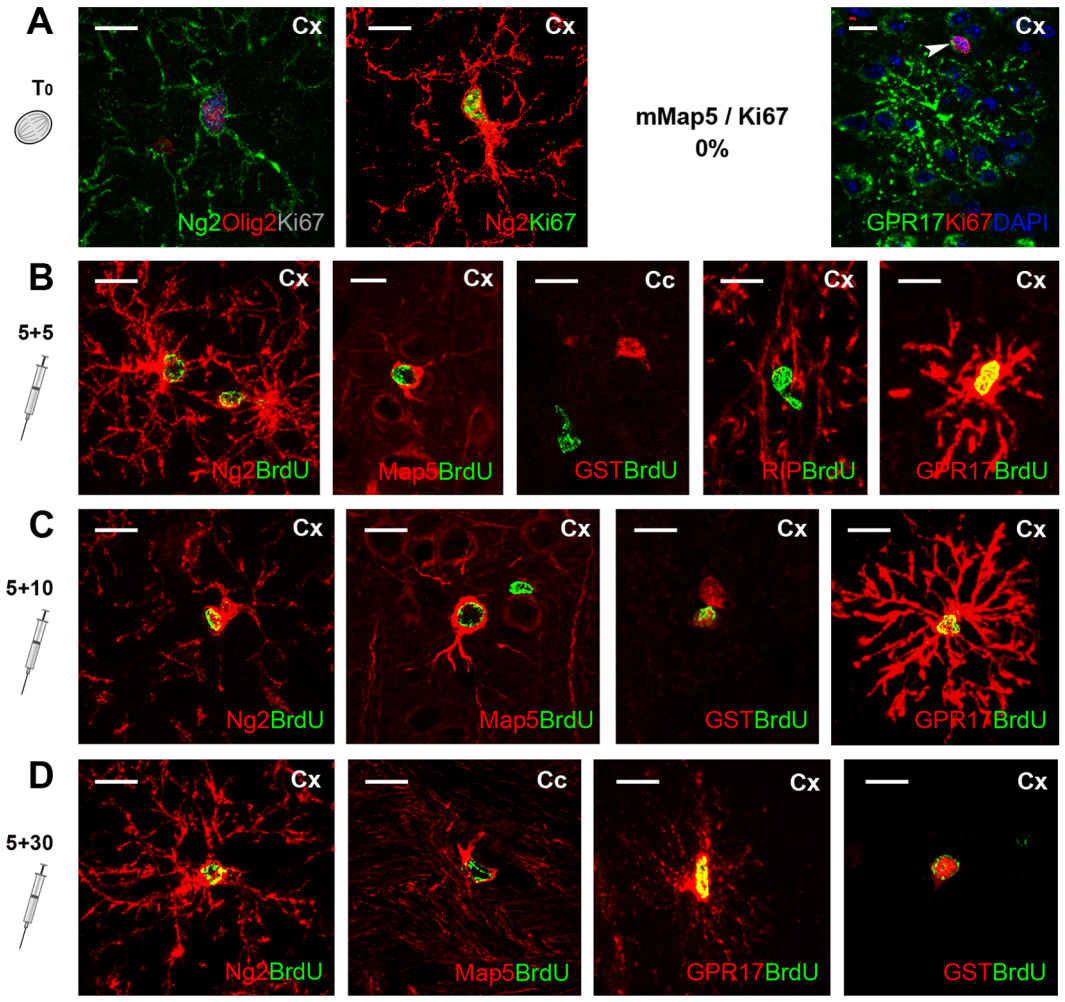

Figure 4. Genesis and differentiation of mMap5 cells in mice. Analyses carried out with endogenous and exogenous cell proliferation markers in double staining with Map5 and other markers of the oligodendroglial lineage. A, No proliferation of mMap5 cells is detectable by using the endogenous cell proliferation marker Ki67, whereas most Ng2+cells are clearly cycling. Rare Ki67+/GPR17+cells are detectable (A, right), their staining being restricted to the Golgi in the cell body (arrowhead). B–D, Analyses carried out with BrdU i.p. administration followed by different survival times (from 10 to 30 days). BrdU/Map5 double staining is detectable starting from 5–10 days in mice. E (top), quantification of newly generated cells expressing Map5, Ng2, GPR17, GST-π, at different BrdU post-injection survival times, in grey (GM, cortex) and white matter (WM, corpus callosum). Data indicate the percentage of BrdU+/marker+ cells out of total BrdU+ nuclei (raw data are reported in Fig. S3). E (bottom), quantification of newly generated mMap5 cells out of the mMap5 cell whole population (analysed in animals with 5+5 days BrdU treatment). Scale bars: 10 μm.
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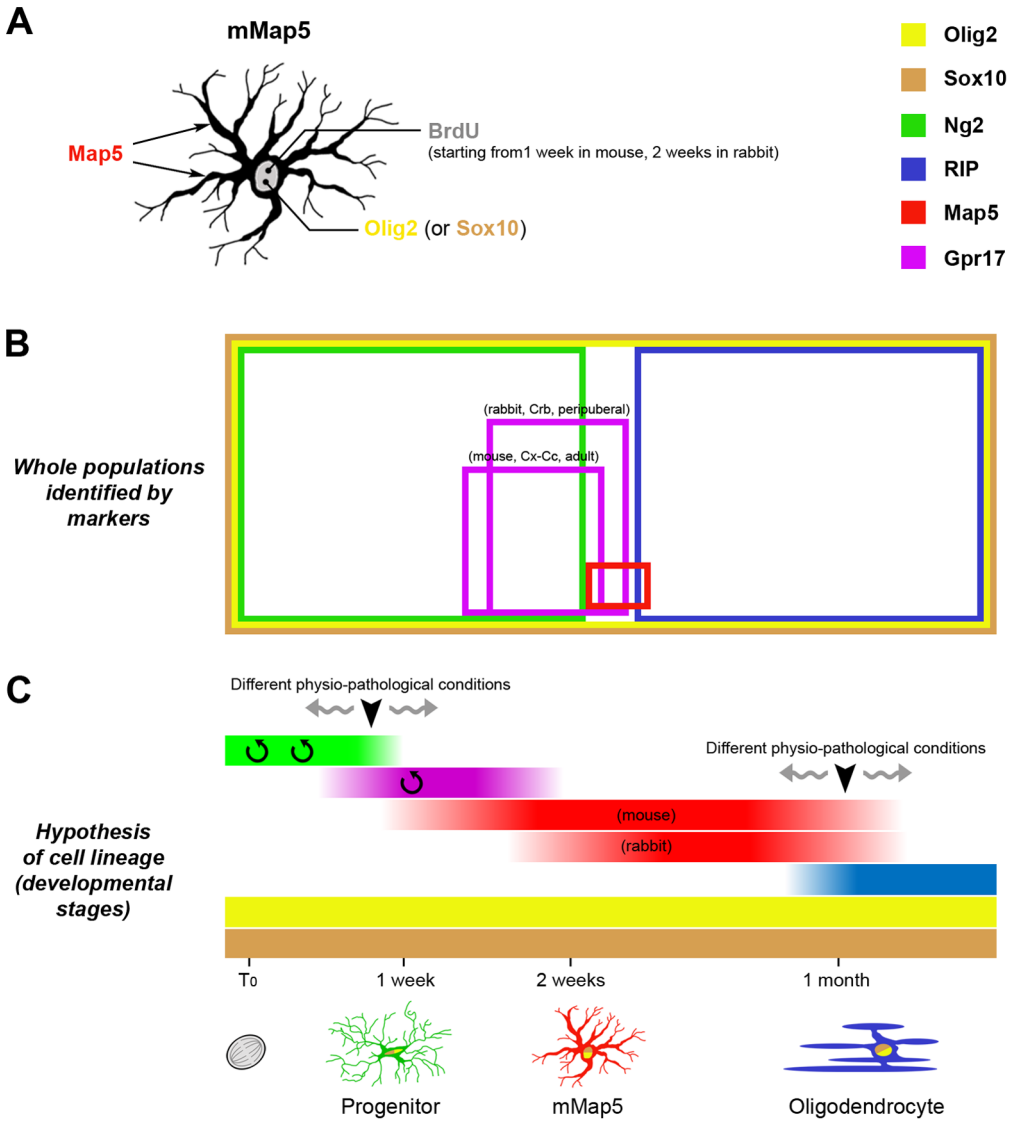

Figure 5. Schematic summary of the spatial and temporal distribution of several markers linked to the mMap5 cell population. A, The mMap5 cell population can be revealed by Map5/Olig2 double staining, and the subset of newly generated mMap5 cells by Map5/Olig2/BrdU triple staining (left). Double and triple staining with different markers (see colors in the legend on the right) can provide information on the spatial (B) and temporal (C) distribution of different subpopulations. B, The relative amount of each cell population revealed by different markers is indicated by colored squares. There is no overlapping between mMap5 and Ng2+cell population, and only a very small overlapping with the GST-π+ oligodendrocytes. C, Hypothesis on the time course for newly generated mMap5 cells by using endogenous and exogenous cell proliferation markers.
doi:10.1371/journal.pone.0063258.g005

amount with age (Fig. 10A), but significant reduction of the mMap5 cell number, especially in the white matter (see graphics in Fig. 10B, and rabbit, mouse regional data in Figs. 6 and 7).

Discussion

In the adult mammalian CNS, beside *de novo* cell genesis of 'classic' neurogenic sites (SVZ and dentate gyrus [62,63]), the occurrence of local, parenchymal progenitors which continue to divide throughout life has progressively became a new area of investigation [1,2,64,65]. In vivo, these cells are different from neural stem cells, since they are not segregated within germinal layer-derived, highly regulated niches [66,67]. Parenchymal progenitors are widespread in the CNS tissue, so that they do not need to migrate to reach the injured sites (see [3] for review). They can react to brain injury in different ways [68,69,70], whereas neurogenesis supported by the stem cell niches appears functionally committed to restricted physiological roles and poorly supportive for repair [71,72].

In spite of the indisputable interest for parenchymal progenitors, our knowledge of their biology remains incomplete due to a remarkable difficulty in defining cell populations/subpopulations which often display regional features and different degrees of differentiation, not always revealed by specific markers. Here we analysed the cellular and molecular features of a subset of multipolar cells immunoreactive for Map5, a microtubule-associated protein expressed in both neurons and glia [40]. In the past, the glial localization of Map5 has been underestimated. This fact could be attributable to the different appearance of

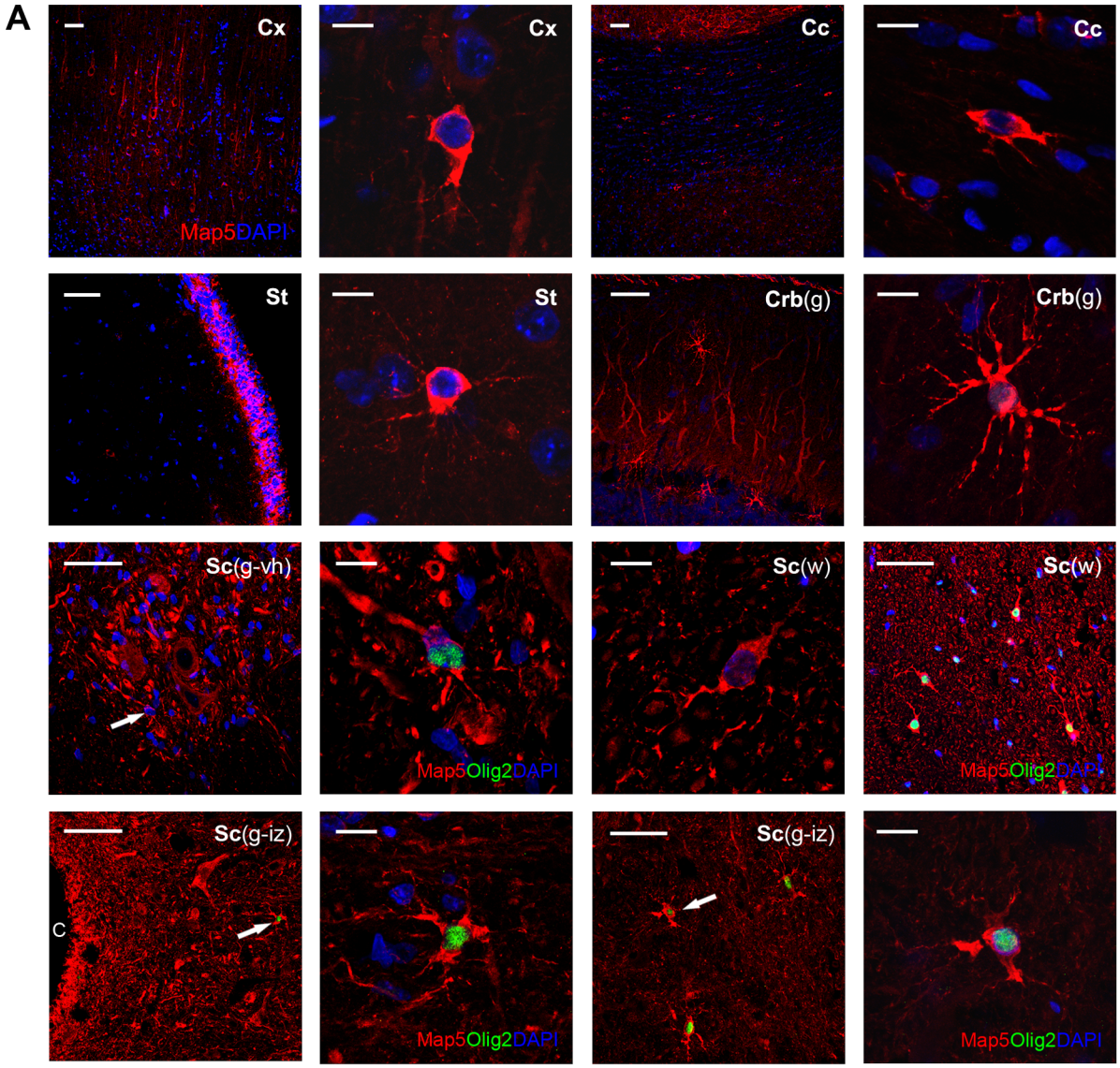

Figure 6. Regional distribution of mMap5 cells in the rabbit CNS. A, Representative images of mMap5 cells in the cerebral cortex (Cx), corpus callosum (Cc), striatum (St), cerebellum (Crb), and spinal cord (Sc). g, grey matter; w, white matter; vh, ventral horn; iz, intermediate zone; c, central canal. B, Quantitative analysis of mMap5 cell density in different regions, at three different ages. Squares indicate the areas in which cell counts have been carried out. Asterisks/dots indicate significant statistical differences in mMap5 cell densities among different regions/ages (two way ANOVA, $p<0,001$). Scale bars: Low magnifications, 50 μm; high magnifications, 10 μm.
doi:10.1371/journal.pone.0063258.g006

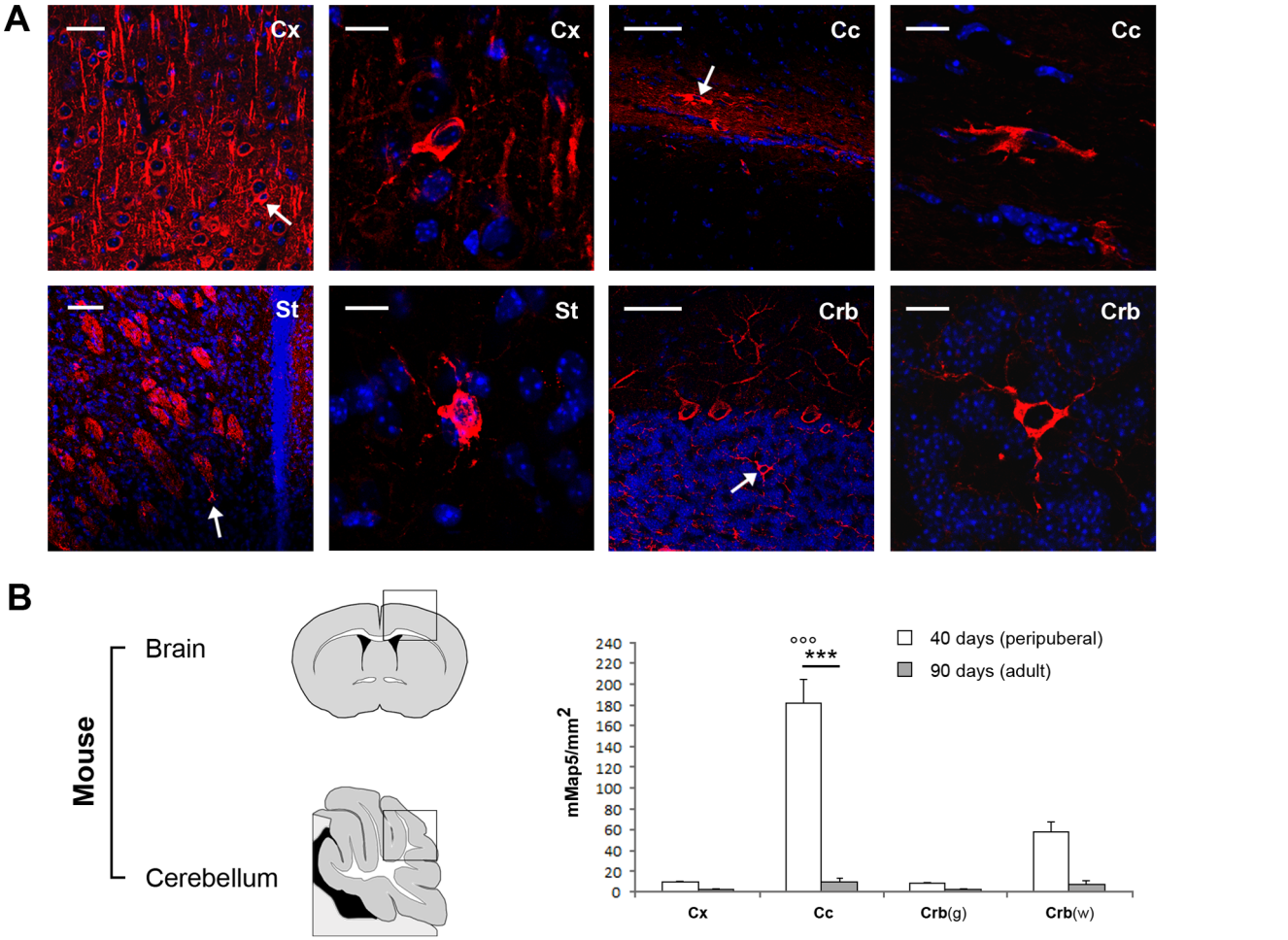

Figure 7. Regional distribution of mMap5 in the mouse CNS. A, Representative images of mMap5 cells in the cerebral cortex (Cx), corpus callosum (Cc), striatum (St), cerebellum (Crb). g, grey matter; w, white matter. B, Quantitative analysis of mMap5 cell density in different regions, at two different ages. Squares indicate the areas in which cell counts have been carried out. Asterisks/dots indicate significant statistical differences in mMap5 cell densities among different regions/ages (two way ANOVA, $p<0,001$). Scale bars: Low magnifications, 50 μm; high magnifications, 10 μm.
doi:10.1371/journal.pone.0063258.g007

mMap5 cells in mice and rabbits described here, which likely contributed to the 'hiding' of mMap5 cells in previous studies carried out on rodents (see for example [13,29]).

mMap5 cells are a population of newly generated, postmitotic parenchymal elements of the oligodendroglial lineage

Although widely expressed in different types of neuronal cells (mature neurons and neuroblasts generated from both neural stem cells and parenchymal progenitors), Map5 also decorates a subset of multipolar glial-like cells (Fig. 1). We show that these multipolar elements, here referred to as mMap5 cells, are not neurons, nor astrocytes or microglia, and consistently express Olig2, Sox10, and, to a lesser extent, Sox9 and Sox2 transcription factors in their nuclei (Figs. 2 and 3). Olig2 is necessary for oligodendrocyte lineage development in multipotent progenitor cells of the embryo and adult [73]. The transcription factors Sox9 and Sox10 jointly occur in cells of the oligodendrocyte lineage, being important for their specification and terminal differentiation [74]. Sox9, also expressed in astrocytes, in the oligodendrocyte lineage marks the stage of immature oligodendrocyte precursors, then being turned off during maturation. Sox10 is not expressed in all CNS glial cells, being restricted to the oligodendrocyte lineage. With the onset of specification, Sox10 starts to be expressed in proliferating oligodendrocyte progenitors and persists in mature, terminally differentiated oligodendrocytes (reviewed in [74]). On the whole, the pattern of molecular expression found in mMap5 cells clearly places them in the oligodendroglial lineage. In addition, some mMap5 cells also express Sox2, a transcription factor involved in neural stem/progenitor cell maintenance [75]. This raises the question if either all mMap5 cells are committed to the oligodendroglial lineage or some of them might retain properties of immature/progenitor cells. To answer this question, we performed a study on cell proliferation dynamics, by using different cell division markers (Fig. 4). Our results obtained with the endogenous cell proliferation marker Ki67 demonstrate that mMap5 cells are not cycling elements (as the Ng2+progenitors are [1,2]). Nevertheless, BrdU injections followed by different survival times showed that, even if postmitotic, when expressing the Map5 molecule a substantial proportion of these cells is newly generated (25% of all the mMap5 cells and 20% of cells which are newly generated at 10–15 days post-injection; data detected with our BrdU injection protocols, which cannot reveal all the newlyborn cells and thus underestimate the total number of newly generated mMap5 cells). It is interesting to note that, unlike Map5 expression