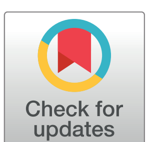
RESEARCH ARTICLE

Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity

Valentina Cerrato[1,2☯], Elena Parmigiani[1☯¤], María Figueres-Oñate[3], Marion Betizeau[4], Jessica Aprato[1], Ishira Nanavaty[1], Paola Berchialla[5], Federico Luzzati[2,6], Claudio de'Sperati[7,8], Laura López-Mascaraque[3], Annalisa Buffo[1,2]*

1 Department of Neuroscience Rita Levi-Montalcini, University of Turin, Turin, Italy, **2** Neuroscience Institute Cavalieri Ottolenghi, Orbassano, Turin, Italy, **3** Department of Molecular, Cellular, and Developmental Neurobiology, Cajal Institute -CSIC-, Spanish National Research Council, Madrid, Spain, **4** Brain Research Institute, University of Zurich Irchel, Zurich, Switzerland, **5** Department of Clinical and Biological Sciences, University of Turin, Turin, Italy, **6** Department of Life Sciences and System Biology, University of Turin, Turin, Italy, **7** Laboratory of Action, Perception and Cognition, Vita-Salute San Raffaele University, Milan, Italy, **8** Experimental Psychology Unit, Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy

☯ These authors contributed equally to this work.
¤ Current address: Department of Biomedicine, University of Basel, Basel, Switzerland
* annalisa.buffo@unito.it



OPEN ACCESS

Citation: Cerrato V, Parmigiani E, Figueres-Oñate M, Betizeau M, Aprato J, Nanavaty I, et al. (2018) Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity. PLoS Biol 16(9): e2005513. https://doi.org/10.1371/journal.pbio.2005513

Academic Editor: Cagla Eroglu, Duke University, United States of America

Received: January 28, 2018

Accepted: September 13, 2018

Published: September 27, 2018

Copyright: © 2018 Cerrato et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files. Matlab data and used codes are available from the authors.

Funding: Local funds of the University of Turin (grant number). Received by AB. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Ministero dell'Istruzione, dell'Università e della Ricerca – MIUR project "Dipartimenti di Eccellenza 2018 – 2022" (grant

Abstract

The morphological, molecular, and functional heterogeneity of astrocytes is under intense scrutiny, but how this diversity is ontogenetically achieved remains largely unknown. Here, by quantitative in vivo clonal analyses and proliferation studies, we demonstrate that the major cerebellar astrocyte types emerge according to an unprecedented and remarkably orderly developmental program comprising (i) a time-dependent decline in both clone size and progenitor multipotency, associated with clone allocation first to the hemispheres and then to the vermis(ii) distinctive clonal relationships among astrocyte types, revealing diverse lineage potentials of embryonic and postnatal progenitors; and (iii) stereotyped clone architectures and recurrent modularities that correlate to layer-specific dynamics of postnatal proliferation/differentiation. In silico simulations indicate that the sole presence of a unique multipotent progenitor at the source of the whole astrogliogenic program is unlikely and rather suggest the involvement of additional committed components.

Author summary

Astrocytes are abundant cells of the brain essential to support and shape neuronal activity. They can be grouped in different subclasses based on their remarkable variety of morphologies, molecular profiles, and specialized functions. Although different astrocyte types likely display specialized interactions with distinct neuron categories, the different classes of astrocytes have only partially been unmasked. How astrocyte heterogeneity is ontogenetically achieved remains largely unknown. Here we approached this question by

number ex L. 232/2016). Received by the Department of Neuroscience "Rita Levi Montalcini". The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Ministry of Economy and Competitiveness of Spain (grant number BFU2016-75207-R). Received by LLM and supporting MFO. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. Network of European Neuroscience Schools Fellowship (grant number). Received by VC. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. SystemsX.ch transition postdoc fellowship (grant number). Received by MB. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Abbreviations: A-P, antero-posterior; BG, Bergmann glia; BLBP, brain lipid–binding protein; BrdU, bromodeoxydine; CNA, cerebellar nuclei astrocyte; D-V, dorso-ventral; E, embryonic day; EdU, 5-ethynyl-2′-deoxyuridine; EGL, external granular cell layer; GEE, generalized estimating equations; GL, granular layer; GLA, granular layer astrocyte; GLAp, granular layer astrocyte precursor; GLAST, glutamate aspartate transporter; HetC, heterogeneous clone; hGFAP, human glial fibrillary acidic protein; HomC, homogeneous clone; IUE, in utero electroporation; mCerulean, monomeric Cerulean; mCherry, monomeric Cherry; mKO, mKusabira Orange; M-L, medio-lateral; MP, multipotent progenitor; mT-Sapphire, monomeric T-Sapphire; NeuN, neuronal nuclei; NFIA, nuclear factor 1 A; NND, Nearest Neighbor Distance; P, postnatal day; PAX2, paired box gene 2; PC, Purkinje cell; PCL, Purkinje cell layer; PCLp, Purkinje cell layer precursor; Pm, paramedian; pVimentin, phosphorylated Vimentin; PWM, prospective white matter; RG, radial glia; SHH, Sonic hedgehog; SOX10, SRY-box 10; Tx, tamoxifen; VC, ventricular ce; VZ, ventricular zone; WMA, white matter astrocyte.

studying the development of the main astrocyte types of the cerebellum. The reconstruction of developmental lineages in the mouse embryo combined with proliferation studies and computational modeling demonstrate that cerebellar astrocyte types emerge according to an unprecedented and remarkably orderly developmental program. Embryonic progenitor cells produce either only a single astrocyte type or more types. These distinct astrocyte lineages display stereotyped architectures and recurrent modularities. Moreover, the generation of astrocytes follows a well-defined spatiotemporal pattern, defined by a time-dependent allocation of astrocytes to distinct cerebellar territories and an inside-out sequence of differentiation, coupled with a decline over time in both progenitor amplification and capability to produce distinct astrocyte types. These results provide the first evidence that an ontogenetic program, tightly regulated in space and time, determines astrocyte heterogeneity.

Introduction

Increasing evidence supports the morphological, molecular, and functional heterogeneity of astrocytes across and within distinct regions of the developing and adult central nervous system (CNS) [1–3]. Although much progress has recently been made, we are still far from understanding how such heterogeneity emerges. Pioneer astrocyte lineage–tracing analyses at the single-cell level revealed that distinct progenitors generate different astrocyte types in the cerebral cortex, suggesting that clonal identity is the basis of astrocyte diversity [4–6]. Nevertheless, it remains unclear to what extent this applies to other brain regions. Most importantly, if and how the gliogenic potential of astroglial progenitors changes during development in terms of lineage composition and size of individual clones has not been explored.

A suitable model to address these questions is the cerebellum, given its simple cytoarchitecture with unique and well-characterized major astroglial types defined by distinct morphology, layering, marker expression, and functions [7,8] (S1 Table). These distinct types comprise fibrous astrocytes of the white matter (WMAs) and, in the overlying cortex, star-shaped bushy velate astrocytes of the granular layer (GLAs) and polarized Bergmann glia (BG), lined up in the Purkinje cell layer (PCL) [8,9]. Cerebellar astroglia are posited to originate from fourth ventricle radial glia (RG) cells [8,10–13] through their direct transformation into BG starting at embryonic day (E)14 and through the amplification of intermediate progenitors populating the nascent cerebellar parenchyma [14,15].

Here, by in vivo clonal analyses, we resolve the lineages of astrocytes in the cerebellum and show that astrogliogenesis occurs according to a well-defined spatiotemporal pattern from precursors whose fate potential declines over time. Our data disclose a stereotyped modularity in clone composition, revealing that unprecedented developmental rules govern astrogliogenesis at the clone level. We further demonstrate that postnatal proliferation ultimately defines clone size and that progenitors in the PCL can generate both BG and GLAs. Finally, in silico modeling suggests that cerebellar astrocyte heterogeneity does not emerge from a unique multipotent progenitor (MP) pool but may also require committed components.

Results

Embryonic waves of astrogliogenesis populate distinct cerebellar regions

To investigate the emergence of astroglial heterogeneity, we employed StarTrack [4], a system based on multiple plasmids that express up to 12 different fluorophores in the tagged

cells and their progeny after piggyBac-driven stochastic integration into the genome. This allowed the classification of cells labeled by the same color combination as clonally related in the vast majority of cases (91%, as estimated by lumping error evaluation; see Methods). We labeled RG in the cerebellar ventricular zone (VZ; S1 Fig) and their astroglial descendants by StarTrack plasmids carrying the human glial fibrillary acidic protein (hGFAP) promoter after in utero electroporation (IUE) in the mouse fourth ventricle (Fig 1A–1C) either at E12, a fully neurogenic phase, or E14, reported as the beginning of gliogenesis [11,13,16,17].

At the end of cerebellar maturation (postnatal day [P]30), tagged cells were identified as astrocytes belonging to all typical cerebellar types comprising WMAs, GLAs, and BG (see Methods; Fig 1A–1C and S2 Fig and S1 Table). Rarely, astrocytes were also observed in cerebellar nuclei (cerebellar nuclei astrocytes [CNAs]). Thus, gliogenic RG populate the cerebellar VZ starting from early embryonic stages. Moreover, the apparent delamination of some E12-tagged hGFAP-expressing ($^{+}$) precursors from the VZ already at E13 (S1B Fig) suggests that cerebellar gliogenesis starts earlier than E14.

Notably, E12- and E14-tagged clones displayed strikingly complementary medio-lateral (M-L) localizations. About 80% of E12-P30 clones settled in the hemispheres, whereas E14-P30 clones were exclusively (100%) in the vermis (Fig 1D). This lateral-to-medial shift in clone settlement is reminiscent of the sequence of birth of Purkinje neurons, which starts in the hemispheres and paravermis at earlier time points [14,16].

Clones also had a specific distribution along the antero-posterior (A-P) axis. E12-P30 clones were found in all folia of the hemispheres with no defined pattern in the paravermis/vermis (S3A Fig and Fig 1E). However, E14-P30 clones were more segregated in the anterior (I–V) and posterior-nodular (VIII–X) vermian lobules and rarely found in the central zone (lobules VI and VII, S3B Fig and Fig 1E). This pattern suggests that another VZ-derived astrogliogenic wave might populate the central structures. In summary, gliogenic RG are already present at E12 and produce astrocytes according to the spatiotemporal pattern of Purkinje cell (PC) genesis, although with a delay.

Gliogenic ventricular progenitors undergo a developmental restriction in their differentiation potential

We identified 2 main clone types: homogeneous clones (HomCs), formed by astrocytes of the same type (Fig 2A–2C), and heterogeneous clones (HetCs), including distinct astrocyte types (Fig 2D and 2E). Both clone types were generated at the 2 examined stages, although with different frequencies. E12 progenitors produced similar proportions of HomCs and HetCs, whereas E14 progenitors predominantly generated HomCs, suggesting restriction in the progenitor differentiation potential (Fig 2F and 2G). HomCs for each astroglial type were found at both time points. However, compared to E12-P30 clones, the E14-P30 WMA HomCs doubled in frequency (Fig 2F and 2G), whereas BG HomCs decreased to one-third. Among E12-P30 HetCs, 17.6% comprised 3 astroglial types (BG+GLA+WMA, triple clones; Fig 2D and 2F), whereas this proportion halved in E14-P30 clones (Fig 2G, 7.8%). On the other hand, HetCs including both BG and GLAs (BG+GLA, double clones, Fig 2E) were similarly represented in both data sets (Fig 2F and 2G). CNA HomCs and other kinds of HetCs including almost any combination of astrocyte types were found at both time points, but in very limited numbers and without overt changes (Fig 2F and 2G). Therefore, they were not considered for detailed quantitative analyses. Interestingly, although HomCs were overall more represented, 90% of all cortical astrocytes belonged to HetCs at both time points (S4 Fig), indicating that HetCs

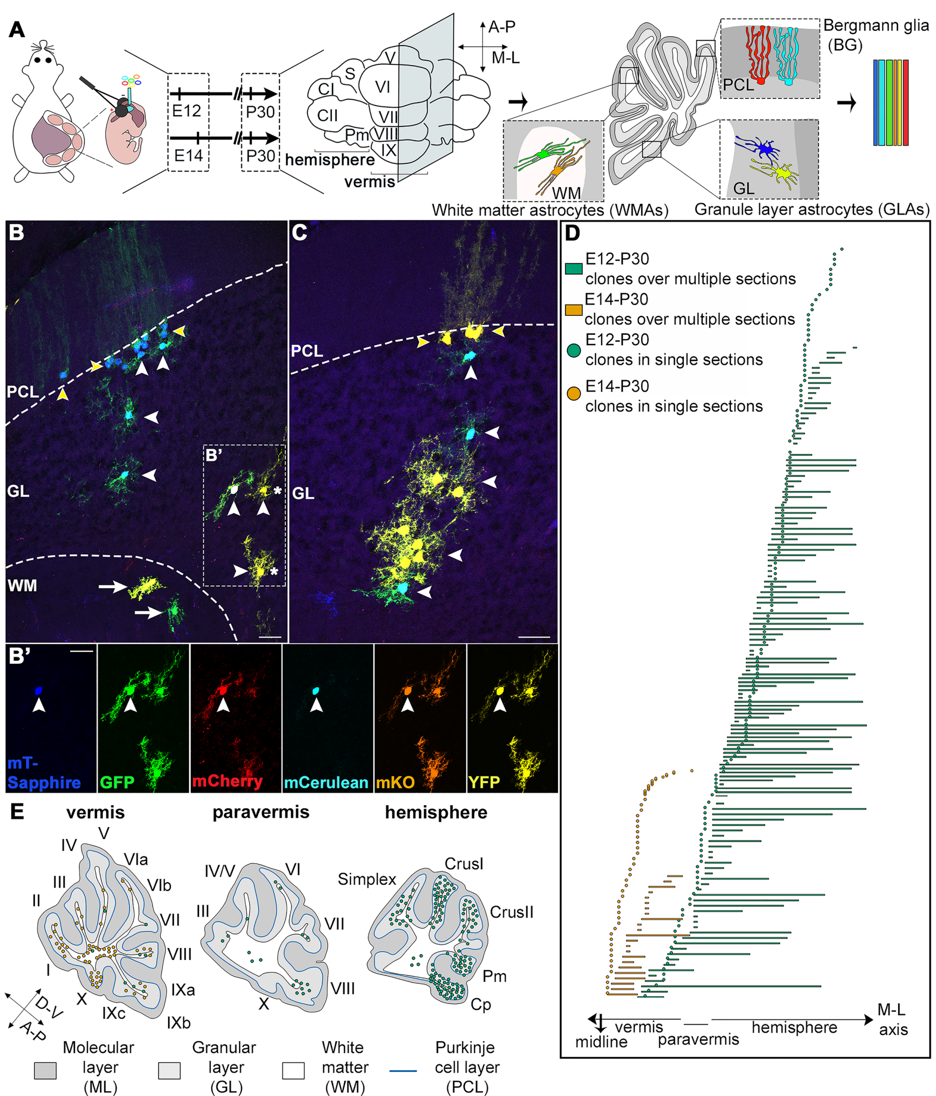


Fig 1. In utero StarTrack electroporations and clone allocation in the cerebellum. (A) Schematic representation of the experimental design. The hGFAP-StarTrack mixture was electroporated at E12 or E14, and clonal analysis was performed at P30. (B,C) StarTrack-labeled astrocytes are found in all cerebellar layers in P30 mice and comprise WMAs (white arrows), GLAs (white arrowheads), and BG (yellow arrowheads). In B', 2 sister GLAs share the same combination of fluorescent proteins (asterisks), whereas the third GLA displays a different color combination, thus deriving from a different progenitor, even though it is very close to the other 2 GLAs. (D) Schematic representation of the relative M-L extension of each clone. E12-P30 clones (green) preferentially settle in the cerebellar hemispheres, whereas E14-P30 families (orange) are exclusively located in the vermis. Based on the cerebellar symmetry around the midline, all clones are projected on one-half cerebellum. The paravermis is defined as that region where lobule IX fades and lobule X is still present. (E) Diagrams are representative of clone distribution along the A-P axis. E12-P30 clones (green) are homogeneously distributed in all lobules of the hemispheres, whereas E14-P30 ones (orange) preferentially occupy the ventral vermis, including both anterior and posterior folia. Each dot corresponds to 1–2 clones. When clones are found in >1 lobule, they are repeatedly represented in each corresponding folium. Scale bars: 30 μm. A-P, antero-posterior; BG, Bergmann glia; CI and CII, CrusI and CrusII; Cp, copula pyramidis; D-V, dorso-ventral; E, embryonic day; GFP, green fluorescent protein; GLA, granular layer astrocyte; hGFAP, human glial fibrillary acidic protein; mCerulean, monomeric Cerulean; mCherry, monomeric Cherry; mKO, mKusabira Orange; M-L, medio-lateral; mT-Sapphire, monomeric T-Sapphire; P, postnatal day; PCL, Purkinje cell layer; Pm, paramedian; S, Simplex; WM, white matter; WMA, white matter astrocyte; YFP, yellow fluorescent protein.

https://doi.org/10.1371/journal.pbio.2005513.g001