Spatio-temporal aspects of information processing in the cerebellar cortex

Thesis submitted for the degree of

“Doctor of Philosophy”

By

Gilad Jacobson

Submitted to the Senate of the
Hebrew University of Jerusalem

January 2009
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This work was carried out under the supervision of

Professor Yosef Yarom
When you set out on your journey to Ithaca,
pray that the road is long,
full of adventure, full of knowledge.
The Lestrygonians and the Cyclops,
the angry Poseidon -- do not fear them:
You will never find such as these on your path,
if your thoughts remain lofty, if a fine
emotion touches your spirit and your body,
The Lestrygonians and the Cyclops,
the fierce Poseidon you will never encounter,
if you do not carry them within your soul,
if your soul does not set them up before you.

Pray that the road is long.
That the summer mornings are many, when,
with such pleasure, with such joy
you will enter ports seen for the first time;
stop at Phoenician markets,
and purchase fine merchandise,
mother-of-pearl and coral, amber and ebony,
and sensual perfumes of all kinds,
as many sensual perfumes as you can;
visit many Egyptian cities,
to learn and learn from scholars.
Always keep Ithaca in your mind.
To arrive there is your ultimate goal.
But do not hurry the voyage at all.
It is better to let it last for many years;
and to anchor at the island when you are old,
rich with all you have gained on the way,
not expecting that Ithaca will offer you riches.
Ithaca has given you the beautiful voyage.
Without her you would have never set out on the road.
She has nothing more to give you.
And if you find her poor, Ithaca has not deceived you.
Wise as you have become, with so much experience,
you must already have understood what Ithaca means.

Constantine P. Cavafy (1911)
Acknowledgements

There is a misconception about the secluded atmosphere in which science is supposedly carried out. Science is a social praxis, and I have many people to thank for help along my PhD path.

I've been extremely fortunate to carry out my doctoral thesis under the guidance of Prof. Yosi Yarom. Yosi is a unique character even in this most diverse jungle of human oddities. His endless generosity and good humour reside alongside pungent criticism. His willingness to judge scientific work in an agnostic manner does not stop him from formulating provocative and far-reaching hypotheses about the nervous system. His hedonistic attitude towards food, drink and diving, is balanced with a frugal, almost hysterical attitude that emerges whenever the purchase of hard-drives is discussed. His apparent credulity towards the human character is only a thin guise for a capacity to sum up people’s strengths and weaknesses within seconds. And whatever his judgement, he is always willing to enjoy a good glass of single malt with you and forget yesterday’s tensions and disputes. Thank you, Yosi, for a great time, and for teaching me that science is not only a process, but also has bottom lines.

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---

\(^1\) בְּבַרְזֶל יָחַד בַּרְזֶל, פְּנֵי יַחַד וְאִישׁ - רֵעֵהוּ

\(^2\) מַתְחַדֵּד סכִּינָה אֵין לְחֵרוֹת, מַתְחַדֵּד חָכָם אֵין מַחְטוֹר
Figure 0. Copperplate illustration of the human cerebellum (coronal section), from de Vieussens’ *Neurographia universalis*, 1684. Appears in Clarke and O’Malley, 1968.
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pray that the road is long,
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\(^2\) אַי סְכִי מַמְחָדָד אֲלָה בִּרְכֵּר הָדוּרֵה, כְּאִי תַּלְמִידֵי חָכִי מַמְחָדָד אֲלָה בּוֹחֵר (בְּרַאֲשֵׁי רָבָּה מִי וּרְאוּ)
Figure 0. Copperplate illustration of the human cerebellum (coronal section), from de Vieussens’ *Neurographia universalis*, 1684. Appears in Clarke and O’Malley, 1968.
Abstract

The cerebellum is a phylogenetically ancient part of the vertebrate brain, which is crucial for accurately timing complex motor tasks. Accumulating evidence suggests that the cerebellum is also involved in other tasks, such as sensory processing and cognitive tasks. The role of the cerebellum in this diverse set of tasks is heatedly debated. The cerebellum has been suggested to play a secondary role of coordination, control or timing of tasks which can be performed – albeit imperfectly – in its absence. The simple, uniform anatomy of the cerebellum suggests that it performs one type of computation, which is then used by a variety of other brain areas for a variety of different tasks.

A common theme uniting many of the tasks the cerebellum has been implicated in is temporal coordination. Cerebellar lesions lead to “asynergy” – a decomposition of complex motor tasks into their individual components, and a loss of temporal coordination between these components on the 10-500 millisecond range. The ability to estimate the duration of sensory stimuli is also compromised, on the same time scale. One mechanism which has been proposed for the ability of the cerebellum to support timing has been a clock mechanism in the inferior olive. Neurons of the inferior olive can generate subthreshold oscillations of their membrane potential. These oscillations generate in turn action potentials that are then transmitted to other brain areas and may thus support timing. This mechanism suffers from several limitations. Most severely, it is unclear how oscillations at 10 Hz, typical of the inferior olive, may underlie timing on intervals as short as 10 milliseconds, significantly shorter than the cycle duration.

My PhD thesis addresses the question whether – and how – the cerebellum and inferior olive may support timing on the entire range implicated by behavioural studies. The work is composed of three parts. The first part deals with neuronal activity in the cerebellar cortex of awake, behaving rats that result from oscillations in the inferior olive. The second part I study the source simple spikes in Purkinje cells, the only output neurons of the cerebellar cortex, using a combination of electrophysiology and voltage-sensitive dye imaging. In the third part, I propose a model for the olivo-cerebellar circuit as a temporal pattern generator.

In the first results chapter I demonstrate for the first time the ability of the inferior olive to support timing on a time range shorter than the cycle duration. Inferior olivary neurons innervate the cerebellar cortex, and especially Purkinje cells. The dendrite of each Purkinje cell is engulfed by the
climbing fibre axon of a single inferior olive neuron. Activity in the climbing fibre is translated into at least one Purkinje cell output spike, and a prolonged depolarisation and calcium influx into its dendrite (a “complex spike”). In the awake, behaving animal it is impossible to record activity directly from the inferior olive. Inferior olivary output can nonetheless be monitored by recording complex spike activity from Purkinje cells. To monitor inferior olive output, I used rats implanted with chronic arrays of 32 tungsten electrodes in the cerebellar cortex. Activity was monitored simultaneously in many sites in the cerebellar cortex, over an area of 1 x 2 mm. To enhance and regularise olivary output, rats were injected with harmaline, a mono-amine oxidase inhibitor that acts directly on olivary neurons and enhances their propensity to oscillate. I employed advanced signal analysis methods to follow the phase of oscillations on the different electrodes. Past reports have mainly demonstrated zero-phase synchronisation between complex spikes of different Purkinje cells. In this chapter, I demonstrate that electrode pairs in the cerebellar cortex can oscillate at the same frequency but with a constant, non-zero phase difference. These phase differences generate the time intervals shorter than a cycle duration necessary for temporal coordination. I also demonstrate that oscillation frequency is modulated synchronously in the entire recorded region on slow time scales, and that frequency elevation is correlated with the behavioural state of the rat. During frequency changes, no significant change occurs in the phase difference between electrode pairs. The invariance of phase differences to frequency changes allows the olivo-cerebellar circuit to generate temporal patterns that may then be replayed at different speeds without distortion. This ability may be crucial for time warping of complex motor tasks.

In the second results chapter, I demonstrate that Purkinje cell simple spikes are intrinsically generated, and can therefore not carry the timing signal of the olivo-cerebellar circuit. Each Purkinje cell receives inputs from about 200,000 parallel fibres. The prevalent dogma is that parallel fibre activity underlies Purkinje cell simple spikes. To study the relationship between Purkinje cell simple spikes and the network activity, I performed extracellular recordings of Purkinje cell activity simultaneously with voltage-sensitive dye imaging of the surrounding cerebellar cortical area, in anaesthetised rats and guinea pigs. Parallel fibres were electrically stimulated as a control for the voltage-sensitive dye signal. This direct stimulation yielded a significant, easily identifiable signal in the fluorescent signal (1% change). To study the relationship between parallel fibre activity and Purkinje cell firing, I collected data simultaneously from the imaging and electrophysiology, and averaged the imaging signal around simple spike times. Such averaging has previously been used to uncover structure of neuronal activity in the visual cortex. Despite the quality of the evoked optical signal and the massive averaging, no spatio-temporal structure was revealed in the average activity preceding simple spikes. This supports the view
that simple spikes are intrinsically generated, and are largely unrelated to the ongoing activity in the parallel fibres. This suggests that the timing signal of the olivo-cerebellar system has a source other than Purkinje cell simple spike firing.

In the third results chapter I present a model of olivo-cerebellar temporal pattern generation. This model runs against the prevalent dogma, according to which the cerebellar cortex determines cerebellar output, while the inferior olive is merely an internal “teacher”. The model suggests, rather, that the cerebellar output is governed by the temporal patterns generated by inferior olive oscillations. The cerebellar cortex, in contrast, is suggested to be an orchestrator of the inferior olive, by dynamically changing the coupling within the inferior olive to generate different output patterns. This model incorporates a variety of physiological observations hitherto unaccounted for, and provides several clear predictions that may easily support or refute it by simply-designed experiments.

In conclusion, my thesis contributes both positive findings (phase differences in olivary activity) and negative findings (simple spikes are independent of parallel fibre activity), that together help to establish a new and testable model of olivo-cerebellar function.
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<th>Abbreviation</th>
<th>Definition</th>
<th>Unit</th>
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<tbody>
<tr>
<td>ACSF</td>
<td>artificial cerebrospinal fluid</td>
<td></td>
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<tr>
<td>BCM</td>
<td>Bienenstock, Cooper, Munro</td>
<td></td>
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<tr>
<td>CF</td>
<td>climbing fibre</td>
<td></td>
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<tr>
<td>CN</td>
<td>cerebellar nuclei</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>complex spike</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
<td></td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>Cx36</td>
<td>connexin-36</td>
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<tr>
<td>EPSP</td>
<td>excitatory post-synaptic potential</td>
<td></td>
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<tr>
<td>ET</td>
<td>essential tremor</td>
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<tr>
<td>FIR</td>
<td>finite impulse response</td>
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<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
<td></td>
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<tr>
<td>GC</td>
<td>granule cell</td>
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<tr>
<td>GoC</td>
<td>Golgi cell</td>
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<tr>
<td>HH</td>
<td>Hodgkin-Huxley</td>
<td></td>
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<tr>
<td>IN</td>
<td>interneuron</td>
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<tr>
<td>IO</td>
<td>inferior olive</td>
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<tr>
<td>i.p.</td>
<td>intra-peritoneal</td>
<td></td>
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<tr>
<td>IPSP</td>
<td>inhibitory post-synaptic potential</td>
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<tr>
<td>LTD</td>
<td>long-term depression</td>
<td></td>
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<tr>
<td>LTP</td>
<td>long-term potentiation</td>
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<tr>
<td>MEG</td>
<td>magneto-encephalography</td>
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<tr>
<td>MF</td>
<td>mossy fibre</td>
<td></td>
</tr>
<tr>
<td>M-L</td>
<td>medio-lateral</td>
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<tr>
<td>ML</td>
<td>molecular layer</td>
<td></td>
</tr>
<tr>
<td>ms</td>
<td>millisecond</td>
<td></td>
</tr>
<tr>
<td>mV</td>
<td>millivolt</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>Purkinje cell</td>
<td></td>
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<tr>
<td>PCA</td>
<td>Principal component analysis</td>
<td></td>
</tr>
<tr>
<td>PF</td>
<td>parallel fibre</td>
<td></td>
</tr>
<tr>
<td>R-C</td>
<td>rostro-caudal</td>
<td></td>
</tr>
<tr>
<td>SNR</td>
<td>signal-to-noise ratio</td>
<td></td>
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<tr>
<td>SS</td>
<td>Simple spike</td>
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</tr>
<tr>
<td>STM</td>
<td>spike-triggered movie</td>
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<tr>
<td>SVM</td>
<td>support-vector machine</td>
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<tr>
<td>VSD</td>
<td>voltage-sensitive dye</td>
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<td>VSDI</td>
<td>voltage-sensitive dye imaging</td>
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1. General Introduction

“... Behind this [the brain], right at the back, comes what is termed the ‘cerebellum’, differing in form from the brain as we may both feel and see.” (Aristotle, The account of animals, I, 16 [1])

Ever since the first documented account in the 4th century BC, and until this very day, the cerebellum acted upon neuroscientists with a siren-like quality, alluring them with the melody of its beautiful anatomy, only to have them crushed against the rocks of contradictory details. Erasistratus of Ceos (304-250 BC), co-founder of the anatomy school of Alexandria, seems to have been intrigued by the cerebellum’s macro-architecture of folding, and indirect quotes suggest that he correlated brain folding with either motor agility or intelligence (quoted in Galen [2]). As intriguing as these early ideas may be, only in the 19th century did enough behavioural and anatomical data accumulate to allow conjectures about its function to be formalised (a thorough account of pre-modern cerebellar thought can be found in [3,4]). Flourens was the first to delicately remove parts of the cerebellar cortex and study the resulting behavioural deficits. This led him to conjecture in 1824 that the cerebellum was crucial for the correct coordination of limb movements but not for their initiation [5]. Around the same time, improvements in achromatic microscopy allowed Jan Purkyně in 1837 to describe for the first time an individual nerve cell - a large neuron of the cerebellar cortex - now named after him [6]. The next two centuries saw a huge advance in knowledge of the cerebellum on the behavioural, anatomical and physiological levels. While the cerebellum has emerged as an extremely simple and repeating neuronal structure, it has evaded a unifying theory of its function and the neuronal computation it performs.

The introduction will provide an up-to-date, albeit incomplete, review of our understanding of the olivo-cerebellar system on the anatomical, physiological and behavioural levels. A short description of current theories of cerebellar function and their limitations will provide the necessary background for my thesis. My main claim is that current ideas about what the olivo-cerebellar circuit computes and how it does so are inconsistent with the accumulating physiological data. This calls for a reconsideration of olivo-cerebellar function. The Results section of my thesis provides a new model of olivo-cerebellar function, and two lines of experimental evidence supporting it.
1.1. **Anatomy of the olivo-cerebellar circuit**

The cerebellum is composed of two main anatomical divisions, the cerebellar cortex and the cerebellar nuclei. In addition, the cerebellum receives the sole output of the inferior olive (IO), a medullary nucleus, with whom it has reciprocal connections as described below.

1.1.1. The Cerebellar Cortex

The cerebellar cortex consists of three layers: The superficial molecular layer (ML), the Purkinje cell (PC) layer, and the innermost granule cell (GC) layer. This structure is remarkably uniform, and varies little throughout vertebrate phylogeny. There are only a handful of cell types in the cerebellar cortex, and these are organised in an almost crystal-like manner. This organisation is schematically demonstrated in Figure 1.1. The sole output of the cerebellar cortex is carried by the GABAergic PC. It has a large soma (20-30 µm in diameter), a large and planar dendritic tree whose dimensions are ~400 µm x 400 µm and organised in a single layer. The PC axon leaves the cerebellar cortex to innervate the cerebellar nuclei, and in addition sends collaterals to other cells in the cerebellar cortex [7], including other PCs [8].

The cerebellar cortex receives two major inputs which differ both quantitatively and qualitatively. The climbing fibre (CF) input arises from neurons in the IO. The axon of each IO neuron bifurcates and synapses onto 3-10 PCs [9,10]. Each PC receives only a single CF input, but this input is extremely strong, as the CF engulfs the lower two-thirds of the PC dendrite and forms multiple contacts with it. The other input to the cerebellar cortex is the mossy fibre (MF) pathway. MFs arise from a variety of sources (brainstem, spinal cord, and indirect cortical inputs via the dorsal pontine nuclei). MFs impinge upon GCs with a significant divergence factor, each MF innervating ~400-600 GCs, with each GC receiving 3-5 MF inputs [11,12]. GCs in turn send their ascending axons directly up into the ML, where they bifurcate to form the parallel fibres (PFs) – thin unmyelinated axons that run along the medio-lateral axis of the ML, perpendicular to the PC dendrites. PCs receive ascending axon inputs from GCs located just underneath, and PF input from more distal GCs. Each PC receives ~150,000-200,000 relatively weak or silent PF synapses [13].
Several local GABAergic interneurons are found within the cerebellar cortex. Stellate and basket cells are situated in the ML, receive mostly PF inputs, and impinge upon PCs. Their orientation (both dendritic tree and axonal field) is parasagittal, and they differ in their PC target: stellate cells synapse onto the PC dendrite, while basket cells envelop the PC soma. These neurons therefore provide disynaptic feed-forward inhibition of PF signals onto PCs.

Figure 1.1. The cytoarchitecture of the cerebellar cortex. A. Parasagittal cut through the cerebellar cortex shows the arrangement of the cell types. Black: Purkinje cells. Blue: granule cells. Green: Golgi cell. Red: stellate cell. Orange: basket cell. Mossy and climbing fibres are shown in brown and purple, respectively. Figure from [14]. B. Confocal image of a rat cerebellar cortex clearly demonstrates the three layers. Granule cell layer (bottom) is characterised by a dense packing of cells (blue labelling – nuclei). PC somata (cyan) are restricted to a mono-layer above the granule cells, and their dendrites (green) extend to the topmost ML. Cyan/green: quantum dot labelling of IP₃R (related to Ca²⁺ dynamics and highly expressed in PCs). Red: quantum dot labelling of GFAP (glial marker). Adapted from [15].
The Golgi cell (GoC) is a large GABAergic neuron whose soma is located at the top of the granule cell layer. It has two dendritic arborisations, a basal dendritic arbour which receives MF synapses, and an apical dendritic arborisation which receives PF synapses. Both of these are radial, violating the general orthogonal organisation of cerebellar cortical elements. The GoC axon bifurcates to form a plexus innervating many GCs, and has been suggested to provide feedback inhibition controlling the excitability of GCs [16].

The cerebellar cortex receives also monoaminergic inputs. Of these, most noteworthy is the serotonergic input arising from the dorsal Raphe nuclei. Serotonergic axons terminate on synapses found on Purkinje cells but mostly as a plexus without any differentiated post-synaptic elements, thus acting mostly through volume transmission [17,18,19]. In recent years, serotonin has been shown to strongly excite the Lugaro cell [20,21], a relatively neglected neuron in cerebellar research. Lugaro cells are as abundant as GoCs (~10% of PCs), and have myelinated axons running along the PF axis. They have been shown to directly inhibit GoCs through mixed GABA/glycinergic synapses, and to inhibit PCs [22]. Their role in cerebellar processing is still poorly understood.

1.1.2. *The cerebellar nuclei*

The cerebellar nuclei (CN) are the sole output stations of the cerebellum (with the exception of the vestibular nuclei). They receive glutamatergic afferent copies of both CFs and MFs on the one hand, and GABAergic feedback from PCs on the other. Usually, three nucleus pairs are recognised in mammals: the fastigial nuclei (or central nuclei) located near the midline and interconnected with the cerebellar vermis; the interpositus nuclei, lateral to the fastigial; and the dentate nuclei, most lateral of all and interconnected with the cerebellar hemispheres.

Three neuronal populations are found in the CN, without a clear spatial segregation: Glutamatergic projection neurons (PNs), projecting to a variety of brain areas including the cortex (via the thalamus); GABAergic PNs, projecting back to the IO; and local GABAergic interneurons.

1.1.3. *The inferior olive*

The inferior olive (IO) lies on the ventral floor of the caudal medulla, and is subdivided into several nuclei: The medial and dorsal accessory olive, the principal olive, and some additional small sub-nuclei related to the vestibular system [23,24,25]. The IO receives excitatory input from a variety of sources, including cerebral innervation via the mesodiencephalic junction [26]. The neurons of the IO are all excitatory, and their axons enter the contralateral cerebellum as CFs, forming large synapses onto
PCs (see 1.1.1) and afferent collaterals on CN neurons. IO neurons do not form chemical synapses with each other, but are coupled electrically through connexin-36 (Cx36) gap junctions, each neuron being directly coupled to ~50 other IO neurons [27]. The site of dendro-dendritic gap junction coupling is surrounded by chemical synapses including GABAergic input from the CN [28,29], forming a distinct structure called the glomerulus. The current flow through the gap junction and thus the effective coupling between IO neurons is under a strong GABAergic control originating from the CN [30].

1.1.4. Loops in the olivo-cerebellar system

Several loops exist within the olivo-cerebellar circuit, and between it and other structures. These are:

- **The IO-CN loop**: The CN inhibits the IO and the IO excites the CN through CF collaterals.
- **The IO-cerebellar cortex loop**: The IO sends excitatory CFs onto PCs, which in turn disinhibit the IO through a disynaptic pathway via the CN.
- **The cerebellum and the cerebral cortex are reciprocally connected.** Cerebral output is relayed to the cerebellar cortex as MFs through the dorsal pontine nuclei, and cerebellar output is relayed to the cerebral cortex via the thalamus.

Anatomical and physiological data suggest that these loops are topographically organised, such that going through all the synapses within a loop, one returns to the same anatomical area. For example, IO neurons innervate PCs that inhibit the same CN neurons that receive their collaterals [31]. CN inhibition targets the area in the IO from which it receives CF collaterals [32,33]. More surprisingly, cerebellar-cerebral connections are also organised into anatomical closed loops [34]. Thus, motor, sensory and prefrontal cortices communicate reciprocally with different areas of the cerebellum, in contrast to the classical idea that the cerebellum receives wide inputs but affects only motor cortex.
1.2. **Physiology of the olivo-cerebellar circuit**

1.2.1. **The cerebellar cortex**

1.2.1.1. **Purkinje cell spiking**

The PCs are the sole output neurons of the cerebellar cortex. PCs are GABAergic neurons, inhibiting the CN neurons. PCs integrate two distinct excitatory inputs. The strong CF input from the IO generates a massive dendritic depolarisation and Ca$^{2+}$ entry termed a complex spike (CS), which in turn usually generates 1-2 axonal Na$^{+}$ spikes [35]. PCs also produce Na$^{+}$ spikes, termed simple spikes (SSs), at a relatively high rate (from 20 to >100 Hz, with huge variability dependent on species, recording conditions and cell-to-cell variability). The source of SS activity in PCs is still debated. SSs are traditionally assumed to arise from coincident MF input delivered through GCs, but it is now clear that PCs can produce SSs even in the absence of any synaptic input [36]. PCs exhibit bi-stable dynamics of their membrane potential [37], with SSs being intrinsically generated in the UP state of the membrane potential and completely absent in the DOWN state [38]. This bi-stability is unique in that it arises from biophysical properties of PCs and is not a network phenomenon. There is an on-going debate about the existence of bi-stability in awake animals, with experimental evidence still not being decisive [39,40,41]. Despite the intrinsic origin of SSs, MF input can still modulate the firing rate of PCs [42,43,44].

1.2.1.2. **Parallel fibre modulation of Purkinje cell activity**

The organisation of PF inputs onto PCs suggests that PCs should integrate inputs from a variety of GCs located along a medio-lateral “beam”. As described below, this seems not to be the case.

A major problem in understanding PC processing is that they seem to be extremely insensitive to PF inputs. This was first demonstrated by Bower and Woolston [45], who showed that PC receptive fields were similar to those of the underlying GCs, and did not integrate the sensory information from GCs further away along the PF “beam”. This result has since been replicated using other methods [46,47], and seems to reflect the fact that the majority (>85%) of PF synapses are silent [13]. The synapses created by the ascending axon of the GC onto the PCs just above it must be stronger than the PF synapse to account for the above results. This may stem from the ascending axon synapse being unique [48] or from the absence of mechanisms for synaptic long-term depression in this synapse [49,50] (but see [51]).
1.2.1.3. Interaction between complex and simple spikes

The two excitatory inputs to PCs interact with each other in several ways. First, the CF-induced CS causes a cessation in SS firing (CS pause) which lasts several tens of ms [52,53,54,55]. Thus, IO activity can influence cerebellar output not only through its direct effect on the CN through CF collaterals, but also by its modulation of PC-CN inhibition. An extreme form of CS influence on SS activity is the toggling effect that CSs have on the state of bi-stable PCs: CSs can shift PCs from the DOWN state to the UP state and vice versa, thus controlling SS output on even longer time-scales of up to seconds [38].

Complementary to its online modulation of SS activity, the CS signal also determines the sign of long-term plasticity of PF-PC synapses. Ito (1982) was the first to demonstrate that coincident PF and CF inputs onto a PC induces long-term depression (LTD) of the PF synapse [56], now known to be postsynaptic [57]. Only in the last decade has the opposing long-term potentiation (LTP) mechanism been demonstrated, by activating PFs alone [58,59]. This leads to the following learning rule at the PF-PC synapse: LTP is induced by the relatively low levels of Ca$^{2+}$ achieved by PF stimulation alone, while the higher CS-induced Ca$^{2+}$ levels result in the co-activated PF synapses undergoing LTD. PC long-term plasticity differs from pyramidal cell plasticity [60] not only in the inverted dependence on Ca$^{2+}$ concentration, but also in the lack of a well-defined timing requirement between the two signals (spike-timing dependent plasticity) [61,62].

1.2.1.4. Molecular layer interneurons

Two local inhibitory interneurons (INs) are located in the ML – basket and stellate cells. Both receive mostly PF inputs and inhibit PCs. It has been shown that ML IN inhibition provides disynaptic inhibition of PF inputs onto PCs, thereby shortening the PC synaptic potentials and shortening the effective time window for synaptic integration [63,64]. This raises the possibility that ML INs serve to turn PCs into “coincidence detectors”, that respond with SSs only to GC inputs arriving within a short time window of ~1 ms. Simple spike times would therefore reflect the times in which coincident inputs arrived through potent GC-PC synapses.

A major problem with the integration window hypothesis is that the ML inhibitory circuit undergoes strong adaptation to repetitive stimuli [65,66]. MF input usually elicits bursts of GC firing, and the ML inhibition is expected to efficiently limit the integration time window only for the first spike, thereafter undergoing suppression and causing integration time window to widen significantly. Another complication is the excitation-inhibition balance. Strong excitatory inputs may overcome the ML
inhibition, and it is reasonable to expect inhibition to scale with the size of the excitatory input to maintain a constant time window of integration. Results in this respect are confusing. On the one hand, *in vitro* results suggest that IN-PC IPSPs undergo LTD when co-activated with CF inputs, thus “balancing” the change in direct excitatory inputs to PCs [67]. On the other hand, *in vivo* work suggests that plasticity at the PF-IN synapse is reciprocal to the PF-PC plasticity [68,69], thus acting in the exact opposite way expected from the integration time hypothesis.

A second possibility, elaborated in the Results (Appendix 4.2), is that ML inhibition serves to control the state of PCs.

1.2.2. *The inferior olive*

The IO is a unique neuronal structure. Its neurons have no chemical synapses connecting them, but they are electrotonically coupled with their neighbours via gap junctions [70,71,72]. Each IO neuron is connected to tens of its neighbours, as corroborated by dye coupling and dual electrical recordings [27]. Coupling coefficients are in the range of 0.2-0.01, and depends on the distance between the two neurons. The lower coupling coefficients may therefore reflect an indirect coupling through an intermediate cell. IO neurons possess a unique combination of conductances, giving rise to two different Ca\(^{2+}\) spikes. Somatic Ca\(^{2+}\) spikes are generated by a low-threshold T-type Ca\(^{2+}\) conductance which requires hyperpolarisation below -70 mV to remove inactivation. Dendritic Ca\(^{2+}\) spikes are high-threshold and are followed by a long after-hyperpolarisation mediated by voltage- and Ca\(^{2+}\)-dependent K\(^+\) conductances [73]. The two different Ca\(^{2+}\) spikes may co-exist as they are mediated by different conductances and spatially segregated. These two conductances interact and allow IO neurons to oscillate [74], and also limit the maximal spike rate of IO neurons to about 10 Hz [75].

1.2.2.1. **IO oscillations and electrotonic coupling**

IO neurons can oscillate in the range of ~5-15 Hz and oscillations tend to be synchronised in neighbouring neurons [76]. There is, though, a disagreement regarding the conditions necessary for these oscillations to manifest themselves. Some evidence suggests that oscillations arise from single IO neuron properties and require no electrotonic coupling [77,78,79]. These studies rely on constitutive knockout of the Cx36 gene or on non-specific pharmacology. New evidence from wild-type rats suggests that areas of the IO infected with a lentivirus that blocks trafficking of Cx36 (and thus decouples the neurons locally) do not exhibit oscillations, while other areas in the same IO that are not infected still oscillate [80]. This adds to a variety of other evidence linking electrotonic coupling with the emergence
of oscillations [81,82,83]. Two mechanisms have been suggested for the network dependence of oscillations. Manor and colleagues [84,85] suggested that there is a heterogeneity of channel density between neurons in the IO. Theoretical work demonstrated that different combinations T-type and leak conductances created qualitatively different behaviours of IO neurons. By coupling two IO neurons that were each non-oscillatory, it was possible under some conditions to shift the two cells into an oscillatory regime. This possibility was corroborated recently by an elegant dynamic clamp study [86]. A second mechanism proposed by Loewenstein and colleagues [87,88] does not require neuronal heterogeneity. The crux of this mechanism is that the tendency of certain internal variables of neurons (e.g. Ca\(^{2+}\) conductance) to oscillate is stabilised by the membrane potential that provides negative feedback. When non-oscillating neurons of this type are coupled, the effective increase in membrane conductance prevents the membrane potential from performing its stabilising role, and the internal propensity to oscillate manifests itself in the voltage.

1.2.2.2. Control of IO oscillations by GABAergic input

An important feature of the olivary network is the fact that dendro-dendritic gap junctions are surrounded by GABAergic synapses originating from the CN (see 1.1.3). Notwithstanding the controversy whether oscillations are a cellular or a network phenomenon, it is clear that GABAergic input from the CN can suppress olivary oscillations [30,89]. This does not result from the hyperpolarising effect of GABA, as GABA applications do not replicate the effect. Rather, it seems that the increased shunt at the gap junction site prevents current flow between cells and decreases the effective connectivity between them.

1.2.2.3. Patterns of olivary activity

IO activity has been characterised both in vitro and in vivo. IO activity in vitro can be monitored directly, using e.g. whole-cell patch recordings from one or more neurons [27,30,78,79,82] or voltage-sensitive dye imaging [76,77,90] which follows the subthreshold membrane activity [91]. In vitro work has demonstrated that an entire patch of IO neurons can oscillate in phase and generate propagating waves [76] and can also exhibit breakdown into small clusters of coherently oscillating IO neurons [90]. The size of these clusters can be extended by blocking GABAergic synapses [90] and be decreased using gap junction blockers [77], suggesting that inhibitory input onto the IO determines the spatio-temporal aspects of the oscillating network with its ability to effectively decouple the network. Accessing the IO in vivo is extremely difficult (but see e.g. [92,93]), especially intracellularly, due to its location on the ventral floor of the caudal medulla. Nonetheless, two recent studies have uncovered the fact that many
IO neurons do indeed oscillate in vivo [94,95]. While providing information on the existence of oscillations (though somewhat different in detail), little can be inferred from these studies about the network level, being still limited to a single cell.

A different approach is usually taken to study IO network activity in vivo. PC complex spikes arise from action potentials of single IO neurons. By monitoring CS activity in many different PCs, it is possible to infer about the underlying dynamics in the IO. This method is limited to the suprathreshold signals that propagate to the cerebellar cortex. Extrapolation from the CS activity to the possible underlying rhythmic activity in the IO presupposes several factors: (1) that spikes are limited to certain phases of the subthreshold oscillation, which has indeed been demonstrated [74,94,95,96], and (2) that the propagation time of spikes from the IO to the cerebellar cortex is relatively constant, which has also been verified [97,98,99,100]. Monitoring of CSs from PC pairs was pioneered in the late ‘60s [101,102], demonstrating that CSs of adjacent PCs tend to be synchronised. In the late ‘80s, this approach was extended to multi-electrode arrays and study of the spatio-temporal patterns of IO output could start in earnest [103,104,105]. For the sake of clarity, I split the discussion of these results into two categories – rhythmicity and synchronisation.

**Rhythmicity:** There is a long and ongoing debate regarding the existence and prevalence of in vivo CS rhythmicity. Multi-electrode recordings from the cerebellar cortex of both awake and anaesthetised rats usually demonstrate statistically significant CS rhythmicity, as evident in the auto-correlation function. It has been demonstrated that this rhythmic activity does not reflect olivary input, but is actually increased in the absence of glutamatergic inputs to the IO, despite an overall decrease in CS rate [106]. Furthermore, it has been demonstrated that CS rhythmicity can be dramatically increased if GABAergic input to the IO is blocked [107,108], and is decreased when the IO is perfused with carbenoxolone, a gap junction blocker [109]. From these studies emerges the following picture: The coupled IO is intrinsically oscillating, creating in its output rhythmic CS activity. This rhythmicity is weak, as a result of two factors: (1) GABAergic input from the CN, presumably suppressing oscillations by weakening the effective coupling, and (2) the fact that some IO spikes arise from external, non-locked events mediated by glutamatergic input.

Sensory-evoked CS responses have also been shown to contain a rhythmic component not present in the stimulus [110,111]. It has also been demonstrated that IO oscillations emerge when an animal performs rhythmic motor activity [92]. This result lends itself to multiple interpretations, as the IO rhythmicity is not necessarily intrinsic, but may reflect rhythmic motor or sensory inputs related to
the motor activity. Other groups have failed to find signs of rhythmic activity in CS activity in relation to motor behaviour [112,113]. This issue is discussed in the Results (section 4.10). Suffice it to say here that the model I propose for olivo-cerebellar timing does not predict clock-like oscillatory activity to emerge during motor tasks, especially not in motor tasks where there is no periodic component to the behaviour [112].

**Synchronisation:** In the earliest reports of dual PC recordings [101,102], it was found that pairs of PCs not only exhibited rhythmic activity, but were time-locked to each other. These findings preceded the understanding of olivary dynamics and coupling, and were interpreted as reflecting a common input [102].

Multi-electrode studies have shown that PC pairs tend to be synchronised above chance level, especially if located in the same parasagittal band, although the actual correlation values may be very low \( \rho = 0.005 - 0.1 \) [105,114]). The spatial organisation of the correlation may be related to the projection anatomy of the CFs: Each CF projects to several PCs restricted to a narrow parasagittal band [9], and nearby neurons in the IO project to the same band [10]. The parasagittal bands of synchrony probably reflect the strong electrotonic between adjacent IO neurons.

Several factors may modulate the extent and identity of the synchronised PCs. It has been shown that during a behavioural task, the number of synchronised PC pairs increases [111], and it has also been demonstrated that different PC ensembles are synchronised in different behavioural contexts [115]. These results suggest that synchrony is a dynamic phenomenon under neuronal control [116,117] (see more in 1.4.3). Blocking GABAergic input indeed increases the spatial extent of the synchronised region [107,118], while perfusion of the IO with carbenoxolone, a gap junction blocker, decreases synchrony [109].

The above results suggest that synchrony and rhythmicity of PC CSs are two facets of the same phenomenon, namely subthreshold oscillations of the membrane potential in groups of coupled IO neurons. A major gap exists between the richness of oscillation patterns described *in vivo* and the limitation to zero-lag synchronisation usually described *in vivo*. Some evidence supporting the existence of non-zero phase differences between distant PCs can be found in the literature (Figure 5b in [119], Figure 7c in [105], Figure 7c in [120]), although never pursued systematically. This topic is the focus of Chapter 2.1 of the Results section.
1.3. **Cerebellum and behaviour**

The early 19th century saw an unprecedented advance the study of brain anatomy which was unmatched by studies of its function. This imbalance spurred a variety of unsubstantiated theories of brain function. For example, Gallen and Spurzheim’s view of the cerebellum as controlling sexual function [121] remained prevalent for decades.

Systematic studies of cerebellar function began in earnest by removing parts of the cerebellum of animals and observing the resultant behavioural impairments. Rolando was the first to report (in 1809) that cerebellar lesions led to deficits in the ipsilateral motor function [122]. Flourens’ later, and much more careful, work (1824) led him to propose that the cerebellum is not an initiator of motor activity, but rather coordinates motor activity. In his own words (translation taken from [3]):

*I have shown that all movements persist following ablation of the cerebellum; all that is missing is that they are not regular and coordinated. From this I have been induced to conclude that the production and the coordination of movements form two classes of essentially distinct phenomena and that they reside in two classes of organs also essentially distinct...*

This view, later adopted by Sherrington (viewing the cerebellum as the “head ganglion of the proprio-ceptive system” [123]) and matching a prevalent view in contemporary cerebellar research, took time to gain foothold, competing with other ideas that have since been rejected. The study of cerebellar function has since advanced immensely, and I shall briefly review the contemporary view of the cerebellar involvement in motor and non-motor function, with a special stress on timing. My claim is that the ability to correctly time events, whether motor, sensory or cognitive, is a common theme uniting diverse cerebellar functions.

1.3.1. **The olivo-cerebellar system and motor function**

The study of cerebellar lesions in animals and in humans pointed to its role in motor function [5,122,123]. The basic symptoms of cerebellar malfunction were noted in Holmes’ seminal paper in 1939 [124], but the controversies of his time retain their fervour to this day (see e.g. review in [125]). It is still disputed whether there is a unique cerebellar role in multijoint coordination or if this can be attributed to its role in controlling single-joint movement [125,126]; whether its role in coordination is related to interaction torques or to timing [127,128]; and whether it is important in motor performance or learning [129,130]. Covering this huge body of literature is beyond the scope of this introduction. I shall focus on
the extensive evidence for a cerebellar role in timing the different components of a compound motor task, which was phrased succinctly by Holmes [124]:

*Delay in the initiation of one component relative to that of another, and excessive range of one element of the movement, particularly at a proximal joint, are the chief causes of "decomposition" [in cerebellar patients]...*

This basic feature of cerebellar impairment (also termed asynergia) has since been described in a variety of motor tasks. One example is ball throwing to a target. In overarm throws that combine several joints, cerebellar patients fail to correctly time finger opening and ball release relative to arm position, resulting in a large error [131,132]. Another example is reach-grasp movements, which exhibit locking between the time of maximal finger aperture and maximal deceleration, which is lost in cerebellar patients [133]. The idea that cerebellar patients suffer from difficulties in motor timing gains more direct support from the experiments of Ivry and colleagues, demonstrating e.g. that variability in rhythmic finger tapping results from a compromise to an internal timing mechanism [134].

It is more difficult to obtain direct evidence for a cerebellar involvement in timing in the intact brain, and the required paradigms may still have to be developed and tested (see Discussion section 6.3). But some evidence has started to accumulate. It has been known since late 19th century that volitional movements contain fluctuations of velocity in the 7-9 Hz range [135,136,137]. These fluctuations have recently been recorded in conjunction with MEG, demonstrating the cerebellar involvement in subserving this rhythm [138]. Interestingly, this is the same frequency range which is exaggerated in patients suffering from Essential Tremor (ET). Patients suffering from ET exhibit excessive tremor which is elevated during volitional movements [139]. Untypically for a neuronal disease, ET is not correlated with any neurodegenerative process. Rather, there is evidence linking it to abnormal activity in the olivo-cerebellar circuit [140]. Olivary hypertrophy has been demonstrated directly in a closely related pathology, palatal tremor [141].

A well-established model of ET in animals is harmaline-induced tremor [142]. Harmaline is a mono-amine oxidase inhibitor, and as such was used in attempts to treat Parkinsonism in the 1960s [143]. Paradoxically, tremor was elevated [144] after harmaline. Later, it was shown that harmaline tremor arises from a direct effect on the IO [119], and that harmaline elevates the propensity of IO neurons to oscillate by changing the kinetics of a low-threshold Ca$^{2+}$ conductance [74]. Thus, a mechanism that has been suggested to allow motor timing, creates, when active abnormally, excess rhythmic timing during movement. Recently, it has further been demonstrated that when the IO is
decoupled unilaterally during harmaline tremor, the accurate relative timing of muscle pairs on the contralateral side of the body is lost [145]. While this is clearly a pathological state, it still hints to the possibility of olivary signals directly controlling movement time.

1.3.2. The olivo-cerebellar system and non-motor function

Evidence has accumulated during the past decades suggesting that the cerebellum is involved in non-motor functions of the nervous system. It has also become clear that separating brain areas into purely motor and purely sensory ones may be an inadequate way of conceptualising brain function. Motor performance depends crucially on the ability to incorporate sensory information into motor planning. The interpretation of sensory information also depends on the ability to incorporate self-induced movements. Two manifestations of this more integrative view are the emerging field of active sensing [146,147], and the subject of cancellation of self-produced (or expected) sensory inputs [148,149].

1.3.2.1. Involvement in sensorimotor function

Perhaps the most convincing example of the cerebellar involvement in a sensorimotor task is classical eyelink conditioning. In this task, an unconditioned stimulus is used that elicits an eyelink: either of the eyelid or of the nictitating membrane found in rodents. This aversive stimulus is coupled during learning to a preceding conditioned stimulus, which is of a neutral nature (e.g. a tone at a specific frequency, a light flash etc.). The animal (or human) learns to associate the conditioned stimulus with the subsequent unconditioned stimulus. During learning, the animal develops a correctly timed conditioned response, i.e. it learns to blink at a time just preceding the expected unconditioned stimulus, and hence to avoid the unpleasant stimulus to the eye. Classical conditioning was first shown in the 1980s to depend on intact cerebellar circuitry [150,151]. Later studies have shown that the cerebellum is crucial for correctly timing the conditioned response if [152]. Recently it has been demonstrated using transgenic mice that when LTD of the GC-PC synapse is impaired, mice can still associate, but the CR is ill-timed [153].

Another example for the cerebellum’s involvement in sensorimotor timing comes from the suppression of sensory information arising from one’s own motion, which underlies the suppression of self-tickling responses [148]. Blakemore and colleagues created delays between the motor action and the sensory input and were able to “rescue” the ticklish feeling, as the subjects received the tactile stimulus displaced in time relative to what was expected from their motor output. Imaging results suggest that the cerebellum is involved in predicting the self-produced sensory signal necessary for such
cancellation [149]. It is tempting to link the cerebellum to sensory prediction: In both passive and active electric sensation, animals must cancel the self-produced electrical signals that dominate sensory input, to be able to detect real sensory events. This computation is performed in a variety of structures which are strikingly similar anatomically to the cerebellum (“cerebellar like structures”) [154,155].

1.3.2.2. Predicting the time of sensory events

The cerebellum receives a variety of sensory inputs from both peripheral sources and from the cerebral cortex, and sensory representations in it have been extensively studied (see e.g. [45,69,156,157]). Classically, these inputs were also thought of as necessary for the cerebellum to execute its “reflexive” motor tasks [123]. But behavioural and imaging studies in both healthy and cerebellar subjects have revealed a direct sensory role for the cerebellum. For example, the cerebellum has been shown to be engaged when a visual target’s velocity has to be estimated, but not when other properties of its motion such as direction are estimated [158,159]. The cerebellum is likewise engaged in estimating the duration of auditory stimuli, and cerebellar patients perform poorly on such tasks [160,161]. There is evidence from human MEG studies that the cerebellum responds in a timely manner to an expected, omitted stimulus within a sequence [162]. Some temporal aspects of speech perception seem also to rely on an intact cerebellum [163,164], and lately it has been suggested that the cerebellum is even involved timing of cognitive tasks [165,166]. The common theme to all these studies is the cerebellar involvement in sensory processing that involves the estimation or prediction of time, in the range of ~10-500 ms. A successful model of olivo-cerebellar timing must provide a mechanism that can produce, upon request, accurate time intervals in this range.

1.4. Theories of cerebellar function

Theories of cerebellar function range from the all-encompassing and ambitious to restricted theories dealing with specific behaviours. Some theories may be inspired by a particular facet of cerebellar anatomy, with the actual function remaining vague, while other theories may tackle a specific function and impose a theoretical framework upon the cerebellum (e.g. control theory), allocating the components of the cerebellar circuit into the required rubrics. Two shortcomings are shared by most cerebellar theories. First, the enormous knowledge accumulated about olivo-cerebellar physiology is at best only partially incorporated. Second, the basic requirement from a theory – to provide clear and decisive tests that can refute it – is rarely fulfilled. My aim in this section is to review several theories of
cerebellar function. Most but not all of these theories, aim at providing a mechanism by which the olivo-
cerebellar circuit can support the correct timing of different tasks.

1.4.1. The Marr theory of the cerebellar cortex

In his seminal paper of 1969, David Marr proposed a theory of the cerebellar cortex [167] that
provides a mechanism for tasks classically attributed to the cerebellum – “conditional” reflexes [123]
(i.e. posture corrections that are conditional on the general state of the body, e.g. activated when
standing but not when sitting) and motor skill learning [168,169]. Marr claims that the cerebellum learns
to generate a specific action in a given context. During learning of a skilled movement, the required
action is explicitly “spelled out” by IO activity arising from afferents of cerebral commands. Context is
carried by the MF signal. Learning is achieved by a Hebb-like plasticity rule [170] that strengthens GC-PC
synapses concurrently activated with the CF synapse, carrying the cerebral command. After learning, the
context alone is enough to activate PCs, and a complex movement flows on because the execution of
each of its components creates the necessary context ( proprio-ceptive, visual etc.) for the next
component. Thus, time is implicitly embedded in Marr’s theory.

Marr’s theory assigns a role for the unique pattern of divergence at the MF-GC level and
subsequent convergence of GC synapses onto PCs. The MF-GC divergence creates an expanded, non-
linear representation of the input, in the form of “codons” [171]. Thus, two overlapping contextual
inputs at the MF level will have a much smaller overlap in the GC “codon representation”. The
convergence of GCs onto PCs allows many PCs (and hence, many actions) to be activated by any context,
since these context-action relationships are not predefined. The orthogonal organisation of PFs with
respect to the PC dendritic tree can thus be viewed as a means of maximising the number of inputs onto
each PC. Other elements of the cerebellar circuitry are also assigned roles in Marr’s theory, but are
beyond the scope of this introduction.

1.4.2. Braitenberg’s theory of cerebellar timing

Braitenberg has proposed several theories of how the cerebellum could allow complex motor
tasks to be carried out [172,173,174,175]. A complex motor task is composed of several motor
primitives, and these have to be combined in a timely manner for the complex movement to be properly
performed. Braitenberg believed the orthogonal organisation of the cerebellar cortex was designed to
allow different PCs along a medio-lateral “beam” to be sequentially activated by the same PFs. The thin,
unmyelinated axons of the PFs suggested that spikes propagate slowly along them, and can thus create
the time intervals necessary for combining motor primitives. It turns out, though, that the propagation
velocity (20-30 cm/s) can only support time intervals of ~10 ms, much shorter than the time intervals required for motor binding and the time range in which the cerebellum has been implicated (see section 1.3). Later, modified versions of the theory (“tidal wave theory”, [175]) put a stress on the ability of PCs to detect accurate temporal sequences in its input: GC activity that propagates along the medio-lateral axis at the PF velocity will efficiently sum up and generate a “tidal wave” input onto PCs, while input at different velocities, or random input, will be much less effective. The evidence for such sensitivity is relatively weak [176,177], and this version is also further removed from concrete tasks the cerebellum must support. The intrinsic origin of PC SSs is a further complication for this theory, because SSs are supposed to encode “tidal waves” carried by the PFs.

1.4.3. The inferior olive as a neuronal clock

Rodolfo Llinás suggested as early as 1974, that the IO may actually be the seat of olivo-cerebellar timing in motor tasks [89]. Three elements were combined by Llinás in forming this theory: (1) The observation of electrotonic coupling between olivary neurons [70]; (2) the oscillatory properties of olivary neurons [119]; and (3) the possibility (not yet confirmed at the time!) that inhibitory inputs surrounding the gap junctions may effectively decouple IO neuron pairs [178]. With the finding that IO neurons can be viewed as conditional oscillators, under the control of GABAergic feedback from the CN (see section 1.2.2), the olivary theory of timing could be refined. Different muscle groups must be synchronised and timed in different movements. The IO supplies the basic mechanism for timing, using a clock at ~10 Hz, and the cerebellum, via the CN, ensures that only the relevant parts of the IO are coupled and oscillating.

This suggestion gained some support from the finding that different subsets of PC CSs are synchronised in different behavioural contexts [115] and that cross-muscle synchrony is compromised in the uncoupled IO [145]. Two objections are usually voiced against this theory. One objection is that the low firing rate of IO neurons would not allow it to dominate cerebellar output through the CN and hence provide a timing signal. A second objection is that it is unclear how a 10 Hz clock could support timing in the entire range the cerebellum has been implicated in (10-500 ms) [161]. Recent evidence, obtained from simultaneous recordings in many cerebellar and non-cerebellar structures, suggests that CN output bears a close resemblance to IO activity [179], so IO may actually be able to determine cerebellar output, either directly via CN collaterals or via CS-induced pauses in PC firing. The question of how olivary oscillations may support timing on intervals shorter than the oscillation cycle is the heart of the Results (section 2).
1.4.4. Granule cell timing hypothesis

Recently, Ohyama et al. [180] (see also Yamazaki and Tanaka [181]) have proposed a novel mechanism by which the cerebellum may create temporal patterns, and hence provide feed-forward control. To achieve this, rich, reproducible spatio-temporal patterns are created in the granule layer in response to a sustained activation of a group of MFs. This is done by a sparse, random wiring between GCs and Golgi cells (GoCs), as yet unsubstantiated. A long activation of MFs leads to alternating patches of activated GCs, because each GC receives feedback inhibition from GoCs at different times. This is the first step in their mechanism – the transformation of a sustained MF input into a reproducible progression of GC subsets active at different latencies relative to the MF activation. In the next step, a “teaching” signal arriving from the IO suppresses specifically GCs that are concurrently activated with it. Thus, only a small subset of PCs will receive a suppressed input, and hence disinhibit the CN at the correct time.

On the one hand, this hypothesis is attractive because it provides a mechanism of cerebellar timing and an underlying learning mechanism that is consistent with the notion of the IO as providing a learning signal. Nonetheless, it suffers from a variety of problems that render it highly improbable. First, it relies on a specific, non-corroborated connectivity scheme between GCs and GoCs. GoC axonal fields are wide and dense, and it is hard to see how the spatio-temporal patterns Ohyama et al. require can emerge. Second, it relies on SSs as the output signal of the cerebellar cortex, and therefore is undermined by the finding that SSs are intrinsically generated. Third, the restriction of IO spikes to specific phases of the subthreshold oscillations, and the mere existence of these oscillations, is a nuisance for this theory.
Results
2. Inferior Olivary Oscillations and their Phase Structure

Based on the article:

*Invariant phase structure of olivo-cerebellar oscillations may underlie cerebellar pattern generation.*

2.1. Abstract

Complex movements require accurate temporal coordination between their components. The temporal acuity of such coordination has been attributed to an internal clock signal provided by inferior olivary oscillations. However, a clock signal can produce only time intervals that are multiples of the cycle duration. Because olivary oscillations are in the range of 5-10 Hz, they can support intervals of ~100-200 ms, significantly longer than intervals suggested by behavioural studies. Here I provide evidence that by generating non-zero phase differences, olivary oscillations support intervals shorter than the cycle period.

Chronically implanted multi-electrode arrays were used to monitor the activity of the cerebellar cortex in freely moving rats. Harmaline was administered to accentuate the oscillatory properties of the inferior olive. Olivary induced oscillations were observed on most electrodes with a similar frequency. Most importantly, oscillations in different recording sites retained a constant phase difference that assumed a variety of values in the range of 0-180°, allowing the olivo-cerebellar system to support time intervals much shorter than the cycle duration. These phase differences were maintained across large global changes in the oscillation frequency.

The inferior olive may thus underlie not only rhythmic activity and synchronization, but also temporal patterns that require specific phase differences. The maintenance of phase differences across frequency changes enables the olivo-cerebellar system to replay temporal patterns at different rates without distortion, allowing the execution of tasks at different speeds.
2.2. Introduction

Cerebellar involvement in timing is well established, with evidence encompassing both normal and pathological conditions. Motor coordination in the 7-9 Hz range has been shown to involve the cerebellum [138], and pathologies associated with the cerebellum can either disrupt motor timing [131,182,183] or exaggerate tremor in this frequency range [139]. The cerebellum also plays a pivotal role in timing of sensory and cognitive functions [159,160,166,184,185,186] (see also Introduction, section 1.3). The olivo-cerebellar system thus seems to be crucial for accurate timing in the range of tens to hundreds milliseconds. As elaborated in the Introduction (section 1.4), two mechanisms have been proposed for cerebellar timing. First, parallel fibres have been suggested to activate Purkinje cells (PCs) with accurate delays [172,174,175] subserving timing. Second, oscillations in the inferior olive (IO) [74,95,187,188] have been proposed to act as a clock signal for timing. Both these mechanisms fail to cover the required range of 10-500 ms, the former because the length of a parallel fibre is exhausted within ~10 ms and therefore can only support shorter time scales, and the latter because an olivary clock signal can only support temporal coordination at multiples of the clock cycle (~100-200 ms) [189]. Time intervals shorter than the cycle duration could be generated by creating phase differences between different sub-groups of IO neurons. While in vitro studies demonstrate that IO oscillations can exhibit rich spatio-temporal dynamics [76,90], the possibility that different parts of the IO may be coupled at non-zero phase difference has largely been overlooked (but see [105] and [190]).

To investigate the phase structure of olivary activity in the cerebellar cortex, arrays of 32 tungsten electrodes were chronically implanted in the rat cerebellar vermis and the complex spike field oscillations were studied in awake, behaving rats. Harmaline was administered to expose the underlying structure of IO oscillatory activity. The results indicate that IO oscillations can retain non-zero phase differences independent of frequency. I suggest that these phase differences endow the olivo-cerebellar system with the capability to time events – both motor and cognitive – across the time range suggested by behavioural observations.
2.3. Materials and Methods

2.3.1. Micro-electrode arrays

Custom made arrays of 32 Isonel-coated tungsten microwires (35 µm in diameter) were arranged in a 4x8 matrix. Electrodes were spaced 250 µm on both axes such that the entire array spanned 1.75 mm x 0.75 mm. Electrode resistance prior to implantation was ~200 kΩ.

2.3.2. Surgical procedure

All procedures were approved by Bar Ilan University institutional animal care and use committee and were performed in accordance with the National Institutes of Health guidelines. The surgical procedures have previously been described in detail [191,192]. In brief, adult Long Evans male rats weighing 420-540g (Harlan, IN and Taconic, NY) were initially sedated by 5% isoflurane and then injected i.m. with ketamine HCl and xylazine HCl (100 mg/kg and 10 mg/kg, respectively). Supplementary injections of ketamine and xylazene were given as required.

The rat’s head was fixed in a stereotaxic frame (Kopf Instruments, CA). A midline incision was made to expose the skull surface up to the occipital ridge posteriorly. Connective tissue was removed and the skull surface cleaned with 3% hydrogen peroxide. Four stainless steel screws (Small parts, FL) were tapped into the skull to serve as ground electrodes and as anchors for the dental cement. A craniotomy, slightly larger than the electrode array, was performed above the occipital bone. The vermal portion of lobule V/VI was exposed and dura removed. The ground wire was rolled around all four screws and an electrode array oriented in the rostro-caudal or medio-lateral axis was slowly lowered into the brain while monitoring the neural activity using a neural recorder system (Plexon Inc., TX). Electrodes were fixed at depths of 0.8-2 mm below surface using a high viscosity cyanoacrylate (RX-gel; Pacer technology, England). The array, anchoring screws and the ground electrode were covered with dental cement, leaving only the connector exposed. Rats were allowed at least 2 weeks of recovery prior to recording.

2.3.3. Location of electrodes

Electrode position within the cerebellar cortex was verified histologically. In brief, electrical lesions were done under anaesthesia by passing an electrical current (10 µA, 8-15 sec; Stoelting Co., IL) between recording electrodes and a ground connected to the screws passing through the rat skull.
Animals were kept under anaesthesia for ~1 h, and euthanized (pentobarbital, i.p.). Saline (NaCl, 0.9%, ~200 ml) was initially perfused through the heart at a rate of 10ml/min. using a peristaltic pump (MasterFlex), and then perfusion was switched to formaldehyde (4%) in PBS (~500 ml, same perfusion rate). The brain was then carefully removed and kept in formaldehyde-PBS solution for 2 days. Para-sagittal slices (75 µm thick) were cut using a vibratome and stained with haemotoxylin. Electrode tracks (Fig. 2.1a, arrow) and lesion sites could be observed on different slices and different cortical layers (Fig. 2.1a,b, stars). In the top panel, the lesion is in the PC layer, while in the lower panel, three lesions are observed, two in the PC / molecular layer, and one in the white matter. The minority of electrodes that did not detect harmaline-induced oscillatory activity were probably located in the granule cell / white matter layers.

**Figure 2.1. Location of electrodes in cerebellum.** a. Para-sagittal slice through cerebellar vermis shows the location of one electrode track (arrow) and lesion site (star). b. A second slice, ~1.5 µm lateral to the one in a, shows three clear lesions, two of them in the PC layer / molecular layer of lobule V.
2.3.4. Data acquisition

Activity was recorded from rats 2-10 weeks post-surgery using a multi-channel acquisition processor system (Plexon Inc., TX). Channels were filtered before sampling between 0.15-9 kHz, and up to 16 channels at a time were recorded continuously at 40 kHz. Activity was then recorded in awake animals, before and after harmaline HCl injection (Sigma-Aldrich, St Louis, MO, 12-15 mg/kg, i.p. injection under light isoflurane anaesthesia). Unless otherwise stated, results presented are from harmaline-injected animals taken after the effect of harmaline stabilised (10-20 minutes).

2.3.5. Data analysis

All data analysis was performed on MATLAB 7 (MathWorks, MA).

**Complex spike analysis.** Spikes were detected using a median threshold [193]. Extracellular complex spikes were identified by their wide waveform (2-8 ms), their low firing rate prior to harmaline administration (< 2 Hz) and were analyzed as such only if their shape was retained during the harmaline condition. When multiple spike shapes were detected on the same electrode, clustering was performed using the projection on the first two principal components of the aligned spikes.

**Extraction of multi-unit envelopes.** After harmaline application, the amplitude envelope of the voltage signal in many electrodes oscillated at slow rates. The carrier frequency range of this slow rate differed across experiments, and exhibited some variability within an experiment. The original voltage signal was band-pass filtered in the carrier frequency range, and the amplitude envelope extracted by low-pass filtering (0-250 Hz) its absolute value. In some cases, bands were wide and could potentially violate the band-limitation requirement [194], and therefore oscillation phase in different frequency bands was compared to ensure that no distortions were introduced.

**Definition of oscillatory channels and spectral analysis.** Envelopes were analysed for rhythmic activity by calculating the power spectrum using Welch’s averaged periodogram method (DPSS window, NW=3). Further analysis was performed only on oscillatory channels, in which the envelope power spectrum after harmaline application exhibited a peak > 3dB above the power in adjacent frequency bins. Coherence between pairs of envelopes and spectrograms were calculated using Welch’s algorithm with similar parameters to power spectrum calculation.
**Phase difference extraction and stability.** To extract phase differences between channel pairs, the two envelopes were filtered to create narrow-band signals [195] (see Figure 2.2). Band-filtered envelopes were then parsed into 10 s windows with 5 s overlap. The cross-correlation of pairs of segments was calculated, and the phase difference was extracted from the cross-correlation by taking the angle of its Hilbert transform at $\Delta t=0$.

---

**Figure 2.2.** Extraction of narrow-band envelope and phase difference. **a.** Cross correlations from four electrode pairs recorded simultaneously, exhibiting a variety of correlation strengths and correlation phases. **b.** Coherence between the above envelope pairs. Vertical red lines demarcate the frequency band used for extracting narrow-band envelopes (similar for all electrodes). **c.** Cross-correlations from the above four electrode pairs, performed on the narrow-band envelope signals. The narrow-band property allows the extraction of relative phase ($\phi$) from each pair.
To quantify the stability of phase differences across all measurements, standard methods from circular statistics [196] were used. The mean phase difference and mean resultant length (MRL, a.k.a. phase locking index factor, PLF [197]) were calculated. The MRL of phase difference values \( \{ \varphi_k \}_{k=1}^N \), where \( 0 \leq \varphi_k < 2\pi \), is defined as:

\[
MRL = \frac{1}{N} \left| \sum_{k=1}^N e^{i\varphi_k} \right|.
\]

MRL values range from 0 (uniform distribution of phase differences on the unit circle) to 1 (constant phase difference). Noise levels of the MRL were assessed using bootstrap statistics. Surrogate data was created with Fourier amplitude similar to the original envelopes but with random phase, and the above procedure for calculating MRL was repeated 200 times to yield bootstrap statistics.

**Global nature of frequency changes.** Bootstrap statistics were used to validate the global nature of oscillation frequency dynamics. Frequency time courses (FTCs) of all resonant electrodes were averaged to yield the mean FTC. The percent variance in each FTC explained by the mean FTC was used as a global similarity index (GSI). To ensure that obtained GSI values were significant, the above procedure was repeated with surrogate FTCs. Surrogate FTCs were created by shifting cyclically each FTC by a random time interval, and repeating all the computation of mean FTC and GSIs. These surrogate FTCs retain all the statistics of the original FTCs, and do not violate the null hypothesis that modulations in each channel were independent. This procedure was repeated 2000 times to yield bootstrap statistics for each experiment.

2.4. Results

2.4.1. The effect of harmaline on cerebellar cortical activity

Harmaline induced rhythmic activity in the cerebellar cortex of awake rats, observed both in single unit complex spike (CS) activity of Purkinje cells (Fig. 2.3a, red) and in the ensemble activity, characterized by the envelope of the multi-unit activity (MUA) (Fig. 2.3b, red). MUA envelope oscillations could be observed on most electrodes (15/16 electrodes in this experiment; on average, 10/14 electrodes per experiment exhibited oscillatory activity), suggesting that nearby neurons that contributed to the MUA oscillated in-phase. The rhythmic character of the neuronal activity, quantified by the auto-correlation of CS and MUA (Fig. 2.3c,d red traces), was absent prior to harmaline administration (Fig. 1c,d black traces). This was true for all electrodes in all experiments. Harmaline was
therefore crucial for robust observable oscillations in freely moving animals [119] (see Discussion). The spectrograms of complex spike activity (Fig. 2.3e) and MUA envelope (Fig. 2.3f) demonstrated that rhythmic activity started with an initial transient period (T<20 min.), after which oscillations frequency remained relatively constant. MUA envelope oscillations could be detected earlier than CS rhythmicity, probably due to integration of activity across several cerebellar units. In this experiment, oscillation frequency was ~8.6 Hz on all 15 electrodes that exhibit oscillatory activity. Across experiments (n=12), stable oscillation frequency was in the range of 4-11 Hz with one exception (20-30 Hz).

Figure 2.3. The effect of harmaline on cerebellar cortical activity. a. Raw data and complex spikes times (vertical bars) taken before (black) and after (red) harmaline application. b. Raw data with its corresponding multi-unit activity (MUA) envelope before (black) and after (red) harmaline application. Data was collected simultaneously with the data in a, and has the same time scale. Envelopes are drawn above the raw data. c. Auto-correlation of the complex spike train reveals rhythmic activity after harmaline application (red) but not before (black). d. The same as c for MUA envelope. Note that the activity after harmaline has the same oscillation frequency of the CS activity in c. e & f. Spectrograms of CS and MUA envelope following harmaline application (T=0). Note the delay in CS oscillation onset and the transient changes in oscillation frequency (arrows).
Within each experiment, oscillation frequency was similar across electrodes, and occasional epochs of frequency deviation (Fig. 2.3e,f, white arrows) occurred simultaneously on all recorded electrodes. These frequency changes were corroborated in the single-unit CS activity (Figure 2.4).

**Figure 2.4.** a. Single unit complex spikes exhibit episodes of frequency elevation simultaneously with the multi-unit activity envelopes. Spectrogram of envelope segment, demonstrating a long epoch of frequency elevation. Black bars mark 40 s segments of baseline (left; ~8.5 Hz) and elevated (right; ~9.7 Hz) frequency analysed in b-d. b. Segments of (1 s) of raw voltage trace (black) and envelope (red) during baseline (below) and elevated (above) frequency. c. Auto-correlation of envelope during baseline (black; left black bar in a) and elevated (red; right black bar in a) frequency. d. Power spectrum of the envelopes in the two frequency ranges. e. High-resolution spectrogram of single-unit CS activity taken form a different electrode simultaneously recorded with the electrode in a-d. f-h. Same as b-d, for the single-unit CS data.
2.4.2. *Channel pairs exhibit a variety of stable relative phase difference values*

Oscillation frequency in each experiment was similar on all electrodes, but different electrodes oscillated with a variety of phase differences. Raw data segments from three electrodes (Fig. 2.5a, black traces) and their envelopes (red traces) demonstrate that oscillatory activity was not necessarily zero phase locked. The bottom two electrodes exhibited in-phase oscillations while the top electrode oscillated out-of-phase. The typical phase difference between pairs of electrodes was extracted from the cross-correlation of their envelopes. The cross-correlations between electrode pairs from Fig. 2.5a are shown in figure 2.5b, exhibiting ~0° and ~180° phase difference. All pairs reverberated at the same frequency, but exhibited a variety of time shifts and a variety of correlation strengths.

To examine the stability of phase differences, each experiment was parsed into 10 s segments and phase difference was calculated separately in each segment. Figure 2.5c illustrates the phase difference distribution across segments for the two electrode pairs shown in Fig. 2.5b. The phase difference distribution width, quantified by the Mean Resultant Length (MRL; see section 2.3.5), is shown in Fig. 2.5d. For phase differences < 90° (49/91 pairs), pairs had very high MRL values (MRL=0.91±0.18), indicating that phase differences were extremely tightly maintained throughout the experiment. Even for large phase differences (>90°), MRL values were relatively high (42/91, MRL=0.54±0.17), and differed significantly from chance level (MRL=0.15±0.1, dotted line). Across all experiments (n=12), a similar trend was observed (Fig. 2.5e), with the majority of phase differences in the range of 0-70° (Fig. 2.5f). These results suggest that oscillations in adjacent parts of the cerebellar cortex (and in this case, in adjacent parts of the IO) are coupled and can maintain a constant non-zero phase difference.
Figure 2.5. Electrode pairs exhibit variety of stable relative phase values. a. Segments of raw data from 3 simultaneously recorded electrodes (black) and their respective envelopes (red). Electrode i is out-of-phase relative to electrodes ii, iii. b. Cross-correlations between two electrode pairs (black) reverberate at the resonant frequency and exhibit different phase differences. Phase difference is extracted from cross-correlation of narrow-band envelopes (red; see METHODS). Cross-correlations are from electrode pairs from a. c. Circular histograms of phase difference for the same electrode pairs as in b (255 segments from a total of 21.5 min.) d. Mean Resultant Length (MRL) of phase difference distribution plotted against phase difference. Each dot represents one electrode pair. Dotted line - chance level. e. Population summary of MRL as a function of phase difference for all experiments (n=12). Phase difference was grouped into 20°-wide bins. f. Probability distribution of phase differences across all pairs, averaged across all experiments (n=12).

2.4.3. Oscillation frequency is modulated synchronously

Changes in the resonant frequency lasting several seconds could be observed even after frequency stabilization. Figure 2.6a shows the MUA envelope spectrogram of three simultaneously recorded electrodes. All three electrodes display a remarkably similar modulation in the oscillation frequency, characterized by occasional rises in oscillation frequency from a baseline of 8.6 Hz to 10 Hz lasting several seconds (arrows). In order to quantify this similarity, I first extracted the time course of oscillation frequency from the spectrogram of each electrode (see section 2.3.5).
Figure 2.6. Oscillation frequency is modulated synchronously. a. Spectrograms of MUA envelope from three different electrodes during the same experiment, demonstrating that frequency changes happen simultaneously. b. Frequency time courses (FTCs, black lines) from all resonant electrodes (n=13) and the global mean FTC (red line). c. The mean global similarity index (GSI) for each experiment ± 1SD (abscissa) are plotted against the GSI obtained using bootstrap methods with surrogate FTCs in the same experiment (ordinate), indicating that in all experiment, the common modulation is much higher than expected by chance.
The frequency time courses (FTCs) of all the electrodes (n=13) are superimposed in figure 2.6b (black lines) together with their mean (red line), and all exhibit almost identical temporal dynamics. Global changes in frequency would result in high similarity of individual FTCs to their mean, and I defined a global similarity index (GSI; see section 2.3.5) to quantify this similarity. In this experiment, GSIs were in the range of 0.55±0.15, indicating that most frequency fluctuations occurred simultaneously in the entire recorded cerebellar region. Frequency variability as a function of time was quantified in each experiment by the coefficient of variation (CV) of the mean FTC ($\sigma_{FTC}/\langle FTC \rangle$). CV values were in the range of 0.05-0.18, i.e. frequency changes had a standard deviation of 5-18% of the mean oscillation frequency. In all experiments (n=13), GSIs were on average 0.48±0.17 (Fig. 2.6c, abscissa), and significantly higher than those obtained using surrogate FTCs (Fig. 2.6c, ordinate; see section 2.3.5). Frequency was thus co-modulated, and can therefore be viewed as uniform in the recorded cerebellar area.

2.4.4. Frequency changes are related to the behavioural state of the rat

The epochs of frequency elevation observed in the oscillatory activity were intermittent in nature and lasted several seconds. To study whether these epochs were related to rat behaviour, rat behaviour was monitored using a single video camera at 30 frames / s. Movies were then manually analyzed, and segmented into epochs during which the rat was still and moving. Tremor was not quantitatively monitored, but was observed in all rats and appeared enhanced during volitional movement. The movies (n=4 rats) were analyzed. After harmaline application, rats spent 19-45% of the time moving around the cage. During movement epochs, changes could be observed in the spectrograms of the MUA (Fig. 2.7a; yellow bars denote epochs). Movement was associated with an average shift of 6.7% in the frequency peak (examples in Fig. 2.7b). All experiments analyzed for movement exhibited shifts in the 6.7-20% range (Fig. 2.7c). Covert changes in the arousal state of the rat, or other behavioural parameters, may more fully account for the frequency variability. These results imply that marked global changes in oscillation frequency are correlated with changes in the behavioural state of the animal.
Figure 2.7. Frequency changes are related to the arousal state of the rat. a. Spectrogram of envelope fluctuations, with arousal periods (animal movement) marked by yellow bars). b. Power spectrum of envelope fluctuations averaged across rest (black) and movement (red) demonstrates the 6.7% frequency elevation observed in this experiment. c. Mean ± 1SD of peak oscillation frequency while the animal was still (abscissa) and moving (ordinate) for all taped experiments (n=4).
2.4.5. Phase differences are retained across frequency changes

The lag in activity between electrode pairs can be interpreted either as phase or as time differences. This distinction becomes significant, if one of these parameters is invariant to frequency changes. Invariance of phase difference to frequency changes has been shown to exist in other oscillating neural systems [198], and enables a system to scale time patterns across varying speeds. Phase and time differences between electrode pairs were calculated throughout the experiments. In Fig. 2.8a, the MUA envelope spectrograms of two electrodes are shown. Both envelopes have a similar pattern of frequency change across time. Fig. 2.8b demonstrates similar FTCs for all oscillatory electrodes (black lines, n=10; red line shows mean FTC; GSI=0.78). The FTC was segmented into regions of low and high frequency (9.3±0.2 Hz and 10.7±0.2 Hz in this experiment). The mean phase difference between each pair of electrodes was extracted in the low frequency range (Δφ, LF) and the high frequency range (Δφ, HF) and were plotted against each other (Fig. 2.8c). The regression line through all electrode pairs (green line) demonstrates that phase difference is well maintained across ~15% frequency change (slope 0.92; statistically indistinguishable from 1). Time delays between electrode pairs in the two frequency ranges demonstrates that time differences are less well maintained (Fig. 2.8d; slope 0.81 differs significantly from 1).

Population data are summarized in Fig. 2.8e (n=10; two experiments were excluded due to negligible frequency changes). Each experiment is represented by the slope of the phase difference plot (such as in Fig. 4c) and by the slope of the delay plot (such as in Fig. 2.8d). All the points in the graph are below the diagonal, indicating that phase differences (abscissa) are maintained much better than delays (ordinate). Invariant time delays would have resulted in phase slopes > 1, which are never observed in the data (see Appendix 2.1). In 6/11 experiments the phase was almost perfectly maintained (statistically indistinguishable from 1). Nonetheless, in all experiments phase differences were better maintained than time differences. The ability of the olivo-cerebellar system to maintain phase differences relatively well during significant frequency changes has important implications for pattern generation [199].
Figure 2.8. Phase is retained across large frequency changes. a. Spectrograms of MUA envelopes from two simultaneously recorded electrodes exhibit similar, almost binary, frequency transitions. b. FTCs of all 10 oscillatory electrodes from the same experiment (black lines), and the global mean FTC (red). c. Summary of phase differences for electrode-pairs in the low frequency range (LF, 9.1-9.5 Hz; abscissa) vs. the high frequency range (HF, 10.5-10.9 Hz; ordinate). Dotted red is the identity line, and dots close to it correspond to electrode pairs whose phase remained constant when frequency changed. Green line is the linear regression through all data points, whose slope value is 0.92 (statistically indistinguishable from 1). Phases were extracted from cross-correlations of narrowband envelopes. d. Summary of delays between electrode-pairs in the LF (abscissa) vs. HF (ordinate). Delay is further away from the diagonal (slope 0.81, significantly different from 1). e. Population summary (n=10) of delay and slope changes. Each star summarizes the slope ratio (HF/LD) of delays (ordinate) vs. the slope ratio of phases (abscissa). Values closer to 1 indicate better maintenance of value. In all experiments, phase was better maintained than delays.
2.5. Discussion

By recording multi-unit activity in the cerebellar cortex of rats, I unravel the phase structure of harmaline-induced oscillations. Changes in oscillation frequency were global in the recorded region, but oscillation in different electrodes retained phase differences. This allows the olivo-cerebellar system to support time intervals shorter than the cycle duration [199], covering the range implicated by behavioural studies [189].

Harmaline-induced oscillatory activity in the cerebellar cortex has previously been shown to depend on intact olivo-cerebellar connectivity [119], manifesting itself as rhythmic CS activity. Indeed, harmaline acts directly on IO neurons, inducing rhythmic activity by shifting the activation curve of their low-threshold Ca$$^{2+}$$ conductance [74]. Hence, harmaline does not change the basic electrotonic coupling between IO neurons, but only their propensity to oscillate and produce spikes.

Without harmaline, IO oscillations are non-stationary and intermittent [94], with only a minority of subthreshold oscillation cycles producing spikes [95]. Recordings in the cerebellar cortex therefore pose severe limitations on the detection of non-zero phase differences. Cross-correlations between PCs using short time windows would not yield enough spikes to detect phase locking. Using large time windows would violate the stationarity requirement for cross-correlation analysis. Furthermore, phase difference invariance implies that the time lag of cross-correlation peak would moves frequency changes, and the resulting cross-correlation would be smeared. This may give rise to the impression that olivary activity is non rhythmic [200]. The enhanced regularity and high firing rate imposed by harmaline help to uncover a phenomenon that most likely occurs during normal behaviour. Indeed, temporal delays between groups of synchronous PCs have been reported in animals performing a repetitive motor task [201], suggesting a role for such non-zero phase differences.

The two significant aspects of the results are the maintenance of non-zero phase between areas of the cerebellar cortex, and their invariance to frequency changes. I suggest that these results should be interpreted within the framework of olivary timing, first proposed by Llinás [89]. The phase structure revealed in this work enables the cerebellum to create complex output patterns. In contrast, a synchronized clock that works with fixed quantum of time – the cycle duration – can produce time sequences that are limited to the cycle duration and its multiples. By combining several clocks with non-zero phase differences, the temporal patterns within each cycle are almost unlimited, and the system can support much shorter time intervals [199]. The shortest interval is set by the accuracy of the olivary spiking mechanism and the longest interval by the cycle duration or multiples thereof (see Chapter 4).
My results are supported by in vitro studies that have demonstrated a wide repertoire of olivary oscillations including stationary phase locked oscillations, breakdown into smaller groups of phase-locked oscillators [90] and travelling waves [76]. Phase differences can appear in vitro within the same olivary area, at distances as small as 100 μm [76]. In contrast, previous work on CS synchrony in vivo has mostly described zero phase synchrony between PC pairs [100,106,118,202], with non-zero phase differences been reported only anecdotally [105,119,120,190,201], and never studied systematically. Nonetheless, kinematic studies in harmaline treated rats suggest that IO coupling is required to maintain tight, non-zero phase differences between muscle pairs, further supporting the results [145]. The mechanism of phase difference maintenance still requires elucidation, but probably relies on direct or indirect coupling between oscillating IO neurons. The recording electrodes were placed in vermal regions of lobule V and VI (and occasionally perhaps in lobule IV), which are all connected to the medial accessory olive and do not cross IO anatomical boundaries [203]. This provides the anatomical substrate necessary for direct and indirect coupling between the IO neurons. The fact that large phase differences were correlated with a decrease in the synchronization measure (MRL), may result from an increase in the possibility of synaptic inputs to the olive to interfere with the coupling between the areas.

A system that maintains phase differences across frequency changes has important implications for temporal sequence generation. For example, complex motor behaviour – from walking to playing the piano – can be performed accurately at different speeds. This requires undistorted scaling of temporal patterns. I propose that the olivo-cerebellar system may underlie this ability through phase difference invariance to frequency changes.
Appendix 2.1—The relationship between phase maintenance and delay maintenance

We have shown (Fig. 2.8) that the phase difference between electrode pairs is well maintained, while time delays are not. How are these two measures related to each other? Assume that the oscillatory activity switches between two discrete values, $F_1$ and $F_2$ ($F_1 < F_2$). Under the assumption of phase constancy, a phase difference value can be attributed to some electrode pair, denoted as $\Delta \varphi$ (assume $\Delta \varphi \neq 0$, as this case has trivial zero phase and delay). This phase difference corresponds to different delays in the two frequency regimes:

\[
\Delta t_1 = \frac{1}{F_1} \cdot \frac{\Delta \varphi}{2\pi} \\
\Delta t_2 = \frac{1}{F_2} \cdot \frac{\Delta \varphi}{2\pi}
\]

Phase constancy therefore implies that the delay:delay graph depicted in Fig. 4d should have a slope of

\[
\frac{\Delta t_2}{\Delta t_1} = \frac{F_1}{F_2} < 1
\]

as seen in the results. The exact delay:delay slope value depends on the percent frequency change between the two discrete frequency values. Non-invariance of the delay does therefore not imply that delay values in the two frequency ranges are uncorrelated, but rather determines a certain slope value < 1. In a similar fashion, delay invariance would have led to phase:phase slope values > 1, a phenomenon never observed in the data.
3. In vivo voltage sensitive dye imaging of cerebellar activity

3.1. Abstract

This chapter describes a study of cerebellar cortical activity in vivo I performed using voltage-sensitive dye imaging (VSDI). The motivation for this work was two-fold:

i. **Technical motivation**: To adapt cutting-edge VSDI methods for in vivo recordings from the cerebellar cortex.

ii. **Scientific motivation**: To study the relationship between the activity of single Purkinje cells (PCs) and the network activity.

The cerebellar cortex is ideally suited for VSDI. It is a shallow structure, allowing convenient optical access, and has a simple micro-circuitry (see Introduction, section 1.1.1). VSDI has previously been applied to the cerebellar cortex in vivo, but with limited temporal resolution and with massive averaging [204,205]. In my host lab, a system for VSDI of an isolated cerebellum-brainstem preparation has been developed and used extensively [46,206,207,208,209]. My goal was to adapt this system for use in an in vivo rodent preparation, and to apply VSDI to the cerebellar cortex at high spatial and temporal resolution.

The scientific drive for in vivo VSDI of cerebellar cortex was to elucidate the relationship between the high rate of simple-spike (SS) firing by PCs, and network activity in the cerebellar cortex. As elaborated in the Introduction (sections 1.2.1.1-2), little evidence exists for an effect of PF activity on PC output. In this chapter, I employ VSDI in vivo combined with single-unit recordings of PC spikes to demonstrate that spontaneous PC activity does not depend on PF inputs. Direct stimulation of PF axons elicits a clear progression of activity along the medio-lateral axis of the cerebellar cortex. In contrast, the average activity preceding PC SSs contains no spatio-temporal structure, even when thousands of SSs are averaged. This result further demonstrates that PF activity has little or no effect of PC firing. This lends further support to the idea that PC SSs result from intrinsic properties of PCs and can be input-independent.
3.2. Introduction

3.2.1. Voltage-sensitive dye imaging

VSDI is a powerful tool for studying the activity of large neuronal ensembles [210]. VSDs were first described in the ‘60s by Tasaki and colleagues [211]. Its applicability for simultaneous monitoring of activity from large neuronal populations was demonstrated in the ‘70s by Cohen, Salzberg, Grinvald and others [91,212,213]. VSDs are organic molecules that bind the external surface of cell membranes and change their optical properties – either absorption or emission - in response to changes in the membrane potential. These changes occur within microseconds and can be monitored using CCD cameras or photo-diode arrays. In the last decade, the Grinvald lab (Weizmann Institute) has developed a line of VSDs that have an absorption spectrum above 600 nm (e.g. RH-1691, RH-1692, RH-1838), far from the haemoglobin absorption peak (530 nm) that impeded the use of previous VSDs in vivo. These dyes were chosen for use in this study. In the future, genetically encoded VSDs [214,215,216,217] and 2-photon imaging [218] may improve VSDI resolution and cell-specificity, although currently their kinetics are much slower than the synthetic VSDs.

3.2.2. Imaging of olivo-cerebellar activity

VSDI of olivo-cerebellar activity began in the 1980s with studies in slices of the skate cerebellar cortex [219]. Successful implementation has so far been limited mostly to in vitro preparations [90,206,209]. VSDs have also been used in the cerebellar cortex in vivo, albeit with limited results. Several papers by the Ebner and colleagues employed VSDI to study in vivo cerebellar responses to PF stimulation [204,205]. They were able to demonstrate the effect of several neurotransmitter agonists and antagonists on the PF response, but their image acquisition rate (30 Hz) posed a severe limitation. In addition, averaging of 200-400 images was necessary to obtain reasonable data, mostly due to noise resulting from motion artefacts.

A different approach is to image changes in Ca\(^{2+}\) concentration using 2-photon imaging. Ca\(^{2+}\) imaging is particularly well suited for studying PC complex spikes, which give rise to large dendritic Ca\(^{2+}\) transients. The shallow location of the PC dendrites allows easy optical access in vivo with the use of 2-photon imaging. This method has begun to yield results during the last year [220,221]. It is unclear whether this method will allow identifying Ca\(^{2+}\) transients resulting from single SSs, as these occur at a high rate and result in small changes in [Ca\(^{2+}\)].
3.2.3. The source of Purkinje cell simple spikes

Purkinje cells (PCs) can generate two different spikes: Complex spikes (CSs) and simple spikes (SSs). CSs result from activation of the climbing fibre axon, whose soma lies in the inferior olive (IO). CSs usually occur at a low rate (0-2 Hz). In contrast, SSs are generated at a high rate (from 20 to >100 Hz), and are typical HH-type Na⁺ spikes. The prevalent dogma is that SSs result from the coincident input of PFs. An authoritative neuroscience textbook (Kandel, Schwartz & Jessell, 2000) states that:

Climbing fibers have unusually powerful synaptic effects on Purkinje neurons. Each action potential in a climbing fiber ... results in .. a complex spike... In contrast, parallel fibers produce a brief postsynaptic potential that generates a single action potential or simple spike. [222]

As elaborated in the Introduction (sections 1.2.1.1-2), accumulating evidence is challenging the second part of this input-output scheme. In brief, SSs are generated at a high rate in the absence of synaptic inputs, both in vitro [37] and in vivo [36]. Furthermore, PCs respond mostly to granule cell (GC) inputs delivered through the ascending axon, and not to the PFs [13,46,47]. Together, these results suggest that most SSs do not result from PF input. This has so far not been tested directly by comparing spontaneous SSs with the ongoing PF activity. In this chapter, I directly compare single PC SSs with the ongoing activity in the surrounding cerebellar area to directly address this issue.

3.2.4. The source of PC simple spikes: A Gedanken experiment

I describe the expected observations if SSs were determined by PF inputs. The time constant for synaptic summation is expected to be ~1 ms, due to the disynaptic inhibition of molecular layer interneurons [63,64].

![Diagram showing Purkinje cell simple spikes arising from coincident parallel fibre inputs](image)

Figure 3.1. Purkinje cell simple spikes arise from coincident parallel fibre inputs. PC (red) receives inputs from granule cells \(G_1, G_2, ..., G_N\) that are located at medio-lateral distances of \(l_1, l_2, ..., l_N\). See text for mathematical details.
The Gedankenexperiment proposed here can be summarised briefly as follows: **If the timing of PC SSs is determined by coincident PF input, then averaging the spatio-temporal activity preceding a SS should reveal a “beam”-like PF signal that increases in amplitude as it approaches the PC.**

Assume that $N$ coincident PF inputs are necessary to generate a SS at an arbitrary time $t_0$. The $N$ GCs giving rise to these PFs are denoted by $G_1, G_2, ..., G_N$. The medio-lateral distances between these GCs and the target PC are denoted by $l_1, l_2, ..., l_N$ (see figure 3.1). If all PF signals arrive simultaneously, then each granule cell $G_i$ had to emit an action potential at time $t_0 - \Delta t_i$. Assuming a constant PF conduction velocity $v$,

\[ (E3.2) \quad \Delta t_i = \frac{l_i}{v} + \delta_i \]

where $\delta_i$ is the propagation time along the ascending axon and shall be ignored for the sake of simplicity.

At a certain point in time $t_0 - \delta t$, activity of the PFs that arise from $\{G_i\}_{i=1}^N$ is spatially localised to $d = \pm \delta t \cdot v$ (i.e. equidistant on both sides of the recorded PC). Its amplitude varies with different choices of $\{G_i\}_{i=1}^N$, but averaging across the different choices and assuming a spatially uniform distribution of GCs on the interval $[-D, D]$ (where $D$ is the length of one PF branch), the expected amplitude $\alpha$ of the PF signal at $d$ is

\[ (E3.3) \quad \alpha = \frac{D - |d|}{D} \cdot N \]

Thus, the average spatiotemporal progression of the signal for $t_0 - \frac{D}{v} \leq t < t_0$ can be summarised by:

\[ (E3.4) \quad x(t) = \pm (t - t_0) \cdot v \]
\[ (E3.5) \quad a(t) = \frac{D - |d|}{D} \cdot N = \frac{D - (t - t_0) \cdot v}{D} \cdot N \]

If spontaneous activity in the GC layer is random, patterns such as depicted in figure 3.2 would be embedded within random, non-repeating patterns. Only by averaging the PF activity across many simple spikes would it be possible to average out the non-repeating random patterns and reveal the repeatable components that lead to PC SSs.
The expected spatio-temporal pattern is presented in figure 3.2. As the PF signals propagate towards the PC (thin vertical line), more PFs are recruited, resulting in an increasing amplitude (smooth and linear after averaging across realisations). After the signals collide above the PC at time $t_0$, they continue travelling away from it while decaying linearly, as the end of successive PFs is reached.

![Figure 3.2](image)

**Figure 3.2.** Average spatio-temporal pattern of PF activity predicted by Gedanken experiment. A PC SS is elicited by the coincident input of many PF inputs. Averaging spatio-temporal activity around SSs should therefore reveal an approaching “beam” of PF activity whose amplitude grows and peaks around the time of the SS ($t_0$) just above the PC, and then decays. M-L: medio-lateral. $D$: length of a single PF branch. $v$: PF velocity.

3.3. Materials and methods

3.3.1. Animal preparation

Research was performed on adult guinea pigs (350-500g) and rats (250-250g). Results were indistinguishable between species. All experiments were performed in accordance with Hebrew University animal maintenance guidelines.

Prior to anaesthesia, animals were injected with atropine methyl nitrate (1mg/kg i.p.; Sigma), a muscarinic cholinergic blocker that does not cross the blood-brain barrier, to minimise tracheal mucus
secretion. After 5-10 minutes, animals were anaesthetised using urethane (1.2g/kg i.p.; Sigma). When necessary, additional doses (10-20% of initial dose) were administered. Procaine (3%; Sigma) was injected subcutaneously over the skull and animals were placed in a custom-built head holder consisting of ear-bars and a mouthpiece. Rectal temperature was monitored and maintained using a heating pad (Harvard Apparatus) driven by a home-made controller. An incision was performed above the skull, and the skull exposed between bregma and the occipital ridge. Muscle was scraped and the surface of the skull cleaned. A dental cement chamber was built on top of the occipital bone with an inner diameter of ∼1-1.5 cm, and the skull underneath drilled to expose a brain area of ∼0.2-1 cm medio-lateral × ∼0.2-0.4 cm rostro-caudal (example in figure 3.3). The opening was usually centred on the midline and performed close to the posterior end of the occipital bone, to avoid the large blood vessels. The dura surface (and later, the brain surface) was kept wet using artificial cerebrospinal fluid (ACSF; in mM: 137.8 NaCl, 2.78 KCl, 1.84 CaCl₂, 2.16 MgCl₂, 2 D-Glucose, 1.6 Na₂HPO₄, 0.3 KH₂PO₄) that was warmed to body temperature. After 10-20 minutes, the dura and arachnoid were carefully removed under a binocular with high (x25) magnification. Arachnoid removal, and sometimes pia mater removal, was found to be crucial for successful diffusion of the dye into the tissue. Care was taken to avoid any damage to surface blood vessels, as these usually resulted in compromises to the tissue integrity, to the optical transparency and to oedema.

**Figure 3.3. Craniotomy and VSD staining.** A dental cement chamber (cham.) is built on the exposed and clean skull. A craniotomy (here, a relatively small ∼2 mm diameter opening) is performed above the cerebellar vermis (in this case, lobule VIa). The “blue” RH-1692 has stained the cerebellar cortex well after two hours of bathing, and has also attached to the surrounding bone. A.P: anterior, posterior.

### 3.3.2. Voltage-sensitive dye application

To record neuronal activity in vivo using VSDs, it is necessary to minimise artefacts resulting from blood cycle, respiration etc. I used two different “blue dyes” developed by Grinvald lab [210,223] that are ideal for this purpose: RH-1838 and RH-1692 (Optical Imaging: Rehovot, Israel). Dye was diluted in ACSF to achieve a final optical density of ∼7 at 580 nm. A dental cement chamber surrounding the skull opening was filled with the dye for 1-2 hours to achieve a deep and uniform diffusion into the tissue (see
Dye solution was changed every 15-20 minutes. RH-1838-stained tissue appeared lighter, but more frequently provided successful imaging. Both dyes reached $\Delta F/F$ of 1% for electrical stimulation of the cerebellar surface (see 3.4.1 below).

3.3.3. Image acquisition

After successful staining of the cerebellar cortex, the opening was covered with low melting point agarose solution (3%) to minimise pulsations and stabilise the recordings. Images were acquired with an array of 464 photodiodes in a hexagonal arrangement (Wu Tech, Potomac, MD). The photodiode array was placed in the focal plane of a microscope (Nikon Optiphot, Japan) equipped with an epi-illumination attachment for fluorescence measurements. Light was transmitted through a filter block (Omega Optical Inc., Brattleboro, VT) with an excitation filter at 630±5 nm (630DF10) and a dichroic mirror of 650 nm (XF2035). A barrier filter of >665 nm was implemented using a Schott glass (3mm, RG665; Schott AG, Mainz, Germany). Light was delivered through a 15 V / 150 W tungsten / halogen lamp, powered by an adjustable, low-noise Kepco power supply. A mechanical shutter (UniBlitz, Rochester, NY) controlled the illumination period. The image was magnified using a 20× lens (NA 0.4, WD 1.8; Leica, Wetzler, Germany). The long WD allowed the insertion of an extracellular recording pipette into the cerebellar cortex to simultaneously record PC activity during optical imaging. Using this magnification, each photodiode sampled an area of 34 µm × 34 µm, and the diameter of the imaged hexagon was 850 µm.

A home-made electronic system composed of two stages was used to convert and amplify the photodiode signal. The first stage converted the current output of the photodiodes to voltage, using a feedback resistor of 100 MΩ. The second stage amplified the 1st stage signal ×100/200/500/1000 (uniform gain for all channels) via AC-coupling with a time constant of 200 ms. The 2nd stage amplification could be bypassed for DC measurements.

The data from each photodiode was acquired at a sampling rate of 5.4-5.6 kHz per channel and 14 bits per sample, using a MicroStar 4400a board (MicroStar Laboratories, Bellevue, WA) equipped with 8 MSXB-018 expansion boards. Electrophysiological data was sampled simultaneously (see below, 4.3.4). Home-made software was developed on LabVIEW 6 (National Instruments, Austin, TX). Software allowed the control of shutter onset, acquisition onset and duration, and electrical stimulation parameters through a MASTER-8 stimulator (A.M.P.I., Jerusalem, Israel). A schematic illustration of the acquisition system is given in figure 3.4.
3.3.4. Electrophysiology

In many experiments, single unit PC recordings were performed simultaneously with VSDI. Sharp glass electrodes were filled with NaCl (2M) and the tip carefully broken, resulting in a resistance of 5-10 MΩ. Electrodes were inserted at a relatively oblique angle, to avoid interference with the optical pathway. The electrode was lowered until PC SSs were detectable. Recordings were performed in voltage clamp tracking mode using an AxoPatch 200B amplifier. The amplifier output was delivered to a vacant input on one of the expansion boards of the optical acquisition system (see above, 4.3.3). The electrode signal was sampled at either 11 or 22 kHz.

SSs appeared as fast, monophasic positive deflections of the current trace. The shape of CSs changed as a function of recording depth. In the ML, CSs appeared as slow, positive, monophasic deflections. When SSs could be detected, CSs were composed of an initial SS-like fast, positive deflection, followed by a slow negative one and a varying number of spikelets. Care was taken to align the tip of the recording electrode to the centre of the imaging field, but this was not achieved at the single-photodiode resolution.

3.3.5. Electrical stimulation

Bi-polar, concentric stimulation electrodes (FHC Inc., Bowdoin, ME) were used for stimulating the surface of the cerebellar cortex to elicit PF responses. The electrodes received a short (0.1-0.2 ms) voltage step from an isolation unit (Devices Sales, Hertfordshire, UK). Stimulation intensity of 5-20 V was usually enough to elicit a strong, optically detectable PF “beam” (see figure 3.6 below).

3.3.6. ECG monitoring

ECG was monitored in all imaging experiments to enable post-hoc subtraction of heartbeat artefacts from the optical signal and as an indication of the animal state. Needles were subcutaneously inserted beneath the right and left forelimbs, and the potential between them filtered (10-1000 Hz), amplified (×1000) and sampled at 5.56 kHz.
Figure 3.4. Scheme of optical / electrophysiological setup. Guinea pig / rat was placed in a head holder, and the brain imaged through an epi-fluorescent microscope equipped with a 20x air lens. Electrical stimulation and recording were performed simultaneously. Signal acquisition is detailed in 3.3.3-3.3.6.

3.3.7. Data analysis

All data analysis was performed on Matlab (Mathworks, Natick, MA) using home-made software.

**Spike detection and sorting**: The electrode signal was analysed using standard techniques. In brief, spike threshold was determined using a function of the signal median [193]. Spike shapes were aligned at a sub-bin resolution using cubic interpolation. Principal component analysis (PCA) was then performed on the aligned spike-shape matrix to obtain the first two principal components (eigenvectors of the spike covariance matrix). The projection of each spike $\tilde{s}_i$ onto these principal components, $\tilde{PC}_1$, $\tilde{PC}_2$ provided two values $a_1^i, a_2^i$, defined by:

$$a_k^i = \langle \tilde{s}_i, \tilde{PC}_k \rangle$$
These values were used to characterise each spike. Spike shapes that could be isolated appeared as clusters on the $\alpha_1: \alpha_2$ plane and were manually delineated. PC SSs were further identified by the co-occurrence of CSs and the existence of a CS pause in SS firing lasting at least 15 ms [52,53].

**Artefact removal from the optical image:** The optical signal was contaminated by two artefacts: Respiration artefacts and heart-beat artefacts. For the large part, these artefacts were additive and repeating. Most of the respiratory artefact was removed by high-pass filtering the optical data of each photodiode $> 2$ Hz. The heart-beat artefact was more variable in shape, probably reflecting the spatial relation of each photodiode to the surrounding blood vessels. Time-domain subtraction of the average heart-beat artefact proved to be superior to other methods. Most importantly, the heart-beat signal could contain high-frequency components overlapping with potentially significant signals, thus ruling out band-pass filtering.

![Figure 3.5. Removal of heartbeat artefacts.](image)

To remove heart-beat artefacts, times of QRS events $t_1^{(QRS)}, t_2^{(QRS)}, \ldots, t_n^{(QRS)}$ were identified in the ECG signal using identical methods to those used in spike identification (figure 3.5a). In most experiments, heart rate was highly regular (see figure 3.5b). For a given photodiode $k$, the optical signal is denoted by $a_k(t)$. The average artefact for each photodiode was calculated by:

$$e_k([0, \Delta t_{\text{min}}]) = \frac{1}{n} \sum_{i=1}^{n} a_k \left( [t_i^{(QRS)}, t_i^{(QRS)} + \Delta t_{\text{min}}] \right)$$  \hspace{1cm} (EQ3.2)

with

$$\Delta t_{\text{min}} = \min \left\{ t_i^{(QRS)} - t_{i+1}^{(QRS)} \right\}$$  \hspace{1cm} (EQ3.3)

and smoothed (0-70 Hz, Hamming-window based FIR filter). This artefact (figure 3.5d) was then subtracted from each heart cycle in the optical signal to create a corrected signal, $\tilde{a}(t)$ (figure 3.5c). The duration of $e_k(t)$ corresponded to the shortest heart cycles. Therefore, to avoid artefacts resulting from
longer heart cycles and discontinuities in the subtraction, spline interpolation was used to
elongate \( e_k(t) \) such that it would merge smoothly with the beginning of the next heart cycle correction.

**Spike-triggered averaging of the optical activity:** Spontaneous optical and electrophysiological
data was collected simultaneously in 5 s blocks, with shutter opening 1-2 s prior to data acquisition. Data
collection segments were separated by 0.5-2 minutes, to minimise phototoxic effects. After artefact
removal and SS identification, segments of ±25 ms were collected around each SS and averaged. This
resulted in a spike-triggered movie (STM), which reflects the typical activity that preceded and followed
each spike. To identify significant events in the STM, bootstrap STMs were generated by repeating the
calculation for surrogate spike-trains. Surrogate spike-trains were generated by maintaining the number
of spikes per block, but placing them in random times within the block.

### 3.4. Results

#### 3.4.1. Responses to direct stimulation of the parallel fibres

Electrical stimulation was applied directly to the cerebellar surface, lateral to the field of view at
a latency of 10 ms after acquisition onset. Activity could be observed propagating laterally from the
stimulation site. Figure 3.6 shows the spatial arrangement of photo-diodes and the time course of
optical activity in each photo-diode. Optical responses can be observed at a latency of 1.5-2 ms after
stimulation. Red traces are those in which a positive peak was above 3SD units of the optical noise
(measured in the initial 10 ms for each trace).

The response progression along the PF axis is demonstrated in figure 3.7 for a different
experiment. Colour and height denote fluorescence changes relative to baseline. Frames are separated
by 0.5 ms. Approximately 1.5 ms post stimulation, the response starts propagating along the medio-
lateral PF axis. Within ~3.3 ms it traverses the entire array, with the front propagating at a velocity of

\[
 v_{PF} = \frac{850 \, \mu m}{3.3 \, ms} \approx 26 cm/s. \]

Across all experiments (n=23\(^1\)), PF velocity was in the range of 15-27 cm/s. Figure 3.8 shows the time course of optical activity for two photo-diodes, one proximal and one distal
relative to the stimulation site. The response is composed of two components, a rapid depolarisation (<1
ms rise-time) followed by a prolonged plateau (20-40 ms). Inhibition [208] was rarely observed in this
preparation.

\(^1\) Guinea pigs: 14; rats: 9.
Figure 3.6. Acquisition of optical activity in the cerebellar cortex using VSD and a photo-diode array. Each trace depicts the time-course of the $\Delta F/F$ signal in a single diode. The 464 diodes are arranged in a hexagon, each photodiode collecting fluorescent signals from an area of 34 $\mu$m $\times$ 34 $\mu$m (hexagon 850 $\mu$m in diameter). PF activity was elicited by a 10 V, 0.1 ms pulse through a bipolar concentric electrode located left (~200 $\mu$m) of the photodiode array on the cerebellar surface at $t = 10$ ms (guinea pig). Red traces exhibited a significant peak (> mean+3SD) between the time interval of 10-20 ms.
PF stimulation was used in all experiments as an indicator of preparation vitality and the efficacy of the VSD in providing signals from neuronal sources. Spontaneous VSDI and electrophysiology was collected and analysed (see following section) only while the evoked PF response was statistically significant.

Figure 3.7. Propagation of parallel fibre signal along the medio-lateral axis. Electrical stimulation (t=0 ms to the left of the imaged area) elicited a PF response whose front propagated at a velocity of 28 cm/s medio-laterally (rat).
3.4.2. Average spatio-temporal activity in the cerebellar cortex related to simple spikes

VSDI data was collected simultaneously with single unit SS activity for long durations. To avoid photo-toxic effects, 5 s acquisition periods were used, at intervals of 0.5-2 minutes. High spontaneous SS rates were observed (20-60 Hz). These resulted in a large total number of SSs (800-2,700) across all experiments (n=7). In each such experiment, 50 ms chunks of optical activity were extracted around each spike (±25 ms). These were averaged across all SSs, to yield the spike-triggered optical activity. I was interested in testing whether spatio-temporal patterns such as those described in figure 3.2 were present in the cerebellar activity. Despite the massive averaging, no such patterns were observed (example in figure 3.9). The activity possessed no detectable spatio-temporal structure, and the deviations from the averaged noise level lacked spatial structure and occurred at a rate not exceeding that expected by the noise statistics. This was verified using bootstrap statistics in which spike numbers were retained while picking random times and repeating the spike-triggered averaging procedure.

Figure 3.8. Temporal characteristics of optical response to surface stimulation of the cerebellar cortex. The rapid onset and slow decay are apparent in both the proximal (black) and distal (red) photo-diodes. Arrow: Time of stimulation. Data shown are average of 5 repetitions.
Figure 3.9. No spatio-temporal pattern in the spike-triggered optical activity. Images were obtained by averaging the VSD activity surrounding each SS.

3.5. Discussion

I have adapted a VSDI system for use in vivo, and shown that high quality VSDI can be achieved on a single trial basis. Evoked parallel fibre (PF) activity could be observed propagating along the medio-lateral axis. In contrast to most VSDI studies, the optical signal observed contained also a rapid, ∼1 ms component, indicating that using photo-diodes (or potentially also fast CCD cameras), VSDI can monitor brain activity on the time scale of spiking activity.

Using a combination of VSDI and electrophysiology in vivo, I have further been able to demonstrate that simple spikes of cerebellar Purkinje cells are not related to any spatio-temporal structure of activity in the cerebellar cortex. This supports the view of SSs being internally generated, and not depending on synaptic input. This runs against the prevalent dogma that views SSs as reporters of SS coincidences [63]. The implications of this result are further discussed in the next chapter (in Appendix 4.2) and in the general Discussion (section 5.1).
3.5.1. How much averaging is necessary to reveal spatio-temporal patterns?

The Gedanken experiment (section 3.2.4) predicts that a pattern of lateral progression should be observed after averaging VSD signals around SSs. Therefore a cardinal issue is the amount of averaging necessary to reveal such patterns. In the evoked experiments (e.g. figure 3.7), the VSD signal noise was typically around 0.2% ΔF/F, although larger values were typically observed in the periphery of the optical field, or in areas that did not stain well with the VSD. This noise level made the detection of the PF response (~1%) possible even on a single trial basis.

In the spike-triggered averaging (section 3.4.2), N=800 to N=2,700 trials were averaged, resulting in a ~30-50-fold (∼√N) noise reduction. Thus, noise level in the centre of the field was ~0.004-0.007% ΔF/F. Is this enough to detect the patterns hypothesised by the Gedanken experiment?

The Grinvald group has demonstrated in the same VSD used by us (RH-1692), that a 3 mV depolarisation results in approximately 0.1% ΔF/F change, and that the dye mostly follows the subthreshold membrane potential (see [224], Supplemental Figure 1). If we assume that just 3 mV depolarisation is enough to bring the PC to threshold, one should expect a ~0.1% ΔF/F signal above the recorded PC. Just before t₀, lateral to the PC, the signal should drop to ~0.05% ΔF/F (because the beams travelling in opposite directions each contribute on average half of the signal), and at a distance of D/2 (> 500 μm), the signal should still be ~0.025% ΔF/F, which would still have an SNR of 4-6 relative to the average noise level and be detected. Therefore, the inability to detect beam-like activity does not stem from the inability to detect a weak signal embedded within a noisy background.

The number of spikes averaged and the evoked signal both suggest that my result is not the consequence of signal-to-noise ratio problems, but rather that PC SSs indeed are unrelated to PF inputs. Even if only a small, undetectable fraction of SS is the result of such coincidences, it is unclear how downstream CN neurons can distinguish the different kinds of PC SSs from each other (see also section 5.1).

3.5.2. Possible sources for the weak effect that PFs exert on Purkinje cells

Mechanistic explanations on several levels have been provided for the insensitivity of PCs to PF inputs. Ekerot and Jörntell have shown that certain stimulation protocols akin to PC LTP protocols [58,59,225] may significantly enlarge the receptive fields of PCs [68], suggesting that most PF-PC synapses are under normal conditions silent. These results are consistent with dual recordings from GCs and PCs, which have demonstrated that most PF-PC synapses (~80%) are silent [13,63], while GC
ascending axon synapses have a much higher probability of being potent. The “privileged” status of ascending axon synapses may arise from their incapacity to undergo LTD [49,50] (but see [51]). While these results shed light on the mechanisms underlying silent PF-PC synapses, they contribute very little to reconciling the discrepancy between the anatomy and physiology, i.e. they do not provide a functional role for the enormous PF system.

The classical framework of Marr [167] and Albus [226] posits that GCs create a redundant and random representation (recoding) of the MF input, allowing PCs to efficiently learn associations (see Introduction, section 1.4.1). Many GCs may thus carry signals that are irrelevant for a certain PC, and plasticity mechanisms will therefore depress many GC-PC synapses. More recently, Brunel and colleagues have argued that adding a reliability requirement to PC associative learning leads to the silencing of weak synapses, thus leading to a large percent of silent synapses [63]. Silent synapses may therefore result from learning mechanisms.
4. A model of the olivo-cerebellar system as a temporal pattern generator

Based on the article:


4.1. Abstract

The olivo-cerebellar system has been implicated in temporal coordination of task components. Here I propose a novel model that enables the olivo-cerebellar system to function as a generator of temporal patterns. These patterns may be used for the timing of motor, sensory and cognitive tasks. The proposed mechanism for the generation of these patterns is based on subthreshold oscillations in a network of inferior olivary neurons, and their control by the cerebellar cortex and nuclei. This model, which integrates a large body of anatomical and physiological observations, lends itself to simple, testable predictions, and provides a new conceptual framework for olivo-cerebellar research.

4.2. Introduction

A mechanistic understanding of cerebellar function is limited to only a handful of simple behaviours, such as the vestibulo-ocular reflex gain adjustment [227] and certain instances of associative learning [228]. These are clearly just the tip of the iceberg: In mammals, the large cerebellar hemispheres communicate with the cerebral cortices, and are assumed to assist a variety of behavioural processes. The exact contribution of the cerebellum to higher brain functions is heatedly debated, and usually relies on suggestive evidence that lends itself to multiple interpretations. This stands in contrast to the crystal-like anatomy and circuitry of the cerebellum (Introduction, section 1.1) and its preservation throughout vertebrate phylogeny, which suggest that it performs a single basic function [167,175,229] (Introduction, section 1.4). One hypothesis is that the olivo-cerebellar circuit produces temporal patterns necessary for timing motor, sensory and cognitive tasks [187]. Here I present a model incorporating the entire olivo-cerebellar circuit that generates such temporal patterns upon request.
Existing models of cerebellar timing assume that temporal patterns are represented by the neuronal activity in the cerebellar cortex. The novelty of this model is that temporal patterns are actually represented by the output of the inferior olive. The role of the cerebellar cortex is to actively reconfigure the inferior olive to generate the correct temporal pattern.

4.2.1. Evidence for a role of the olivo-cerebellar system in timing

Evidence for a cerebellar involvement in timing is presented at length in the Introduction. In brief, converging evidence suggests that the cerebellum is crucial for accurate timing in the 10-500 ms range, in a variety of tasks, ranging from motor coordination (section 1.3.1) to sensory estimation (section 1.3.2). This suggests that the olivo-cerebellar system is the site of an internal timing mechanism in the tens to hundreds of millisecond range. A successful model of olivo-cerebellar timing must therefore provide a mechanism that can produce, upon request, accurate temporal patterns in this time range.

4.2.2. Current theories of cerebellar function

Current theories of cerebellar function are usually variations on one prevailing dogma [222]. According to this dogma, cerebellar output through the cerebellar nuclei (CN) is determined by Purkinje cell (PC) simple spikes, which in turn are determined by the parallel fibre input. The complex spikes, on the other hand, serve as an error / teacher signal that drives synaptic plasticity in the cerebellar cortex, eventually modulating simple spike output. In line with this dogma, many studies have documented simple spike rate modulation linked to sensory stimulation or motor activity [42,43,44] and complex spike activity that could be interpreted as an error signal [230,231,232].

Some theories adhering to this dogma, most noticeably the Braitenberg theories of cerebellar timing [173,175], claim that the structure of the cerebellum is tailored to represent time intervals. In these theories, the granule cells axons – the parallel fibres - act as delay lines, sequentially activating different PCs along a parallel fibre “beam”. Accumulating evidence seems to overrule this possibility (see Introduction, section 1.4.2). Other theories of cerebellar timing attribute timing to feedback loops in the granule-cell – Golgi cell pathway, but rely on shaky assumption (see Introduction, section 1.4.4).

An alternative mechanism, suggested by Llinás and colleagues and not adhering to the prevalent dogma, exploits the oscillations in the inferior olive (IO) as a means for creating a timing signal [116,137]. These subthreshold voltage oscillations [74,76,82,84], recently shown to exist episodically in vivo [94,95] (see Appendix 4.1), have been suggested to provide a clock signal for timing and synchronization. As will
be described below, these subthreshold oscillations can operate as a precise, modifiable pattern generator.

4.3. A novel model of olivo-cerebellar function

The proposed mechanism for the generation of a timing signal is based on subthreshold oscillations in a network of inferior olivary (IO) neurons, and their control by the cerebellar cortex and nuclei. I shall first describe the mechanism for creating temporal patterns in the IO. I shall then go on to describe how the cerebellar cortex via the CN can control the IO to generate different temporal patterns.

4.3.1. Olivary activity determines the olivo-cerebellar output pattern

Temporal patterns are carried by the spikes of IO neurons, and delivered to their targets in the brain via the cerebellar nuclei (CN) through direct olivary excitation and through complex-spike mediated suppression of PC inhibition. Four principles underlie the pattern generation capabilities of the olivary circuit:

- The timing of olivary spikes is locked to the subthreshold oscillations of olivary neurons.
- Olivary neurons oscillate in a coordinated fashion only within a functionally coupled network, within which all neurons are either directly or indirectly coupled.
- The oscillations propagate within the network, generating a variety of phase differences.
- The functional network is determined by decoupling GABAergic input from the CN (see Appendix 4.1).

According to these principles, an input from the CN will form a functionally coupled network that will start to oscillate with internal phase differences that determine the output pattern. Changing the functionally coupled network will produce a different temporal pattern. The duration of one pattern can be as short as a single cycle, in which case it will be non-repeating and include only the intervals determined by phase differences between the network units.
The generation of a temporal pattern and its control by the inhibitory input from the CN is schematically presented in Figure 4.1. The input to the IO is determined by the identity of the active CN inhibitory projection neurons (Figure 4.1A, light blue) and the inactive ones (dark blue), and I assume that this configuration remains constant during the generation of one temporal pattern. The active CN neurons functionally decouple their target IO neurons and hence suppress their oscillatory activity (Figure 4.1B, light grey), while the inactive CN neurons allow a sub-population of IO neurons to oscillate (Figure 4.1B, dark grey). The oscillations propagate within the sub-group of coupled IO neurons, creating phase differences (Figure 4.1C). These manifest themselves as time intervals between the output spikes of the neurons. The signals from all olivary neurons in the functional network are relayed to the CN, where they sum up to form the required output (Figure 4.1D). The spatial organization of CN activity and of IO coupling is thus transformed into a temporal pattern reflecting the phase differences between the neurons in the functional network.

**Figure 4.1. The transformation from a spatial input into a temporal pattern.** Blue rectangle represents the spatial organization of the input level (inhibitory projection neurons in the CN), with dark blue representing the inactive input (A). Grey rectangle represents the organization of coupling in the IO. Only neurons in the dark grey area, which did not receive decoupling inhibitory input, are effectively coupled and oscillating (B). The activity in the four representative neurons in the coupled area is shown in red traces (C).
Most components of the above schematic model are well established (see Appendix 4.1). Here I provide evidence for the ability of neuron pairs in the IO to maintain non-zero phase differences. This ability is crucial for creating time intervals shorter than the cycle duration. Pairs of IO neurons in vitro demonstrate subthreshold oscillations that maintain a non-zero phase (Figure 4.2a) [30,76]. The typical phase between the neurons in this figure can be extracted from the cross-correlation (Figure 4.2b) and has a value of $80^\circ$. Similar results have been demonstrated using voltage-sensitive dyes, showing propagating waves within the IO slice [76]. I further demonstrate that complex spikes in awake, harmaline treated rats, can also exhibit non-zero phase coupling between pairs of electrodes (Figure 4.2c,d). The non-zero phase differences endow this model with the ability to support sub-cycle intervals. The richness of the potential patterns arises from the ability to combine multiples of the cycle duration with these sub-cycle intervals.

![Figure 4.2. Phase differences between olivary units.](image)

**Figure 4.2. Phase differences between olivary units.** a. Dual recordings from 2 olivary neurons in slice that oscillate out-of-phase (adapted from Devor and Yarom [76]). b. Cross-correlation of the recordings from A demonstrate that the typical phase difference is $80^\circ$ and a time difference of $\sim 50$ ms. c. Recording of complex spike activity from two cerebellar sites in awake, harmaline-treated rats with chronically implanted multi-electrode arrays (Jacobson et al., unpublished results). d. Cross-correlation of the complex spikes times between the two cells exhibits a $135^\circ$ phase difference and a $\sim 45$ ms time difference.
4.3.2. The Cerebellar Cortex Controls Olivary Activity

The role of the cerebellar cortex is to reconfigure the oscillating parts of the IO to generate the required output pattern. Figure 4.3 schematically illustrates this process. The mossy fibre input, which can be viewed as a request for a certain temporal pattern (Figure 4.3A) configures PC activity to a specific arrangement of firing states. Some PCs will be excited and shift to the UP state, while others will be inhibited and shift to the DOWN state (Figure 4.3B; see also Appendix 4.2). Inhibitory projection neurons of the CN convey this configuration to the inferior olive (Figure 4.3C). By selectively decoupling parts of the IO a specific functionally coupled network is created, and the required temporal pattern emerges (Figure 4.3D). This pattern is then delivered to its targets via the excitatory projection neurons in the CN (Figure 4.3E).

Figure 4.3. The olivo-cerebellar model of pattern generation. Blue: Purkinje cells and their bi-stable firing pattern. Magenta: Inhibitory projection neurons of the cerebellar nuclei and their firing patterns. Activity in the inferior olive is limited to the functional network (red), where spikes are generated on top of subthreshold oscillations. The decoupled parts of the inferior olive (grey) are quiescent. Black: Excitatory projection neurons of the cerebellar nuclei convey the olivary output to other brain areas.
The mossy fibre input that controls PC state may also influence its firing rate within the UP state. I argue though that the effect of state switching is more dramatic and exerts a stronger effect on the downstream CN neurons.

Neuronal signals are composed of spikes organized in time, and are by their very nature temporal patterns. What, then, is unique about the model I propose? In this model, the entire olivo-cerebellar circuit works in unison to continually transform contextual inputs into temporal patterns. Most crucially, these contextual inputs are defined by their spatial organization, i.e. the identity of the activated mossy fibres whose activity does not contain any internal temporal structure. The temporal structure is generated de novo by the olivary network.

4.4. Reversing the roles of the inferior olive and the cerebellar cortex

The crux of the model presented here is the reversal of roles between the cerebellar cortex and the IO. In this model, patterns generated by the IO constitute the output signal of the entire olivo-cerebellar circuit. This stands in sharp contrast to the prevalent dogma in which the IO is assumed to provide an internal teaching signal for the cerebellar cortex. The role of the cerebellar cortex is likewise changed. In the prevalent dogma, PC simple spikes carry the cerebellar output, while in this model their role is to reconfigure the functional network in the IO, and can therefore be viewed as an internal signal of the olivo-cerebellar circuit.

The need for a role reversal is driven by the inability of the prevalent dogma to account for the intrinsic origin of simple spikes, and its difficulty in deciphering the Purkinje cell output. The latter results from the convergence of hundreds of PCs [233,234] onto a single CN neuron. The rate of synaptic inputs onto a single CN neuron is therefore expected to be on the order of 10³/s. Changes in the firing rate of a single PC are well beyond the ability of any neuron to resolve. Correlations between simple spikes of different PCs may potentially overcome part of this readout problem. But, the mossy fibre input which may underlie such correlations is expected to affect mostly PCs lying along the parallel fibre beam, while PCs that converge onto a single CN neuron are organized in parasagittal bands [203]. These physiological and anatomical observations cast a significant shadow on the prevalent dogma, and a radical conceptual revision is necessary.
4.5. **Purkinje cell signalling**

The temporal patterns created in the IO contain time intervals spanning tens to hundreds of milliseconds. An inhibitory input to the IO must maintain persistent activity for the entire duration of this pattern. Therefore, in this model, inhibitory projection neurons in the CN are viewed as binary elements, either active (decoupling their IO targets) or inactive (retaining IO coupling). As shown in Figure 4.3, CN neurons do not require internal mechanisms for such persistent activity, but merely reflect the state of upstream PC simple spike firing. Purkinje cells possess the biophysical mechanisms necessary for supporting these long time scales. Membrane potential bi-stability (see Appendix 4.2) is reported downstream by simple spikes that are only produced during the UP state. Simple spikes therefore serve as an internal state-reporting signal for reconfiguring the IO via the CN, and therefore in this model their fine temporal details are immaterial.

4.6. **Phenomena not directly addressed in the proposed model**

There are several properties of the olivo-cerebellar circuit that the hypothesis does not directly address. The most important one is the role of excitatory inputs to the IO. In this hypothesis, the role of the IO is to generate temporal patterns of activity. However, IO neurons also receive significant excitatory input from a wide range of peripheral sources and cerebral sources via the mesodiencephalic junction [26,235,236]. What role do these inputs have? One possibility is that the oscillatory activity by itself is insufficient to evoke olivary output, and that the role of these inputs is to provide a background level of excitation upon which the subthreshold oscillatory activity can trigger action potentials. Another possibility is that the IO is capable of producing temporal patterns with accurate relative times, but requires an external, phase-resetting input, to initiate the pattern at the correct absolute time. Last, unexpected events may activate IO neurons outside their typical phase. Strong climbing fibre activation may change the PC configuration, allowing the system to deal with the unexpected situation.

The mossy fibre collaterals that innervate neurons in the CN [237] are also not assigned a specific role in this model. Recent work suggests that it exerts a weaker effect on CN firing than does the IO output [179].

Last, future work would have to address the apparent conflict between the two roles attributed to the olivary output – a signal that switches between depression and potentiation of parallel fibre synapses, and the olivo-cerebellar output in this model. The issue is addressed in a recent paper [238].
4.7. Associating contextual inputs with a temporal pattern

The major task of the cerebellar cortex in this model is to associate a specific contextual input, carried by the mossy fibre signal, with a specific reconfiguration of the IO. This association, I propose, is not hard-wired, but acquired through experience. To achieve this, the cerebellar cortex must learn to associate a certain mossy fibre input with a certain configuration of PC states. The well documented bi-directional plasticity at the parallel fibre synapses [56,58,225,239] can serve to create the required association. The parallel fibre synapses onto PCs that must be shifted to their UP state are potentiated, such that when the contextual input arrives, the depolarization is strong enough to induce this shift (see Appendix 4.2). To shift the other PCs to their DOWN state, it is not enough to depress the corresponding parallel fibre synapses onto them. The depression of direct excitatory synapses from parallel fibres onto PCs must be accompanied by a potentiation of the di-synaptic inhibition [68,69], and I suggest that the potentiated inhibitory input is strong enough to shift PCs to their DOWN state (see Appendix 4.2).

4.8. Comparison to other cerebellar timing hypotheses

In what way is this theory novel, compared to other theories of cerebellar timing? This theory provides a biophysical and systems-level mechanism that can account for cerebellar timing on the relevant time scales. The Braitenberg theories [173,175] of timing fail on account of their short time scales (< 10 ms), and the Llinás theory of an olivary clock [116,137] can only support timing on multiples of the clock cycle (~100 ms). This theory provides a biophysical mechanism for covering the entire time range attributed to the cerebellum. The subthreshold oscillation cycle provides a clock signal, whose multiples cover the long range of supported times. Phase differences within the olive divide each cycle into shorter intervals that cover the short range of supported times. This theory further provides a systems level description of how the cerebellum and the inferior olive may interact to transform contextual inputs into temporal patterns. The olivo-cerebellar interaction “prunes” olivary areas that generate erroneous phases, while retaining only the correct ones.

In a recent review, Kitazawa and Wolpert [200] addressed the problem of complex spike patterns, that under different conditions appear to be either rhythmic, random and possibly also chaotic. I suggest that the different accounts of complex spike firing reflect the dynamic control of olivary oscillations. Olivary output would be rhythmic in areas that are actively supporting rhythmic tasks, and would appear much more random in areas involved in intermittent tasks, because the underlying functional network would be constantly changing. To resolve the conflicting accounts it
would be necessary to measure complex spike activity simultaneously from many neurons that are causally related to a specific task.
Appendix 4.1 - Inferior olive oscillatory activity is a network phenomenon

Neurons of the inferior olive (IO) can exhibit subthreshold oscillations of the membrane potential in the range of ~5-15 Hz. A controversy exists as to whether these oscillations require electrotonic coupling between olivary neurons [70,72,74,109] or whether they also occur in decoupled neurons [77]. This controversy has little bearing on this hypothesis, because the mechanism I suggest for suppressing olivary oscillations is GABAergic inhibition, whose shunting effect goes beyond effective decoupling and is known to suppress oscillations [30].

Oscillations in neighbouring olivary neurons tend to be correlated (Figure 4.4a) [76]. The existence of IO oscillations in vivo has for a long time been a source for debate, partly because it is usually monitored indirectly by observing complex spikes in Purkinje cells. Two recent papers demonstrate directly that IO neurons exhibit oscillatory activity in vivo (Figure 1b, top panel), although it may be highly non-stationary (Figure 4.4b, lower panel) [94,95]. Action potentials are locked to the peak of the oscillation but are elicited in only a minority of the cycles. The non-stationary nature of the oscillations, together with their limited representation in the spike output, may be the source for the conflicting reports regarding in vivo IO oscillations.

The dependence of IO oscillations on functional electrotonic coupling was demonstrated recently by Placantonakis et al. [80]. A mutated gap junction gene was virally introduced to the IO of adult rats, causing a sub-population of IO neurons to become electrotonically isolated. Only IO neurons that maintained coupling to their neighbours exhibited voltage oscillations (Figure 4.4c, right panel; red blobs indicate dye-coupled somata), while oscillations were absent in uncoupled IO neurons (Figure 4.4c, left panel). This observation was replicated by application of the gap junction blocker carbenoxolone.

The effective coupling between neighbouring IO neurons can be modulated by inhibitory input. By elevating the local membrane conductance, the inhibitory input effectively shunts the current flow between the coupled cells (Figure 4.4d) [30,89]. Indeed, transient application of GABA immediately terminates the subthreshold oscillations (Figure 4.4d). It is thus likely that the Inhibitory projection neurons of the cerebellar nuclei that preferentially target the dendro-dendritic gap junctions between IO neurons [29] act in a similar way, modulating the subthreshold oscillations.

In conclusion, IO oscillations arise from network properties which can be controlled by inhibitory inputs from the cerebellar nuclei. The IO is thus viewed in this model as a controllable oscillator, and the cerebellar cortex, via the cerebellar nuclei, is viewed as its controller.
Figure 4.4. a. Dual recording from two olivary neurons in slice (top). Both cells oscillate at ~4 Hz. Zoom in (bottom left) shows that these cells oscillate in phase. The cross-correlation (right) peaks at 0 ms phase lag. Adapted from Devor and Yarom [76]. b. Intracellular recording in vivo from an olivary neuron demonstrates epochs of subthreshold oscillations (top). The voltage spectrogram (below) demonstrates the non-stationary character of the signal. Adapted from Chorev et al. [94]. c. Bottom: Two examples of olivary cells (black) surrounded by dye-coupled cells (red) and virally-infected cells (green). The virus prevents cells from expressing functional gap junctions. Amplitude of subthreshold oscillations decreases with viral infection. Top: The power spectrum corresponding to each of the two cells. Adapted from Placantonakis et al. [80]. d. GABA suppresses subthreshold oscillations in olivary neurons. Top: Schematic illustration of the shunting effect of GABA conductance on coupling. G.J.: Gap junction, connecting the dendritic spines of two IO neurons. Arrows denote size and direction of current flow. Bottom: Whole cell recording from an olivary neuron demonstrates the ability of GABA to suppress oscillations. GABA application is denoted by a bar. Adapted from Devor and Yarom [30].
Appendix 4.2 – Purkinje cell bi-stability

The high rate of simple spike activity in Purkinje cells (PCs) is well documented and is usually assumed to reflect the input arriving from mossy fibres via the granule cells. However, recent studies challenge this classical view, by demonstrating that spontaneous simple spike activity is mostly independent of synaptic input and is of intrinsic origin [37,38,240]. Furthermore, PCs exhibit bi-modal dynamics of firing rate, switching between epochs of high rate of spontaneous activity and quiescence (Figure 4.5a, top). Bi-stability of PC membrane potential has been shown to underlie this bi-modal firing pattern (Figure 4.5a, middle). The spontaneously active state reflects depolarization of membrane potential (UP state) whereas the quiescent state is associated with membrane hyperpolarization (DOWN state). The intrinsic origin of membrane potential bi-stability is demonstrated by the ability to induce state transitions with brief intracellular current injections. Persistent Na⁺ currents are crucial for this bi-stability, but other currents (e.g. Ih, Ca²⁺-activated K⁺) and neuromodulators can modulate both the membrane potential within each state and the threshold for transition between them [37].

Bi-modal firing of PCs was first noted by Bell and Grimm in the late 1960s [101], but its relevance to the behaviour of awake animals is still a matter of heated debate. Recently, Schonewille et al. [39] reported that PCs in awake restrained mice fire continuously, suggesting that they operate in the UP state only. Conversely, preliminary results obtained in freely moving rats [41] and awake restrained cats [40] demonstrate that at least 50% of PCs exhibit bi-modal dynamics. This controversy would be resolved once the membrane potential of PCs from awake, behaving animals would be recorded.

The ability to control the timing of state transitions may endow bi-stability with functional significance. Indeed, Loewenstein et al. [38] have demonstrated that complex spikes can toggle between the two states. Here it is demonstrated for the first time that mossy fibre input can shift PCs to the UP state via direct excitation from granule cells and to the DOWN state by indirect inhibition via molecular layer interneurons (Figure 4.5b). I therefore propose that PCs are designed to function in two discrete states and that the role of synaptic input is to switch between these states rather than to govern the accurate timing of simple spikes.
Figure 4.5. a. Firing rate bi-stability of a PC recorded from an anaesthetized guinea pig. Above: Trace of extracellular recording. Middle: Whole-cell recording from a different cell. Below: Histogram of extracellular firing rate (left) demonstrates that the PC operates in two different firing rate regimes, and histogram of membrane potential (right) from whole-cell recording demonstrates membrane potential bi-stability. b. Synaptic input controls the PC state in a rat cerebellar slice. Electrical stimulation of granule cells evokes an EPSP in the overlying PC, while stimulation of molecular layer interneurons located sagittally to the recorded PC evokes an IPSP (upper traces). The two traces below demonstrate the ability of these inputs to control the PC state.
5. General Discussion

In my thesis, I have presented results that support the view of the inferior olive (IO) as a temporal pattern generator, and a novel model that describes how the cerebellum and IO may act in concert to generate temporal patterns upon request. Here I discuss the possible implications of my results. I then devote a section to a neglected aspect in the model – how can the cerebellum associate a certain contextual input, arising from mossy fibre activity, with a certain temporal pattern? I will argue that the apparent rift between olivary timing theories (e.g. the Llinás theory of an olivary clock and the model of chapter 4) and more classical theories of cerebellar learning can be reconciled under certain assumptions.

5.1. Simple spikes: Their role and read-out mechanisms

The prevailing dogma states that Purkinje cell (PC) simple spikes (SSs) result from coincident parallel fibre (PF) input. The fact that SSs prevail in the absence of synaptic inputs and that they cannot be correlated with any spatio-temporal structure of activity in the cerebellar cortex (chapter 3) calls for a reconsideration of their role.

5.1.1. Can spontaneous SS activity be attributed to coincident PF input?

The VSDI results presented in chapter 3 point against a role of PF activity in determining the of PC SS timing. High rates of SSs were not correlated with any average spatio-temporal progression of activity towards the active PC, even when averaging thousands of SSs. But other considerations also hint that under my recording conditions, SS activity cannot be attributed to tight coincidence of PF activity.

Chadderton et al. [16] report that the average spontaneous rate of GCs under urethane anaesthesia is 0.5 Hz. Isope and Barbour report that approximately 80% of the PF-PC synapses are silent [13,63]. Taken together, the overall rate of efficient PF synapses impinging upon a single PC can be calculated:

\[(EQ5.1) \quad R = N_{PF} \cdot 0.5 \cdot 0.2 = 0.1 \cdot N_{PF} \text{ sp/s}.\]

Assuming that \(N_{PF} = 200,000\), then \(R = 20,000 \text{ sp/s}\), or \(20 \text{ sp/ms}\). I denote the number of PF spikes arriving at a PC within 1 ms by \(M\). Assuming that \(\theta\) PF spikes must arrive within 1 ms to elicit a SS, and
that GC firing is Poisson\(^2\), the probability of causing a PC to fire is given by the tail of the Poisson distribution, which can be calculated numerically from the incomplete Gamma function [241]:

\[
P(M \geq \theta) = e^{-R} \sum_{k=\theta}^{\infty} \frac{R^k}{k!} = \frac{1}{\Gamma(\theta)} \int_0^R t^{\theta-1}e^{-t} dt.
\]

For a threshold of \(\theta = 150\) [63], the probability of spontaneous PC firing \((P(M \geq 150) \sim 10^{-77})\) is practically 0. This implies that PF inputs under these conditions are incapable of driving PC SS output and that PCs must be silent. This is certainly not the case, as PCs fire at high spontaneous rates.

![Figure 5.1. Probability of spontaneous PF coincidences eliciting PC firing. The probability of threshold crossing per 1 ms bin is colour coded as a function of two parameters: The PC spiking threshold \(\theta\) and the population rate \(R\) (in sp/ms).](image)

The functional dependence of PC firing on PF population rate and spiking threshold is shown in figure 5.1. The rate, \(R\), is the effective population rate per 1 ms (see EQ5.1). Several features of this graph should be noted. First, for low GC rates typical under anesthesia, the probability of reaching PC spiking threshold is extremely low, even if taking much lower estimates of \(\theta\). Second, high overall rates would result in a continuous generation of coincidences at a high rate. Only in a narrow range of rates distributed around \(R = \theta\) can the sensitivity to coincidences be maintained without continuously detecting them in the noise.

This caricature of PF activity being unable to drive PCs is obviously flawed in many respects. First, evidence suggests that PCs do not operate far from threshold. Cerminara and Rawson [36] have demonstrated \textit{in vivo} that even in the absence of excitatory synaptic inputs, PCs continue to fire SSs at a

\(^2\) I assume time-invariant spontaneous activity. The spatio-temporal patterns required to elicit PC responses can therefore be ignored, and the analysis be collapsed to EQ5.2.
high rate. The high firing rate seems indeed to be a property of the “UP” state of PC membrane potential [37,38]. If PCs can emit SSs irrespective of PF inputs, it suggests that PCs operate close to, or above their spiking threshold. Under such conditions, the view of the PC as a perceptron [63,167,226] seems inadequate. Neurons in the cerebellar nuclei (CN), downstream of the PC, could therefore not interpret SSs as saying anything obvious about PF inputs.

Could SSs that did result from coincident PF inputs have some “privileged” readout mechanism? One way this could happen is if many PCs responded with SSs to the same PF input, and impinged upon the same cells in the CN. This runs against our knowledge of the corticonuclear pattern of innervation. PCs receiving the same PF inputs lie along the medio-lateral axis, while parasagittal bands of PCs converge upon the same CN site [31]. Thus, there is no anatomical substrate for convergence of PF-responding PCs onto a single CN neuron.

I therefore suggest that the SS signal does not carry exact temporal information of PF activity. Information may either be carried in the overall rate of PF activity, or more extremely - in the absence or existence of spikes, as determined by the bi-stable dynamics of PCs and their control by external inputs.

5.1.2. Simple spike rate code?

Simple spike firing has been shown to be a bad candidate for reporting PF coincidences, and my experimental results argue against it. SSs may still carry information relating to the granule cell (GC) input, including PF input, through rate modulation. This view is in line with the many reports of SS rate modulation as a function of sensory input or motor activity (e.g. [42,43,44]).

Could GC-driven rate modulation be efficiently read out by downstream CN neurons? This is difficult to answer. Hundreds of PCs impinge upon a single CN neuron [233,234]. Rate modulation of a single PC is thus likely to be diluted and impossible to discern. Rate co-modulation of many PCs lying along a parasagittal plane may be more efficiently transmitted to CN neurons. Receptive fields in the cerebellar cortex do indeed have some rostro-caudal extent beyond that of a single PC. Future work should address the question of rate co-modulation in groups of PCs that have common CN projection sites.
5.1.3. Simple spikes as reporters of PC internal state

Another possibility, explored in chapter 4, is that SSs serve to report that PCs are in the “UP” state of their membrane potential. This possibility is consistent with the intrinsic source of SSs, and with the bi-stable dynamics of PCs. This raises several questions:

i. Why is PC state an important property to report? I suggest that the cerebellar cortex, via the CN, controls dynamically the coupling, and hence the oscillatory properties, of the IO (see Results, section 4.5.2). Bi-stable dynamics endow PCs with the ability to retain the IO in a certain configuration on the required long time-scales (hundreds of ms to seconds).

ii. Who determines the PC state? Two signals have been shown to influence PC state. CSs have been shown to “toggle” PCs between UP and DOWN states [38]. Because the same signal is capable of moving a PC both to its UP and to its DOWN state, it is unclear how CSs can deterministically control PC states, which will unavoidably depend on its past configuration. Furthermore, CSs can be viewed as self-interfering signals: Once the IO is in a certain configuration, it will generate CSs that will distort the required PC configuration via toggling, perhaps before the termination of the entire pattern.

Another possibility, elaborated in Results (Appendix 4.2), is that PC state can be controlled deterministically by PF input. Direct PF excitation can shift PCs to the UP state, while disynaptic inhibition can shift them to the DOWN state (figure 4.5). This provides a better method for deterministically controlling PC state without history-dependence.

iii. Can CN inhibitory projection neurons reliably report PC state to the IO? In the model proposed in chapter 4, inhibitory projection neurons of the CN should reliably report PC state to the IO. One problem is that the PC-CN synapse undergoes a strong short-term depression (STD) [242,243], thereby failing to reliably transmit the upstream PC states for long durations. It has still to be shown whether STD is uniform throughout the CN, or is – as the model of chapter 4 would suggest – absent in the inhibitory PNs.

Another problem is that CN neurons typically possess transient components in their spiking activity, most noticeably rebound firing following hyperpolarisation. These components may interfere with the simple coding scheme proposed in chapter 4: That PC quiescence translates into CN inhibition and IO decoupling, while PC firing inhibits CN neurons, thereby retaining coupling in target IO areas.
A recent report suggests that the rebound properties attributed to CN neurons rarely manifest themselves in vivo [244], which implies that CN neurons are better suited for transmitting PC state.

5.2. An “olivary clock” or olivary patterns?

The idea that IO oscillations could act as a neuronal clock is more than three decades old [89] (see Introduction, section 1.4.3) and has been related to the fact that motor activity appears to be segmented in the 7-9 Hz range [135,136,137,138] (Introduction, section 1.3.1). Despite this, and despite the evidence from Essential Tremor (ET), this proposal must tackle two serious problems: The lack of regularity reported in many in vivo studies of olivary activity [94,113,200], and the inability of a ∼10 Hz clock to cover the short time range in which the cerebellum has been implicated [161].

The proposal raised in chapter 4 is that coupled networks within the IO are assembled ad hoc for different tasks. These configurations of the IO network may persist for hundreds of ms to seconds. Therefore, the propensity of the IO to oscillate does not manifest itself as regular oscillations. Constant inhibitory input from the CN serves to “prune” the olivary coupling and retain it only in the parts necessary for generating a certain temporal pattern. This view of IO oscillations can potentially resolve the discrepancy between the conflicting reports. The existence of olivary patterns (which need not be regular) must be tested in the context of a behaviour requiring timing capabilities in the 10-500 ms range. Some evidence in this direction has been provided for repetitive licking [115], but in general this is work that has yet to be done.

Timing in the < 100 ms range can be solved by my finding that different parts of the IO oscillate with a non-zero phase difference, and maintain this phase difference for long durations and across frequency changes. For a given frequency, a non-zero phase difference translates into a time interval which is necessarily shorter than the cycle duration. This stretches the time range which the IO can support down to the 10 ms range.

5.3. The read-out of olivary activity in the cerebellar nuclei

The output of the olivo-cerebellar system reaches its targets in other brain areas via the excitatory PNs in the CN. Neurons in the CN receive several inputs: excitatory collaterals of both mossy and climbing fibres, and convergent inhibition from many PCs. Can excitatory PNs report reliably the IO activity, as necessary by the model proposed in chapter 4?
A recent paper from Dieter Jaeger’s group demonstrates that in vivo, CN neuronal activity has the closest resemblance to IO output [179]. Thus, the cerebellar output is capable of transmitting the olivary signal. This can be done through several mechanisms. First, several nearby IO neurons, whose oscillations are in-phase, may converge upon a single CN neuron, generating a large response [245]. Second, IO neurons influence CN firing indirectly via the CS-induced pauses in SS firing. Such pauses may release CN neurons from inhibition and allow them to report IO activity.

5.4. Testing the model

The differences between the proposed model of chapter 4 and the prevalent dogma are large, and some of the major differences are listed in Table 5.1. These differences lead to several testable predictions which potentially have the power to distinguish between competing theories of cerebellar function.

The major prediction of the model regards the role of the IO in the execution, and not only the learning, of tasks requiring timing. In the prevalent dogma, IO spiking is mostly viewed as a “teacher” or “error” signal which drives learning in the PF-PC synapse (and at other synaptic sites) through LTD/LTP. Thus, the execution of learnt tasks is not expected to be hampered by single-trial perturbations of IO activity. The pattern generation model posits that IO output is the signal that controls accurate timing after learning. Thus, perturbing IO activity on a single-trial basis should result in increased time variability.

Because the model claims that the IO generates spatio-temporal patterns required for accurate temporal execution of tasks, it is crucial to create paradigms in which accurate timing in the 10-500 ms range is required for successful execution of the task. I suggest two possible tasks that are well suited for distinguishing between prevalent hypotheses and the hypothesis presented in chapter 4 of the Results. Both tasks have a simple design and may be applied in rodents, hopefully making the IO interventions suggested below possible. One task requires a motor implementation of timing, and the other sensory estimation of time.
Prevalent view of cerebellar function | Temporal pattern generator model
--- | ---
Role of simple spikes | Report coincident GC inputs, constitute the cerebellar cortical output. To report the state of PC and hence configure the IO coupling via CN inhibitory PNs.
Role of IO spikes | Internal “teacher” / “error” signal of the cerebellum, driving learning in PCs. Dominate cerebellar output and carry the required temporal patterns.
Bi-stability | Ignored. Enables PCs to maintain a required output for long durations (the duration of the required patterns).
ML interneurons | In some theories: Provide disynaptic inhibition and shorten PC time window for integration. Switch PC firing to the DOWN state.
IO oscillations and electrotonic coupling | Usually ignored. Enable the generation of reproducible spatio-temporal patterns.

*Table 5.1. Major differences between proposed model (chapter 4) and prevalent view of cerebellar function.*

5.4.1. *Motor task: Operant conditioning*

In operant conditioning, a subject learns to associate a certain action with a certain outcome which has a behavioural valence. For example, rats may learn to associate lever pressing or nose poking with a food reward. I suggest a slight modification of the usual operant conditioning paradigm, in which subjects will learn that only a *pair* of lever presses at a required time interval results in reward. My claim is that to properly perform the well-timed pair of lever presses, the motor system will rely on an accurate timing signal arising from the IO. Thus, by interfering with IO activity after acquisition of the task, the lever presses will exhibit larger timing variability.
5.4.2. Sensory estimation: Classical conditioning

In classical conditioning, a subject learns to associate an indifferent, “conditioned” cue, with an “unconditioned” stimulus, e.g. an irritating signal such as an air-puff to the eye. An unconditioned stimulus elicits a response, e.g. an eyeblink. After acquisition, a conditioned response emerges. This response is similar to the unconditioned one, but starts before the expected arrival of the unconditioned stimulus (i.e. it is a predictive response), peaking at the expected arrival time. For certain inter-stimulus intervals (~100-500 ms), the cerebellum plays a crucial role in classical conditioning [151,152,153].

I suggest an experimental design akin to the operant conditioning above. Subjects will learn to associate a tone pair with a certain inter-stimulus interval with the unconditioned stimulus. Subjects will learn that tone pairs with a certain jitter do predict the unconditioned stimulus, while a larger jitter does not. As above, the model predicts that performance will be hampered by perturbing IO activity, while in the prevalent view IO activity is required for the learning phase only.

5.4.3. Perturbing IO activity on a single-trial basis

The method chosen for perturbing IO activity is of utmost importance. It has been shown that pharmacological manipulations of IO excitability can result in extinction of acquired conditioned responses or to their maintenance despite employment of an extinction paradigm [246]. This implies that synaptic mechanisms may “kick in” quite fast. I therefore suggest that perturbation tests should be done on a single trial basis, and interleaved with non-perturbed trials.

Mere hyperpolarisation of the IO may yield paradoxical effects, as olivary oscillations may actually be amplified [82]. Perfusion with GABA antagonists may persist for too long and interfere with learning processes, and not only execution. A possible perturbation method would be to activate inhibitory PNs in the CN on a single-trial basis, either using electrical stimulation or optogenetics [247]. This would lead to a temporal decoupling of the target area in the IO with single trial resolution, and allow addressing this important issue.
5.5. Reconciling cerebellar learning and olivary timing

Two views of the olivo-cerebellar system have for several decades stood in apparent contradiction. One view stresses error-driven learning in PCs, where the IO is viewed as the source of an internal cerebellar “error signal” that drives GC-PC plasticity. Another view is that olivary oscillations and CS synchrony reflect the existence of an intrinsic neuronal clock that coordinates and synchronises components of complex motor tasks (see Introduction, section 1.4.3).

For the most part, the proponents of each of the above views have either overlooked or plainly discarded the other view. For example, proponents of olivary timing have questioned the relevance of plasticity mechanisms in the cerebellum for learning and behaviour [248]. Proponents of the learning view tend to overlook the emerging physiological data, such as intrinsic SS firing, olivary oscillations etc. (e.g. [63]). In this section, I present in outline a synthesis of these apparently contradictory views.

5.5.1. Cerebellar learning: Mapping contextual inputs into a state-map of Purkinje cells

In the model presented in chapter 4, MF inputs determine which PCs are in the UP state and which are in the DOWN state. This UP/DOWN configuration in turn configures olivary coupling to produce a specific temporal pattern. The olivo-cerebellar circuit thus performs a spatial-to-temporal transformation.

I suggest that cerebellar learning is the association of a certain MF input with the required UP/DOWN configuration of PCs. Learning takes place through a change in the balance of excitatory vs. inhibitory GC input onto PCs. The determinant of plasticity direction in both cell types seems to be the complex spike [68,225]. For learning of MF-PC state associations to be consistent with olivary pattern generation, CS activity must act both as an output and as a teacher. Is such a dual role for IO output possible? A positive answer to this question can be obtained in a certain framework under several assumptions.

5.5.2. A framework for complex motor task learning

The learning paradigm considered here is the automatisation of complex motor tasks. Automatisation has two aspects: (1) Motor primitives that are initially performed individually in a decomposed manner are merged into a continuous flow and into one flowing movement, and (2) the acquisition of accurate timing. These two aspects are obvious to anyone who has tackled e.g. a piano piece. Initially, notes are played in separation, each one explicitly performed. After learning, playing
progresses smoothly, and the need for attending to each note is lost while flow and temporal acuity are gained.

*Figure 5.2. Olivo-cerebellar learning leads to the accurate temporal execution of otherwise variable movements.* Three different finger movements (top) must be combined in an accurate temporal order (x-axis). Cerebral commands (middle) have large temporal variability across repetitions (y-axis) but with the correct mean value. IO signal after learning has low temporal jitter and renders individual cerebral commands unnecessary.

I shall denote the required sequence of actions and action times of a complex motor task as an ordered set of pairs $\{(a_k,t_k)\}_{k=1}^\theta$. Before automatisation, motor cortex attempts to perform explicitly the individual components. This probably requires attentional resources. Furthermore, I assume that motor cortex is incapable of accurately producing time intervals, and therefore the actual performance suffers from trial-to-trial variability, i.e. the actual times $\hat{t}_k$ are noisy: $\hat{t}_{k+1} - \hat{t}_k = t_{k+1} - t_k + \delta_k$, where $\delta_k$ is a noisy jitter with zero mean, and a variance which reflects the maximal temporal acuity possible without the cerebellum [161, 185]. After learning, the cerebral cortex only provides a “contextual” input to the cerebellum, thereby lowering the attentional load and “outsourcing” the relative timing of movement components to the olivo-cerebellar circuit (figure 5.2).
5.5.3. Cerebellar learning: Assumptions

Several assumptions are necessary for the learning paradigm presented below to be consistent with olivary pattern generation. These assumptions are consistent with our knowledge of cerebellar and olivary anatomy and function, but are sometimes of a speculative nature.

i. The structure of IO maps. The model assumes that different elementary actions are represented in the IO. An action can be a motor primitive (e.g. left index flexion), a sensory tag (e.g. “the tone has ended”) and is defined by the hard-wiring of IO inputs and outputs. Each representation in the IO is wired to a CN “module” and can drive the appropriate action. This is in line with Marr’s view of olivary activity as eliciting basic motor primitives [167]. The added feature is the assumption that for each action $i$, there exist multiple representations in the IO: $a_{i1}, a_{i2}, \ldots, a_{iN}$. This multiple, distributed representation is inspired by the fractured, repeating maps of sensory receptive fields in the cerebellar cortex (figure 5.3a). I further assume that different representations of the same action maintain a certain constant phase difference when oscillating (figure 5.3b).

![Figure 5.3](image-url)  
**Figure 5.3.** Maps in the cerebellar cortex and the inferior olive. a. Fractured somatotopy of cerebellar cortex, with each body parts represented multiple times. Taken from [23]. b. Putative “action map” in the IO. Each action is represented multiple times, and different representations oscillate with phase differences.
ii. **Structure of cerebellar input:** The cerebellar input contains three components:

a. **A noisy “teacher” signal:** A set of action-time pairs \(\{(a_k, t_k)\}_{k=1}^{Q}\) sequentially activates the different IO actions.

b. **A contextual input:** A persistent input through the MF that is unique to each motor task.

c. **Phase reset signal:** At the start of each trial, a cerebral input resets olivary phase. Not all IO representations are reset to the same value, and therefore different representations of the same action retain their typical phase differences. The phase of \(a_{i1}\) (the first representation of an action \(i\)) is assumed to be reset to \(180^\circ\) (see figure 9 in [76]).

5.5.4. *The learning process*

The aim of the learning process is to automate the complex motor sequence. The automatisation manifests itself as a loss of the noisy cortical signal \(\{(a_k, t_k)\}_{k=1}^{Q}\) and its replacement by an accurate olivary signal \(\{(a_k, t_k)\}_{k=1}^{Q}\). At the end of learning, a combination of a contextual input and a phase reset signal is enough to elicit the learnt sequence.

To simplify the explanation of the learning process, I choose to explain a relatively simple compound movement, composed of two actions, \(a_{1}\) and \(a_{2}\). I ignore the question of movement onset, which I assume continues to be controlled by the cerebral cortex and its phase reset signal. The cerebellum, then, has to learn the correct time interval \(\Delta t \equiv t_2 - t_1\). The training set is \(\{(a_1, t_1^{(i)}), (a_2, t_2^{(i)})\}_{i=1}^{P}\).

The basic sequence of events during learning is depicted in figure 5.4. A cortical command arriving at the IO manifests itself as an EPSP on top of the olivary oscillations. The IO oscillations are assumed to be subthreshold, or to eliciting very few suprathreshold spikes. The EPSP summates with the oscillatory membrane potential and elevates the probability of producing a spike. I assume that the probability of an EPSP producing a spike is close to 0 when the oscillation phase is \(180^\circ\) (trough) [249], and high for phase values close to \(0^\circ\) (peak). The sign of PF-PC plasticity depends on the arrival of CSs. In the PC connected to the \(180^\circ\) representation, the contextual PF input leads to LTD, due to the paucity of CSs generated at that phase. In other representations, there are more CSs and learning favours LTD. As elaborated above (Results chapter 4), these may lead after learning to a mapping of the PF input into specific configuration of PC UP/DOWN states (figure 5.5, top). This configuration, in turn, decouples all olivary representations that assumed \(180^\circ\) at wrong timings (figure 5.5, bottom).
Figure 5.4. Inferior olive oscillations gate teacher signal to PCs. From bottom to top: Caricature of phase values in the IO At the correct time $t_2$. Each representation of action $a_2$ (blue circles) oscillates at a different phase. Cerebral motor commands arriving at this time elicit complex spikes except for the olivary representation which is at 180° (blue arrow). The CS signal switches direction of PF-PC plasticity from LTP (green) to LTD (red). This leads to LTP only of the PC connected in loop with the 180° IO representation.

After learning, complex spikes are produced only by the coupled parts of the IO. Thus, the learning process can be viewed as a process of pruning all oscillators in the IO that fail to predict the required time.

It may initially seem strange that the temporal pattern is carried by oscillators that are at 180°, and hence incapable of generating IO spikes. This is the reason that this hypothesis posits that the olivocerebellar circuit subserves only relative timing. At a delay of half cycle duration, the coupled parts of the olive produce the same temporal patterns in their peaks, where spikes are more readily produced. Therefore, during actual motor performance, MF activity and phase reset activity must precede the required execution time, and absolute timing may have to remain a cerebral task.
Figure 5.5. PC control of IO oscillations. After learning, the PF context switches PCs that underwent LTD to the DOWN state, and PCs that underwent LTP to the UP state. SS firing is high only in UP PCs (top arrows), inhibiting inhibitory PNs in the CN and retaining IO coupling in its target (white background). DOWN PCs allow CN neurons to inhibit the IO and decouple the erroneously timed representations of $a_2$.

5.5.5. Predictions of the hypothesised synthesis

The major prediction of this hypothesis is that IO activity gradually acquires an accurate temporal profile necessary for task execution. At the same time, the cerebral cortex is released from the need to explicitly define each movement component. This leads to the prediction that cerebral activity should lose self-generated components of neuronal activity that are related to individual movement components. Cerebral activity may still contain timing signals arising from olivo-cerebellar feedback.

Another prediction is that in the IO should resemble that of the cerebellar cortex: It should contain multiple representations of the same “action”, but with different phase of oscillation (which may imply that they are spatially segregated).
The hypothesis further predicts that IO spikes would be crucial for both learning and performance. During learning, IO spikes determine the sign of plasticity changes in PCs. After learning, they report the temporal pattern generated by the “pruned” olive, which contains only those representations of actions that are well timed. Spatio-temporal IO patterns are therefore expected to emerge that mimic the cerebral activity prior to learning, albeit with superior temporal fidelity.

5.5.6. Problems with the hypothesised synthesis

The most severe problem with the hypothesis is how learnt temporal patterns are maintained. During execution of an acquired motor sequence, IO emits spikes that constitute the olivo-cerebellar output, but these return to their interconnected PCs and may act to depress the potentiated synapses! There are at least two ways this difficulty may be overcome: First, learning may gated by neuromodulatory signals (as has been demonstrated in cerebral cortex [250]) that are present only during learning. Second, LTD may require an excess of IO spikes [246]. Mere oscillations, without superimposed cerebral inputs to the IO which were lost during learning, may retain synaptic efficacies. Indeed, the loss of cerebral commands itself may stabilise the learning.

The proposed synthesis also presupposes several unsubstantiated phenomena. First, while phase differences in olivary activity were described on average (chapter 2), it is unclear how phase differences behave after phase reset. Second, evidence regarding loops between the IO and the cerebellar cortex still lack the resolution to determine whether small groups of neurons that share the same phase and “action” communicate reciprocally with a corresponding small group of PCs and CN interneurons.

5.6. Concluding remarks

After decades of cerebellar research, the olivo-cerebellar circuit still generates more questions than answers on the systems level. A plethora of theories exist, few of which build their way up from experimental data.

I have provided herein evidence that suggests the inferior olive can provide temporal patterns more diverse than previously thought. I have also provided evidence for the irrelevance of parallel fibre activity to the generation of Purkinje cell simple spikes. I combined these facts with other aspects of olivo-cerebellar physiology, into a model of olivo-cerebellar function.
Time (again!) will tell – through rationally constructed experiments - whether the suggested hypotheses survive the scientific process. They provide - whether they turn out to be entirely correct or flawed - a driving force for further research.
Bibliography

32. De Zeeuw CI, Van Alphen AM, Hawkins RK, Ruigrok TJ (1997) Climbing fibre collaterals contact neurons in the cerebellar nuclei that provide a GABAergic feedback to the inferior olive. Neuroscience 80: 981-986.


86. Chorev E, Manor Y, Yarom Y (2006) Density is destiny--on the relation between quantity of T-type Ca2+ channels and neuronal electrical behavior. CNS Neurol Disord Drug Targets 5: 655-662.


122. Rolando L (1809) Saggio sopra la vera struttura del cervello dell'uomo e degli'animali, e sopra le funzioni del sistema nervoso. Sassari: Stampa Privileg.


171. Uhr L, Vossler H A pattern recognition program that generates, evaluates and adjusts its own operators. Proc WJCC 19: 555-569.


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249. Mathy A, Ho S, Davie JT, Clark BA, Hausser M. Cerebellar climbing fiber axons transmit bursts of spikes; 2007; San Diego.

הזרקתי הקיטוב בצבענים פעילות בסיב פורקינייה. אני טונגסטן פוטנציאל עצב כי זמני פורג בלואר ייחודי, בתיאום וב.mainloop תיאום של פורקינייה (p) המזינים לשריון המזימים את העפעורים, מסוגים מזוויות מוסיפים, בいろ אל בורר, ביניהם מזימים, בין מזימים אטלס והActionButton. פורקינייה p של תאים פורקיניים, יאמית והיוויה של קיפוף המזימה, פעילות הפירוקינייה בינו תאים ומשורות הפעילות של פורקינייה, פorest שמייצרים פעילות בגרעין, בנימה בחללים שלויות במזימה ובזיהוי. קליפת ncleus בתא, מחזור אחד, ביחס לזוות פעולות הפרוקינייה של התא, בודק傾י העבר של עיצורים. בודק傾י העבר של עיצורים. הפעילות פורקינייתית של מכונת-CN לסהデザイン פורקינייה, יאמית, יממה ולזרק, בודק傾י העבר של עיצורים. בודק傾י העבר של עיצורים. הפעילות פורקינייתית של מכונת-CN לסהデザイン פורקינייה, יאמית, יממה ולזרק, בודק傾י העבר של עיצורים. בודק傾י העבר של עיצורים. הפעילות פורקינייתית של מכונת-CN L安全事故 לסהデザイン פורקינייה, יאמית, יממה ולזרק, בודק傾י העבר של עיצורים. בודק傾י העבר של עיצורים. הפעילות פורקינייתית של מכונת-CN L安全事故 לסהتصميم פורקינייה, יאמית, יממה ולזרק, בודק傾י העבר של עיצורים. בודק傾י העבר של עיצורים. הפעילות פורקינייתית של מכונת-CN L安全事故 L安全事故 L安全事故 L安全事故
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וכל חום חשב על איתקה
כיﯨיושד או לוחיג שבום.
אף על קל חתו כי פוקח.
שיטוב שופך שיים רбот.
ишגרי כי היא שלק יומ.
שᙻיר בכלי שעך יברך.
אל האמת שאיתקה השגינו כי עשה.

איתקה העניה כי מקשים יפה
אלכליאו כי לא חקית כלל יוצר קלך.
ויור מנה כי לא חוכלophobic.

זיהנו כי🐟בשארה עינה - לא זמחהواجه איתקה.
Mother Goddess, Flora and Fauna.

1911, מיווש: יודר ברונובסקי

אם תצא בוץ אל איתקה
שלא כי יוצקך עד מקד.
מלאת בכרפטאותו, מקלאה בשצה.
אל חרי את מלקסימוניוו (או מתכוקלופיס).
אל חרי את פסידורי המלを使った.
לעלם לא תמגמה על דרכו.
ככל עד מאשיבוריך לשואות, גורשו מעליה.
פעמים אתי בפשש לאות ופקך מקה.
לא דפקול בלקסימוניוו במקוקלופיס.
לא פקודיוו חומס, אלא אס ב.
תסמיד קלאי פשך.

שואל כי יוצקךнесен תואר.
כי בקרביוו ביבס של קוח קלב.
בקובר, כליליאו כי כל.
לא קולס של ארי במלעה.
בקותר-מקושר פיגודון פטר
תקות סרובות מכתבון לבר.
פונים אוכלים, באגרה.
מצרים שואים על ביצורי טובי.
כלשך שוכק תקווה ביצורי טובי.
עברית זו נעתשת בהדרכתו של פרופסור יוסי י THAN
מאפייני מרחב- zaman בעיבוד מידיע
בקליפת המהות הקטן

ไฮובר לשמ קבלת תואר
דוקטור לפילוסופיה

מאת
גלווד יעקובסון

הוגש לסנט האוניברסיטה העברית בירושלים, ה-
טבת תשמ"ט
מאפייני מרחב-זמן בשיבוץ מידה
בוקליפט המהקה הקטן

תירור לשם كبלי תואר
דוקטור לפילוסופיה

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הוגש לסנט האוניברסיטי העברי בירושלים,
בת התשס"ט