

## ***CEREBELLAR CLASSIC***

### **PART I. THE COMPLEX SPIKES AS ONE OF THE CEREBELLAR SECRETS**

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The large majority of neurons of the inferior olivary nucleus project to the cerebellum contralaterally via the olivocerebellar tract. This bundle gathering the axons of the inferior olive is unique from a morphological, physiological and developmental standpoint [1]. A single olivocerebellar axon gives rise to multiple climbing fibers (CFs) and each CF makes hundreds of synapses across the highly branched dendrites of adult Purkinje neurons, wrapping around the proximal dendrites [2]. The multiple CFs originating from a single axon are arranged spatially in a narrow longitudinal band-shaped area, whereas the mossy fiber axons show a transversal distribution [1]. Overall, a triangular topographic loop of neuronal connections is formed between areas of the inferior olive, cerebellar cortex and cerebellar nuclei [1,3]. The olivary projection to the cerebellar nuclei is strictly reciprocal to the nucleo-olivary projection [3]. The olivonuclear projection, the organization of the climbing fiber projection to the cerebellar cortex, and the corticonuclear projection establish an appealing micromodular organization of connectivity in the brain [4]. As summarized by Ruigrok and Voogd [3], « When both the inferior olive and the cerebellar nuclei are considered as folded but continuous sheets of grey matter, the complete nucleo-olivary projection can be described as a simple transformation ». Another fascinating point is that cerebellar modules fit with the cortical compartments defined at a molecular level in Purkinje neurons [5].

The olivocerebellar projection displays anatomical plasticity especially in young ages. The unilateral transection of the cerebellar peduncle in newborn rats led to a complete degeneration of the contralateral inferior olive. In that instance, axons from the ipsilateral medial accessory olive recrossed the cerebellar midline and partially innervated the deprived hemicortex by terminating in the molecular layer and by observing a sagittal striped pattern as typical climbing fibers [6]. On the other hand, when pedunculotomy was performed on postnatal day 7, climbing fibers were found in the cerebellar hemisphere contralateral to the lesion, also arranged in sagittal bands and topographically organized, but entering through the ipsilateral inferior cerebellar peduncle, i.e., through a pathway not present in the adult animal [7].

Early autoradiographic tracing studies had shown retrograde labeling of inferior olivary neurons after injection of tritiated D-aspartate into the rat cerebellar cortex or nuclei; the observed decrease in aspartate release after 3-acetylpyridine-induced destruction of the inferior olive led to the hypothesis that aspartate was the neurotransmitter of climbing fibers [8]. However, later experiments with electron microscopic immunocytochemistry in rats and baboons showed that neurons of the inferior olive were uniformly labeled for both aspartate and glutamate, whereas only glutamate-like immunoreactivity in climbing fiber terminals was

similar to that in the parent cell bodies, suggesting that glutamate should be considered as a more likely neurotransmitter candidate than aspartate in the climbing fibers [9-10]. Further, double-labeling immunocytochemical studies in rats showed glutamate-immunopositivity in axons with the morphological characteristics of climbing fibers in close apposition to principal Purkinje cell dendrites or in synaptic contact with Purkinje dendritic spines [11].

In vitro experiments in slices of newborn rat cerebellum co-cultured with slices of inferior olive from 4-day-old rats indicated that voltage-gated conductances, which are substantially involved in the generation of the complex spike, are gated by the climbing fiber synaptic depolarization rather than directly by the climbing fiber neurotransmitter [12]. Moreover, decreased neurotransmitter release was found to be a major cause of paired-pulse depression at cerebellar climbing fiber-Purkinje cell synapses in rat cerebellar slices examined under voltage-clamp [13]. Purkinje cells in the rat appear to have an intrinsic tendency to develop postsynaptic assemblies of excitatory types, including glutamate receptors, over the entire dendritic territory [14]. In the adult mouse cerebellum, *N*-methyl-D-aspartate (NMDA) receptors of climbing fiber-Purkinje cell synapses contribute to the spontaneous complex spike activity by enhancing complex spike-evoked inward currents and after-hyperpolarization currents [15].

The synapse between CF and Purkinje neuron is considered as one of the most powerful excitatory synapses in the brain. The initial large amplitude spike is followed by small-amplitude spikelets. The complex spikes are much less abundant than the simple spikes which correspond to standard sodium-potassium action potentials and explain the spontaneous tonic mode of firing of Purkinje neurons [16]. The oscillatory activities of the inferior olivary neurons are modulated in particular by excitatory inputs originating in the vestibular system, the somatosensory system, and midbrain areas, resetting oscillations to trigger firing [17]. Adjacent inferior olivary neurons project to a narrow longitudinal striped area in the cerebellar cortex, often corresponding to a single aldolase C stripe [18]. This explains why Purkinje neurons arranged in a longitudinal band show a synchronicity in the firing of complex spikes.

Electrical stimulation of the parallel fibers triggers a release of glutamate which is followed by small depolarizing potentials at numerous sites on the dendritic arbors of the Purkinje neuron [19]. The mechanism of spatio-temporal summation causes a discharge of simple spikes at the level of the proximal axon. The summation is influenced by ionic-channel conductances, expression of calcium/potassium channels, and is conditioned by the morphology of the Purkinje neuron [19]. By comparison, the complex spike is mainly

mediated by sodium channels, and to some extent by calcium channels. It is followed by a hyperpolarization via calcium-activated-potassium channels, with a role of T-type calcium channels in the waveform of the complex spike and a role of potassium channels for spikelet generation [20]. Historically, the generation of the complex spikes has been a subject of discussion in the cerebellum community for several decades. The first spike is produced in the axon, as for the simple spike. CF input triggers dendritic spikes. Direct evidence that the spikelets within the complex spike of Purkinje neurons originate in the axon was provided in 2008 [21]. The propagation of dendritic spikes towards the soma is weak and fails to trigger additional axosomatic spikes due to threshold mechanisms and refractory period issues.

At a first glance, it may look « simple » to identify complex spikes visually and distinguish them from simple spikes. However, visual inspection even performed by the eye of an expert is tedious and may involve errors [16]. Deep learning algorithms based on action potentials and local field potential signals may become particularly useful to detect complex spikes reliably and fastly [16]. The advent of artificial intelligence will be very helpful to grasp the patterns of discharges of Purkinje neurons at a macro-scale level, taking into account bursting, tonic and silent patterns of discharges [19].

Overall, the olivocerebellar system is optimally structured to launch precise patterns of complex spikes and simple spikes during the acquisition and consolidation of motor skills [20]. It is likely that these spatiotemporal patterns complement rate coding to improve the accuracy of motor and cognitive processing in order to tune coordination and participate in predictions which are at the core of the currently prevailing internal model theory. For instance, CF inputs to the cerebellum may carry informations of reward-related predictions. The high frequency burst of spikes elicited by CF activation is critical for the operations handled by the cerebellar cortex. The elucidation of the roles of complex spikes has major impact on our appraisal of the roles of the cerebellar cortex in learning, motor control, cognitive tasks, and the processing of emotions. The error-driven plasticity conveyed by the olivocerebellar tract is the foundation of the original motor learning theory of the cerebellum and is currently considered as the main driver for the adaptation of internal models [22]. In addition to the Marr-Albus theory, there is also evidence that the olivocerebellar tract modulates the spiking of cerebellar nuclei and is also involved in the synchrony of Purkinje cells [23]. Therefore, complex spikes not only act as a teaching signal for Purkinje cells, but they also contribute to a complex function of common temporal discharges for which Purkinje neurons act as surrogate teachers for cerebellar nuclei neurons [24].

In this Cerebellar Classic, Christian Hansel discusses the physiological findings of

John C. Eccles, Rodolfo R. Llinás and Kazuo Sasaki using intracellular recordings, putting in perspective the critical importance of the complex spikes for cerebellar processing of neural information. The cerebellar circuitry still holds numerous secrets, and the coding of the complex spikes in relationship to motor, cognitive, social or affective operations is one of them.

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**CONFLICTS OF INTEREST**

The authors declare no conflict of interest.

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