Microcircuitry and function of the inferior olive

Chris I. De Zeeuw, John I. Simpson, Casper C. Hoogenraad, Niels Galjart, S.K.E. Koekkoek and Tom J.H. Ruigrok

The inferior olive, which provides the climbing fibers to Purkinje cells in the cerebellar cortex, has been implicated in various functions, such as learning and timing of movements, and comparing intended with achieved movements. For example, climbing-fiber activity could transmit error signals during eye-blink conditioning or adaptation of the vestibulo-ocular reflex, or it could carry motor command signals beating on the rhythm of the oscillating and synchronous firing of ensembles of olivary neurons, or both. In this review, we approach the controversial issue of olivocerebellar function from the perspective of the unique organization of the microcircuitry of the olivary neuropil. The characteristic glomeruli are formed by a core of long dendritic or axonal spines, each of which is innervated by both an inhibitory terminal derived from the hindbrain and an excitatory terminal derived from either an ascending or descending input. The dendritic spines, which originate from dendrites with varicosities carrying dendritic lamellar bodies, are coupled by gap junctions. By drawing a comparison with a computational model by Segev and Rall, which might be applicable to the typical olivary spine with its unique morphological features and combined excitatory and inhibitory input, we propose that the microcircuitry of the inferior olive is capable of functioning both in motor learning and motor timing, but does not directly compare intended with achieved movements.

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DESPITE DECADES of investigation, the issue of the function of the olivocerebellar system is still a matter of fervent debate[1]. While some older hypotheses such as the comparator hypothesis still receive attention[2], the two major propositions in this field emphasize the role of the inferior olive in learning and timing of motor behavior[3–8]. In the present study, we approach this issue from the perspective of the local network inside the inferior olive. Does the unique organization of the olivary microcircuitry give us a clue as to what the olive is really doing?

The cerebellum and the inferior olive

The cerebellum receives information about peripheral events and central processes through numerous precerebellar systems, which terminate in different layers of the cerebellar cortex, predominantly as climbing and mossy fibers. The inferior olive gives rise to all the climbing fibers innervating the Purkinje cells, while the Purkinje cells themselves are the sole source of the ouput signals of the cerebellar cortex that reach the central cerebellar and vestibular nuclei. The mammalian inferior olive is composed of the principal olive, the dorsal and medial accessory olives, and several smaller subnuclei, such as the ventrolateral outgrowth, dorsal cap of Kooy, Beta-nucleus and dorsomedial cell column[9–11]. In general, each olivary subnucleus projects contralaterally to one or more longitudinal zones of Purkinje cells and gives off collaterals to the central cerebellar nucleus that receives its main Purkinje-cell input from the same zone (or zones)[12–14]. Since the cerebellar nuclei in turn project to that olivary subnucleus from which they receive collaterals[15–19], the direct connections between them are reciprocally and topographically organized[20]. The anatomical unit consisting of a particular Purkinje-cell zone with its specific olivary input together with their innervation of the associated cerebellar or vestibular nucleus, has been named a cerebellar module[13] (for details see Ref. 21).

The olivocerebellar mesodiencephalic loop

In addition to the olivocerebellar loops, which have their basis in the cerebellar modules, there is another three-element loop, which is superimposed on the olivocerebellar system: the olivocerebellar mesodiencephalic loop (Fig. 1). An important aspect of this loop is that the cerebellar nuclei contain both inhibitory and excitatory projection neurons. The inhibitory neurons provide a GABAergic feedback to the inferior olive, while the excitatory neurons are the mediators via which the cerebellum influences motor behavior[22–25]. One of the major targets of these excitatory neurons is the mesodiencephalic junction. This area incorporates a variety of nuclei, including the nucleus of Darkschewitsch, red nucleus, nucleus interstitialis of Cajal, nucleus of Bechterew, tegmental field of Forel, zona incerta, subparafascicularis nucleus, and the prerubral reticular formation[25–27]. Some of these nuclei, such as the magnocellular red nucleus, project directly to motoneurons and interneurons in the spinal cord affecting motor activity[28], whereas others such as the parvocellular red nucleus, the nucleus of Darkschewitsch, and the nucleus

Chris I. De Zeeuw, Casper C. Hoogenraad, S.K.E. Koekkoek and Tom J.H. Ruigrok are at the Dept of Anatomy, and Casper C. Hoogenraad and Niels Galjart are at the MGC Dept of Cell Biology and Genetics, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR, Rotterdam, The Netherlands; John I. Simpson is at the Dept of Physiology and Neuroscience, New York University School of Medicine, 550 First Avenue, New York, NY 10016, USA.

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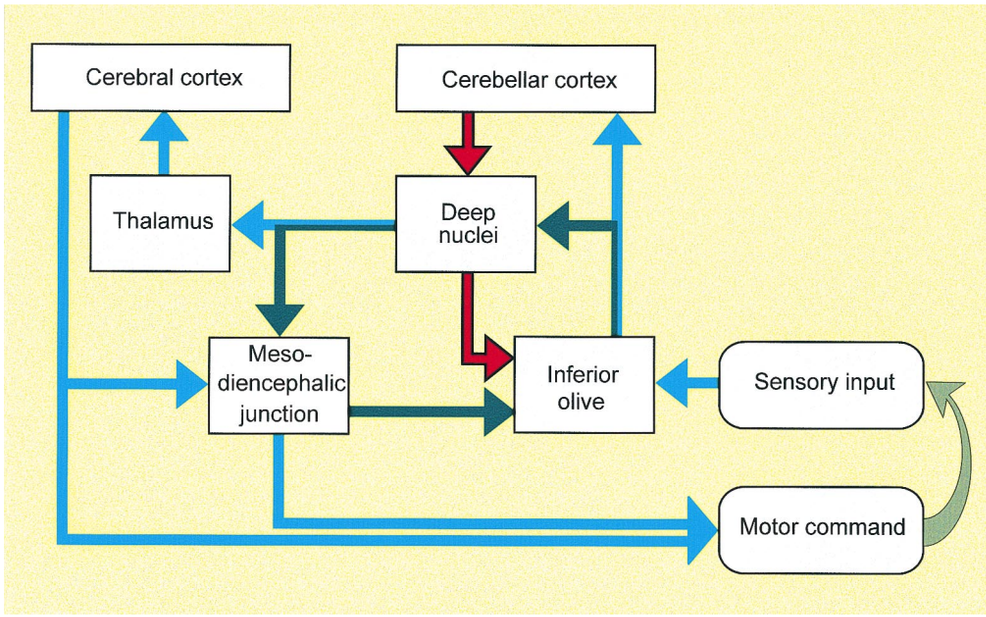

Fig. 1. ***The three-element network (dark blue) composed of the mesodiencephalic junction, deep cerebellar nuclei, and inferior olive forms an important loop in sensorimotor control.*** *Red arrows indicate inhibitory pathways and (light and dark) blue arrows mark excitatory systems.*

of Bechterew project to the inferior olive[29]. Recently, it was demonstrated that cerebellar terminals in the nucleus of Darkschewitsch indeed directly innervate those neurons that project to the inferior olive[30]. Thus, the olivocerebellar mesodiencephalic loop is formed by the projections from the olivary collaterals to the cerebellar nuclei, from the cerebellar nuclei to the mesodiencephalic junction, and from the mesodiencephalic junction back to the inferior olive. Similar to the organization of the olivocerebellar modules, the olivocerebellar mesodiencephalic loop appears to be topographically organized. For example, the rostral medial accessory olive projects to the posterior interposed cerebellar nucleus, which in turn innervates the nucleus of Darkschewitsch, which projects to the rostral medial accessory olive, while the principal olive projects to the dentate cerebellar nucleus, which in turn projects to the parvocellular red nucleus and nucleus of Bechterew, which project to the principal olive.

All three elements of the olivocerebellar mesodiencephalic loop described above are excitatory. This potentially reverberating loop could be controlled by local inhibitory interneurons in the cerebellar nuclei and mesodiencephalic junction, by the GABAergic feedback from the cerebellar nuclei to the inferior olive, and by the Purkinje cell input to the cerebellar nuclei neurons. Importantly, the Purkinje cell input, which is entirely GABAergic[31], affects both the excitatory and inhibitory neurons in the cerebellar nuclei[32,33]. In fact, individual Purkinje-cell axons can innervate both the excitatory neurons in the cerebellar nuclei that project to the mesodiencephalic junction and the inhibitory neurons that provide a GABAergic input to the olive. Thus, the Purkinje cells can control simultaneously the excitatory reverberating olivocerebellar mesodiencephalic loop and the inhibitory feedback that could also partly control this loop (see also Fig. 2).

Cytology and ultrastructure of the inferior olive

Although the corpora olivares were first identified and so named by Gabriel Fallopius near the middle of the sixteenth century[34], more than 300 years passed before Vincenzi[35] characterized the inferior olive nerve cells and portrayed their highly ramified dendritic trees. Von Kölliker[36] and van Gehuchten[37] elaborated upon the cytological features of the inferior olive, but it was Ramón y Cajal[38,39] who most thoroughly described this brainstem area using the Golgi method.

The population of olivary neurons is rather homogeneous. Apart from a few interneurons (<0.1%), which can be GABAergic[40,41], it is composed of two types of neurons. The main type has a spherical cell body with a diameter of 15–30 μm and an arbor of complex spine-bearing dendrites, which are highly branched and

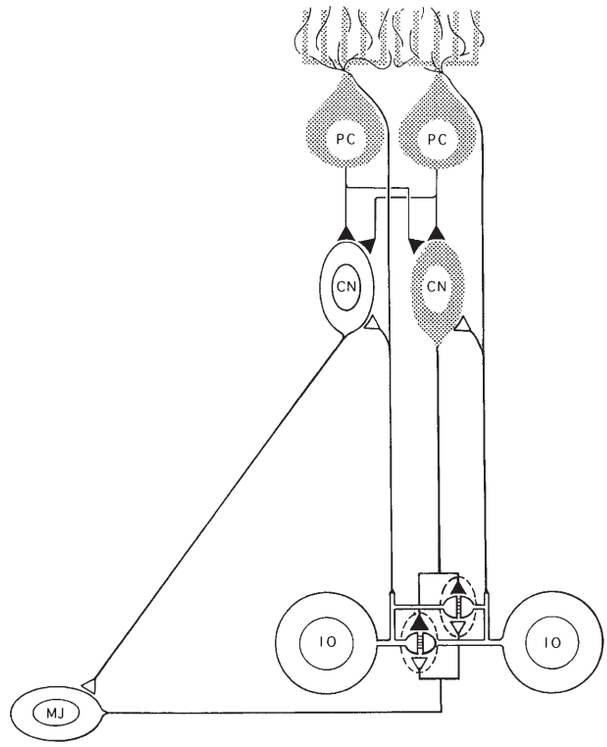

Fig. 2. ***Diagram of the neuropil in the medial accessory olive and principal olive*** **(bottom)**, ***and its relation with the cerebellum*** **(top)** ***and mesodiencephalic junction (MJ,*** **left side)**. *All olivary spines (half circles) are located within glomeruli (dotted circles), and innervated by both an excitatory mesodiencephalic and an inhibitory cerebellar terminal (white and black triangles, respectively). The olivary axons provide climbing fibers to the Purkinje cells (PC) in the cerebellar cortex and collaterals to both the GABAergic and excitatory cerebellar nuclei (CN) neurons. The GABAergic projection neurons in the cerebellar nuclei project exclusively to the inferior olive (IO), while a substantial part of the excitatory projection neurons in these nuclei innervate the neurons in the mesodiencephalic junction that in turn project back to the inferior olive. The excitatory and inhibitory neurons in the cerebellar nuclei can receive input from the same Purkinje cell axon. Small lines between olivary spines indicate the dendrodendritic gap junctions by which they are electrotonically coupled.*

Box 1. Localization of dendritic lamellar bodies and their putative association with dendrodendritic gap junctions, CLIP-115, and Williams Syndrome

Olivary neurons contain hundreds of dendritic lamellar bodies, which occur exclusively in their bulbous dendritic appendages (Fig. A,B). The function of this organelle remains to be elucidated, but several observations suggest it is related to that of dendrodendritic gap junctions[a]. For example, during development dendritic lamellar bodies and dendrodendritic gap junctions arise simultaneously, while during adulthood dendritic lamellar bodies are present in all brain areas where gap junctions between dendrites of neurons are prominent. Moreover, the densities of both dendritic lamellar bodies and dendrodendritic gap junctions in the inferior olive can be down-regulated concomitantly by removal of the cerebellar GABAergic inputs to their electrotonically coupled spines, and the density of dendritic lamellar bodies in the different olivary subnuclei can be correlated to the level of synchronous firing of their neurons[b]. More recently, it was demonstrated that the vesicle transport along microtubules in dendritic shafts that might give rise to the dendritic lamellar bodies is probably mediated by a cytoplasmic linker protein of 115 kDa, CLIP-115 (Ref. c), and that the gene (*CYLN2*) encoding this protein is hemizygously deleted in Williams Syndrome patients (Fig. C,D) (Hoogenraad, C., unpublished observations). These findings raise the interesting possibility that some of the motor coordination deficits that can be observed in Williams Syndrome patients[d] might be due to a malfunction of olivary dendritic lamellar bodies and the putatively associated gap junctions. Another aberration of the expression or localization of olivary gap junctions probably occurs in olivary hypertrophy (see Box 2).

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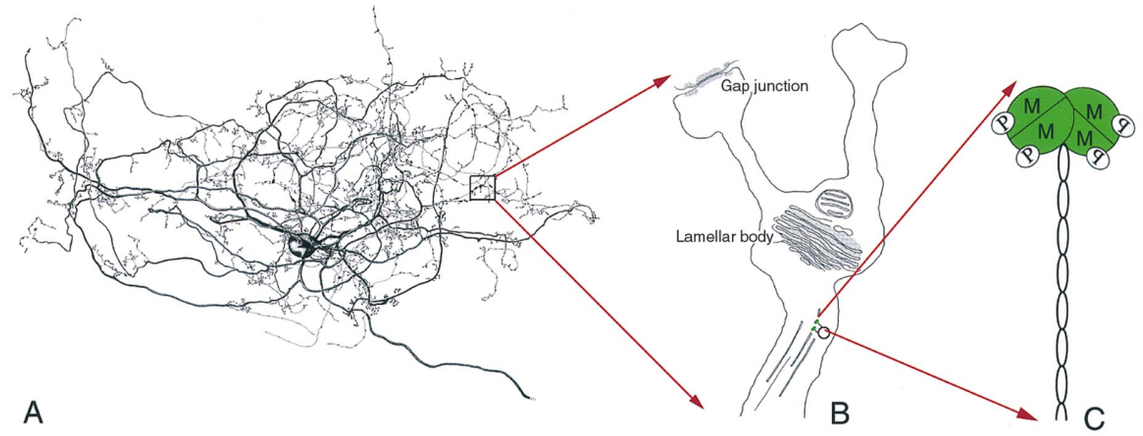

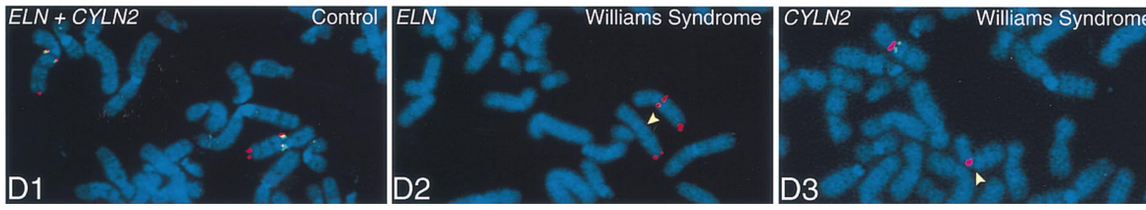

Fig. (A) ***Reconstruction of a typical neuron from the inferior olive in the cat following intracellular injection of HRP.*** *Note the extensive dendritic arbor of the olivary neuron and its large number of dendritic varicosities.* **(B)** *Enlargement of the inset of* (A)*, an example of a dendritic varicosity. Such a dendritic varicosity often contains a dendritic lamellar body and frequently it gives rise to a spine that is coupled to another spine by a dendrodendritic gap junction. Transport of the membranous vesicles that ultimately form the cisternae of the lamellar body is probably specifically mediated in part by a cytoplasmic linker protein, CLIP-115 (Ref. c).* **(C)** *CLIP-115, which contains two microtubule-binding domains (M) with multiple phosphorylation sites (P), probably forms dimers through its large coiled-coil region.* **(D)** *Cytogenetic analysis of* CYLN2*, the gene encoding CLIP-115, by fluorescence* in situ *hybridization indicates that* CYLN2 *is located in the 7q11.23 Williams Syndrome critical region and hemizygously deleted in Williams Syndrome patients (Hoogenraad, C., unpublished observations).* (D1) *Probes for Elastin* (ELN*, red), which is also known to be located in the Williams Syndrome critical region*[e]*, and* CYLN2 *(*CYLN2*, green), and a 7q36-specific cosmid (red) were hybridized to metaphase chromosomes from unaffected individuals. The hybridization signals for Elastin and CLIP-115 are close to each other on 7q11.23.* (D2) *Williams Syndrome patients show hybridization signals for the Elastin cosmids on one chromosome-7 homolog, but not on the other (arrowhead). Both chromosomes 7 do show specific signals for the chromosome 7q36-specific cosmid.* (D3) *Williams Syndrome-affected individuals show hybridization signals for the* CYLN2 *gene on one chromosome-7 homolog, but not on the other (arrowhead).*

tend to turn back toward the soma, at times creating spirals (Box 1, Fig. A). This type of neuron, which occupies a relatively small volume, is the predominant cell type in the principal olive, the rostral part of the medial accessory olive and the dorsal accessory olive. The other type of neuron has relatively long, diffuse, sparsely branched, spiny dendrites radiating away from the soma and occupying a large dendritic field[19,42]. This type occurs mainly in the caudal part of the medial accessory olive. Both cell types are probably projection neurons, that is, they give rise to the climbing fibers, because the axons of both types of cells

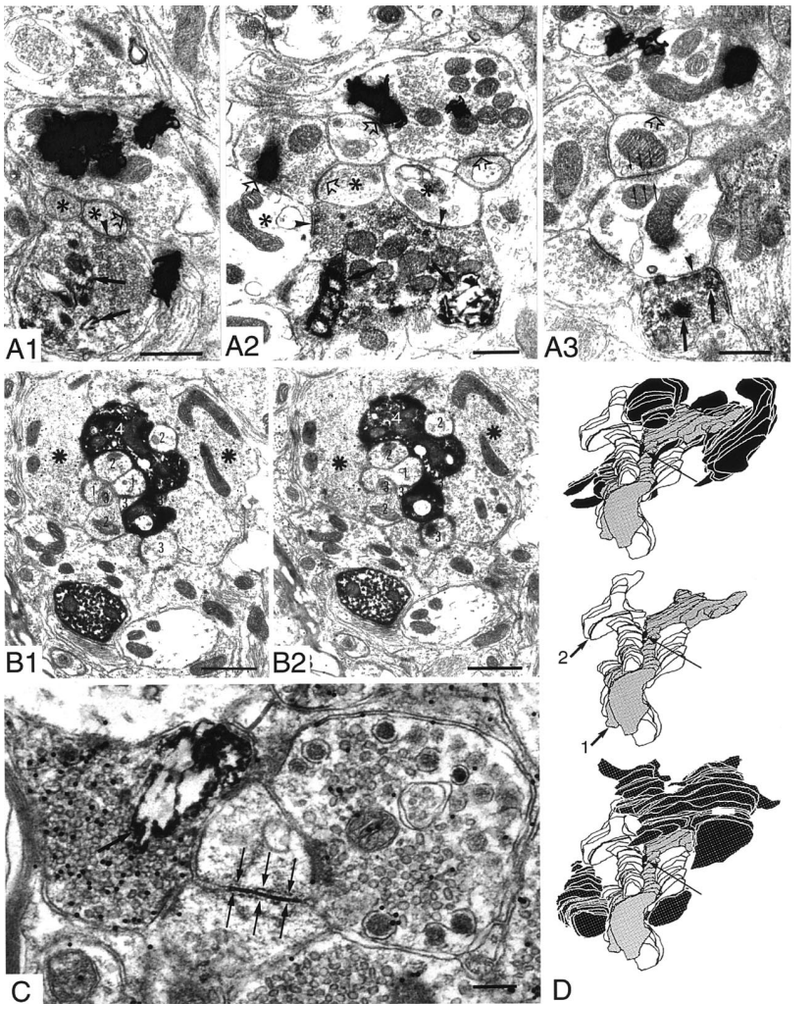

Fig. 3. ***All olivary spines are located in glomeruli and receive both an excitatory and an inhibitory input: a demonstration with three different electron microscopic double-labeling techniques.*** **(A1–3)** *Three glomeruli, which all contain both WGA-HRP (black arrows) anterogradely labeled terminals from the cerebellar nuclei and [^{3}H]leucine anterogradely labeled terminals from the mesodiencephalic junction* (from Ref. 48). *The cerebellar and mesodiencephalic terminals display the morphological characteristics typical of inhibitory and excitatory terminals: pleiomorphic vesicles and symmetric synapses (arrowheads) versus round vesicles and asymmetric synapses (open arrows), respectively. Asterisks indicate examples of dendritic spines innervated by both types of terminals.* **(B1,2)** *Serial section analysis of a glomerulus with four dendritic spines (numbers 1–4) following intracellular injection of HRP combined with postembedding GABA–immunocytochemistry* (from Ref. 49*). In this study we demonstrated that all dendritic spines are innervated by both a GABAergic (asterisks) and a non-GABAergic terminal.* **(C)** *The GABAergic terminals apposed to the dendritic spines that are coupled by gap junctions are derived from the cerebellar nuclei and vestibular complex* (from Refs 22,60). *In this example, the electron micrograph is taken from the dorsal cap of Kooy following injection of WGA-HRP in the nucleus prepositus hypoglossi combined with postembedding GABA-immunocytochemistry.* **(D)** *Reconstruction [from the material used for the experiments described in* (B)*] of a GABAergic (bottom) and non-GABAergic (top) terminal innervating two dendritic spines (numbers 1 and 2) that are electrotonically coupled by a gap junction. Thin arrows in* (A3), (C) *and* (D) *indicate dendrodendritic gap junctions. Scale bars, 0.4 μm* (A1–3), *1.2 μm* (B1,2) *and 0.25 μm* (C).

leave the neuropil of the adult inferior olive without giving off collaterals[43,44].

The ultrastructure of the mammalian olivary neuropil has been described in many studies of various animals[45]. The segments of olivary dendrites as well as the hillocks of olivary axons bear pedunculated club-shaped or racemose spiny appendages[44,46–50]. Whereas it is clear that the dendritic spines are frequently electrotonically coupled by gap junctions[22,47,49,51], it remains to be demonstrated whether this also holds true for the axonal spines[50]. Both the dendritic and axonal spines are characterized by unusually long spine necks. Because of their long necks the spine heads can cluster together and form the core of what is the most characteristic feature of the olivary neuropil: the glomerulus[46,47,49,52]. In general, a glomerulus contains a core of five or six dendritic and axonal spiny appendages, derived from different neurons, that is surrounded by four or five terminals and several glial sheaths[45,49,50]. Serial-section analysis has demonstrated that virtually all spines are located in glomeruli (Fig. 3).

Several attempts have been made to estimate the extent of electrotonic coupling of olivary neurons via gap junctions, which themselves are relatively difficult to demonstrate with standard electron microscopic techniques. Benardo and Foster[53] were able to demonstrate the existence of clusters of six to eight coupled neurons by intracellular injection of Lucifer yellow in slices of the inferior olive of the guinea pig. However, with the application of harmaline and picrotoxin, Llinás and colleagues have demonstrated that in the intact inferior olive synchronous firing can be induced in coupled cellular aggregates of hundreds of neurons[54–56]. In fact, bilateral multiple-unit recordings from the cerebellar cortex demonstrated that an ensemble of coupled olivary neurons in the rat can even extend beyond the midline[57]. In the same study, it was estimated that one olivary neuron can have 500–1000 gap junctions, and that two individual olivary neurons can be coupled by 10–20 gap junctions[57].

Another prominent, possibly related, feature of the olivary neuropil is the presence of dendritic lamellar bodies[51]. This recently discovered organelle consists of stacks of membranous cisternae with electron-dense deposits in between, and it occurs exclusively in the varicose dilatations that are abundant in the peripheral olivary dendrites just outside the glomeruli (Box 1, Fig. A,B). Although we cannot exclude other possible functions, such as intracellular Ca^{2+} control[58], or the exchange of excitable dendritic membranes (see below), various lines of evidence suggest that, as outlined in Box 1, the dendritic lamellar bodies might serve to control the turnover or assembly of dendrodendritic gap-junction channels. The fact that the density of dendritic lamellar bodies in the inferior olive is higher than in any other area of the brain[51], indicates the importance of electrotonic coupling between olivary neurons.

We conclude that the glomeruli, gap junctions and dendritic lamellar bodies are the characteristic elements of the olivary neuropil that make it unique. Although the densities of these structures as well as the complexity of the associated spines vary among the different olivary subdivisions[22,23,47,53,57,59,60], they are present throughout the entire inferior olive[45,51,57].

The combined excitatory and inhibitory input to the olivary glomeruli

The general morphological characteristics of the inputs to the olivary glomeruli have been best explored for the principal olive and rostral medial accessory olive[22,23,45,49,50,61], but subsequent studies have indicated that the same principles hold true for other olivary subdivisions, such as the dorsal accessory olive (De Zeeuw, C., unpublished observations) and the Beta-nucleus, dorsal cap of Kooy, ventrolateral outgrowth and dorsomedial cell column[53,57,62]. Even so, we will restrict ourselves in this review to the mesodiencephalic and cerebellar

inputs to the glomeruli of the principal olive and rostral medial accessory olive.

All mesodiencephalic terminals in the olive are excitatory and display the corresponding morphological characteristics, consisting of rounded vesicles and asymmetric synapses. In contrast, all the cerebellar terminals are GABAergic and have pleiomorphic vesicles and symmetric synapses[63]. Approximately half of both types of terminals in the inferior olive contact dendritic elements inside glomeruli[22,23,45,49,50,61]. Most of the remaining terminals contact the proximal and intermediate dendrites, while relatively few terminate on the somata and axon hillock; presynaptic axo–axonal contacts have not been observed in the inferior olive. The innervation of the inferior olive by the non-GABAergic mesodiencephalic terminals and GABAergic cerebellar terminals is apparently random, because neither type of terminal has a preference for either the extra- or intraglomerular neuropil, and there is no obvious pattern in the distribution of the two types of terminals within the individual glomeruli[23]. As illustrated in Figs 2 and 3, multiple-tracer experiments and intracellular labeling combined with immunocytochemistry have revealed that every spine on the dendrites and axon hillock of all olivary neurons in the principal olive and rostral medial accessory olive receives a synaptic input from both an excitatory mesodiencephalic terminal and an inhibitory cerebellar terminal. Since in most regions of the central nervous system the vast majority of dendritic spines are contacted solely by asymmetric synapses[64–67], the ubiquitous, combined excitatory and inhibitory input to the olivary spines can also be considered as unique.

Functional implications of the microcircuitry of the inferior olive

As outlined above, several morphological features distinguish the neuropil of the inferior olive from that of other regions in the central nervous system. The shape of the dendritic arbor and the morphology of the dendritic and axonal spines are unusual, the combination of an excitatory and inhibitory input to these spines is remarkable, and the prominence of dendrodendritic gap junctions and associated dendritic lamellar bodies is equally striking. What functional implications can be drawn from the design of the olivary dendrites and glomeruli? Although only about half of the terminals in the inferior olive are located inside glomeruli, we evaluate the possible functions of the olive from the perspective of the organization of its glomeruli, because the electrical activities generated in the glomerular spines constitute the unique electrophysiological features of the olivary neuropil. Computational studies have shown that the depolarizations in the cell body and dendritic shafts can be amplified several times by the presence of action potentials in the spines[68]. Thus, the synaptic input to the dendritic shafts probably does not overrule that to spines, and the efficacy of the dendritic input could depend largely on the activity of the inputs to the glomerular spines.

Timing hypothesis

The notion that the inferior olive can function as an oscillating clock providing the appropriate timing of command signals for the appropriate motor domains has been promoted by Llinás and collaborators[7,56,69–71]. This hypothesis can be dissected into three components: (1) olivary neurons have a propensity to fire rhythmically; (2) olivary neurons are dynamically electrotonically coupled by gap junctions so that different synchronous firing patterns can be generated by chemical synaptic inputs; and (3) synchronous olivary activity can be correlated to the initiation and performance of movements.

The intrinsic conductances of olivary neurons, which are differentially distributed over the dendritic and somatic membrane, have been claimed to underly the tendency of olivary neurons to show subthreshold oscillations and to fire rhythmically[72–74]. Extensive rhythmic firing of olivary neurons *in vivo* can be observed during rhythmic vibrissal or tongue movements[7,75], following application of harmaline or picrotoxin[4,56], or, as described in Box 2, following stimulation of the mesodiencephalic projection to the inferior olive[25]. In contrast, relatively little rhythmic activity can be observed during compensatory eye movement responses to vestibular or visual stimulation in rabbits (unpublished observations, Ref. 76, but see Refs 77,78), or during trained wrist movements in monkeys[79]. Thus, rhythmic olivary activity can occur, but so far the function of this activity remains to be demonstrated for natural nonrhythmic motor behavior.

Several physiological studies indicate that the formation of ensembles of synchronously firing olivary neurons is a dynamic process that is controlled by the cerebellar GABAergic input to the glomeruli. Llinás and Sasaki[4] and Lang and colleagues[55] have demonstrated that the degree of synchronization of complex spike (that is, climbing fiber) activity in Purkinje cells can be increased in the mediolateral direction and to a lesser extent in the rostrocaudal direction by applying GABA-receptor antagonists to the inferior olive or by lesioning the central nuclei of the cerebellum. These data suggest that the cerebellar GABAergic terminals that contact the glomerular spines linked by gap junctions are involved in the regulation of electrotonic coupling and serve to dynamically reassemble functional olivary networks. The morphological data reviewed here fully support this concept in that, indeed, all coupled spines in the olive receive a GABAergic input from the hindbrain. However, the question arises whether the mesodiencephalic terminals, which are also located within the glomeruli next to the gap junctions, also affect the efficacy of the coupling. Although no conclusive experimental evidence is available, we assume that an excitatory input to the coupled spines will produce, just as for an inhibitory input, a local conductance increase and thereby a shunt, but as the injected charge as well as the corresponding battery will be positive, this activation will probably not cause decoupling. Thus, the cerebellar GABAergic input, but not the mesodiencephalic innervation, might determine which olivary neurons fire synchronously and thereby act as a pattern generator.

Nonetheless, the mesodiencephalic terminals do conribute to the generation of olivary action potentials (see Box 2), and the prominent termination site of the mesodiencephalic terminals in the glomeruli must be physiologically relevant. The major consequence of this termination site is that the spines inside the glomeruli are innervated by both the inhibitory cerebellar and the excitatory mesodiencephalic terminals. Here, we refer to a computational model by Segev and Rall[68], and propose that such a combined inhibitory and excitatory

Box 2. Intracellular recordings of neurons in the normal and hypertrophic inferior olive

Recently, we have investigated the roles of the mesodiencephalic and cerebellar input, to olivary neurons by intracellular recording[a,b]. By applying short-lasting stimulus trains to the medial part of the ipsilateral mesodiencephalic junction (MDJ) or the contralateral superior cerebellar peduncle (SCP) we were able to distinguish the excitatory effects of stimulating the midbrain from the combined effects of stimulating the excitatory nucleo–midbrain–olivary pathway in conjunction with the inhibitory nucleo–olivary pathway. MDJ stimulation usually resulted in a short-latency action potential (SLAP) suggestive of a monosynaptic activation (mean latency 7 ms) and a long-latency rebound action potential (LLAP; mean latency 180 ms). In contrast, SCP stimulation usually resulted solely in a SLAP and the latency of this potential, which was on average 13 ms, was in accordance with a disynaptic pathway. A short-latency hyperpolarization that would suggest a monosynaptic inhibitory postsynaptic potential was never observed. Thus, the most prominent difference was the propensity of olivary neurons to discharge rebound action potentials after MDJ stimulation and not after SCP stimulation (Fig. B). Interestingly, lesions of the nucleo–olivary pathway in the ventrolateral tegmentum increased the number of rebounds after both MDJ and SCP stimulation[a]. In this situation, some stimulations could even evoke long-latency rebound potentials without a short-latency activation (not shown). From these studies it was concluded that, although lesions of the cerebellar nuclei do increase the excitability of olivary neurons[c], the nucleo–olivary pathway does not directly elicit a robust inhibitory effect on the olivary cells under the stimulus conditions employed. However, since activation of this pathway did influence the incidence of rebound firing and since rebound oscillatory properties have been shown to be directly related to the strength of electrotonic coupling[d,e], these data suggest that the nucleo–olivary pathway might be especially suited to controlling the levels of conjunct membrane oscillations and, hence, affecting the propensity of olivary units to discharge synchronously. The glomerular localization of the electrotonic coupling and its control by the GABAergic nucleo–olivary terminals might be important in the apparent failure of the inhibitory postsynaptic potentials prominently to affect somatic excitability as shown in the above-mentioned paradigm. Moreover, well-balanced interactions between coupled glomerular or distal dendritic compartments, or both, and long-term levels of excitation and inhibition in the proximal dendrites and somata might play a key role in establishing the frequency and depth of the subthreshold oscillatory capacity of the membrane potential[d,f]. Thus, local regulation of electrotonic coupling within the

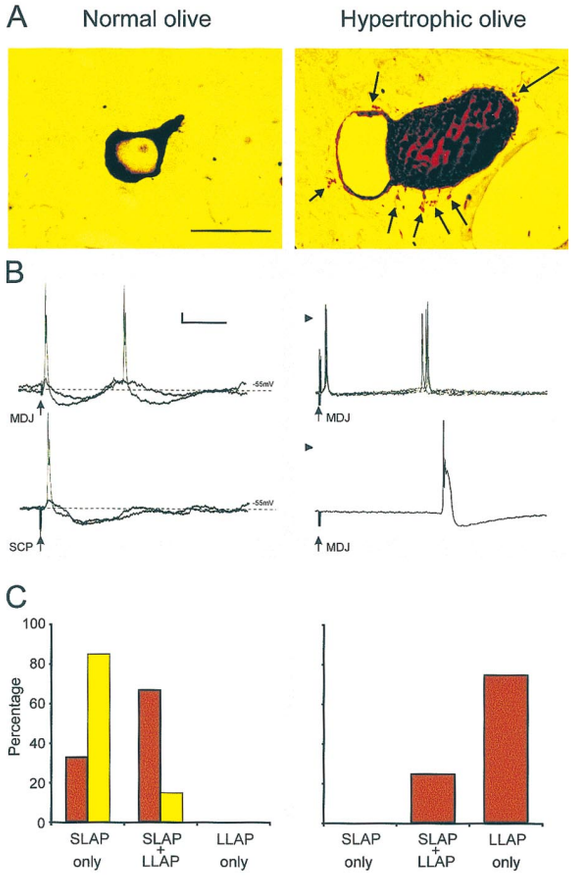

Fig. (A) ***Microphotographs of an intracellularly HRP-labeled normal* (left) *and hypertrophic* (right) *cat olivary neuron (1 μm section; same magnification).*** *Note the high incidence of somatic spines (arrows) and the large vacuole in the hypertrophic cell; scale bar, 25 μm* (from Ref. b). **(B)** *Left-hand panels: typical response patterns of an olivary cell to stimulation (3 pulses at 660 Hz) of the medial mesodiencephalic junction (MDJ, top left-hand panel) and of the superior cerebellar peduncle (SCP, bottom left-hand panel). Note that a rebound of the membrane potential is only seen after MDJ stimulation. Also note that in both situations subthreshold activations still result in a hyperpolarizing membrane potential that has the same time course as the large after-hyperpolarization that follows the characteristic olivary action potential, and which is suggestive of electrotonic coupling with neighboring activated olivary units[a]. Horizontal scale bar, 100 ms; vertical scale bar, 10 mV. Right-hand panels: intracellular recordings from cat hypertrophic olivary neurons responding to MDJ stimulation* (from Ref. b). *Upper panel shows the short latency and rebound activation of 'simple' somatic action potentials (3 superimposed traces). Bottom panel: intracellular activation of another hypertrophic olivary neuron showing a consistent long-latency activation following MDJ stimulation. Note that the resulting action potential did show a very pronounced (>30 ms) after-depolarizing potential. With direct intracellular stimulation (current injection, not shown), however, this after-depolarizing potential could not be triggered. Horizontal scale bars, 50 ms; vertical scale bar, top panel, 20 mV, bottom panel, 10 mV.* **(C)** *Incidence of short latency activation (SLAP) and of rebound firing (LLAP) of intracellularly recorded normal and hypertrophic olivary units resulting from either MDJ (brown columns, respectively) or SCP (yellow columns, only for normal cells) activation. A rebound activation after a SLAP is common after MDJ stimulation, but is seldom triggered after SCP stimulation. However, hypertrophic cells often respond with an action potential after 100–250 ms (LLAP) without a preceding SLAP.*

input to the glomerular spines is suited to a nucleus that is supposed to dynamically generate synchronous time patterns of activation. Segev and Rall showed that the effect of synaptic inhibition can be enhanced in dendritic spines that are contacted by both excitatory and inhibitory synapses, and that this enhanced inhibitory effect can be extremely sensitive to the timing between both types of inputs, with a temporal resolution well below 100 μs[80]. These properties hold true especially for spines whose heads contain a significant number of voltage-dependent channels. At present, it is not known whether olivary dendritic spines have

glomeruli appears to be a more prominent function of the cerebellar GABAergic input than its role in determining the olivary firing frequency. On the other hand, it should be noted that we did not investigate what the effects are of the timing of SCP stimulation with respect to that of MDJ stimulation. Such experiments are crucial for evaluating our hypothesis on the putative function of the olivary spines with their combined excitatory mesodiencephalic and inhibitory cerebellar input that the timing between these two inputs is essential for the efficacy of the inhibitory input.

The importance of the glomerular localization of the vast majority of gap junctions in the normal inferior olive can be illustrated by investigating the electrophysiological properties of hypertrophic olivary neurons, in which most of the gap junctions are located on the soma[g]. Olivary hypertrophy, which can occur in humans after lesions of the central tegmental tract, dentate nucleus, or superior cerebellar peduncle, or both[h,i], can be experimentally induced after hemicerebellectomy in the cat[j] [for comparison between a normal (left) and hypertrophic (right) olivary neuron in the rostral medial accessory olive of the cat, see Fig. A]. Intracellular recordings of hypertrophic neurons have revealed that MDJ stimulation usually failed to trigger the characteristic dendritic after-depolarizing potential[b] (Fig. B). However, the widespread dendritic tree of hypertrophic neurons is still capable of discharging depolarizing potentials, as was occasionally noted when these cells fired spontaneously, or in the rebound phase after MDJ stimulation. Since the somata of hypertrophic cells have numerous spiny appendages (Fig. A), which also show gap junctions[g], the hypertrophic neurons are probably directly coupled to each other at the level of the cell bodies. This somatic coupling might dissociate, in part, the electrical activities in the cell bodies from those in the dendrites, so that the hypertrophic neurons fire mostly with solely somatic Na^+ spikes and no dendritic afterpotentials. This explanation also clarifies why hypertrophic olivary neurons can reach a much higher maximum firing frequency following MDJ stimulation than normal olivary neurons[b]. Thus, on the basis of our investigations of the hypertrophic inferior olive and comparison with the normal inferior olive we conclude that the dominant dendritic localization of the gap junctions is essential for the timing and integrative operations in the inferior olive as well as for the cerebellar inhibitory input to regulate the coupling.

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such excitable channels, but since olivary neurons possess a variety of complex and interacting conductances[72–74,81], some of which might occur solely within the dendrites (high-threshold noninactivating Ca^{2+} channels), it seems an attractive hypothesis that the spines of olivary dendrites indeed have excitable channels. Moreover, several morphological features of the olivary neurons support the assumption of excitable channels within their spines[68]: (1) olivary spines are complex with extremely long and thin spine stalks; these are thought to be favorable assets for excitable spines (high-input resistance plus partial electrical decoupling of the synapse from dendritic impedance load, combined with sufficiently available synaptic current); (2) most olivary spines are located at secondary or tertiary dendrites; these are the most likely candidates for carrying excitable spines, which might function as current boosters to compensate for the attenuation of the potential from distal dendritic regions; and finally (3) olivary spines are coupled by gap junctions, which can provide the synchronous activation that will enhance the properties of excitable spines. Thus, it is attractive to consider the model of Segev and Rall[68] in the context of the olivary spines. If we do so, then it appears that the excitation of olivary cells can only be stopped when the inhibitory cerebellar terminals are firing within a specific, relatively short period of time related to the activity of the excitatory mesodiencephalic terminals. As such, the olivary spines might function as the substrate of a precise time filter that determines whether or not particular signals are being transmitted at particular moments in time[48,57]. Therefore, we agree that it is possible that the olivary spines with their combined excitatory and inhibitory inputs serve as the interlocking 'gears' of the olivary clockwork that might function as a timing device in which spatiotemporal activity patterns are regulated.

The final component of the timing hypothesis is that the generated patterns of synchronous climbing fiber activity influence directly the activity of cerebellar nuclei neurons and thereby the initiation and performance of appropriate muscle synergies. The direct impact of complex spike activity on central nuclei neurons is still a matter of debate. So far, attempts to correlate the activity of Purkinje cells to the activity of the cerebellar nuclei neurons that are innervated by the same Purkinje cells have failed to distinguish the effects of the complex spikes from those of the simple spikes[82,83]. Since it has recently been demonstrated in awake behaving animals that simple spike synchrony can occur, and that it tends to increase as the complex spike synchrony increases[76], this lack of distinction, in fact, raises the possibility that complex spike synchrony elicits its effects via a synchrony in simple spike activity (Fig. 4).

The initial evidence for a correlation between (synchronous) olivary activity and motor performance was provided by the findings that lesions of the inferior olive induce movement decomposition, intention tremor and agonist–antagonist cocontraction[84,85], and that application of harmaline induces synchronous oscillations of olivary neurons and a tremor in phase with this activity[4,55]. More recently, Welsh *et al.* (Refs 7,86) were able to correlate the synchronous climbing fiber activities of specific dynamic ensembles of Purkinje cells to specific components of rhythmic tongue movements. However, the number of studies correlating synchronous climbing fiber activity with movements is so far rather limited and contradicting studies exist. We have, for example, been able to demonstrate synchronous climbing fiber activity in the flocculus of the awake rabbit, but we have been unable to relate it to eye movement performance during natural visual or vestibular stimulation[76] (Fig. 4).

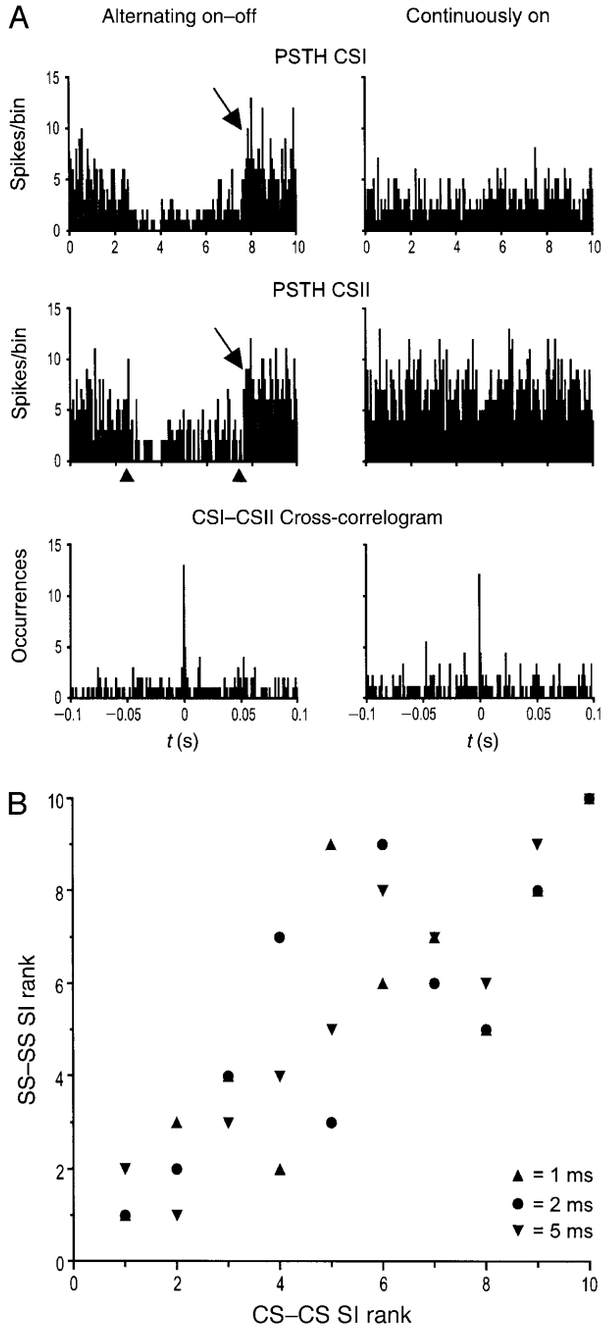

Fig. 4. ***Complex spike synchrony and simple spike synchrony in floccular Purkinje cells of the awake rabbit during optokinetic stimulation.*** **(A)** *Peristimulus time histograms (PSTH, binwidth 50 ms) and cross-correlograms (binwidth 2 ms) of the complex spike (CS) activities of two Purkinje cells that responded optimally to rotational optokinetic stimulation about the vertical axis* (from Ref. 76). *The left and right panels show the complex spike activities of the same Purkinje cells during alternating excitatory–inhibitory (on–off) optokinetic stimulation and continuous excitatory (on) stimulation, respectively. For both paradigms, the cross-correlograms were composed from the same number of spikes. The synchrony during continuous excitatory stimulation was not lower than during alternating stimulation, indicating that the transients of the complex spike activities (arrows), which occur at the turn-around (arrowheads) during alternating on–off stimulation but not during continuous stimulation, cannot be the sole factor responsible for the synchrony.* **(B)** *Correlation between the synchrony indices (SIs) of the complex spike (CS) and simple spike (SS) responses of ten Purkinje cell pairs (for details see Ref. 76). For this graph we ranked and plotted the SIs of the complex spike activities of each pair against the SIs of their simple spike activities. Analyzed at binwidths of 1 ms, 2 ms and 5 ms, the correlation coefficients for the SIs of the complex spike and simple spike responses of these ten pairs were 0.79, 0.81 and 0.86, respectively.*

Thus, from evaluation of the three major components of the timing hypothesis from the perspective of the organization of the olivary network, we conclude that it is well designed to function as a clock in that its inputs might generate synchronous activity patterns of olivary neurons, but whether this feature is indeed necessary for the short-term initiation and performance of motor activities awaits further elucidation and confirmation.

Learning hypothesis

The learning hypothesis, which was originally proposed by Ito, Marr and Albus[3,87,88], states that the climbing fibers of the inferior olive provide the Purkinje cells of the cerebellum with an error signal that indicates inadequate motor activity. The complex spike activity evoked in the Purkinje cells by this climbing-fiber input should lead to a long-term depression of the simple spike response elicited by the parallel fibers of the granule cells, which, in turn, could correct motor performance. Thus, for this hypothesis, it is important that the olivary climbing fibers function as teachers providing the error signals to the cerebellar cortex, and that the olivary circuitry mediates the transmission of these error signals when they are needed and inhibits them when the learning process has to be blocked.

Ample evidence exists for all olivary subdivisions that many of their climbing fibers can mediate internally or externally generated signals that could be used as error signals. For example, somatosensory maps can be created for all major subdivisions[2,89,90] and visual or vestibular error signals can be attributed to the climbing fibers derived from the smaller olivary subdivisions, such as the dorsal cap of Kooy, ventrolateral outgrowth, Beta-nucleus, and dorsomedial cell column[91–95]. Figure 4A provides an example of robust complex spike modulation of two floccular Purkinje cells in an awake rabbit during natural optokinetic stimulation (from Ref. 76). Such activity, which seems to encode retinal slip, has been claimed to serve as the error signal necessary for adaptation of the vestibulo–ocular reflex[3]. An important point regarding transmission of olivary error signals is that if their function is restricted to that of a teacher, their firing frequency should be substantially lower than that of the simple spike response so that the effects of the simple spike responses on the cerebellar nuclei neurons are not dominated by the complex spikes, that is, a teacher instructs his students (simple spikes) to perform the task, but does not carry it out himself. As a consequence of their intrinsic conductances, olivary neurons have, indeed, a relatively low average and maximum firing frequency of approximately 1 Hz and 10 Hz, respectively. When activated, the olivary cell generates, in addition to the usual fast Na^+ action potential, a prolonged (about 15 ms) dendritic after-depolarizing potential and a long-lasting (about 100 ms) large after-hyperpolarizing potential, which in turn de-inactivates a low threshold Ca^{2+} conductance[72,73] (see also Box 2). It appears, therefore, that the afterpotentials of olivary neurons not only underly their presumptive oscillatory properties (see above), but also their low maximum firing frequency. Thus, we can conclude that the nature and frequency of the signals transmitted by the olivary neurons are appropriate for a nucleus that is supposed to function as a teacher in a motor-learning process.

Although it must be acknowledged that the inhibition in the olivary glomerulus is probably only effective

within a relatively small time window with respect to the activity of the excitatory inputs (see explanation above and in Box 2), it still is reasonable to assume that effective inhibition can occur[56,96,97]. Therefore, the olivary circuitry with its combined inhibitory and excitatory inputs to the spines should be capable of selecting and transmitting error signals when they are needed and of inhibiting them when the learning process has to be blocked. Several studies have demonstrated that olivary cells that are highly responsive to a cutaneous stimulus applied to the paw under resting conditions fail to respond when a cat stimulates its own paw, although the same cells do respond if a stimulus interrupts the trained movement (Refs 14,90,98,99, cf. Ref. 100). This observation indicates that precisely timed mechanisms must be available for modulating the sensory responsiveness of olivary cells. Since, as explained above, the interactions between the excitatory and inhibitory inputs are highly sensitive to the timing between them, the olivary glomeruli might function as the substrate for this filtering process and determine when and when not an input signal can be considered as an error signal, and accordingly transmit it or not. More recently, Hesslow and Ivarsson[101] and Kim *et al.* (Ref. 102) demonstrated that the cerebellar GABAergic input to the olive can block the transmission of the excitatory unconditioned stimulus signal in the olive during the continuation of eye-blink conditioning. Thus, the olivary glomeruli with their combined excitatory and inhibitory inputs might not only initially select the error signals out of the pool of incoming sensory signals, but also determine at the end of the learning process when the signal originally encoding an error can no longer be considered as an error signal and has to be stopped. Apparently, an excess of error signals can confound the final motor performance, and a blocking process induced by the cerebellar GABAergic input to the glomeruli could serve to circumvent this problem.

One can go one step further and suggest that the olivary spines themselves might influence the blocking process by altering their geometry or stem resistance. Differences in these parameters seem to be relevant variables for controlling the effectiveness of synaptic inputs[103–105]. The olivary dendritic elements would be excellent candidates for plastic substrates, both from the morphological point of view with their immense spiny network, and from the cell-physiological point of view with their conductances that could modulate their electroresponsive and integrating properties in the long-term[81]. However, because most studies of the olivocerebellar system have focussed on the long-term changes that occur at the parallel fiber–Purkinje cell synapses[3,8,106], or at the synaptic input to the central nuclei neurons[5,107–110], evidence for plastic changes associated with learning processes within the inferior olive is fairly limited[111,112].

One of the prominent features of the olivary neuropil that has so far been constantly neglected by most researchers in the field of cerebellar motor learning is the presence of electrotonic coupling by gap junctions. This feature should also be addressed if one assumes that the major function of the cerebellar GABAergic input to the olive is to block redundant transmission of 'error' signals, because this process, which results directly from an interaction with the excitatory terminals in the glomeruli, is probably influenced by the level of electrotonic coupling. For example, Yarom[70] showed, by connecting an analog simulator with an olivary neuron, that synaptically induced but intrinsically maintained activities and oscillations of electrotonically coupled olivary neurons could be stopped more readily when they were disconnected from each other. Thus, in addressing the role of the olivocerebellar system in motor learning, account should be taken of the phenomenon of electrotonic coupling, which might be more extensive in the inferior olive than in any other area of the brain[51].

Comparator hypothesis

The comparator hypothesis of olivary function, initially advanced by Oscarsson[2,113], proposes that the olivocerebellar system compares intended with performed movements to provide error detection (for review, see Ref. 1). The error-signaling part of this hypothesis has experimental support (see above), but with regard to the function of the olivary microcircuitry, the question is where does the comparison resulting in the error signal occur? In the context in which the comparator hypothesis was originally formulated, the inferior olive was a 'comparator' of command signals from higher centers with the activities these signals elicited at lower levels in the spinal cord, with the presumption that the descending paths from the motor cortex and midbrain and the ascending paths from the spinal cord converged at the level of the olive[113]. However, since anatomical tracing studies over recent years have demonstrated that the descending and ascending projections to the olive generally do not converge on the same olivary neurons (for review, see Ref. 45), it appears unlikely that the signals of intention and achievement are directly compared inside the inferior olive. From the anatomical point of view, a comparison inside the olive is much more likely to happen between the excitatory ascending and descending inputs on the one hand and the inhibitory projections derived from the hindbrain on the other hand. As reviewed above, each dendritic spine of an olivary neuron receives both an inhibitory input from one of the hindbrain regions, which include the cerebellar nuclei, vestibular nuclei, nucleus prepositus hypoglossi, and solitary nucleus, and an excitatory input from the spinal cord, brainstem, mesodiencephalic junction or cerebral cortex. Thus, if the inferior olive itself functions as a comparator, it might do this by comparing the excitatory ascending and descending inputs with the inhibitory inputs from the hindbrain, but this concept diverges substantially from the original hypothesis proposed by Oscarsson, and is more in accord with the blocking process proposed in relation to the learning hypothesis described above.

Concluding remarks

Historically, the inferior olive has been proposed to serve specific functions in the timing hypothesis, the learning hypothesis and the comparator hypothesis. Here, we conclude that the microcircuitry of the inferior olive and the electrophysiological properties of its neurons are most suited to contributing to timing and learning operations in the olivocerebellar system. Although some of the most characteristic morphological features of the olivary neuropil, such as the glomeruli with their dendrodendritic gap junctions, seem to favor the timing hypothesis, other unique characteristics, such as the combined excitatory and inhibitory input to the olivary spines, are compatible with

both the timing and learning hypotheses. Similarly, some of the cell-physiological properties of olivary neurons can be used to support both hypotheses. For example, the intrinsic properties of the olivary membrane underly both their presumptive oscillatory behavior and their low firing frequency, which are important components of the timing and learning hypothesis, respectively. The timing hypothesis would benefit substantially from experiments that directly demonstrate the importance of olivary rhythmicity and synchrony for the timing of nonrhythmic movements in the awake animal, whereas advocates of the learning hypothesis should clarify what role they attribute to the extensive electrotonic coupling in the inferior olive. In sum, we conclude that the olivary microcircuitry supports both the timing and learning hypotheses, but not the original comparator hypothesis. Further investigations will have to elucidate whether all components of the timing and learning hypotheses are valid, to what extent the different components coexist, and to what extent the components of both hypotheses can be integrated.

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