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EDITORIAL

Editorial on the Honorary Cerebellum Issue for the Retirement of Enrico Mugnaini

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Throughout his career, Enrico Mugnaini has been an outstanding neuromorphologist, a generous colleague, and a very knowledgeable and skillful tutor (Fig 1). He did excellent work on a great variety of topics, many of which were related to the distribution and projections of GABAergic neurons [1], the cyto-architecture of various parts of the nervous system, in particular the auditory system [2, 3] and the cerebellum [4], culminating in the work on the morphology and function of unipolar brush cells (UBCs) [5]. The variety of these topics is also reflected in the contributions of Enrico's colleagues and friends in the current issue for The Cerebellum: four papers largely focused on the development, electrophysiology, or systems function of GABAergic neurons [6–9], five papers on the structure and function of UBCs [10–14], while the remaining papers addressed either hyperplastic and degenerative aspects of cerebellar pathology [15, 16], or the interregional connectivity within the auditory system [17, 18]. The formats range from those of commentaries or opinion papers to those of extensive reviews or original articles.

Since Enrico was one of the first to use antibodies against glutamic acid decarboxylase (GAD), the producing enzyme of GABA [1], he triggered a lot of research in this area, particularly in the cerebellar field. Constantino Sotelo, who got to know Enrico back in the seventies at Harvard when both of them spent their postdoc in the lab of Sandy Palay, explains how the GABAergic interneurons of the cerebellar cortex develop, which transcription factors determine their fate, and how they may operate in the adult as electrotonically coupled networks [6]. I myself, Chris De Zeeuw, who got to know Enrico in the nineties at the University of Connecticut (UCONN) when I was doing a postdoc in his lab on the discovery of the dendritic lamellar body [19], describe how responses of interneurons of different parts of the cerebellar

Fig. 1 Picture of Enrico Mugnaini, taken at the Lake of Chicago in August 2013, courtesy of Marco Martina

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cortex look like in the whole-cell in vivo preparation [7]. Next, Neal Barmack, another collaborator of Enrico at UCONN, provides his opinion on data indicating that modulation of interneurons in the vestibulocerebellum can, to a large extent, be determined by activity in the climbing fibers originating in the inferior olive [8], while Piergiorgio Strata, one of the major colleagues from his home country, Italy, outlines the literature addressing the issue as to what extent the cerebellum and its inhibitory connections might also play a role in emotional responses [9].

Running around in his lab in the nineties, it was clear to me that one of the greatest excitements Enrico had in his career was the rediscovery of the UBC. Although these cells had been described as “pale cells” by Altman and Bayer [20] and reported subsequently by others, e.g., [21, 22], it wasn’t until Enrico examined them using Golgi staining [23], electron microscopy [24], and electrophysiology [25] that the UBC avenue of research opened up widely. The first detailed UBC paper in 1994 describing the peculiar brush-like dendritic tree [23] is highlighted in two commentaries in this issue [10, 11]. Enrico and his co-workers further showed that UBCs come in two main flavors, type I calretinin-positive UBCs and type II mGluR1α-positive UBCs [26, 27], and that they give rise to a large proportion of mossy fibers in the vestibulocerebellum [28], which should allow them to provide feedforward signaling. In this issue, we confirm at the cell-physiological level that UBCs can provide extensive, intrinsic forward signaling, bridging gaps of hundreds of milliseconds [13]. Recent data also indicate that the two UBC subtypes may respond differently to a mossy fiber input, type II mGluR1α-positive UBCs showing an excitatory (ON) response and increased firing and type 1 UBCs showing an inhibitory (OFF) response after mossy fiber activation [29]. Gabriella Sekerkova and Marco Martina, both of whom became major colleagues and friends of Enrico after he moved to Chicago, and worked with him on the further characterization of UBCs, showed here that the mossy fiber terminals of UBCs are a VGluT1-positive mossy fiber and are markedly enriched in α-synuclein [12]. Finally, Tom Ruigrok, John Simpson, and collaborators describe how UBCs can be distinguished in vivo in the rabbit flocculus while responding to sigmoidal visuo-vestibular stimulation sometimes indeed revealing latencies of up to 0.5 seconds [14].

Calretinin-positive UBC’s are not only present in the cerebellum, but also in the dorsal cochlear nucleus of the auditory system [30], the organization of which Enrico considered analogous to that of the cerebellum [31]. The connections of the auditory system, in particular those of the olivocochlear system, are highlighted in the current reviews by Doug Vetter and Enrique Saldaña. Doug Vetter highlights the original immunocytochemical work of Enrico, revealing the GABAergic and cholinergic cyto-architecture of the cochlear nuclei, and then describes their ascending projections to the auditory nuclei in higher brainstem regions [18]. Enrique Saldaña, on the other hand, highlights the descending projections within the auditory system, reviewing in detail the projections from the cerebral cortex to downstream nuclei such as the medial geniculate body, inferior colliculus, nucleus of lateral lemniscus, superior olivary complex, and cochlear nuclei [17].

The final two chapters of this issue focus on mechanisms underlying cerebellar disease, a topic at the heart of Enrico as he proudly obtained an MD title in Pisa in Italy. First, Dr. Kathleen Millen and colleagues mapped the so-called tippy mutation to a 2.1 MB region of the distal chromosome 9, not encompassing any gene previously implicated in cerebellar development or neuronal degeneration, and they show that this spontaneous ataxic mouse suffers from a novel type of non-apoptotic Purkinje cell degeneration [15]. This chapter includes Enrico himself as one of the co-authors and has been cross-linked to one of the earlier published issues of the Cerebellum this year. Second, Quentin Pittman, Richard Hawkes, and co-workers show compelling evidence that cerebellar hyperplasia with alterations in zonal patterning as well as motor and social behaviors may be induced by maternal immune activation [16], highlighting the potential relevance of critical periods in cerebellar development and proper regulation of programmed Purkinje cell death.

In addition to these topics, which together cover large parts of Enrico’s favorite research areas, he has been studying many other subjects. For example, after moving to Chicago in 1996, he started to collaborate with Teepu Siddique on Amyotrophic lateral sclerosis, contributing to a paper in Nature on UBQLN2 mutations causing an X-linked form of ALS [32]. Consistent with the style of Enrico, this paper is illustrated with more than ten full-page panels of high quality light-microscopic micrographs, typical for neuroanatomical journals like the Journal of Comparative Neurology or Journal of Neurocytology. The abundance of high-quality photographs also marked his old lab at UCONN, hidden in a corner of the campus at Storrs, where all walls were covered with electron and light photomicrographs.

Enrico probably is one of the most general and outstanding neurocytologists, who can recognize the precise brain area he is studying just by looking at a fraction of the tissue of this area under the electron microscope. In fact, we used to joke in the lab that EM (i.e., Enrico Mugnaini) was born for the EM (i.e., electron microscope) as soon as his father gave him his name. His outstanding capacity as a neurocytologist does not only hold now, but probably was even true in the old days as witnessed by stories from older dinosaurs like Jan Voogd and Rodolfo Llinas.

Jan: I am among the many whose lives were touched by Enrico Mugnaini, as a colleague, a tutor, a scientist, but mostly as a friend. I first met Enrico in 1969 at Chicago’s O’Hare airport, when I set my first steps on American soil. We were on our way to the Llinas meeting on Neurobiology of Cerebellar Evolution and Development. Enrico was in the company of Jan Jansen, the senior professor of anatomy of the Anatomical Institute in Oslo, where he had spent some years as a post-doc learning electron microscopic techniques from Fred Walberg, and where he met his future wife, Erna. I didn't see much of Enrico during that particular meeting, as most of the time he was writing in his hotel room on a chapter on the cytology of the cerebellar cortex in birds [33]. The cyto-architecture of the cerebellum was a recurrent theme in his research, but it was difficult to name any brain structure or organelle that didn't receive his attention at one time or another. The dorsal cochlear nucleus figured in many of his early papers with Kirsten Osen, a colleague from his days in Oslo [34]. The cerebellum-like nature of this nucleus was emphasized by its "Purkinje cells": the cartwheel neurons [3]. More recently the acoustic system recurred in the paper with Enrique Saldaña on the striking tonotopic organization of the inferior colliculus [35]. GAD histochemistry identified GABAergic neurons in structures as diverse as the amygdala and olfactory bulb [1] and it allowed him to study the cerebellar nucleo-olivary pathway as a GABAergic system [36]. The cholinergic innervation and receptors of the cerebellum were studied with Dick Jaarsma [37], while the UBC appeared as a neglected interneuron in a paper with Alessandra Floris in 1994 [23], but developed in the best-known cerebellar interneuron in a large series of Enrico's papers. "He has always time for others and he was never prepared to accept the second best", words with which Enrico and his colleagues epitomized Sandy L. Palay [38], but which equally apply to Enrico Mugnaini. The papers in this volume by his friends and colleagues pay homage to 50 years of inspiring neuroscience by one of the peers in this realm.

Rodolfo: As in Jan's case I first encountered Enrico at the AMA Meeting on the Neurobiology of Cerebellar Evolution and Development, in 1969 in Chicago. Enrico was direct and had beautiful anatomy to offer on chick cerebellar development at both the light and ultrastructural level. All this was accompanied by excellent drawings of his morphological results. Above all he had the charm of the wonderful Northern Italians and the cool confident posture derived from the work he and his colleagues were doing at the famous Anatomical Institute in Oslo. From that time on, the sparks generated by that meeting, on a topic that was considered at that time somewhat recondite, the cerebellum, served as a constant stimulus for both sincere friendship and often heated discussions. Enrico always made his case with clarity of word and eloquent morphology. Quite unique in discussion was his Italian body postures and movements delivered in clear English with a most delightful Northern Scandinavian accent.

One of the reasons why Enrico has always been an excellent morphologist is that he also is an excellent chemist, able to create and mix the proper concentrations at the right time for the right duration. When I came to do the first immuno-runs in his lab on the lamellar bodies and things were far from working, Enrico looked at the sections and said, while raising his Italian two fingers and thumb: "well I would do the primary 11 minutes longer at 5 degrees higher temperature, use a higher concentration of donkey milk as a blocker and try two different fixatives at a lower pH." At first I thought he was just joking or trying to impress me, but when it turned out to be just perfect, I started to recognize his genius as a chemist. This impression was only further enhanced when he made me wonderful steaks with the most incredible mixture of herbs at his home late at night after coming back from the lab. Along the same line, the authors of this editorial feel the best closure comes from the hand of Enrico himself, who used to write poems on occasion, one of which was pointed out by John Simpson. It seems outdated, but is also valid nowadays.

Poem by Mugnaini published in The Cerebellum–New Vistas in 1982 [39]:

The method that shows you too little here
May show you too much there.
HRP, which we did not have before,
Will always show you that there is more.
It may then take another touch
To help decide what is too little and what is too much.

Enrico, true when you wrote it at a meeting in Washington, D.C. in 1980, still true today and tomorrow. Enrico, we want to show you here our appreciation of everything you did for us.

Cheers, Your Friends

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