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Supplementary information

Revisiting the neurovascular unit

In the format provided by the authors and unedited

Supplemental Methods

The count matrix and cluster annotations are from http://dropviz.org[1]. The dataset is composed of 15,985 endothelial cells from 9 sequenced libraries corresponding to 9 regions in the adult mouse brain.

We used the Seurat integration pipeline to remove batch effects between each library[2]. In brief, the 9 libraries (composed of 3-7 replicates per region) were log-normalized independently using a size factor of 10,000, and the 2,000 most variable genes were extracted. Default parameters were used to identify anchor correspondences between biologic replicates and return an integrated expression matrix for all cells. After scaling, we computed a principal component analysis, and used the first 20 components to cluster the cells with the *FindClusters* Seurat function. Visualization of the integrated data was performed by t- distributed stochastic neighbor embedding implemented in Seurat (*tsne.method = 'FIt-SNE' , resolution= 0.4*). The MAST method (Vers. 1.12.0) was used to calculate cluster-specific markers *(logfc.threshold = log(1.5))*, and cell type identity was manually assigned to clusters using previously established marker genes [3–5]. This workflow produced 7 global clusters of endothelial cells across the 9 different brain regions, enabling the libraries to be jointly analyzed. We generated an expression heatmap to plot the top markers for each cluster *(average logfc value > log(1.5), percentage of cells expression > 0.5)*.

Comparative analysis of frontal cortex and hippocampal endothelial cells was performed by subsetting these cells from the dataset. This subset was integrated and clustered again with the same parameters described above. For each endothelial cell type, the MAST test was used to identify the differentially expressed genes between both regions *(logfc.threshold = log(1.5))*. Following this, we generated scatter plots of the log-normalized average expression of both frontal cortex and hippocampal endothelial cells genes (gray dots), to highlight the differentially expressed genes upregulated in frontal cortex and hippocampus (light blue). The top 10 most regulated genes are labeled and indicated in dark blue.

Data analysis was performed in the R (Vers. 4.02) statistical environment, and *ggplot* (Vers. 3.2.1) R/tidyverse and Seruat (Vers. 3.2.0) packages. All gene ontology terms were derived from the biological process subset of MSigDB's v7.1 GO gene sets (C5) for *Mus musculus*. All significant differentially expressed genes were used for analysis and resulting pathways with a FDR q-value <0.05 are presented.

References

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Supplemental table 1: Enriched GO terms in cortical and hippocampal endothelial cells. The gene ontology (GO) terms were derived from the biological process subset of MSigDB's v7.1 GO gene sets (C5) for Mus musculus. All significant differentially expressed genes were used for analysis and resulting pathways with a FDR q-value <0.05 are presented.

Subtype	Region	GO Term	Description	*Genes*
Arterial 1	Frontal Cortex	N/A		
Arterial 1	Hippocampus	Regulation of cell death	Any process that modulates the rate or frequency of cell death. Cell death is the specific activation or halting of processes within a cell so that its vital functions markedly cease, rather than simply deteriorating gradually over time, which culminates in cell death. [GOC:dph, GOC:tb]	*Rgcc, Igfbp3, Bnip3, Zbtb16, Serinc3, Timp3, Tsc22D3, Ier3*
Arterial 1	Hippocampus	Cell motility	Any process involved in the controlled self-propelled movement of a cell that results in translocation of the cell from one place to another. [GOC:dgh, GOC:dph, GOC:isa_complete, GOC:mlg]	*Slc3A2, Slc7A5, Rgcc, Igfbp3, Adgrg1, Fam107A, Plat, Esam*
Arterial 1	Hippocampus	Branched chain amino acid transport	The directed movement of branched-chain amino acids into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. Branched-chain amino acids are amino acids with a branched carbon skeleton without rings. [GOC:ai, GOC:bf]	*Slc3a2, Slc7a5*

Subtype	Region	GO Term	Description	*Genes*
Arterial 2	Frontal Cortex	N/A		
Arterial 2	Hippocampus	Regulation of cell population proliferation	Any process that modulates the frequency, rate or extent of cell proliferation. [GOC:jl]	*Clu, Nr1d1, Adgrg1, Adarb1, Zbtb16, Cdh13, S100a6*

Subtype	Region	GO Term	Description	*Genes*
Arterial 3	Frontal Cortex	Cell cell signaling	Any process that mediates the transfer of information from one cell to another. This process includes signal transduction in the receiving cell and, where applicable, release of a ligand and any processes that actively facilitate its transport and presentation to the receiving cell. Examples include signaling via soluble ligands, via cell adhesion molecules and via gap junctions. [GOC:dos, GOC:mah]	*Snap25, Map1A, Nrgn, Mef2C, Map1B, Syt1, Mbp*
Arterial 3	Frontal Cortex	Vesicle fusion to the plasma membrane	Fusion of the membrane of a vesicle with the plasma membrane, thereby releasing its contents into the extracellular space. [GOC:aruk, GOC:bc, ISBN:0071120009, PMID:18618940]	*Snap25, Syt1*
Arterial 3	Frontal Cortex	Cell projection organization	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of a prolongation or process extending from a cell, e.g. a flagellum or axon. [GOC:jl, GOC:mah, http://www.cogsci.princeton.edu/~wn/]	*Snap25, Map1A, Mef2C, Map1B, Syt1*
Arterial 3	Frontal Cortex	Regulation of secretion	Any process that modulates the frequency, rate or extent of the controlled release of a substance from a cell or a tissue. [GOC:ai]	*Snap25, Mef2C, Syt1, Mbp*
Arterial 3	Frontal Cortex	Regulation of transport	Any process that modulates the frequency, rate or extent of the directed movement of substances (such as macromolecules, small molecules, ions) into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. [GOC:ai]	*Snap25, Mef2C, Map1B, Syt1*
Arterial 3	Hippocampus	Membrane organization	A process which results in the assembly, arrangement of constituent parts, or disassembly of a membrane. A membrane is a double layer of lipid molecules that encloses all cells, and, in eukaryotes, many organelles; may be a single or double lipid bilayer; also includes associated proteins. [GOC:dph, GOC:tb]	*Pmaip1, Bcl2L1, Cav2, Ndrg1, Tbc1D4, Tfrc, Cd59, Fnbp1L*
Arterial 3	Hippocampus	Regulation of cell death	Any process that modulates the rate or frequency of cell death. Cell death is the specific activation or halting of processes within a cell so that its vital functions markedly cease, rather than simply deteriorating gradually over time, which culminates in cell death. [GOC:dph, GOC:tb]	*Pmaip1, Bcl2L1, Fas, Timp3, Serinc3, Net1, Id1, Ndrg1, Zbtb16, Tsc22D3*
Arterial 3	Hippocampus	Response to oxygen levels	Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus reflecting the presence, absence, or concentration of oxygen. [GOC:BHF, GOC:mah]	*Pmaip1, Fas, Ndrg1, Slc2A1, Plat*
Arterial 3	Hippocampus	Vesicle organization	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of a vesicle. [GOC:mah]	*Cav2, Tbc1D4, Cd59, Fnbp1L*

Subtype	Region	GO Term	Description	*Genes*
Arteriolar-Capillary	Frontal Cortex	Regulation of membrane potential	Any process that modulates the establishment or extent of a membrane potential, the electric potential existing across any membrane arising from charges in the membrane itself and from the charges present in the media on either side of the membrane. [GOC:jl, GOC:mtg_cardio, GOC:tb, ISBN:0198506732]	*Mef2C, Nrxn1, Atp1A2, Rgs4, Ntrk2, Gria3, Sez6, Gabra1, Ank3, Scn2A, Kcnh7*
Arteriolar-Capillary	Frontal Cortex	Regulation of transport	Any process that modulates the frequency, rate or extent of the directed movement of substances (such as macromolecules, small molecules, ions) into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. [GOC:ai]	*Mef2C, Nrxn1, Atp1A2, Rgs4, Snap25, Syt1, Camk2A, Slc1A2, Pclo, Gria3, Myo5A, Mbp, Vsnl1, Ank3, Scn2A, Kcnh7*
Capillary Arterial	Frontal Cortex	Secretion	The controlled release of a substance by a cell or a tissue. [GOC:ai]	*Mef2C, Nrxn1, Snap25, Slc1A3, Syt1, Camk2A, Slc1A2, Pclo, Ntrk2, Myo5A, Mbp, Vsnl1, Aldoc, Pcdh7, Scg3*
Arteriolar-Capillary	Hippocampus	Branched chain amino acid transport	The directed movement of branched-chain amino acids into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. Branched-chain amino acids are amino acids with a branched carbon skeleton without rings. [GOC:ai, GOC:bf]	*Slc3a2, Slc7a5*

Subtype	Region	GO Term	Description	*Genes*
Capillary	Frontal Cortex	Cell cell signaling	Any process that mediates the transfer of information from one cell to another. This process includes signal transduction in the receiving cell and, where applicable, release of a ligand and any processes that actively facilitate its transport and presentation to the receiving cell. Examples include signaling via soluble ligands, via cell adhesion molecules and via gap junctions. [GOC:dos, GOC:mah]	*Snap25, Map1a, Nrgn, Mef2c, Map1b, Syt1, Mbp*
Capillary	Frontal Cortex	Vesicle fusion to the plasma membrane	Fusion of the membrane of a vesicle with the plasma membrane, thereby releasing its contents into the extracellular space. [GOC:aruk, GOC:bc, ISBN:0071120009, PMID:18618940]	*Snap25, Syt1*
Capillary	Frontal Cortex	Regulation of microtubule polymerization or depolymerization	Any process that modulates the frequency, rate or extent of microtubule polymerization or depolymerization by the addition or removal of tubulin heterodimers from a microtubule. [GOC:mah]	*Map1a, Map1b*
Capillary	Hippocampus	Membrane organization	A process which results in the assembly, arrangement of constituent parts, or disassembly of a membrane. A membrane is a double layer of lipid molecules that encloses all cells, and, in eukaryotes, many organelles; may be a single or double lipid bilayer; also includes associated proteins. [GOC:dph, GOC:tb]	*Pmaip1, Bcl2L1, Cav2, Ndrg1, Tbc1D4, Tfrc, Cd59, Fnbp1L*
Capillary	Hippocampus	Regulation of cell death	Any process that modulates the rate or frequency of cell death. Cell death is the specific activation or halting of processes within a cell so that its vital functions markedly cease, rather than simply deteriorating gradually over time, which culminates in cell death. [GOC:dph, GOC:tb]	*Pmaip1, Bcl2L1, Fas, Timp3, Serinc3, Net1, Id1, Ndrg1, Zbtb16, Tsc22D3*

Capillary	Hippocampus	Response to oxygen levels	Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a stimulus reflecting the presence, absence, or concentration of oxygen. [GOC:BHF, GOC:mah]	*Pmaip1, Fas, Ndrg1, Slc2a1, Plat*
Capillary	Hippocampus	Vesicle organization	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of a vesicle. [GOC:mah]	*Cav2, Tbc1D4, Cd59, Fnbp1L*

Subtype	**Region**	**GO Term**	**Description**	***Genes***
Capillary-Venular	Frontal Cortex	Cell cell signaling	Any process that mediates the transfer of information from one cell to another. This process includes signal transduction in the receiving cell and, where applicable, release of a ligand and any processes that actively facilitate its transport and presentation to the receiving cell. Examples include signaling via soluble ligands, via cell adhesion molecules and via gap junctions. [GOC:dos, GOC:mah]	*Snap25, Mef2C, Map1A, Nrgn, Map1B, Syt1, Celf4, Pclo, Edn3*
Capillary-Venular	Frontal Cortex	Regulation of secretion	Any process that modulates the frequency, rate or extent of the controlled release of a substance from a cell or a tissue. [GOC:ai]	*Snap25, Mef2C, Syt1, Pclo, Edn3*
Capillary-Venular	Frontal Cortex	Regulation of transport	Any process that modulates the frequency, rate or extent of the directed movement of substances (such as macromolecules, small molecules, ions) into, out of or within a cell, or between cells, by means of some agent such as a transporter or pore. [GOC:ai]	*Snap25, Mef2C, Map1B, Syt1, Pclo, Edn3*
Capillary-Venular	Frontal Cortex	Regulation of cell projection organization	Any process that modulates the frequency, rate or extent of a process involved in the formation, arrangement of constituent parts, or disassembly of cell projections. [GOC:mah]	*Snap25, Mef2C, Map1B, Syt1*
Capillary-Venular	Hippocampus	Negative regulation of endothelial cell differentiation	Any process that stops, prevents, or reduces the frequency, rate or extent of endothelial cell differentiation. [GOC:go_curators]	*Xdh, Id1*
Capillary-Venular	Hippocampus	Wound healing	The series of events that restore integrity to a damaged tissue, following an injury. [GOC:bf, PMID:15269788]	*Maff, Adipor2, Plat, Cd59, Dock9*
Capillary-Venular	Hippocampus	Positive regulation of extrinisic apoptotic signaling pathway via death domain receptors	Any process that activates or increases the frequency, rate or extent of extrinsic apoptotic signaling pathway via death domain receptors. [GOC:TermGenie, PMID:17245429]	*Pmaip1, Id1*
Capillary-Venular	Hippocampus	Response to hormone	Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a hormone stimulus. [GOC:jl]	*Adipor2, Slc2A1, Junb, Timp3, Tbc1D4, Fam107A*

Subtype	**Region**	**GO Term**	**Description**	***Genes***
Venous	Frontal Cortex	Regulation of cell population proliferation	Any process that modulates the frequency, rate or extent of cell proliferation. [GOC:jl]	*Ptn, Dusp1, Edn3, Tgfb2, Nes, Camk2N1, Nampt*

Venous	Frontal Cortex	Cell chemotaxis	The directed movement of a motile cell guided by a specific chemical concentration gradient. Movement may be towards a higher concentration (positive chemotaxis) or towards a lower concentration (negative chemotaxis). [GOC:dph]	*Ptn, Dusp1, Edn3, Tgfb2*
Venous	Frontal Cortex	Membrane organization	A process which results in the assembly, arrangement of constituent parts, or disassembly of a membrane. A membrane is a double layer of lipid molecules that encloses all cells, and, in eukaryotes, many organelles; may be a single or double lipid bilayer; also includes associated proteins. [GOC:dph, GOC:tb]	*Ptn, Tgfb2, Syt1, Snap25, F5*
Venous	Frontal Cortex	Vesicle fusion to the plasma membrane	Fusion of the membrane of a vesicle with the plasma membrane, thereby releasing its contents into the extracellular space. [GOC:aruk, GOC:bc, ISBN:0071120009, PMID:18618940]	*Syt1, Snap25*
Venous	Hippocampus	Cellular transition metal ion homeostasis	Any process involved in the maintenance of an internal steady state of transition metal ions at the level of a cell. A transition metal is an element whose atom has an incomplete d-subshell of extranuclear electrons, or which gives rise to a cation or cations with an incomplete d-subshell. Transition metals often have more than one valency state. Biologically relevant transition metals include vanadium, manganese, iron, copper, cobalt, nickel, molybdenum and silver. [GOC:mah, ISBN:0198506732]	*Lcn2, Mt1X, Fth1, Mt1E, Cp*
Venous	Hippocampus	Response to cytokine	Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a cytokine stimulus. [GOC:sl]	*Lcn2, Mt1X, Ptgs2, Xbp1, Vcam1, Cd14, Ifitm1, Osmr, Timp3, Ackr1*
Venous	Hippocampus	Regulation of cell population proliferation	Any process that modulates the frequency, rate or extent of cell proliferation. [GOC:jl]	*Fth1, Ptgs2, Xbp1, Vcam1, Ifitm1, Osmr, Lrg1, Grem1, Apod, Zbtb16, Scgb3A1*
Venous	Hippocampus	Cell motility	Any process involved in the controlled self-propelled movement of a cell that results in translocation of the cell from one place to another. [GOC:dgh, GOC:dph, GOC:isa_complete, GOC:mlg]	*Ptgs2, Xbp1, Vcam1, Ifitm1, Fam107A, Grem1, Apod, Plat, Enpp2, Ch25H*
Venous	Hippocampus	Response to toxic substance	Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a toxic stimulus. [GOC:lr]	*Lcn2, Mt1X, Mt1E, Ptgs2, Vcam1, Cd14*
Venous	Hippocampus	Regulation of response to stress	Any process that modulates the frequency, rate or extent of a response to stress. Response to stress is a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a disturbance in organismal or cellular homeostasis, usually, but not necessarily, exogenous (e.g. temperature, humidity, ionizing radiation). [GOC:dhl]	*Ptgs2, Xbp1, Cd14, Osmr, Apod, Serinc3, Plat, Map3K6, Ier3*
Venous	Hippocampus	Apoptotic signaling pathway	A series of molecular signals which triggers the apoptotic death of a cell. The pathway starts with reception of a signal, and ends when the execution phase of apoptosis is triggered. [GOC:mtg_apoptosis]	*Lcn2, Ptgs2, Xbp1, Cd14, Timp3, Serinc3*
Venous	Hippocampus	Blood vessel morphogenesis	The process in which the anatomical structures of blood vessels are generated and organized. The blood vessel is the vasculature carrying blood. [GOC:jid]	*Ptgs2, Xbp1, Lrg1, Grem1, Apod, Enpp2*

Venous	Hippocampus	Regulation of cell adhesion	Any process that modulates the frequency, rate or extent of attachment of a cell to another cell or to the extracellular matrix. [GOC:mah]	*Xbp1, Vcam1, Fam107A, Grem1, Apod, Zbtb16*
Venous	Hippocampus	Inflammatory response	The immediate defensive reaction (by vertebrate tissue) to infection or injury caused by chemical or physical agents. The process is characterized by local vasodilation, extravasation of plasma into intercellular spaces and accumulation of white blood cells and macrophages. [GO_REF:0000022, GOC:mtg_15nov05, ISBN:0198506732]	*Ptgs2, Vcam1, Cd14, Osmr, Ackr1, Apod*