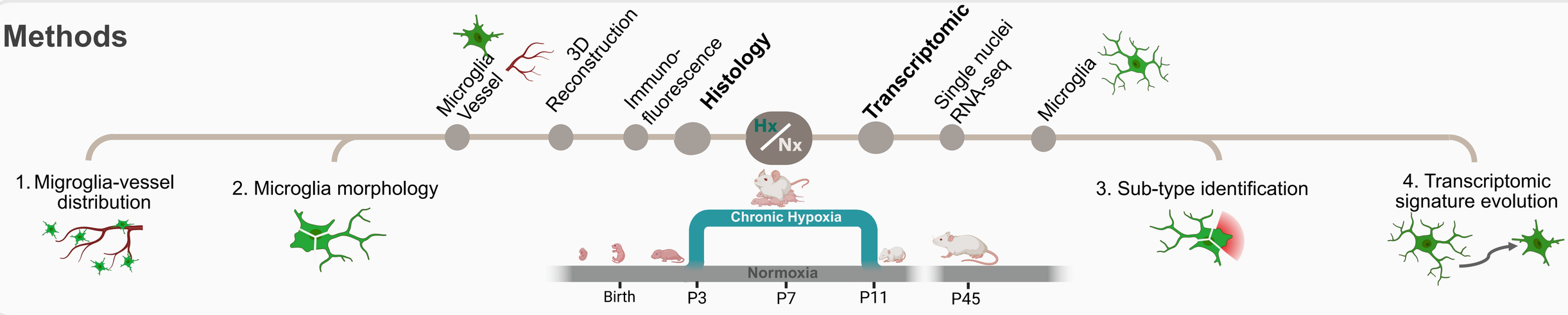
Effect of Hypoxia on Microglial morphology and transcriptomic signature in a mouse model of great prematurity

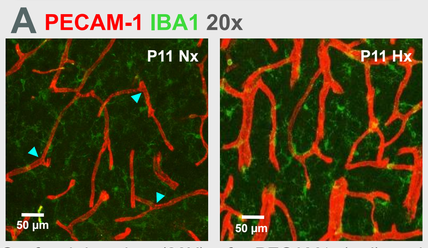
Jeanne Gaudicheau Aubry[1], Alexane Andrieux[1], Camille Barbara[1], Thomas Gagnieu[1], Jean-François Ghersi-Egea[2], Olivier Raineteau[1]

Brain development relies on tightly coordinated interactions between microglia and the vascular network. Early-life disturbances such as extreme prematurity can disrupt this interplay and contribute to neurodevelopmental disorders. Using a neonatal mouse model of chronic hypoxia that reproduces key features of great prematurity, we investigated the spatial and temporal dynamics of microglial reactivity.

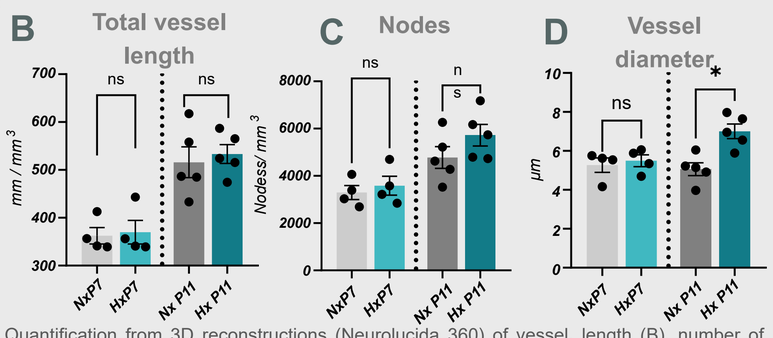
Methods



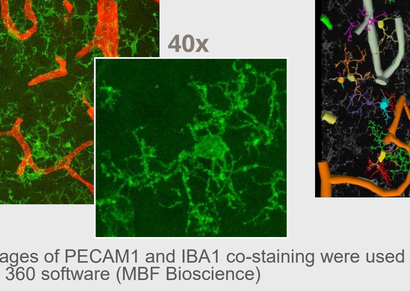
1 Chronic hypoxia alters blood vessel diameter and microglial distribution without impacting vascular density



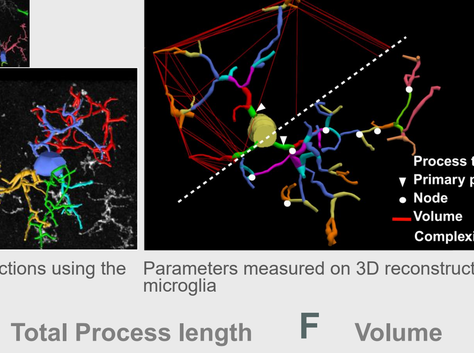
Confocal imaging (20X) of PECAM1 (red) and IBA1 (green) stainings. Nodes are indicated by blue arrows



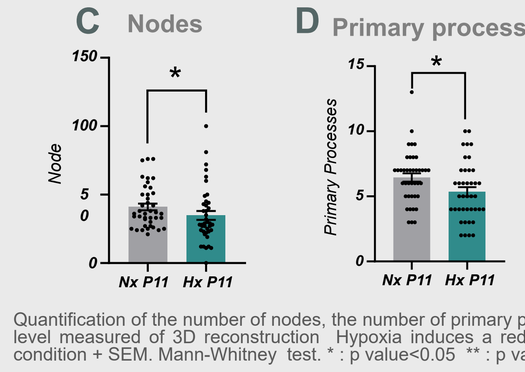
Quantification from 3D reconstructions (Neurolucida 360) of vessel, length (B), number of node (C) and diameter (D) in both normoxic and hypoxic conditions at P7 and P11. Note that only the vessel diameter is impacted by hypoxia n=5 animal in each condition + SEM. Mann-Whitney test. * : p value<0.05



Confocal imaging (40X) of PECAM1 (red) and IBA1 (green) staining. Microglia are indicated by the yellow arrows.
Dotted white lines represent the microglial classification group considering distance to the closest blood vessel

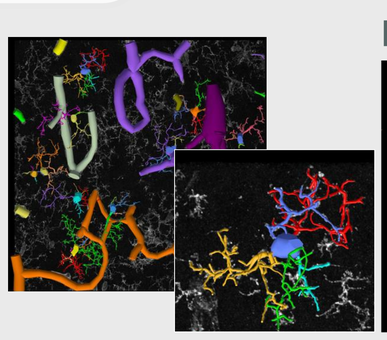


Microglial soma quantification in each distance group illustrates a delay in microglia dispersion. n=5 animals in each condition + SEM. Mann-Whitney test. * : p value<0.05



Quantifications of microglial density (IBA1+; G) and proliferating microglia (IBA1+/Ki67+; H). Transient reduction of microglia density (P7) appears to be compensated by increased proliferation at P11. n=5 animals in each condition + SEM. Mann-Whitney test. * : p value<0.05

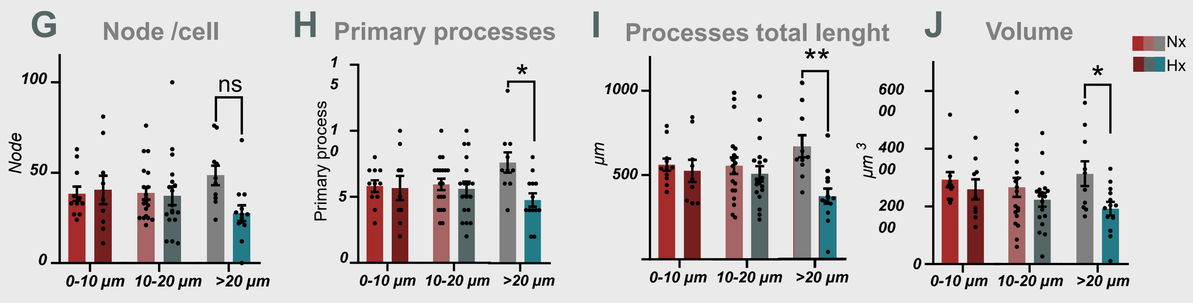
2 Hypoxia induces heterogenous morphologic alteration limited to a subpopulation of microglia distant from blood vessel



Confocal images of PECAM1 and IBA1 co-staining were used for 3D reconstructions using the Neurolucida 360 software (MBF Bioscience)

Parameters measured on 3D reconstructed microglia

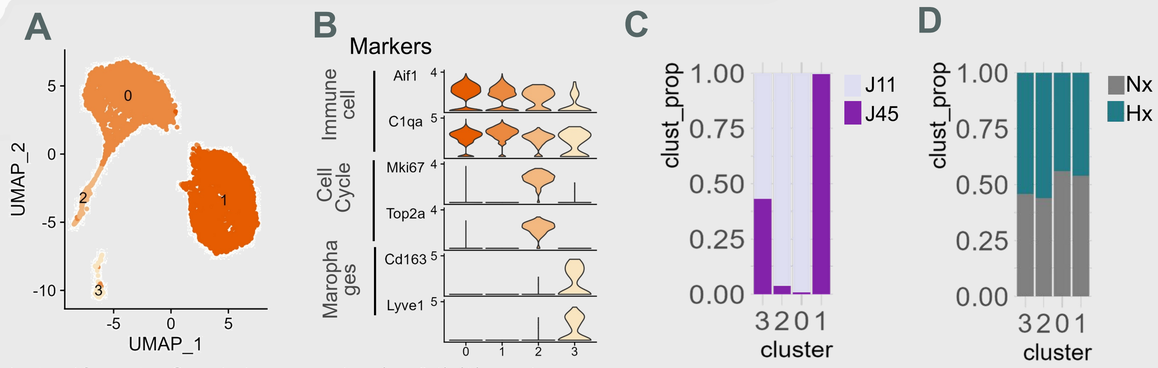
Quantification of the number of nodes, the number of primary processes, total process length and volume of microglia at the single cell level measured of 3D reconstruction Hypoxia induces a reduction of several microglia morphological features. N=80 cell in each condition + SEM. Mann-Whitney test. * : p value<0.05 ** : p value < 0.001



Quantifications of number of nodes, number of primary processes, total process length, and microglial volume of individual microglia, measured from 3D reconstructions in each distance group from blood vessels. Morphological alterations are specific to a microglial subtype distant from blood vessels. N=80 cell in each condition + SEM. p-value was calculated with a Mann-Whitney statistical test. * : p value<0.05 ** : p value < 0.001

1.Univ Lyon, Université Claude Bernard Lyon1, Inserm, Stem Cell and Brain Research Institute U1208, Bron, France.
2.Fluid Team, Lyon Neurosciences Research Center, INSERM U1028, CNRS, UMR5292, University Lyon-1, Lyon, France.

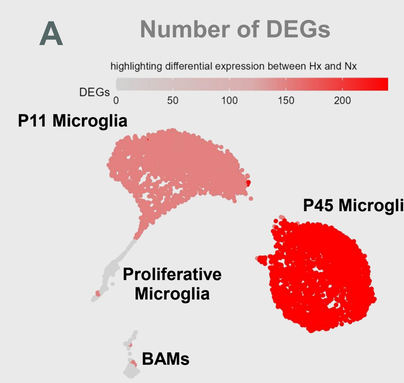
3 Microglial transcriptomic signature evolves through time



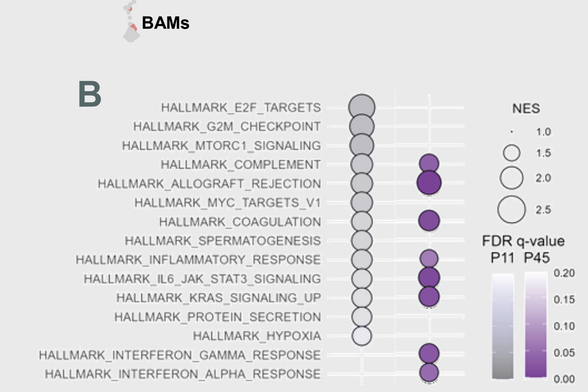
Cluster identified using Seurat at low resolution (i.e. 0.1) (A), cell types were identified (B) through markers of immune cell (Aif1, C1qa), cell cycle (Mki67, Top2a) and macrophages (Cd136, Lyve 1).

Proportion of Nuclei at P11 and P45 and from normoxic and hypoxic conditions within each cluster.

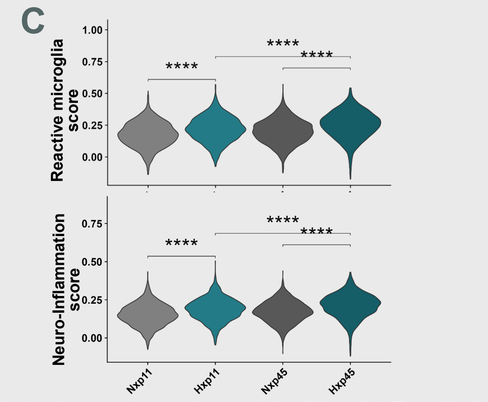
4 Transcriptomic signatures highlight a persisting microglial reactivity



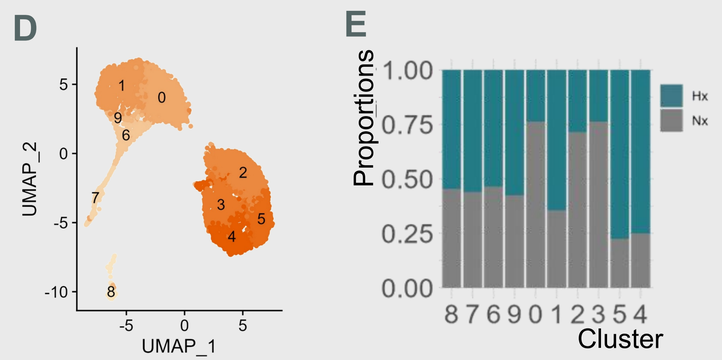
UMAP of DEG induced by hypoxia in all clusters. Hypoxia-induced transcriptional dysregulation are mainly observed in postmitotic microglia cells and are prevalent at P45. BAM : Brain associated macrophages



GSEA analysis highlight hallmark gene sets induced by hypoxia at P11 and P45 associated to inflammation. NES: Normalized Enrichment Score.



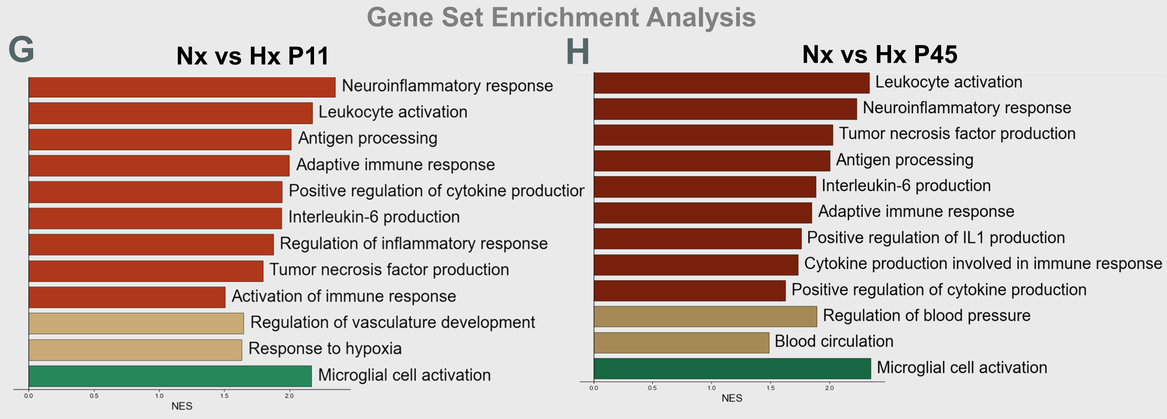
Module Score of genes involved in " Microglial Reactivity" and "Neuro-inflammation" identify an Hypoxia induced inflammatory response that persist at P45.



Cluster identified using Seurat at high resolution (i.e. 0.6, D) and proportion of hypoxic and normoxic nuclei in each cluster (E). Clusters 1, 4, and 5 are predominantly composed of Hypoxic microglia nuclei

UMAP showing module score for "Microglial Reactivity" gene-set enriched in clusters 1, 4, and 5.

Gene Set Enrichment Analysis



Selection of gene sets enriched by hypoxia at P11 (G) and P45 (H) identified by GSEA analysis. Note that numerous gene sets are linked to Inflammation (red), vasculature (yellow), and microglial reactivity (green). Inflammatory gene set enrichment persists at P45, such as Microglial reactivity. Vasculature gene sets evolve from "developmental" to "regulatory" ones. NES: Normalized enrichment score

Conclusion

Short and long-term effects of neonatal chronic hypoxia on microglia