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Oligodendrocyte heterogeneity in the mouse juvenile and adult central nervous system

Sueli Marques[#1], **Amit Zeisel**[#1], **Simone Codeluppi**[1,2], **David van Bruggen**[1], **Ana Mendanha Falcão**[1], **Lin Xiao**[3,4], **Huiliang Li**[3], **Martin Häring**[1], **Hannah Hochgerner**[1], **Roman A. Romanov**[1,5], **Daniel Gyllborg**[1], **Ana Muñoz Manchado**[1], **Gioele La Manno**[1], **Peter Lönnerberg**[1], **Elisa M. Floriddia**[1], **Fatemah Rezayee**[1], **Patrik Ernfors**[1], **Ernest Arenas**[1], **Jens Hjerling-Leffler**[1], **Tibor Harkany**[1,5], **William D. Richardson**[3], **Sten Linnarsson**[1,*], and **Gonçalo Castelo-Branco**[1,*]

[1]Laboratory of Molecular Neurobiology, Department Medical Biochemistry and Biophysics, Karolinska Institutet, SE-17177 Stockholm, Sweden

[2]Department of Physiology and Pharmacology, Karolinska Institutet SE-17177 Stockholm, Sweden

[3]Wolfson Institute for Biomedical Research, University College London, Gower Street, London WC1E 6BT, United Kingdom

[4]Institute of Neuroscience, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, China

[5]Department of Molecular Neurosciences, Center for Brain Research, Medical University of Vienna, Austria

[#] These authors contributed equally to this work.

Abstract

Oligodendrocytes have been considered as a functionally homogenous population in the central nervous system (CNS). We performed single-cell RNA-Seq on 5072 cells of the oligodendrocyte lineage from ten regions of the mouse juvenile/adult CNS. Twelve populations were identified, representing a continuum from *Pdgfra*+ oligodendrocyte precursors (OPCs) to distinct mature oligodendrocytes. Initial stages of differentiation were similar across the juvenile CNS, whereas subsets of mature oligodendrocytes were enriched in specific regions in the adult brain. Newly-formed oligodendrocytes were found to be resident in the adult CNS and responsive to complex motor learning. A second *Pdgfra*+ population, distinct from OPCs, was found along vessels. Our study reveals the dynamics of oligodendrocyte differentiation and maturation, uncoupling them at a transcriptional level and highlighting oligodendrocyte heterogeneity in the CNS.

[*]Correspondence to: sten.linnarsson@ki.se, goncalo.castelo-branco@ki.se.

Additional Author notes:
SM, AZ, HL, WDR, SL and GC-B designed the experiments. PE, EA, JH-L, TH, WDR, SL and GC-B, senior authors, obtained funding. SM, AZ, SC, HH, RAR, DG, MH, AMM, GLM, FR, HL, LX, EF performed experiments. LX, HL and WDR have priority of observation of the rapid differentiation of oligodendrocytes in the complex motor wheel paradigm. SM, AZ, DvB, AMF, GLM, PL analysed data. SM, AZ, SL and GC-B wrote the paper, with the assistance and proofreading of all authors.

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Oligodendrocytes ensheath axons in the CNS, allowing rapid saltatory conduction and providing metabolic support to neurons. While a largely homogeneous oligodendrocyte population is thought to execute these functions throughout the CNS (1), these cells were originally described as morphologically heterogeneous (2). It is thus unclear if oligodendrocytes become morphologically diversified during maturation through interactions within the local environment, or if there is intrinsic functional heterogeneity (3–5). We analyzed 5072 transcriptomes of single cells expressing markers from the oligodendrocyte lineage, isolated from ten distinct regions of the anterior-posterior and dorsal-ventral axis of the mouse juvenile and adult CNS (Fig. 1A and 1B). Biclustering analysis (6) (Fig. S1B and S15), hierarchical clustering (Fig. 1C) and differential expression analysis (Supporting File Supplementary Excel S1 and S2) led to the identification of thirteen distinct cell populations. t-Distributed Stochastic Neighbour Embedding (t-SNE) projection (Fig. 2A) indicated a narrow differentiation path connecting OPCs and myelin-forming oligodendrocytes, diversifying into six mature states, which was supported by pseudo-time analysis (Fig. S2A-B).

OPCs co-expressed *Pdgfra* and *Cspg4* (Figs. 2B, S1B and S10) and 10% co-expressed cell cycle genes (Fig. S2E-F), consistent with a cell division turnover of 19 days in the juvenile cortex (7). Several genes identified in OPCs were previously associated with astrocytes/radial glia (6) (*Fabp7* and *Tmem100*, Figs. S1B, S3 and S10), consistent with the origin of OPCs from radial glia-like cells, and their capacity to generate astrocytes in injury paradigms (8).

Differentiation-committed oligodendrocyte precursors (COPs) were distinct from OPCs (lacking *Pdgfra* and *Cspg4*), and expressed *Neu4* and genes involved in keeping oligodendrocytes undifferentiated (*Sox6*, *Bmp4* and *Gpr17*) (9–11) (Figs. 2B, S1B, S4 and S10). They presented lower levels of cell cycle markers (Fig. S2E-F), while expressing genes involved in migration (*Tns3* and *Fyn*) (Fig. S10). Newly-formed oligodendrocytes (NFOL1-2) expressed genes induced at early stages of differentiation (12–14) (*Tcf7l2*, *Casr,* Fig. S10). While *Gpr17* expression decreased in these cells, *Tcf7l2*, involved in oligodendrocyte differentiation (15), peaked (Fig. S10).

Myelin-forming oligodendrocytes (MFOL1-2) expressed genes responsible for myelin formation (*Mal, Mog, Plp1, Opalin* and *Serinc5*, Fig. S1A-B). Single-molecule fluorescence RNA *in situ* hybridization (smFISH) showed that myelin-forming populations (*Ctps*+) were distinct from mature oligodendrocytes (*Klk6*+), (Fig. 2C, S4D). Mature oligodendrocytes (MOL1-6) expressed late oligodendrocyte differentiation genes (12) (*Klk6*, *Apod*) and genes present in myelinating cells (*Trf* and *Pmp22*) (Fig. S1B).

We identified a second *Pdgfra+* population, vascular and leptomeningeal cells (VLMCs), distinct from OPCs and segregated from all oligodendrocyte lineage cells (Fig. 1C and 2A). This population was also found when sorting GFP+ cells from *Pdgfra*-H2B-GFP (16) and *Pdgfra*-Cre-RCE (LoxP-GFP) mice (17) (Fig. S2C). These cells exhibited low levels of *Cspg4* (NG2) (Fig. 2B), specifically expressed *Lum* (Fig. 2B and S4), markers of the pericyte lineage (*Vtn, Tbx18*; Fig. 2B, S1B and S2D) and laminins and collagens characteristic of the basal lamina. *Pdgfra*+/*Sox10*- VLMCs were localized on blood vessels

(Fig. 2D, S4 and S11A-B) and meninges (Fig. S11A-C). In contrast, COL1A1-/PDGFRA+ OPCs were distributed in the parenchyma, in close association but not overlapping with the vasculature (Fig. 2D and S11B)(18). VLMCs specifically exhibited markers present in transcriptomes of OPCs isolated based on PDGFRA + immunoreactivity (Fig. S3) (14), most likely previously assigned to OPCs due to co-purification.

We retrieved the fifty genes that better differentiate every branch of the dendrogram plot (Fig. 1C) and investigated their putative function by Gene Ontology (GO) (Fig. S6-9, Supporting File Supplementary Excel S1 and S2). Differentiation-committed oligodendrocyte precursors were enriched in cell fate commitment and adhesion genes, while newly-formed oligodendrocytes (1-2) already presented genes involved in steroid biosynthesis, ensheathment of neurons and cell projection organization (Fig. S7). These populations exhibited unique expression of *Tcf7l2*, *Itpr2, Tmem2* and *Pdgfa* (Fig. 3A and S4). ITPR2, encoding an intracellular Ca2+ channel, was more specific to oligodendrocytes than TCF7L2 and exhibited close to 100% overlap with SOX10 positive cells (Fig. S5A, D). We observed that ITPR2 immunoreactive cells were distinct from PDGFRA+ OPCs (Fig. S5B), and lineage tracing confirmed that ITPR2+ cells are the progeny of OPCs (Fig. S2C and S5C). 22±2% and 25±1.5% of the OPC-derived *Pdgfra*-H2B-GFP+ cells were ITPR2+ in the somatosensory (S1) cortex and CA1 hippocampus at P21 respectively, while 43±3.7% double positive cells were found in the corpus callosum (Fig. S5C). The percentage of ITPR2+/Sox10+ cells in the corpus callosum remained within the same range at P7 (47±4%) and P21 (37±1%, Fig. 3C). 77±4% and 48±7% of SOX10+ oligodendrocytes were ITPR2+ at P7 in the CA1 hippocampus and S1 cortex, respectively, and decreased to less than 20% thereafter (Fig. 3B-C). This distribution of ITPR2+ oligodendrocytes correlates with active and prolonged differentiation in the juvenile rat corpus callosum (19). These tissues still maintained 10-20% ITPR2+ cells at adult stages (P90, Fig. 3C).

To investigate the potential function of the ITPR2+ population in the adult brain, we analysed their dynamics in the corpus callosum of mice engaged in motor learning in the complex wheel paradigm, a process that requires active myelination (20). In this paradigm, running on the wheel leads to an increase in the number of proliferating OPCs after 4 days, followed by an increase in oligodendrocytes after 8 days (20). However, increased motor skills were already apparent after 2 days in wild type mice, but not in mutant mice that were unable to synthesize new myelin (20), suggesting that oligodendrocyte lineage cells contribute to learning already within the first 2 days. We found that the number of ITPR2+/SOX10+ was increased ~50% in mice that ran on the complex wheel for 2 days, relative to non-runners (Fig. 3D-E). Thus, novel motor activity might trigger rapid differentiation of OPCs into ITPR2+ committed precursors/newly-formed oligodendrocytes that contribute to early learning by facilitating electrical transmission, either through the initiation of myelination or some other pre-myelinating function.

We were unable to identify region- or age- (juvenile versus adult) specific subpopulations of OPCs in our dataset (Fig. 2A, 4A and 4B). Nevertheless, 16% of the juvenile OPCs were in the cell cycle (as determined by the simultaneous expression of more than 2 cell cycle markers, Fig. S2F), compared to ~3% of the adult OPCs. Similarly, differentiation-committed oligodendrocyte precursors and newly-formed oligodendrocytes were present in

all regions in juvenile mice (Fig. 1C and 4A), revealing a common trajectory of differentiation between the different regions (Fig. 2A). These populations were also observed in the adult corpus callosum and S1 cortex, albeit in considerably lower numbers compared to juvenile mice (Fig. 4B). Based on the distribution of cell types in the juvenile mice, we classified regions as immature (anterior regions such as amygdala and hippocampus), intermediate (corpus callosum, zona incerta, striatum and hypothalamus) and mature (cortex and posterior regions such as dorsal horn and SN-VTA) (Fig. 4A and S12). These regional differences could result from different timing of oligodendrocyte maturation during post-natal development (21, 22). Indeed, myelination first starts in the rat in posterior regions (dorsal horn) around P7, while in anterior regions of the CNS (amygdala, hippocampus, striatum and cortex) it occurs between P21 and P28 (23).

Different regions of the CNS were populated by diverse mature oligodendrocytes (Fig. 1C and Fig. S12). While some populations, such as MOL5, were present throughout the regions, other MOLs where enriched in certain regions (Fig. S12). Some of these mature oligodendrocyte populations might be intermediate stages or have specific functions in juvenile mice but then disappear in adulthood. Subsets of MOL5 and MOL6, were mainly present in S1 cortex and corpus callosum in the adult mice (Fig. 4B). Since MOL5 was already present in several regions of the juvenile CNS (Fig. 1C and Fig. S12), final maturation of oligodendrocyte might already be achieved in the juvenile mice in certain regions, such as the dorsal horn, but only in adulthood in others, such as the corpus callosum.

GO analysis indicated a divergence already at the stage of myelin formation (Fig. S8 and Supporting File Supplementary Excel S1, S2). Although mature oligodendrocyte populations shared the expression of many genes, some were differentially enriched within populations (Fig S8, Supporting File Supplementary Excel S1, S2) indicating segregation of MOL1-4, enriched in lipid biosynthesis and myelination genes (*Far1* and *Pmp22*), from MOL5-6 (adult), enriched for synapse parts such as *Grm3* (metabotropic glutamate receptor, mainly enriched in MOL6) and *Jph4.* We confirmed the presence of GRM3 in the oligodendrocyte lineage (*Pdgfra*-Cre-RCE) and specifically in CC1+ mature oligodendrocytes in the juvenile cortex (Fig. S11D). Even within MOL1-4, enriched in myelin-related genes, specific populations, such as MOL3, are more likely to be involved in synaptic activity (Fig. S9 and Supporting File Supplementary Excel S1, S2). Optic nerve oligodendrocytes can form axon-myelinic synapses, responding to axonal action potentials via glutamate ionotropic NMDA receptors (24). We analysed the expression of ionotropic and metabotropic glutamate receptors and other ions channels, including TRP (25) and potassium channels (Fig. S14). Although most glutamate receptor subunits were expressed throughout oligodendrocyte lineage cells, there was preferential expression in some populations, with single cells displaying combinations of subunits that might determine function. Potassium channels and TRPs were also expressed in a cell type-specific manner, displaying a scattered distribution within populations (Fig. S14). Thus, the communication of mature oligodendrocytes with neighbouring neurons might be mediated through specific receptors and channels, following synaptic input or vesicular release.

Our study provides a high-resolution view of the transcriptional landscape of a single neural subtype across multiple regions of the CNS, and indicates a transcriptional continuum between oligodendrocyte populations, with a subset representing distinct but nevertheless connected stages in the maturation path from an OPC to mature oligodendrocytes (Fig. S16). Initial differentiation was uniform throughout the CNS, while mature oligodendrocyte subtype specification occurred later at postnatal stages and in a region specific manner. Each brain region appears to optimize its circuitry by representation of unique proportions and combinations of mature oligodendrocytes. Our data also indicate that ITPR2+ oligodendrocytes are involved in rapid myelination in complex motor learning and thus might be relevant in other active maturation/myelination processes such remyelination in disease/lesion paradigms. Non-proliferative *Nkx2*.2+ precursors with a profile consistent with these cells (Fig. S10) have been observed in lesions of multiple sclerosis patients (26). Therefore our study, by establishing oligodendrocytes as a transcriptionally heterogeneous cell lineage, might lead to new insights into the aetiology of myelin disorders such as multiple sclerosis and suggest novel targets for their treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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One Sentence Summary

Transcriptional heterogeneity of oligodendrocytes across the juvenile and adult mouse central nervous system

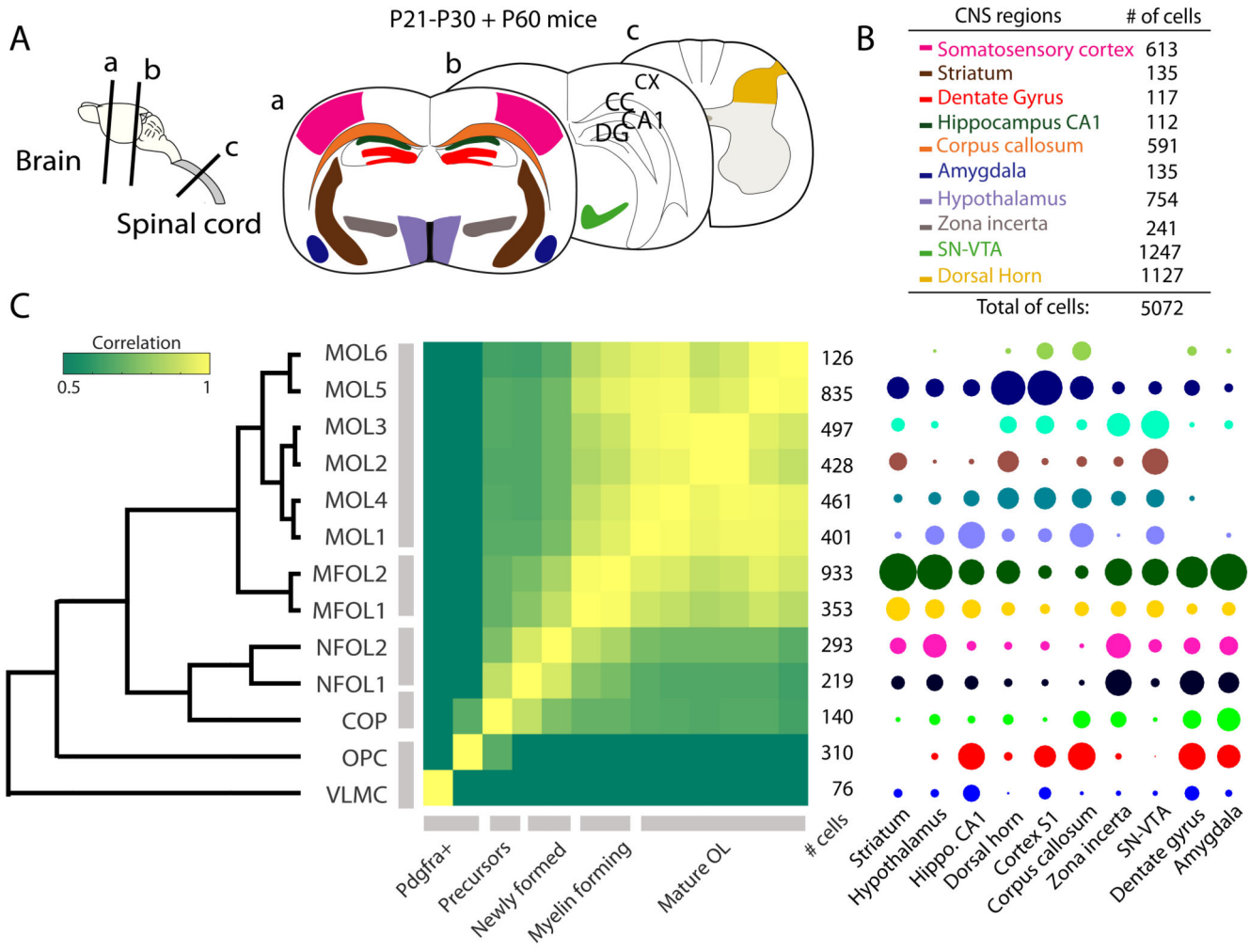

Figure 1. Single cell RNA-Seq analysis of 5072 cells expressing markers of the oligodendrocyte lineage in ten regions of the mouse CNS.

(A) Targeted regions. (B) Number of cells analysed for each region. (C) Hierarchical clustering (left), correlation matrix (middle) and subclass abundances by region (right).

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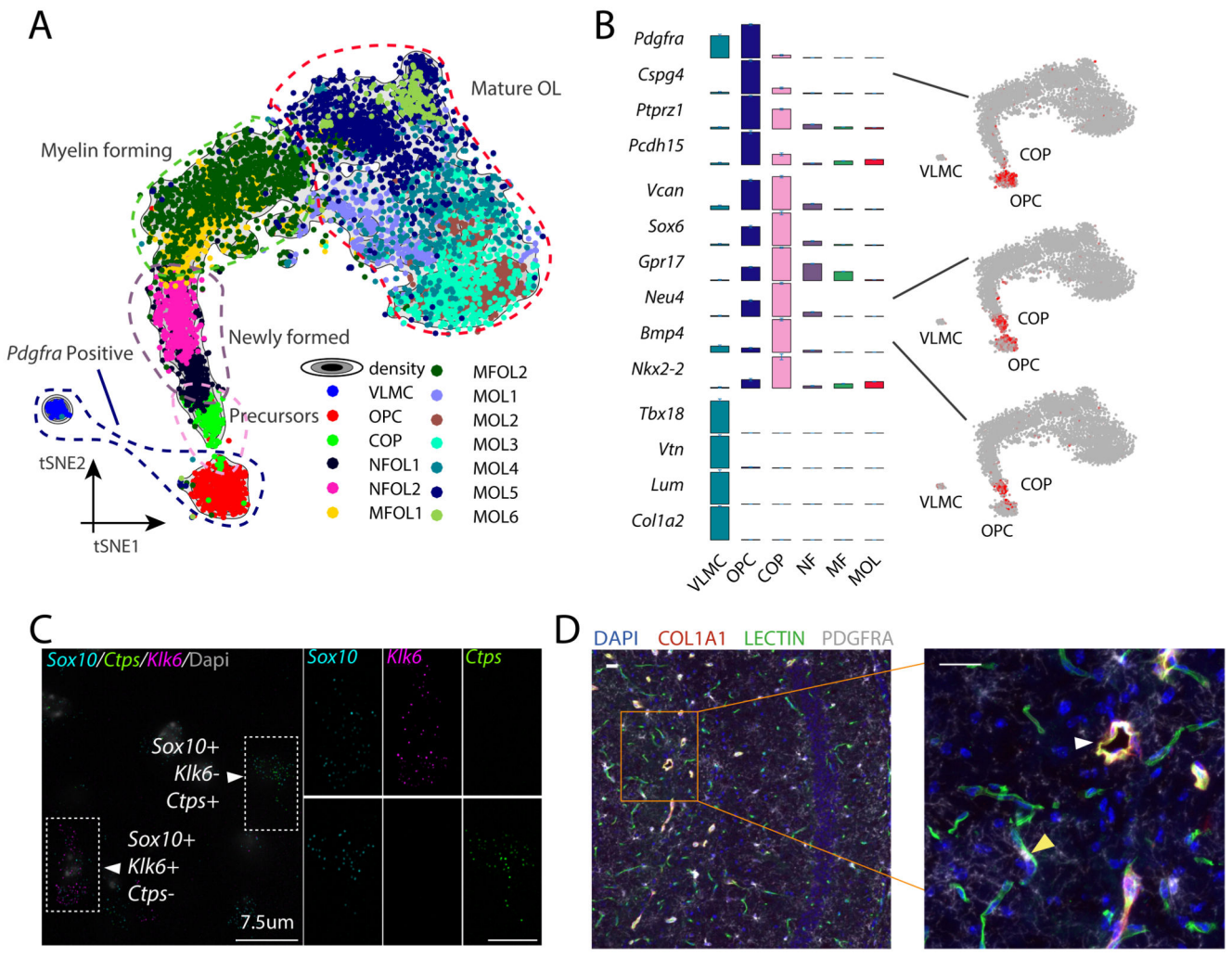

Figure 2. Oligodendrocyte cell states in the continuous maturation process from precursors to mature cells.

(A) t-SNE projection showing the trajectory from OPCs to mature oligodendrocytes. (B) Average (±s.e.m.) expression of marker genes for OPCs, COPs and VLMCs. Representative markers are overlayed on the tSNE map (gray, low expression; red, high expression). (C) smFISH for *Sox10, Ctps* (MFOL marker) and *Klk6* (MOL marker) confirm that these populations are distinct. (D) Immunohistochemistry of COL1A1 (VLMCs), PDGFRA (OPCs and VLMCs) and Tomato LECTIN (blood vessels) in P21 brain. White arrowhead - VLMCs; yellow arrowhead- OPCs (COL1A1-). Scale bar 25μm.

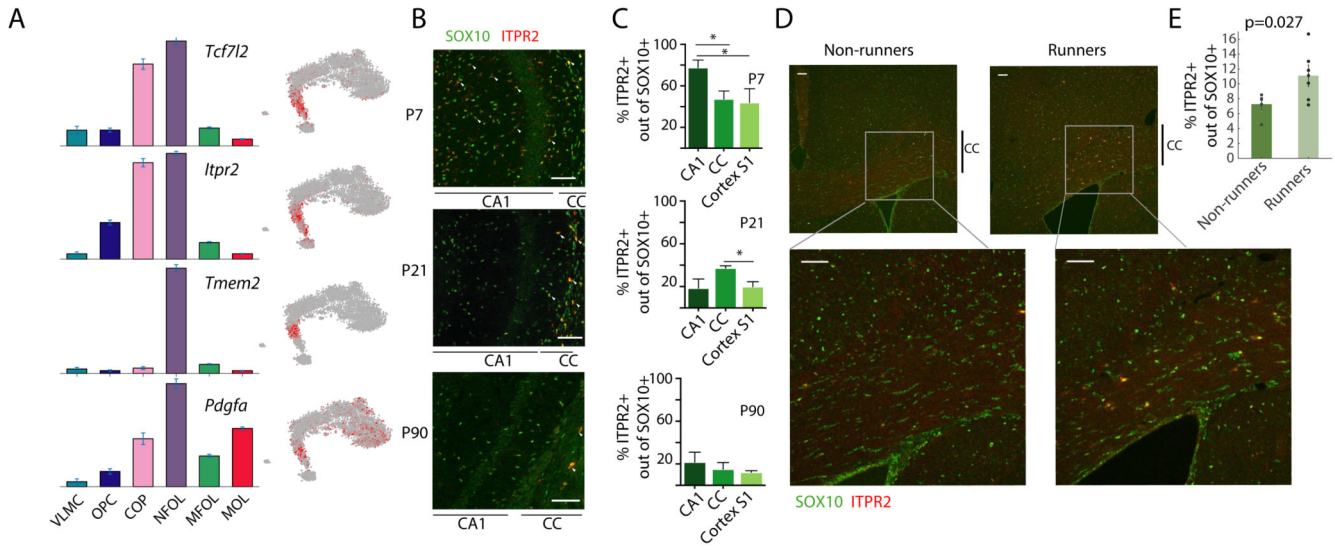

Figure 3. ITPR2+ oligodendrocytes are present in regions of active differentiation and increase in mice undergoing learning in the complex wheel paradigm.

(A) Average (±s.e.m.) expression level of *Tcf7l2*, *Itpr2*, *Tmem2* and *Pdgfa* along the oligodendrocyte lineage. (B-C) Immunohistochemistry and quantification of ITPR2+/SOX10+ cells in P7, P21 and P90 brain. One-way ANOVA with Tukey´s multiple comparison test **P*<0.05, *n*=3. (D-E) Immunohistochemistry and quantification of ITPR2+/SOX10+ cells in corpus callosum of P60 non-runners versus runners after 2 days in the complex wheel-learning paradigm (one-tailed Student's t test). Scale bars 75 μm.

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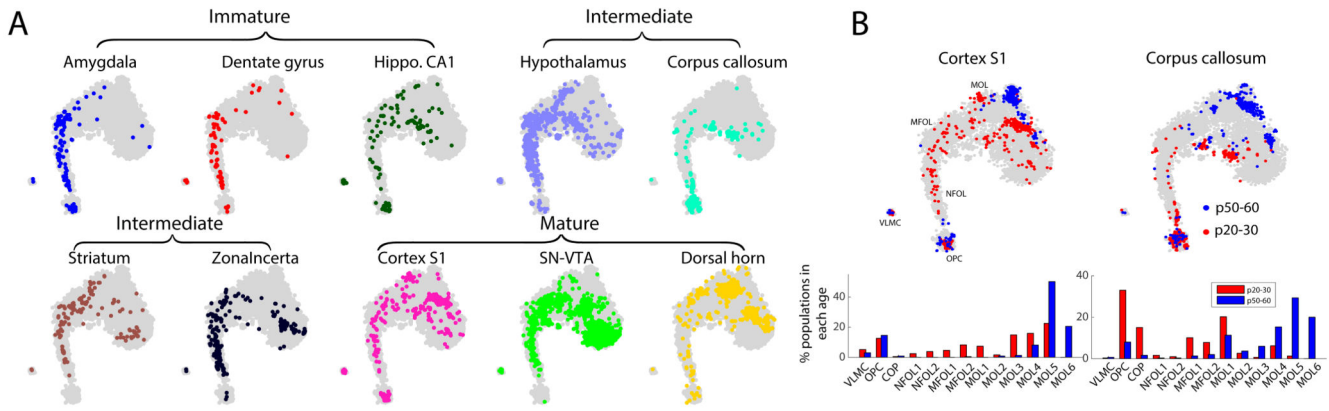

Figure 4. Region and age specific distribution of mature oligodendrocytes

(A) t-SNE projections as in Fig. 2A with colored dots representing cells from each of the ten CNS regions analyzed. (B) Age distribution of OL populations in cortex S1 and corpus callosum. Bar plots show the percentage of each population by age. Red- juvenile brain; blue- adult brain.

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