression of the 5-HT$_{1A}$ receptor has been studied in great detail in the cerebellar cortex of rodents. Its mRNA is transiently expressed by Purkinje cells and most likely by the molecular layer interneurons from the end of the embryonic stage to P15 (Matthiessen and others 1992; Miquel and others 1994). A peak of expression around P0 and undetectable mRNA levels in the adult have also been observed in humans (Burnet and others 1995). The quantity of receptor, assessed by binding or immunohistochemistry, is maximal between P5 and P10 and decreases to undetectable levels by P15 in rats (Vergé and others 1993; Kung and others 1994; Laporte and others 1994; Mathis and others 1994).

The mRNA for 5-HT$_{1B}$ receptors is expressed by the Purkinje cells but not by granule cells or by the molecular layer interneurons (Boschert and others 1994; Doucet and others 1995). Binding sites with the pharmacological profile of 5-HT$_{1B}$ receptors have not been detected in the cerebellar cortex but are very abundant in the deep cerebellar nuclei, putatively on Purkinje cell axons. A similar pattern in other central structures has led to the hypothesis that 5-HT$_{1B}$ receptors are major presynaptic regulators of the output projection of principal neurons (Boschert and others 1994). In the guinea pig cerebellar cortex, the 5-HT$_{1D}$ receptor mRNA is expressed in the granule cell layer but the protein may be expressed at low densities because binding of specific ligands was not detected (Bonaventure and others 1998).

In situ hybridization studies have revealed low levels of 5-HT$_{2A}$ receptor mRNA in the rat (Pompeiano and others 1994; Wright and others 1995) and human (Burnet and others 1995) cerebellar cortices. Immunohistochemical data are controversial. Polyclonal antibodies raised against a 20–amino acid N–terminal peptide of the receptor stain neurons in the brain but yield only a nonspecific granular staining in the young (before PN28) rat cerebellum (Morilak and others 1993). Another set of affinity-purified polyclonal antibodies stained the main aspiny dendrites of Purkinje cells but no other cellular component of the adult rat cerebellar cortex (Maeshima and others 1998). In line with this result, a monoclonal antibody raised against all 72 N-terminal amino acids of the 5-HT$_{2A}$ receptor stains the Purkinje cell bodies and main dendritic branches, as well as large interneurons (probably Golgi cells) in the

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

Table 1. Expression of the metabotropic serotonin receptor subtypes in the cerebellar cortex

5-HT Receptor Subtype	mRNA Expression	Specific Binding	Immunohistochemistry of Protein Expression
5-HT_{1A}	Purkinje cells and molecular layer interneurons until P15	Not detected after P15 in rat	Purkinje cells and molecular layer interneurons until P15
5-HT_{1B}	Purkinje cell layer	Not detected in the cortex but high in cerebellar nuclei	
5-HT_{1D}	Granule cell layer	Not detected	
5-HT_{2A}	Low levels		Aspiny dendrites of Purkinje cells Golgi cells? Astroglial and Bergman glial cells?
5-HT_{2B}			Purkinje cells and deep cerebellar nuclei
5-HT_{2C}	Not detected	Lowest of all brain	
5-HT_{4}	Not detected		
5-HT_{5a}	Granular layer (mouse) Granular layer and Purkinje (human)		
5-HT_{5b}	Not detected		
5-HT_{6}	Low levels probably in the granular layer		High staining of the molecular layer
5-HT_{7}	Purkinje cell layer		

granular layer (Cornea-Hébert and others 1999). With the commercial form of the same antibody (Pharmingen), and using cryopermeabilization (a technique that might better preserve glial cells), a staining was found only in Bergmann radial glia and granular layer astrocytes (Dieudonné unpublished results). This glial staining would be consistent with the presence of a glial expression sequence in the promoter of the 5-HT_{2A} receptor gene (Ding and others 1993). In conclusion, 5HT-2A receptors may be expressed in Purkinje cells, Golgi cells, and glial cells of the cerebellar cortex.

Although the 5-HT_{2B} receptor is mainly expressed outside of the central nervous system, immunohistochemical data suggest a strong expression of this receptor in Purkinje cells and deep cerebellar nuclei (Choi and Maroteaux 1996; Duxon and others 1997). The mRNA for 5-HT_{2C} receptors could not be detected by in situ hybridization in the adult cerebellum (Molineaux and others 1989; Hellendall and others 1993; Pompeiano and others 1994), and the density of binding sites for mesulergine in the rat cerebellar cortex is the lowest of the brain (Pranzatelli 1993). 5-HT_4 (Eglen and others 1995; Gerald and others 1995) and 5-HT_{5b} (Matthes and others 1992) receptor mRNAs were not detected in the cerebellum, whereas 5-HT_{5a} mRNA is found in the granular layer of the mouse cerebellar cortex (Matthes and others 1992) as well as in the granular layer and Purkinje cells of the human cerebellar cortex (Pasqualetti and others 1998). 5-HT_6 receptor mRNA is detected at low levels in the cerebellum by RT-PCR (Gerard and others 1996) and Northern blot (Ruat and others 1993) and seems to be localized in the granular layer (Ward and others 1995). The protein may be highly expressed in the molecular layer (Gerard and others 1997), suggesting a localization in parallel fibers or in Purkinje cell dendrites. Finally, the mRNA for the 5-HT_7 receptor has been detected by in situ hybridization in the Purkinje cell layer.

In many central structures, serotonin acts as a fast excitatory transmitter at morphologically classical synapses where the ionotropic 5-HT_3 receptor is expressed. 5-HT_3 receptors are often found in a postsynaptic position at axo-axonic synapses, where they modulate transmitter release from the axon that is postsynaptic to the serotonergic synapse. This mode of action is probably very limited in the cerebellar cortex because few varicosities containing serotonin are engaged in classical synapses and because the expression of 5-HT_3 receptor mRNA in the cerebellum is very low (Miquel and others 1995). Nevertheless, calcium increases induced by the activation of 5-HT_3 receptors have been reported in a preparation of cerebellar synaptosomes, and immunoreactivity for 5-HT_3 receptors is present in some of these synaptosomes (Nayak and others 1999). This suggests that, in the cerebellum too, serotonin might control synaptic transmission through the activation of presynaptic 5-HT_3 receptors.

Physiological Effects of Serotonin on Purkinje Cells and Their Synaptic Inputs

Because serotonergic neurons innervating the cerebellum were first believed to be located in the raphe nuclei, early attempts at elucidating the role of serotonergic modulation in the cerebellar cortex involved stimulating the raphe and recording simple spikes from Purkinje cells in anaesthetized rats (Strahlendorf and others 1981). Raphe stimulations evoked a burst of spikes with

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

a short latency, followed by a silent period. This is to be expected from the fact that most of the raphe neurons projecting to the cerebellar cortex are not serotonergic but glutamatergic and give rise to mossy fibers. Therefore, these experiments could not demonstrate conclusively the action of serotonin in the cerebellar cortex and local iontophoretic applications of serotonin were used.

In Vitro and In Vivo Modulation of Purkinje Cell Simple Spike Activity

The spontaneous firing rate of Purkinje cells recorded extracellularly in the anaesthetized rat is modulated by iontophoretic applications of serotonin (Strahlendorf and others 1984; Lee and others 1986, 1987; Strahlendorf and others 1988; Strahlendorf and others 1989; Strahlendorf and others 1991). Simple spike frequencies can be increased or decreased or undergo biphasic modulation with a transient decrease preceding the increase. The direction of the modulation depends on the initial firing rate: Units with a low spontaneous firing rate are excited, and rapidly firing units are mostly depressed (Strahlendorf and others 1984). The direction also depends on the nature of the anaesthetic used (Strahlendorf and others 1988) and on the animal species, because in the cat, only depression has been recorded (Kerr and Bishop 1992). The variability of the serotonergic effects might be due in part to real differences in the serotonergic innervation and serotonergic control of the cerebellar cortex in different lobules and different species (see "Organisation of the Cerebellar Serotonergic Afferents"). Alternatively, serious concerns can be raised concerning the iontophoresis technique: It changes the local pH (see Hicks and others 1989), produces substantial currents that may affect the membrane potential of the cell recorded, and uses very high concentrations of drugs that can lead to nonspecific activation of receptors around the ejection site in addition to specific activation at more remote locations. Despite these concerns, the modulation of the firing of Purkinje cells is unlikely to be artifactual, because it has a pharmacology consistent with the known serotonin receptor subtypes. The inhibitory effect of serotonin in the rat and cat was attributed to 5-HT_{1A} receptors (Darrow and others 1990; Kerr and Bishop 1992), because it can be mimicked by applications of (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and ipsaspirone and because it is blocked by spiperone. However, the effect is almost certainly mediated by another receptor with a similar pharmacology, as many studies have shown that the 5-HT_{1A} receptor is not expressed in the adult cerebellum. The 5-HT_7 receptor, which is present in Purkinje cells, is a good candidate because it has a high affinity for serotonin, 8-OH-DPAT, and spiperone. In most rat Purkinje cells, coapplication of serotonin and spiperone unmasks a net excitatory effect of serotonin, even in the cells that were inhibited by serotonin before spiperone application (Darrow and others 1990). 5-HT_{2A} receptors might be involved in this excitatory effect.

The cellular mechanisms of the modulation of Purkinje cell firing by serotonin have been sought both in vivo and in slices. In many parts of the CNS, serotonin changes the excitability of its target neuron through the modulation of their voltage-dependent conductances. In slices, bath-applied serotonin affects the resting membrane conductance of Purkinje cells, producing a net inward current under single electrode voltage-clamp and a depolarization under current-clamp. This effect was observed in rat (Wang and others 1992; Li and others 1993) and turtle (Lu and Larson-Prior 1996) slices, has a 5-HT_2 pharmacology (Li and others 1993), and could therefore account for the serotonin-induced excitation of Purkinje cells in vivo. In addition, bath-applied serotonin inhibits a transient (I_A-like) component as well as a more sustained component of the potassium currents activated by depolarization (Wang and others 1992). In the same experimental conditions, serotonin shifts the activation curve of the cationic current activated by hyperpolarization (I_h) toward more hyperpolarized potentials (Li and others 1993). I_h is involved in the genesis of the spontaneous oscillatory firing activity of Purkinje cells (Chang and others 1993), and a shift in its activation curve together with a decrease of the hyperpolarization due to I_A may explain the inhibitory effects of serotonin on the firing of Purkinje cells in vivo. In summary, the firing behavior of Purkinje cells may switch from an oscillatory mode dependent on I_h to a tonic firing mode induced by serotonin-driven depolarization and dependent on non-inactivating sodium currents. These intrinsic modulations and the synaptic inputs received by Purkinje cells may interact in vivo to produce the variable pattern of modulation observed.

Serotonergic Modulation of GABAergic Inhibition of Purkinje Cells

An interaction of serotonin with the GABAergic inhibitory control of Purkinje cells was first suggested by the finding that pentobarbital, an anaesthetic that potentiates GABAergic inhibition, increases the inhibitory effect of serotonin on Purkinje cell firing (Strahlendorf and others 1988). Serotonin was later found to have a synergistic inhibitory effect with applications of GABA (Strahlendorf and others 1991; Kerr and Bishop 1992), muscimol (a $GABA_A$ agonist), and baclofen (a $GABA_B$ agonist) (Strahlendorf and others 1991) when ejection currents at which serotonin alone did not produce an effect were used. Nevertheless, when ejection of serotonin produced an effect by itself (higher ejection currents), it mainly summated with the inhibitory effect of iontophoretic applications of GABA. It produced an increased GABA-induced inhibition of Purkinje cells with high firing rates but a reduced GABA-induced inhibition of Purkinje cells firing at low frequencies (Strahlendorf and others 1989). A GABAergic mechanism for the action of serotonin in vivo is made unlikely

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

by the fact that bicucculline, an inhibitor of $GABA_A$ receptors, is unable to block the modulation of Purkinje cell firing by serotonin (Lee and others 1987). In addition, serotonin does not affect the response to iontophoretic application of GABA recorded in voltage-clamped Purkinje cells in slices (Mitoma and Konishi 1999).

The modulation by serotonin of the molecular layer inhibitory interneurons that control Purkinje cells (basket and stellate cells) was studied in acute slices of the rat cerebellum. In young animals, the inhibitory transmission at synapses made by basket cells and stellate cells on both Purkinje cells (Dieudonné unpublished data) and Golgi cells (Dieudonné and Dumoulin 2000) was depressed by nanomolar concentrations of serotonin. This effect disappeared after postnatal day 15 in Golgi cells and was very reduced in older animals in Purkinje cells. This effect had a typical 5-HT_{1A} pharmacology: It was mimicked by low nanomolar concentrations of 8-OH-DPAT and was insensitive to ketanserin (10 µM). The time window during which this inhibitory effect is observed is similar to the one during which 5-HT_{1A} receptors are expressed in the molecular layer of the cerebellar cortex. It must be noted that P15 is also the age around which the maturation of basket and stellate cell synapses is accompanied by a dramatic decrease in the amplitude of the spontaneous and unitary ipscs recorded from Purkinje cells (Pouzat and Hestrin 1997). Thus, serotonin may reduce the strength of immature basket cell and stellate cell synapses, through the activation of transiently expressed 5-HT_{1A} receptors.

Using the same experimental preparation, Mitoma and others (1994) reported opposite effects. According to these authors, a single application of serotonin (10 µM) leads to a reversible presynaptic facilitation of synaptic transmission at basket/stellate cell synapses onto Purkinje cells. Repetitive applications of serotonin produce an irreversible facilitation (Mitoma and Konishi 1999). The discrepancy with the results of Dieudonné and Dumoulin (2000) might come from the higher concentrations of 5-HT used (10 µM versus 1 µM) or from differences in the control of the postsynaptic cell (internal solution, holding potential, access resistance). The low concentrations of calcium buffer (0.1 mM EGTA) and the holding potential of –50 mV used by Mitoma might allow high resting concentrations of intracellular calcium, as well as large transient concentrations, which could activate calcium-dependent biochemical processes or retrograde messenger systems (Vincent and others 1992). These biochemical pathways may in turn be modulated by serotonin. Alternatively, the absence of blockers of voltage-dependent conductances, the relatively high access resistance, and the holding potential of –50 mV used by Mitoma (at which both potassium conductances and persistent calcium and sodium conductances are activated) might have resulted in a poor spatial control of the potential in the dendrites of the recorded cells. Because low internal chloride concentrations were used by Mitoma and others (1994) and Mitoma and Konishi (1999), ipscs were small and were recorded close to their reversal potential. Thus, the known inhibition by serotonin of the voltage-dependent conductances present in Purkinje cell dendrites could have promoted a better control of distal dendrites and have resulted in an apparent increase in the frequency of spontaneous ipscs and in the amplitude of evoked ipscs. Therefore, a direct modulation of inhibitory inputs to Purkinje cells by serotonin in the adult cerebellar cortex still remains to be demonstrated.

Serotonergic Modulation of Glutamatergic Transmission

The iontophoresis of serotonin in the rat and cat in vivo antagonizes the increase in the firing frequency of Purkinje cells induced by several agonists of non-NMDA ionotropic glutamate receptors (Lee and others 1986; Kerr and Bishop 1992; Netzeband and others 1993). This effect was observed whether serotonin alone increased or decreased the firing rate of the recorded units. In rat slices, serotonin was also found to depress the firing of Purkinje cells induced by glutamate iontophoresis and the inward current induced by quisqualate in voltage-clamped Purkinje cells (Hicks and others 1989). The pharmacology of these effects is not well established. However, bath-applied serotonin did not depress parallel fiber and climbing fiber synaptic currents evoked by electrical stimulation in rat slices (Mitoma and Konishi 1999) and had a modest (30%) inhibitory effect on disynaptic epscs evoked by stimulation of the mossy fibers in the turtle (Lu and Larson-Prior 1996). In addition, calcium transients induced by climbing fiber stimulations in rat Purkinje cells were not affected by serotonin (Pisani and Ross 1999).

The effects of serotonin on the release of glutamate (Maura and others 1986; Raiteri and others 1986; Maura and others 1988; Maura, Carbone, and others 1991; Maura and Raiteri 1996) and aspartate (Maura, Barzizza, and others 1991) and on the signaling processes downstream of glutamate receptor activation (production of nitric oxide and cyclic GMP) (Maura and others 1995; Maura and Raiteri 1996; Marcoli and others 1997; Marcoli and others 1998) have also been studied extensively by biochemical methods in cerebellar slices and preparations of cerebellar synaptosomes. 5-HT_{2C} receptors inhibit the release of glutamate from purified putative mossy fiber synaptosomes (Maura and others 1988; Maura, Carbone, and others 1991), whereas 5-HT_{1D} receptors seem to inhibit the release from parallel fibers in synaptosomes and slices (Raiteri and others 1986; Maura and others 1988; Maura and Raiteri 1996). Finally, 5-HT_{1A} and 5-HT_{2C} receptors appear to control the production of nitric oxide and cyclic GMP induced by bath application of NMDA or AMPA on cerebellar slices (Maura and Raiteri 1996; Marcoli and others 1997; Marcoli and others 1998). The

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

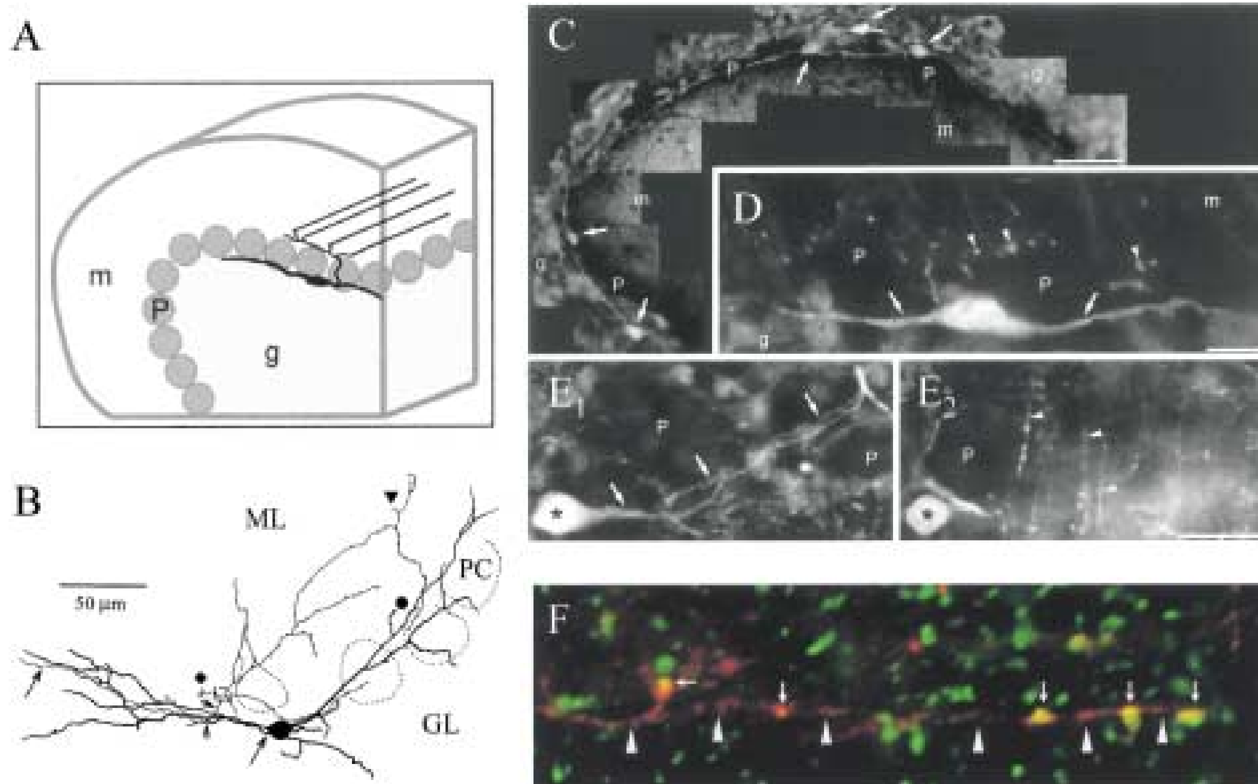


Fig. 2. Morphology of the Lugaro interneurons of the cerebellar cortex. *A*. Bloc diagram of a cerebellar lobule showing the transverse extension of the cell body and dendrites, in the parasagittal plane, under and inside the Purkinje cell layer, and the longitudinal extension of the parallel axons in the molecular layer. *B*. Parasagittal projection of a biocytin-filled Lugaro cell recorded from rat cerebellar slices. The parasagittal axon is visible in the molecular layer, and the longitudinal axons are marked by symbols. *C*. Numerous Lugaro cells stained for calretinin (a calcium-binding protein) in the curvature of a cerebellar lobule (parasagittal cut). *D*. Details of a calretinin positive Lugaro cell. Note the numerous longitudinal axons transected in the lower molecular layer (arrowheads). *E*. Calretinin-positive Lugaro cells in a plane cut tangential to the Purkinje cell layer. Note the mostly transverse orientation of Lugaro cell dendrites in the Purkinje cell layer (E_1) and the longitudinal extension of the axons in the immediately adjacent molecular layer (E_2). *F*. A longitudinal Lugaro cell axon stained for the neuronal glycine transporter GlyT2 (red, arrowheads) and for GAD (green) in its varicosities (arrows). Adapted from Dieudonné and Dumoulin (2000) (© 2000 by the Society for Neuroscience). GL = granule cell layer; ML = molecular layer; PC = Purkinje cell.

identification of 5-HT_{2C} receptors in these studies remains putative and is somewhat in contradiction with the very low level of 5-HT_{2C} receptor mRNA and binding sites in the cerebellum. Functional conclusions are hard to draw from these results because the stimulations used are not physiological and may induce excitotoxicity and/or several classes of regenerative processes involving both neurons and glia. Indeed, as already mentioned, no modulation of parallel fiber transmission was found when tested electro-physiologically.

The Lugaro Cell Is a Major Effector for Serotonin in the Cerebellar Cortex

The Lugaro cell was described for the first time as a distinct cell type of the cerebellar cortex in the late 19th century (Lugaro 1894). Since then, a handful of publications have been devoted to this cell (reviewed in Lainé and Axelrad 1998). The cell body of Lugaro cells is found in the granule cell layer, and their dendrites extend in the Purkinje cell layer and occasionally in the granule cell layer but never in the molecular layer (Fig. 2*B*–2E_1). The axons of Lugaro cells ramify in the molecular layer, and occasionally in the granule cell layer, where they develop a sparse plexus in the same parasagittal plane as the somatodendritic compartment (Fig. 2*B*). In contrast to all other inhibitory interneurons of the cortex, Lugaro neurons generate a second plexus of axons composed of thick (1–2 µm diameter) fibers running parallel to the parallel fibers (Fig. 2*A*, 2E_2, and 2*F*). These longitudinal Lugaro cell axons are myelinated (Dieudonné and Dumoulin 2000) and constitute, with parallel fibers, the only axonal system of the cerebellar cortex able to transfer information between distant parasagittal modules formed by parasagittally organized Purkinje cells and inhibitory interneurons.

In the only electrophysiological study of Lugaro cells to date, Dieudonné and Dumoulin (2000) demonstrated that these interneurons do not fire spontaneous action potentials in rat cerebellar slices. Submicromolar concentrations of serotonin induce the rhythmic firing of all

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

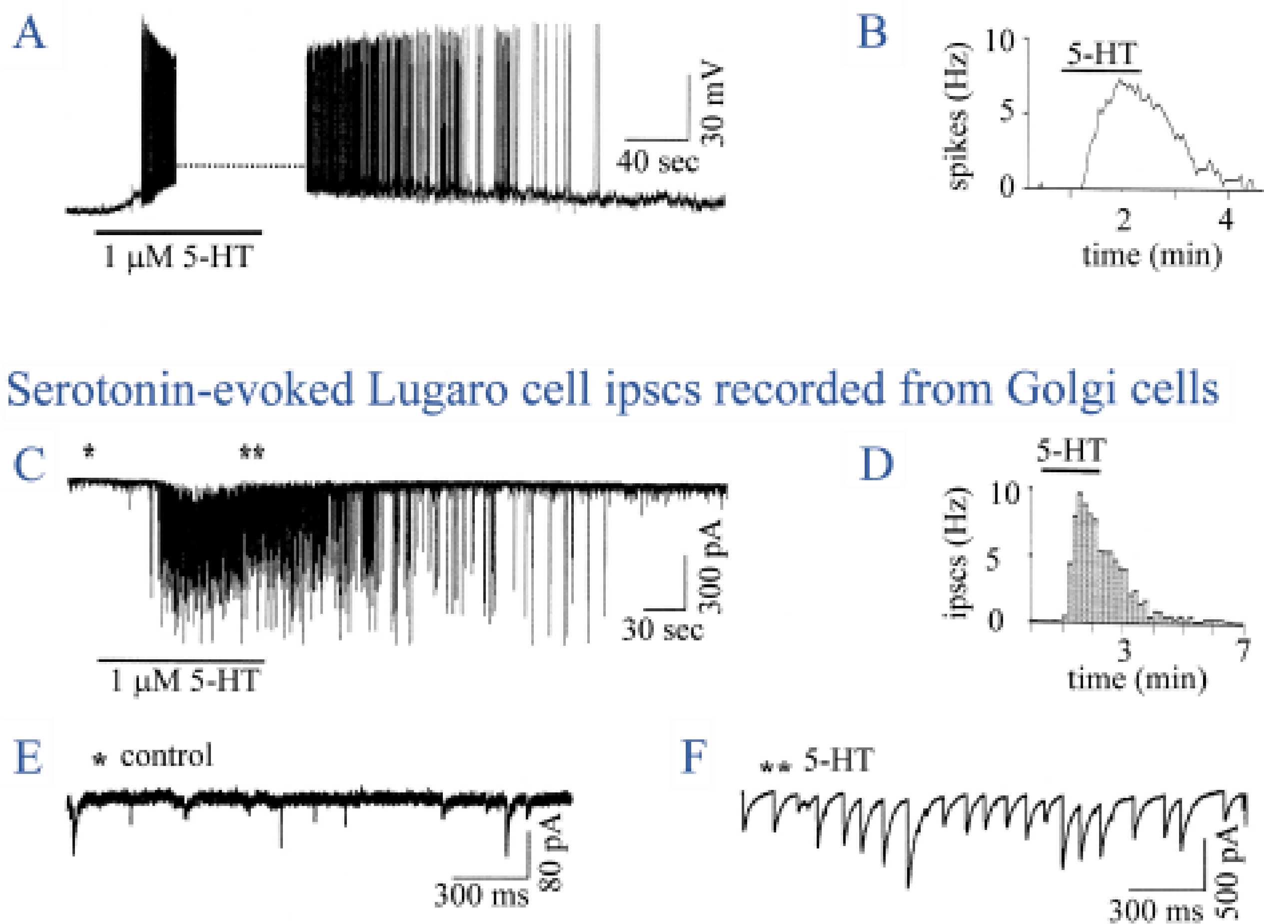


Fig. 3. Serotonin induces the repetitive firing of Lugaro cells. *A*. Current-clamp recording of a Lugaro cell in rat cerebellar slices. The spontaneously inactive Lugaro cell is reversibly excited by serotonin, which induces a sustained spike firing. *B*. Summary of the effect of serotonin on the spike firing frequency in another Lugaro cell. *C*. The serotonin-induced activation of Lugaro cells evokes large ipscs in Golgi cells, which dominate the spontaneous synaptic activity. *D*. Summary of the effect of serotonin on the frequency of large-amplitude ipscs in the same Golgi cell. *E* and *F*. Faster time scale of portions of the recording in *C*. Note the rhythmic occurrence of the ipscs. Adapted from Dieudonné and Dumoulin (2000) (© 2000 by the Society for Neuroscience).

Lugaro cells at steady-state frequencies ranging from 5 to 15 Hz (Fig. 3*A* and 3*B*). As Lugaro cells receive very little glutamatergic excitatory input and are controlled by a very intense GABAergic inhibition (Dieudonné unpublished results), serotonin is the principal neuromediator able to activate these cells. The excitation of Lugaro cells by serotonin can be mimicked by α-methyl-5-HT and 5-metoxytryptamine at 10-fold higher concentrations than serotonin. Concentrations of 8-OH-DPAT, up to 10 μM, and of 5-carboxamidotryptamine (5-CT), up to 1 μM, and of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), up to 0.5 μM, did not produce the effect, neither did they desensitize the preparation to simultaneous application of serotonin. Ketanserin 10 μM (but not 1 μM), if preincubated in the bath, was able to inhibit the effect of 1 μM 5-HT (all pharmacological data unpublished). This suggests that the serotonin receptor involved is not a member of the 5-HT_1, 5-HT_3, or 5-HT_4 families. It may be a 5-HT_{2C} receptor, although DOI should have produced an effect at the concentration used. It could also be of the 5-HT_6 subtype, which is known to have a low affinity for serotonin and no affinity for DOI. This would be in agreement with the low level of mRNA coding for this protein in the cerebellum (since the Lugaro cells are few), but not with report(s) that its expression is highest in the molecular layer.

The distribution of the serotonergic plexus in the cerebellar cortex suggests that Lugaro cells are the primary targets for these fibers. The paucity of fibers in the molecular layer (particularly in the cat) argues against a strong effect on excitatory and inhibitory synapses in this layer, as demonstrated electrophysiologically. The

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

location of Lugaro cells and serotonergic fibers varies in parallel across species. In the rat the plexus of serotonergic fibers is densest in the upper granular layer, where all the cell bodies and most of the dendrites of Lugaro cells are located (Lainé and Axelrad 1996; Dieudonné and Dumoulin 2000). In contrast, the distribution of serotonergic fibers in the granular layer is more homogeneous in the cat, where it has been shown that Lugaro cells are found in the whole depth of the granular layer (Sahin and Hockfield 1990). Interlobular variations in the density of serotonergic fibers also seem to be correlated with the distribution of Lugaro cells. In the rat vermis Lugaro cells and serotonergic fibers are more abundant in the posterior lobules (VII to X) (Dieudonné and Dumoulin 2000). Finally, longitudinal serotonergic fibers in the molecular layer might be in relation with longitudinal Lugaro cell axons, as axo-axonal contacts have been described in this layer. Double immunocytochemical detection of serotonergic fibers and Lugaro cells will be necessary to assess more directly their spatial relationship.

What is the consequence of Lugaro cell firing on other cell types? It was recently demonstrated by morphological (Lainé and Axelrad 1998), electrophysiological (Dieudonné and Dumoulin 2000), and immunohistochemical methods (Dumoulin, Triller, and Dieudonné, in preparation) that inhibitory interneurons (basket, stellate, and Golgi cells) are the only postsynaptic targets of Lugaro cell axons (Fig. 3*C*–3*F*). The ipscs evoked in molecular layer interneurons by the serotonergic activation of Lugaro cells are mediated by $GABA_A$ receptors (Dieudonné unpublished results) and come in large part from contacts made by the parasagittal axonal plexus of Lugaro cells (Dumoulin, Triller, and Dieudonné, in preparation). In contrast, serotonergic activation of Lugaro cells evokes large-amplitude ipscs in Golgi cells (Dieudonné and Dumoulin 2000), which are mediated by both glycine and $GABA_A$ receptors (Dumoulin, Triller, and Dieudonné, in preparation). Immunohistochemical staining confirmed that the Lugaro cell is a mixed GABAergic/glycinergic inhibitory interneuron (Dumoulin, Triller, and Dieudonné, in preparation) (Fig. 2*F*), perhaps explaining why the inhibitory effect of serotonin on Purkinje cells firing in vivo is blocked by picrotoxin (a nonspecific $GABA_A$ and glycine receptor blocker) but not by bicucculline (a more specific $GABA_A$ receptor blocker) (Lee and others 1987). At least part of the synapses of Lugaro cells onto Golgi cells are formed by the longitudinal axons of Lugaro cells (Dumoulin, Triller, and Dieudonné, in preparation). It was estimated that each Golgi cell is contacted by about 10 Lugaro cells, whereas each Lugaro cell may contact as many as 150 Golgi cells over a parasagittal extension of 2 mm (Dieudonné and Dumoulin 2000). As each Golgi cell contacts an estimated 100,000 granule cells, the activity of the granule cells presynaptic to a given Purkinje cell may be indirectly controlled by one, or a few, Lugaro cells.

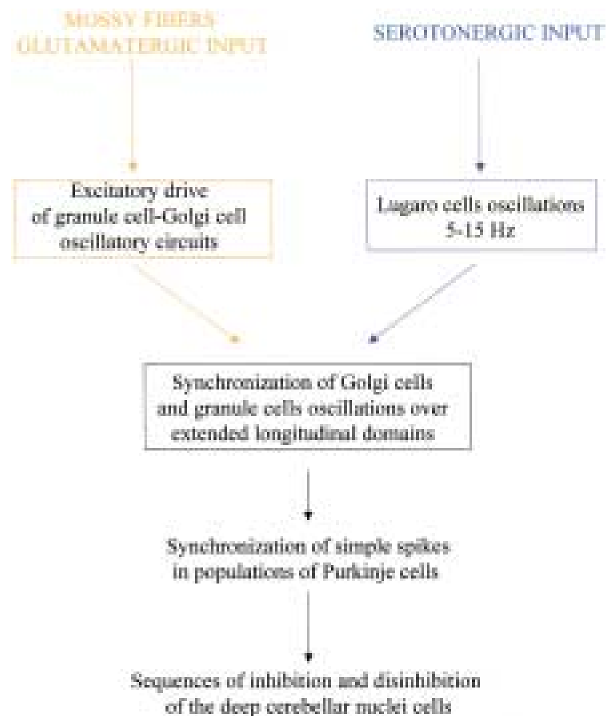


Fig. 4. Schematic diagram of the effect of serotonergic activation of Lugaro cells on synaptic processing in the cerebellar cortex.

Serotonin as an ON/OFF Switch of the Cerebellar Cortex: A Hypothesis

It is well-known that the firing of serotonergic raphe neurons is correlated with motor tasks and falls virtually to zero during rapid eye movement sleep, which may account for the strong depression of descending motor control during this phase. Furthermore, the firing of each cell in a particular raphe nucleus is modulated by a specific set of tasks, probably in accordance with its zones of projection (Jacobs and Fornal 1997). Although serotonergic cells innervating the cerebellar cortex are more dispersed in the brainstem reticular formation, they seem to follow the same functional organization. Serotonergic cells are located in or around every precerebellar nuclei, and the topography of their projection appears to parallel that of the precerebellar glutamatergic neurons. In addition, the amount of serotonin released in the cerebellar cortex at different times in the day, as measured by microdialysis, is strongly correlated to the intensity of motor activity and arousal, but not to stress (Mendlin and others 1996). Therefore, serotonin is probably released in the cerebellar cortex where and when sensorimotor information carried by mossy fiber glutamatergic input has to be processed.

How does serotonin neuromodulation affect this processing (see Fig. 4)? By activating Lugaro cells, serotonin may allow 5- to 15-Hz oscillations to be generated in the cerebellar cortex or may allow the cerebellar cortex to follow 5- to 15-Hz oscillations transmitted from

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

precerebellar nuclei. It is notable that this is the range of frequencies of spontaneous oscillations in the inferior olive. Synapses of Purkinje cells on Lugaro cells (Dumoulin unpublished results) may link temporally serotonin-induced Lugaro cell oscillations to oscillations of the inferior olive. Lugaro cells would in turn synchronize large populations of Golgi cells through their longitudinal axons. Synchronization would be facilitated by the ability of the retroinhibitory circuit between granule cells and Golgi cells to behave as an oscillator (Dieudonné 1998; Maex and Schutter 1998). Such synchronizations of Golgi cells and granule cells over large domains may be at the origin of the 7- to 18-Hz oscillations recorded from the granular layer of the paramedian lobule of the monkey (Pellerin and Lamarre 1997) and Crus IIa of the rat (Hartmann and Bower 1998). Interestingly, these oscillations are linked to the motor arousal state of the animal and occur at specific stages of the motor output preparation. The synchronization of large populations of granule cells over longitudinal domains would be of crucial importance for Purkinje cell signal processing, as it would create bursts of excitatory inputs coming from the parallel fibers. In addition, the effects of serotonin on Purkinje cell intrinsic properties might be important to stop the spontaneous firing of Purkinje cells and adapt their responsiveness to synchronous rhythmic excitatory inputs. A synchronization of single spikes over local populations of Purkinje cells would also likely result from this mode of operation. This would parallel the synchronization of complex spikes, which is observed in populations of Purkinje cells located in parasagittal stripes, and which is known to convey significant motor information (Welsh and others 1995). Indeed, asynchronous firing of Purkinje cells will inhibit deep cerebellar nuclei cells, whereas synchronous firing will produce an inhibition-disinhibition sequence, known to lead to rebound firing of the deep cerebellar nuclei cells.

In conclusion, serotonin-induced activation of Lugaro cells may cause the synchronization of Golgi cell, granule cell, and Purkinje cell simple spikes. This network-level action of serotonin may operate as an ON/OFF switch for the processing of mossy fiber information in the cerebellar cortex and for the control of deep cerebellar nuclei.

References

Batini C. 1993. The cerebellar projection from the neurons of the raphe nuclei and the origin of the serotoninergic innervation of the cerebellum. In: Trouillas P, Fuxe K, editors. Serotonin, the cerebellum, and ataxia. New York: Raven. p 51–61.

Beaudet A, Sotelo C. 1981. Synaptic remodeling of serotonin axon terminals in rat agranular cerebellum. Brain Res 206:305–29.

Bishop GA, Ho RH. 1985. The distribution and origin of serotonin immunoreactivity in the rat cerebellum. Brain Res 331:195–207.

Bishop GA, Ho RH, King JS. 1985. Localization of serotonin immunoreactivity in the opossum cerebellum. J Comp Neurol 235:301–21.

Bishop GA, Ho RH, King JS. 1988. A temporal analysis of the origin and distribution of serotoninergic afferents in the cerebellum of pouch young opossums. Anat Embryol 179:33–48.

Bishop GA, Kerr CW, Chen YF, King JS. 1993. The serotoninergic system in the cerebellum: origin, ultrastructural relationships, and physiological effects. In: Trouillas P, Fuxe K, editors. Serotonin, the cerebellum, and ataxia. New York: Raven. p 91–112.

Bonaventure P, Voorn P, Luyten VHML, Jurzak M, Schotte A, Leysen JE. 1998. Detailed mapping of serotonin 5-HT_{1B} and 5-HT_{1D} receptor messenger RNA and ligand binding sites in guinea-pig brain and trigeminal ganglion: clues for function. Neuroscience 82:469–84.

Boschert U, Aït Amara D, Segu L, Hen R. 1994. The mouse 5-hydroxytryptamine$_{1B}$ receptor is localized predominantly on axon terminals. Neuroscience 58:167–82.

Burnet PWJ, Eastwood SL, Lacey K, Harrison PJ. 1995. The distribution of 5-HT1A and 5-HT2A receptor mRNA in human brain. Brain Res 676:157–68.

Chang W, Strahlendorf JC, Strahlendorf HK. 1993. Ionic contributions to the oscillatory firing activity of rat Purkinje cells in vitro. Brain Res 614:335–41.

Chan-Palay V. 1975. Fine structure of labelled axons in the cerebellar cortex and nuclei of rodents and primates after intraventricular infusions with tritiated serotonin. Anat Embryol 148:235–65.

Choi DS, Maroteaux L. 1996. Immunohistochemical localisation of the serotonin 5-HT2B receptor in mouse gut, cardiovascular system, and brain. FEBS Lett 391:45–51.

Cornea-Hébert V, Riad M, Wu C, Singh SK, Descarries L. 1999. Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. J Comp Neurol 409:187–209.

Cumming-Hood PA, Strahlendorf HK, Strahlendorf JC. 1993. Effects of serotonin and the 5-HT2/1C receptor agonist DOI on neurons of the cerebellar dentate/interpositus nuclei: possible involvement of a GABAergic interneuron. Eur J Pharmacol 236:457–65.

Darrow EJ, Strahlendorf HK, Strahlendorf JC. 1990. Response of cerebellar Purkinje cells to serotonin and the 5-HT_{1A} agonists 8-OH-DPAT and ipsapirone in vitro. Eur J Pharmacol 175:145–53.

Dieudonné S. 1998. Submillisecond kinetics and low efficacy of parallel fibre-Golgi cell synaptic currents in the rat cerebellum. J Physiol 510:845–66.

Dieudonné S and Dumoulin A. 2000. Serotonin-driven long-range inhibitory connections in the cerebellar cortex. J Neurosci 20:1837–48.

Ding D, Toth M, Zhou Y, Parks C, Hoffman BJ, Shenk T. 1993. Glial cell-specific expression of the serotonin 2 receptor gene: selective reactivation of a repressed promoter. Mol Brain Res 20:181–91.

Doucet E, Pohl M, Fattaccini CM, Adrien J, El Mestikawy S, Hamon M. 1995. In situ hybridization evidence for the synthesis of 5-HT1B receptor in serotoninergic neurons of anterior raphe nuclei in the rat brain. Synapse 19:18–28.

Duxon MS, Flanigan TP, Reavley AC, Baxter GS, Blackburn TP, Fone KC. 1997. Evidence for expression of the 5-hydroxytryptamine-2B receptor protein in the rat central nervous system. Neuroscience 76:323–9.

Eglen RM, Wong EHF, Dumuis A, Bockaert J. 1995. Central 5-HT4 receptors. Trends Pharmacol Sci 16:391–7.

Gardette R, Krupa M, Crepel F. 1987. Differential effects of serotonin on the spontaneous discharge and on the excitatory amino acid-induced responses of deep cerebellar nuclei neurons in rat cerebellar slices. Neuroscience 23:491–500.

Gerald C, Adham N, Kao H-T, Olsen MA, Laz TM, Schechter LE, and others. 1995. The 5-HT_4 receptor: molecular cloning and pharmacological characterization of two splice variants. EMBO J 14:2806–15.

Gerard C, El Mestikawy S, Lebrand C, Adrien J, Ruat M, Traiffort E, and others. 1996. Quantitative RT-PCR distribution of serotonin 5-HT6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. Synapse 23:164–73.

Gerard C, Martres MP, Lefevre K, Miquel MC, Vergé D, Lanfumey L, and others. 1997. Immuno-localization of serotonin 5-HT6

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

receptor-like material in the rat central nervous system. Brain Res 746:207–19.
Hartmann MJ, Bower JM. 1998. Oscillatory activity in the cerebellar hemispheres of unrestrained rats. J Neurophysiol 80:1598–604.
Hellendall RP, Schambra UB, Liu JP, Lauder JM. 1993. Prenatal expression of 5-HT1C and 5-HT2 receptors in the rat central nervous system. Exp Neurol 120:186–201.
Hicks TP, Krupa M, Crépel F. 1989. Selective effects of serotonin upon excitatory amino acid-induced depolarizations of Purkinje cells in cerebellar slices from young rats. Brain Res 492:371–6.
Höckfelt T, Fuxe K. 1969. Cerebellar monoamine nerve terminals, a new type of afferent fibers to the cortex cerebelli. Exp Brain Res 9:63–72.
Jacobs BL, Fornal CA. 1997. Serotonin and motor activity. Curr Opin Neurobiol 7:820–5.
Kerr CW, Bishop GA. 1991. Topographical organization in the origin of serotoninergic projections to different regions of the cat cerebellar cortex. J Comp Neurol 304:502–15.
Kerr CW, Bishop GA. 1992. The physiological effects of serotonin are mediated by the $5HT_{1A}$ receptor in the cat's cerebellar cortex. Brain Res 591:253–60.
Kitzman PH, Bishop GA. 1994. The origin of serotoninergic afferents to the cat's cerebellar nuclei. J Comp Neurol 340:541–50.
Kitzman PH, Bishop GA. 1997. The physiological effects of serotonin on spontaneous and amino acid-induced activation of cerebellar nuclear cells: an in vivo study in the cat. Prog Brain Res 114:209–23.
Kung MP, Zhuang ZP, Frederick D, Kung HF. 1994. In vivo binding of [^{123}I]4-(2′-methoxyphenyl)-1-[2′-(N-2″-pyridinyl)-p-iodobenzamido-]ethyl-piperazine, p-MPPI, to 5-HT1A receptors in rat brain. Synapse 18:359–66.
Lainé J, Axelrad H. 1996. Morphology of the Golgi-impregnated Lugaro cell in the rat cerebellar cortex: a reappraisal with a description of its axon. J Comp Neurol 375:618–40.
Lainé J, Axelrad H. 1998. Lugaro cells target basket and stellate cells in the cerebellar cortex. Neuroreport 9:2399–403.
Laporte A-M, Lima L, Gozlan H, Hamon M. 1994. Selective in vivo labelling of brain $5\text{-}HT_{1A}$ receptors by [^{3}H]WAY 100535 in the mouse. Eur J Pharmacol 271:505–14.
Lee M, Strahlendorf JC, Strahlendorf HK. 1986. Modulatory action of serotonin on glutamate-induced excitation of cerebellar Purkinje cells. Brain Res 361:107–13.
Lee M, Strahlendorf JC, Strahlendorf HK. 1987. Picrotoxin but not bicuculline antagonizes 5-hydroxytryptamine-induced inhibition of cerebellar Purkinje neurons. Exp Neurol 97:577–91.
Li SJ, Wang Y, Strahlendorf HK, Strahlendorf JC. 1993. Serotonin alters an inwardly rectifying current (I_h) in rat cerebellar Purkinge cells under voltage clamp. Brain Res 617:87–95.
Lidow HGW, Molliver ME. 1982. An immunohistochemical study of serotonin neuron development in the rat: ascending pathways and terminal fields. Brain Res Bull 8:389–430.
Lu H, Larson-Prior LJ. 1996. Serotoninergic modulation of evoked responses in turtle cerebellar Purkinje cells. J Neurophysiol 76:3102–13.
Lugaro E. 1894. Sulle connessioni tra gli elemente nervosi della corteccia cerebellare con considerazioni generali sulsignificato fisiologico dei rapporti tra gli elementi nervosi. Rivista Sperimentale di Freniatria 20:297–331.
Maeshima T, Shutoh F, Hamada S, Senzaki K, Hamaguchi-Hamada K, Ito R, and others. 1998. $Serotonin_{2A}$ receptor-like immunoreactivity in rat cerebellar Purkinje cells. Neurosci Lett 252:72–4.
Maex R, Schutter ED. 1998. Synchronization of Golgi and granule cell firing in a detailed network model of the cerebellar granule cell layer. J Neurophysiol 80:2521–37.
Marcoli M, Maura G, Tortarolo M, Raiteri M. 1997. Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in rat cerebellum: involvement of $5\text{-}hydroxytryptamine_{2C}$ receptors. J Neurochem 69:427–30.
Marcoli M, Maura G, Tortarolo M, Raiteri M. 1998. Tradozone is a potent agonist at $5\text{-}HT_{2C}$ receptors mediating inhibition of the N-methyl-D-aspartate/nitric oxide/cyclic GMP pathway in rat cerebellum. J Pharmacol Exp ther 285:983–6.
Mathis CA, Simpson NR, Mahmood K, Kinahan PE, Mintun MA. 1994. [11C]WAY 100635: a radioligand for imaging 5-HT1A receptors with positron emission tomography. Life Sci 55:403–7.
Matthes H, Boschert U, Amlaiky N, Grailhe R, Plassat JL, Muscatelli F, and others. 1992. Mouse 5-hydroxytryptamine5A and 5-hydroxytryptamine5B receptors define a new family of serotonin receptors: cloning, functional expression, and chromosomal localization. Mol Pharmacol 43:313–19.
Matthiessen L, Daval G, Bailly Y, Gozlan H, Hamon M, Vergé D. 1992. Quantification of $5\text{-}hydroxytryptamine_{1A}$ receptors in the cerebellum of normal and X-irradiated rats during postnatal development. Neuroscience 51:475–85.
Maura G, Barzizza A, Folghera S, Raiteri M. 1991. Release of endogenous aspartate from rat cerebellum slices and synaptosomes: inhibition mediated by a 5-HT2 receptor and by a 5-HT1 receptor of a possibly novel subtype. Naunyn-Schmiedebergs Arch Pharmacol 343:229–36.
Maura G, Carbone R, Guido M, Pestarino M, Raiteri M. 1991. $5\text{-}HT_2$ presynaptic receptors mediate inhibition of glutamate release from cerebellar mossy fibre terminals. Eur J Pharmacol 202:185–90.
Maura G, Guadagnin A, Raiteri M. 1995. Low nanomolar serotonin inhibits the glutamate receptor/nitric oxide/cyclic GMP pathway in slices from adult rat cerebellum. Neuroscience 68:455–63.
Maura G, Raiteri M. 1996. Serotonin $5\text{-}HT_{1D}$ and $5\text{-}HT_{1A}$ receptors respectively mediate inhibition of glutamate release and inhibition of cyclic GMP production in rat cerebellum in vitro. J Neurochem 66:203–9.
Maura G, Ricchetti A, Raiteri M. 1986. Serotonin inhibits the depolarization-evoked release of endogenous glutamate from rat cerebellar nerve endings. Neurosci Lett 67:218–22.
Maura G, Roccatagliata E, Ulivi M, Raiteri M. 1988. Serotonin-glutamate interaction in rat cerebellum: involvement of 5-HT1 and 5-HT2 receptors. Eur J Pharmacol 145:31–8.
Mendlin A, Martín FJ, Rueter LE, Jacobs BL. 1996. Neuronal release of serotonin in the cerebellum of behaving rats: an in vivo microdialysis study. J Neurochem 67:617–22.
Miquel CC, Emerit MB, Gingrich JA, Nosjean A, Hamon M, El Mestikawy S. 1995. Developmental changes in the differential expression of two serotonin 5-HT3 receptor splice variants in the rat. J Neurochem 65:475–83.
Miquel MC, Kia HK, Boni C, Doucet E, Daval G, Matthiessen L, and others. 1994. Postnatal development and localization of $5\text{-}HT_{1A}$ receptor mRNA in rat forebrain and cerebellum. Dev Brain Res 80:149–57.
Mitoma H, Kobayashi T, Song SY, Konishi S. 1994. Enhancement by serotonin of GABA-mediated inhibitory synaptic currents in rat cerebellar Purkinje cells. Neurosci Lett 173:127–30.
Mitoma H, Konishi S. 1999. Monoaminergic long-term facilitation of GABA-mediated inhibitory transmission at cerebellar synapses. Neuroscience 88:871–83.
Molineaux SM, Jessell TM, Axel R, Julius D. 1989. 5-HT1C receptor is a prominent serotonin receptor subtype in the central nervous system. Proc Natl Acad Sci U S A 86:6793–7.
Morilak DA, Garlow SJ, Ciaranello RD. 1993. Immunocytochemical localization and description of neurons expressing $serotonin_2$ receptors in the rat brain. Neuroscience 54:701–17.
Nayak SV, Rondé P, Spier AD, Lummis SCR, Nichols RA. 1999. Calcium changes induced by presynaptic 5-hydroxytryptamine-3 serotonin receptors on isolated terminals from various regions of the rat brain. Neuroscience 91:107–17.
Netzeband JG, Weathers LB, Strahlendorf HK, Strahlendorf JC. 1993. Serotonin depresses excitatory amino acid-induced excitation of cerebellar Purkinje cells in the adult rat in vivo. Brain Res 608:145–9.
Palkovits M, Brownstein M, Saavedra JM. 1974. Serotonin content of the brainstem nuclei in the rat. Brain Res 80:327–49.
Pasqualetti M, Ori M, Nardi I, Castagna M, Cassano GB, Marazziti D. 1998. Distribution of the 5-HT5A serotonin receptor mRNA in the human brain. Brain Res Mol Brain Res 56:1–8.
Pellerin JP, Lamarre Y. 1997. Local field potential oscillations in primate cerebellar cortex during voluntary movement. J Neurophysiol 78:3502–7.

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009

Pisani A, Ross WN. 1999. Weak effect of neuromodulators on climbing fiber-activated $[Ca^{2+}]_i$ increases in rat cerebellar Purkinje neurons. Brain Res 831:113–8.

Pompeiano M, Palacios JM, Mengod G. 1994. Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. Brain Res Mol Brain Res 23:163–78.

Pouzat C, Hestrin S. 1997. Developmental regulation of basket/stellate cell→Purkinje cell synapses in the cerebellum. J Neurosci 17:9104–12.

Pranzatelli MR. 1993. Regional differences in the ontogeny of 5-hydroxytryptamine-1C binding sites in rat brain and spinal cord. Neurosci Lett 149:9–11.

Raiteri M, Maura G, Bonanno G, Pittaluga A. 1986. Differential pharmacology and function of two $5\text{-}HT_1$ receptors modulating transmitter release in rat cerebellum. J Pharmacol Exp ther 237:644–8.

Ruat M, Traiffort E, Arrang JM, Tardivel-Lacombe J, Diaz J, Leurs R, and others. 1993. A novel rat serotonin ($5\text{-}HT_6$) receptor: molecular cloning, localization and stimulatioin of cAMP accumulation. Biochem Biophys Res Commun 193:268–76.

Sahin M, Hockfield S. 1990. Molecular identification of the Lugaro cell in the cat cerebellar cortex. J Comp Neurol 301:575–84.

Strahlendorf JC, Lee M, Netzeband JG, Strahlendorf HK. 1988. Pentobarbital augments serotonin-mediated inhibition of cerebellar purkinje cells. Neuroscience 27:107–15.

Strahlendorf JC, Lee M, Strahlendorf HK. 1984. Effects of serotonin on cerebellar Purkinje cells are dependent on the baseline firing rate. Exp Brain Res 1984;56:50–8.

Strahlendorf JC, Lee M, Strahlendorf HK. 1989. Modulatory role of serotonin on GABA-elicited inhibition of cerebellar Purkinje cells. Neuroscience 30:117–25.

Strahlendorf JC, Lee M, Strahlendorf HK. 1991. Serotonin modulates muscimol- and baclofen-elicited inhibition of cerebellar Purkinje cells. Eur J Pharmacol 201:239–42.

Strahlendorf JC, Strahlendorf HK, Barnes CD. 1981. Comparative aspects of raphe-induced modulation of evoked and spontaneous cerebellar unit activity. Prog Clin Biol Res 68:217–25.

Sur C, Betz H, Schloss P. 1996. Immunocytochemical detection of the serotonin transporter in rat brain. Neuroscience 73:217–31.

Takeuchi Y, Kimura H, Sano Y. 1982. Immunohistochemical demonstration of serotonin-containing nerve fibers in the cerebellum. Cell Tissue Res 226:1–12.

Tolbert DL, Bantli H, Bloedel JR. 1976. Anatomical and physiological evidence for a cerebellar nucleocortical projection in the cat. Neuroscience 1:205–17.

Tolbert DL, Bantli H, Bloedel JR. 1978. Multiple branching of cerebellar efferent projections in cats. Exp Brain Res 31:305–16.

Triarhou LC, Ghetti B. 1991. Serotonin-immunoreactivity in the cerebellum of two neurological mutant mice and the corresponding wild-type genetic stocks. Chem Neuroanat 4:421–8.

Trouillas P, Fuxe K. 1993. Serotonin, the cerebellum, and ataxia. New York: Raven.

Vergé D, Matthiessen L, Kia HK, Gozlan H, Hamon M, Bailly Y, and others. 1993. $5\text{-}HT_{1A}$ receptors in the olivocerebellar complex. In: Trouillas P, Fuxe K, editors. Serotonin, the cerebellum, and ataxia. New York: Raven. p 179–90.

Vincent P, Armstrong CM, Marty A. 1992. Inhibitory synaptic currents in rat cerebellar Purkinje cells: modulation by postsynaptic depolarization. J Physiol (Lond) 456:453–71.

Wang Y, Strahlendorf JC, Strahlendorf HK. 1992. Serotonin reduces a voltage-dependent transient outward potassium current and enhances excitability of cerebellar Purkinje cells. Brain Res 571:345–9.

Ward RP, Hamblin MW, Lachowicz JE, Hoffman BJ, Sibley DR, Dorsa DM. 1995. Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. Neuroscience 64:1105–11.

Welsh JP, Lang EJ, Suglhara I, Llinas R. 1995. Dynamic organization of motor control within the olivocerebellar system. Nature 374:453–7.

Wright DE, Seroogy KB, Lundgren KH, Davis BM, Jennes L. 1995. Comparative localization of serotonin 1A, 1C and 2receptor subtype mRNAs in rat brain. J Comp Neurol 351:357–73.

Downloaded from http://nro.sagepub.com at PRINCETON UNIV LIBRARY on November 16, 2009