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CEREBELLAR CLASSICS XI

Commentary on "E. Mugnaini and A. Floris, The Unipolar Brush Cell: A Neglected Neuron of the Mammalian Cerebellar Cortex. J Comp Neurol, 339:174–180, 1994"

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One of the accomplishments of Enrico's laboratory in the late 1980s—then at the University of Connecticut in Storrs—was demonstrating that cerebellar-like neuronal microcircuits exist in the acoustic brainstem [1]. This was done by coupling classic neuroanatomical methods (such as electron microscopy and tract tracing) to what were then two novel techniques: immunocytochemical cell-specific neuronal labeling and the use of transgenic mice. Thus, in the early 1990s, the laboratory focused on clarifying the precise organization, input and output, and evolutionary significance of the recently identified cerebellar-like microcircuit in the mammalian dorsal cochlear nuclear complex (DCN). At the same time, Enrico's laboratory continued its long-standing interest in cerebellar organization and development (Enrico's cerebellar grant was continuously funded for what might be a record-setting 38 years [2]).

Like most serendipitous scientific discoveries, the unipolar brush cell's (UBC's) identification was both unforeseen and unplanned, but nevertheless the by-product of a sagacious and prepared mind. Because the Purkinje cell markers used in the DCN studies were mostly calcium-binding proteins (calbindin, PEP-19, and parvalbumin), Enrico constantly reached out to potential collaborators who worked with this family of proteins. Among them was David Jacobowitz from the NIH, who sent us antibodies to the calcium-binding protein calretinin, which turned out to be a cell-marker for what we now know as one of the UBC subtypes [3]. As a beginning graduate student, I had no notion of what we had found. But Enrico immediately put it into context, recalling studies describing novel cerebellar neurons primarily localized to the vestibulocerebellum: Altman and Bayer's pale cells [4], Susan Hockfield's Rat-302 cells [5], Munoz' monodendritic neurons [6], and the secretogranin-positive cells described by Cozzi et al. [7].

Bringing to bear his expertise and encyclopedic knowledge of classic neuroanatomy and electron microscopy, Enrico turned his attention to the UBC's ultrastructure and synaptology [8] and quickly realized that previous studies (including his own) had already described UBC features but mistakenly attributed them to other cerebellar cell types. For example, the "hairy dendrites" and ringlet subunits that he had previously described for Golgi cells of the cat cerebellum [9], and the giant mossy fiber "en marron" synapse previously described on the postsynaptic Golgi II neuron by Chan-Palay and Palay [10]: all of these turned out to be UBC hallmarks. Other telltale features of the UBC revealed by a combination of pre- and post-embedding immunoelectron microscopy included a high density of large dense-cored vesicles; an abundance of high molecular weight neurofilament protein, whose dephosphorylated variant was later identified as the Rat-302 protein [11]; a postsynaptic microfilamentous actin web under the giant mossy fiber-UBC synapse [12]; and the

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presence of peculiar ringlet subunits later referred to as the "botrysome," an organelle regulated by visual experience in cat visual cortex [13].

Having gained an appreciation of the comparative anatomy approach from his days in the Department of Anatomy at the University of Oslo, Enrico, with Alessandra Floris, searched for UBCs in his collection of archival Golgi sections from different species that had previously been used in the DCN studies. Observations from these Golgi sections—the crux of the current Cerebellar Classic paper—showed that in all mammalian species studied up to that time, the UBC features remain remarkably similar [14]. However, because incomplete metallic impregnation (especially of the myelinated axon) is an inherent weakness of the Golgi technique, Mugnaini and Floris were able to follow UBC axons for only 100–300 microns from the point of emergence: this did not sit well with reviewers and delayed publication of this paper.

Not long thereafter, as Enrico's laboratory was preparing to move to Chicago, David Rossi, from the Slater laboratory at Northwestern, back-filled UBCs with Lucifer yellow after patch-clamping to verify the identity of the cells that he had been recording from. To their surprise (and ours), they saw that the UBC axon gave rise to large mossy fiber terminals that meandered within the granular layer of the sagittal slice of the cerebellar vermis [15]. Thus, one of our initial collaborative projects was the challenge to identify the optimal experimental conditions that would allow us to preserve and visualize the ultrastructure of those cells that had been recorded from. With enough determination and tweaking, we eventually showed that the back-filled rosette-shaped enlargements were the central component of glomeruli where UBC axon terminals formed synapses not only with granule cells but also with other UBCs. Although we were not able to get paired recordings from a UBC and its postsynaptic target, we were able to identify granule cells that responded to white matter stimulation with long-latency repetitive bursts of polysynaptic activity [16].

Subsequently, Grazia Nunzi and others from the Slater laboratory, using organotypic cultures and electrophysiological techniques, showed that the intrinsic UBC mossy fibers are glutamatergic and estimated that in the nodulus at least 50 % of mossy fiber terminals arise from UBCs [17]. Taken together, these results suggest that this intrinsic network of UBC axons generate a feed-forward amplification of single mossy fiber afferent signals that would reach the overlying Purkinje cells via ascending granule cell axons and their parallel fibers [18]. These finding also require that Fig. 4 in the paper by Mugnaini and Floris (the schematic diagram of the presumed wiring of the UBCs [14]) be updated. Nevertheless, it should be pointed out that the intrinsic network described above are based on cerebellar slice preparations, leaving the question of whether UBC axons project beyond the granule cell layer still unanswered,

Collaborations with the laboratories of Ryuichi Shigemoto in Japan and Robert J. Wenthold at the NIH not only allowed us to localize various glutamate receptor subunits within and around mossy fiber-UBC synapse [19, 20], but also paved the way for identifying the distinct mGluR1-alpha subset [21]. Using a combination of tract tracing and immunocytochemistry, we found that primary [22] and secondary [23] cholinergic vestibular afferents form synapses with UBCs.

Now, 20+ years after the identification of this cell type, UBCs are becoming the focus of interest for an increasing number of cerebellar investigators. This is the consequence of several intriguing findings obtained within the last few years. It was shown that UBCs form two separate cell populations with different distribution and functional properties [24, 25]. While the potential impact of this discovery for the understanding of the organization of the cerebellar network still needs to be fully appreciated, a series of papers have suggested that UBC research may harbor a remarkable and previously unanticipated translational impact. In addition, the laboratory of Thomas Brozoski has suggested that UBCs may contribute to the generation of deafferentation-induced tinnitus [26]. This hypothesis nicely matches the observation that UBCs are capable of intrinsic firing even in the absence of synaptic inputs [24, 27]. At about the same time, collaborative work involving the Mugnaini lab led to the hypothesis that UBC loss may contribute to ataxic phenotype [28, 29].

While on one coast of the Atlantic ocean, evidence was being gathered as to the translational value of UBC investigation; on the other coast, the laboratory of Chris de Zeuuw was finding that the peculiar time course of the synaptic transmission at the mossy fiber-UBC terminal endows UBCs to reliably represent time intervals in the cerebellar cortex, suggesting that these cells may be critical for adaptive control of behavior [30]. Such an important role finds further support in the paper by Lee et al. (in this issue of *The Cerebellum*) in which the authors show that in lobule X more than 50 % of all the mossy fiber terminals are not extrinsic mossy fibers, but UBC axons, thus supporting the idea that these neurons can heavily influence the function of cerebellar networks. In the light of these recent developments, it is safe to suggest that the UBCs is a cell type that has finally come to age and will merit ever increasing attention in the future.

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