of synantocytes (see also red arrows in panel A). These cells are better visualized in rabbit than in mouse by immunocytochemistry, since staining in mice reveals to a lesser extent the ramifications of cell processes (schematically showed by drawings on the right; total length of cell processes quantifications in the two mammalian species is showed in Fig. S2A). Crb, cerebellum; ML, molecular layer; Cx, cerebral cortex; Cc, corpus callosum; SVZ, subventricular zone; LV, lateral ventricle; Sc, spinal cord; Dg, dentate gyrus of the hippocampus. Scale bars: A, 30 μm; B, 10 μm.
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cells, with a peak during DNA synthesis and mitosis [57]) were consistently negative (Fig. 4A). By contrast, and as expected, double staining between Ki67 antigen and Ng2 frequently revealed cycling Ng2+progenitors (Fig. 4A). Ki67+/GPR17+cells were rare and, when occurring, they showed a typical staining of the Golgi (restricted to the cell body and absent in processes [53]; Fig. 4A). By analysing BrdU-injected mice killed at different survival times (from 1 week to 1 month, see methods; Fig. 4B–D), the first BrdU+/Map5+double labelling was observed starting from 6–10 days (Fig. 4B). In parallel experiments, carried out on rabbits, the first BrdU+/Map5+double labeling was detectable at 11–15 days survival (not shown). Hence, mMap5 cells are newly generated in the adult mammalian CNS, yet postmitotic when expressing this microtubulin protein, which appears 1 or 2 weeks after cell division, depending on the animal species. Counting of the number of BrdU+ mMap5 cells out of all BrdU+ nuclei (percentage of newly generated mMap5 cells out of all newly generated cells) at different survival times, in the cerebral cortex

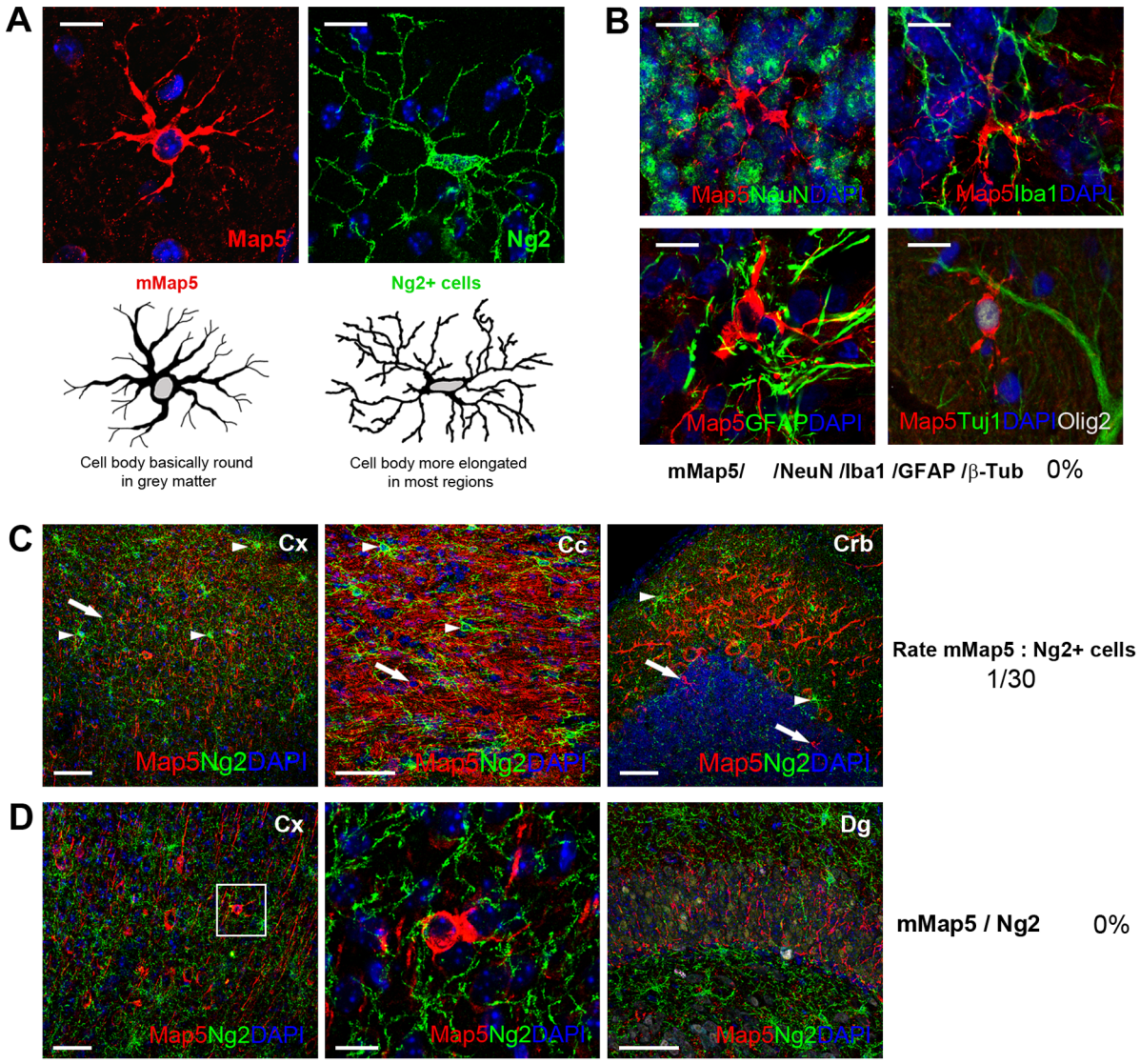

Figure 2. Morphological and phenotypic characterization of mMap5 cell population. A, Despite a substantially similar appearance, mMap5 cells are slightly different from Ng2+cells as to the shape of their cell body (quantifications of cell soma diameters is showed in Fig. S2A). The photographs are representative of two extremes: round-shaped and elongated cell bodies. B, Double staining with neuronal and glial antigens indicate that mMap5 cells are not neurons nor astrocytes or microglia. C, mMap5 cells (arrows) are ramified elements with a morphological appearance similar to Ng2+cells (arrowheads) but far less numerous. D, mMap5 cells and Ng2+cells belong to two distinct, non overlapping populations. Crb, cerebellum; Cx, cerebral cortex; Cc, corpus callosum; Dg, dentate gyrus of the hippocampus. Scale bars: A,B, 10 μm; C,D, 50 μm; inset in D, 10 μm.
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Table 2. Primary antibodies used in this study.

Antigen	Antibody/antiserum	Host	Dilution	Source	Species tested
Map5	mono, clone AA6	mouse	1:5000	Millipore	R,C,GP,S,Mn,H +, M −
	poly	goat	1:300	Santa Cruz	M,R +
Cell proliferation					
Ki67	mono	mouse	1:500	BD Pharmigen	M,R +
	poly	rabbit	1:1500	Novocastra	M +, R −
BrdU	mono, BU1/75	rat	1:300	AbDSerotech	M,R,C +
Progenitors cells					
SOX2	poly	rabbit	1:1500	Millipore	M,R +
	poly	goat	1:400	Santa Cruz	M,R +
SOX9	poly	rabbit	1:1000	Millipore	M,R +
Neuroblasts/Neurons					
DCX	poly	guinea pig	1:1000	Abcam	M,R +
	poly	rabbit	1:1000	Abcam	M,R +
NeuN	mono, A60	mouse	1:200	Millipore	M,R +
βIII-Tub	Mono (Tuj1)	mouse	1:100	Millipore	M,R +
βIII-Tub	Poly (Tuj1)	rabbit	1:1000	Covance	M,R +
Astrocytes					
GFAP	mono	mouse	1:300	Millipore	M,R +
	poly	rabbit	1:2000	Dako	M,R +
Microglia					
Iba1	poly	rabbit	1:1000	Wako	M,R +
Ependyma					
S-100β	poly	rabbit	1:10000	Swant	M,R +
Oligodendroglia					
Precursor/immature					
Ng2	mono	mouse	1:200	Chemicon	M,R −
	mono	mouse	1:500	US Biological	M,R −
	poly	rabbit	1:200	Chemicon	M +, R−
	mono	mouse	1:200	Sigma	M,R −
	mono	mouse	1:200	Upstate	M,R −
OLIG2	poly	rabbit	1:500	Millipore	M,R,C +, GP,S,Mn,H −
	poly	goat	1:400	R&D System	M,R,C +, GP,S,Mn,H −
SOX10	poly	goat	1:1000	Santa Cruz	M,R,C,GP,S,Mn +, H −
GPR17	poly	rabbit	1:10000	Marta Fumagalli	M,R +
PDGFRα	poly	rabbit	1:1000	Santa Cruz	M +, R −
		rat	1:100	BD Pharmigen	M +, R −
JAM-A	poly	rabbit	1:200	Santa Cruz	M −, R −
Mature/myelinating					
GST-π	poly	rabbit	1:400	MBL	M +, R −
RIP (CNPase)	mono	mouse	1:400	Chemicon	M,R +

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and corpus callosum (Fig. 4E, top and Fig. S3) revealed that they are stable at low levels in the grey matter, whereas they increase in the white matter at 1–2 weeks, then decreasing again at 1 month. Then, we counted the percentage of BrdU+ mMap5 cells out of all mMap5 cells, at the time they start to be detectable as newly generated cells in mice (end of the first week; see Fig. 4E, bottom). We found that 13% (7 Map5+/BrdU+ cells out of 53 Map5+cells, counted from 3 mice) and 25% (16 Map5+/BrdU+ cells out of 63 Map5+cells, from 3 mice) of the mMap5 cells are newlyborn, respectively in the grey and white matter. Finally, we quantified the percentage of newly generated cells immunoreactive for Ng2, GPR17, or GST-π (number of BrdU+/marker+ cells out of all BrdU+ nuclei) in order to compare them with those of the BrdU+ mMap5 cells (data summarized in Fig. 4E). As expected, most of the Ng2+cells and a substantial percentage of the GPR17+cells display active proliferation and appear to be newly generated during the first two weeks. At the same time, GPR17 expression is high (thus providing a link between Ng2+and mMap5 cells), then

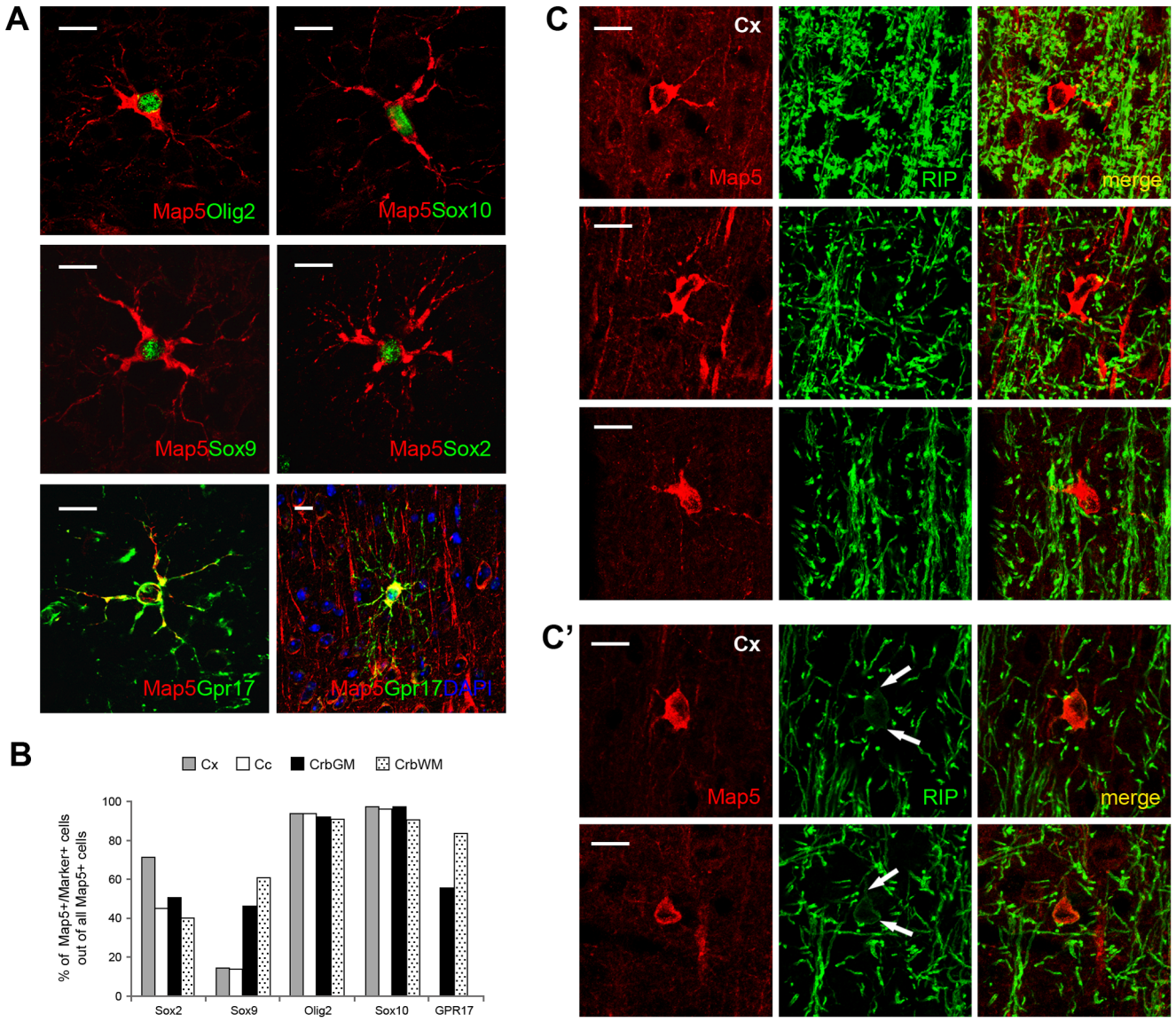

Figure 3. Phenotypic characterization of mMap5 cells in the oligodendrocytic lineage. A, Double staining between Map5 and different markers of the oligodendroglial lineage in mMap5 cells. B, Quantitative evaluation of mMap5 cells expressing different markers in different brain regions. C,C′, Very low overlapping between mMap5 cells and the mature oligodendrocytic population. Double staining with Map5 and mAb anti-RIP (CNPase) in the mouse cerebral cortex (Cx) generally showed no co-expression (C); a faint overlapping of the two antigens could be detectable only in some cells (C′, arrows). Scale bars: 10 μm.
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droppig fastly. At 1 month survival, beside a high persistence of newlyborn Ng2+cells (more evident in the grey than white matter, wherein they drop from 86% to 57% to 32%; see Fig. 4E) only a few newlyborn GPR17+cells can be found (4% in both grey and white matter). Conversely, only a few cells with features of mature oligodendrocyte are detectable at short periods after BrdU injection, whereas they dramatically increase at 1 month. Taken together, results from the time course experiments show that mMap5 cells are largely newly generated, postmitotic elements of the oligodendroglial lineage. Furthermore, they indicate that some mMap5 cells can differentiate into mature oligodendrocytes (especially and more rapidly in the white matter) or maintain the Map5 staining for undetermined times (at least 1 month; see Figs. 4E and 5C).

Regional distribution of mMap5 cells in the CNS of rabbits and mice

In order to verify if mMap5 cell occurrence and distribution is widespread in the CNS and if it parallels the heterogeneity reported in literature for the synantocyte/polydendrocyte populations, qualitative and quantitative analyses were carried out in several CNS regions of the rabbit (cerebral cortex, corpus callosum, striatum, cerebellum, spinal cord; Fig. 6) and mouse (cerebral cortex, corpus callosum, cerebellum; Fig. 7). These regions were chosen since they include both white matter tracts and grey matter parenchyma. As stated above (see also Fig. 1B), mMap5 cells are far less detectable in mouse tissue with respect to rabbit, due to a different staining generally more restricted to the cell body and primary processes in rodents (Fig. S2A). For this reason, in parallel with regional analysis in mice (Fig. 7), we

performed a more widespread study in different areas of the rabbit CNS (Fig. 6). Morphological variations in the overall cell shape were observed, with particular differences in white and grey matter. Most mMap5 cells had a round soma from which 3–4 main processes radiate in all directions. In grey matter they were arranged among the neuronal cell bodies, and their processes occupied a roughly spherical territory (Figs. 6 and 7). In the cerebral cortex (both six- and three-layered regions) the mMap5 cells were often resting on neuronal somata and processes (Fig. 1A, bottom). Quantification of mouse cell soma diameters in this region is reported in Fig. S2B. In the cerebellum they were associated with Purkinje cell somata, in this case assuming a more flattened shape, or widespread in the cortex with a soma prevalently round-shaped (quantification of mouse cell soma diameters in Fig. S2B). In white matter (e.g., corpus callosum and spinal cord fiber tracts), the cell bodies were more flattened/elongated with few short processes starting from the two poles and trapped in-between myelinated axons (Figs. 1B and 6A; quantification of mouse cell soma diameters in Fig. S2B), which are reminiscent of the GPR17+cells detected by Lecca et al. [58]. The most ramified, typically stellate morphology was frequently detectable in the granule layer and molecular layer of the cerebellum, and to a lesser extent in the striatum. mMap5 cells in the cerebral cortex and spinal cord grey matter were more irregular/heterogeneous in their overall shape, frequently endowed with three main processes (Fig. 6A, bottom). In the spinal cord grey matter mMap5 cells were more abundant in the intermediate zone, less abundant in the dorsal horn, and rare in the ventral horn (Fig. 6A, bottom). The wider variety of different shapes was detectable in the spinal cord white matter, with many bipolar and neuronal-like, pyramidal morphologies (Fig. 6A, bottom). These shapes were unusual with respect to the multipolar aspect of mMap5 cells in other regions.

Hence, some differences (more flattened in white matter, more multipolar in grey matter) substantially reflect those described for the Ng2+cells [1]. Nevertheless, our analysis of cell soma diameters revealed higher heterogeneity for mMap5 cells in some CNS areas with respect to that described for Ng2+cells (see Fig. S2B).

In order to assess the number of mMap5 cells in different CNS regions, cell densities were counted in all the areas examined, at different ages. Quantification results are reported in histograms of Fig. 6 (rabbit) and 7 (mouse). In general, mMap5 cells are quite numerous in the white matter with respect to grey matter, both in rabbits and mice (see statistics in Figs. 6 and 7). Yet, this difference is remarkable only in young animals, since the number of mMap5 cells in the white matter falls after puberty (see statistics in Figs. 6 and 7, and below for age-related considerations). This substantial reduction of mMap5 cell density in the shift from the peripuberal to adult period is particularly evident in large bundles of axons of the corpus callosum and spinal cord dorsal columns. The age-related drop is more evident in mice, due to a higher initial cell density in rodents with respect to lagomorphs. At subsequent ages the cell densities seem quite stabilized, even in old animals (e.g., in 1-year old mice; value referred to a different strain).

Comparative analysis of mMap5 cells occurrence in other mammals

In addition to the systematic analysis in most regions of the rabbit CNS and mouse brain, we analyzed brain tissue sections from other mammalian species characterized by increasing brain size and neocortex complexity (guinea pig, cat, sheep, monkey, human; Fig. 8). Different regions, including cerebral cortex, striatum, cerebellum, and different ages, up to 70 years in humans, were considered. Multipolar Map5+cells were found in all species, regions and ages investigated. In most cases, compatibly with tissue fixation/antibodies successful staining, these cells showed co-expression with markers tested in rabbit and mouse, thus confirming they belong to the mMap5 cell population (Fig. 8). The wide range of presence of mMap5 cells in the CNS of different mammals indicates a common role/distribution in phylogeny, including humans.

mMap5 cell distribution in chronic neurodegenerative damage and in acute trauma

Although many reports indicate the general involvement of Map5 molecule in different pathological conditions [40], the participation of mMap5 cells is still unknown. To gain more insight into the role of mMap5 cells in chronic damage we extended our analysis to a mouse transgenic model of neurodegeneration (APPPS1, a model of Alzheimer's Disease; [59]. Notably, the density of Olig2+mMap5 cells was significantly increased in the APPPS1 cortical grey matter compared with age-matched WT controls ($2{,}1\pm0{,}4$ cells/mm^2 in APPPS1 versus $0{,}3\pm0{,}1$ cells/mm^2 in WT, $p<0.001$; Fig. 9A). The number of Olig2+mMap5 cells was also significantly increased in the white matter of the corpus callosum (4 ± 1 cells/mm^2 in APPPS1 versus $0{,}5\pm0{,}5$ cells/mm^2 in WT, $p<0.05$; Fig. 9A). To address the issue of a possible involvement of mMap5 cells in acute trauma conditions, we analyzed the cerebral cortex and corpus callosum of mice after stab wound (SW) injury. At 15 days post-lesion the density of Olig2+mMap5 cells in the grey matter was similar to that detectable in intact mice ($2{,}6\pm0{,}2$ cells/mm^2 in SW, versus $1{,}9\pm0{,}4$ cells/mm^2 in control, $p>0.05$; Fig. 9B). By contrast, the number of Olig2+mMap5 cells was significantly increased in the underlying white matter ($10{,}8\pm1{,}8$ cells/mm^2 in SW versus $4{,}9\pm1{,}7$ cells/mm^2 in control, $p<0.05$; Fig. 9B). This result is consistent with previous studies reporting that GPR17, which is also expressed by these Olig2+mMap5 cells, is significantly increased in both the APPPS1 model and after induction of SW in rodents [53].

Hence, our data show that a substantial increase of mMap5 cells does occur in chronic neurodegenerative conditions (both in grey and white matter), and, to a lesser extent, after traumatic injury (in the white matter only).

Map5 expression and mMap5 cells at different ages

Since the amount of polydendrocytes remarkably varies with age [60,61], we analysed the behavior of mMap5 cells at different postnatal (peripuberal and adult) stages (Fig. 10). First of all, western blots of protein lysate from total brain of mouse (40 days and 3 months old), and rabbit (3,5 months and 1 year old), probed with anti-Map5 and normalized with anti-vinculin antibodies, were performed. Values (from older animals expressed relatively to young animals of the same species) indicated that total level of Map5 did not change significantly with respect to age (Fig. 10A). This result must be read as the total amount of neuronal and glial Map5, although we know that it is prevalently expressed in neurons (see first paragraph of the Result section and Fig. 1). By contrast, counting of the mMap5 cells at different ages clearly indicated their progressive reduction from peripuberal to adult stages, more dramatic in the white matter (regional data in rabbit and mouse are reported in Figs. 6 and 7; the trend of average data from all regions examined is summarized in Fig. 10B). Their level then results stabilized during adulthood and in old individuals (the latter data are referred to 1 year old mice of the C57BL/6 strain). On the whole, by comparing Map5 expression in peripuberal and young/adult rabbits with age matched mice, in both species we observed no significant reduction of the total Map5 molecule