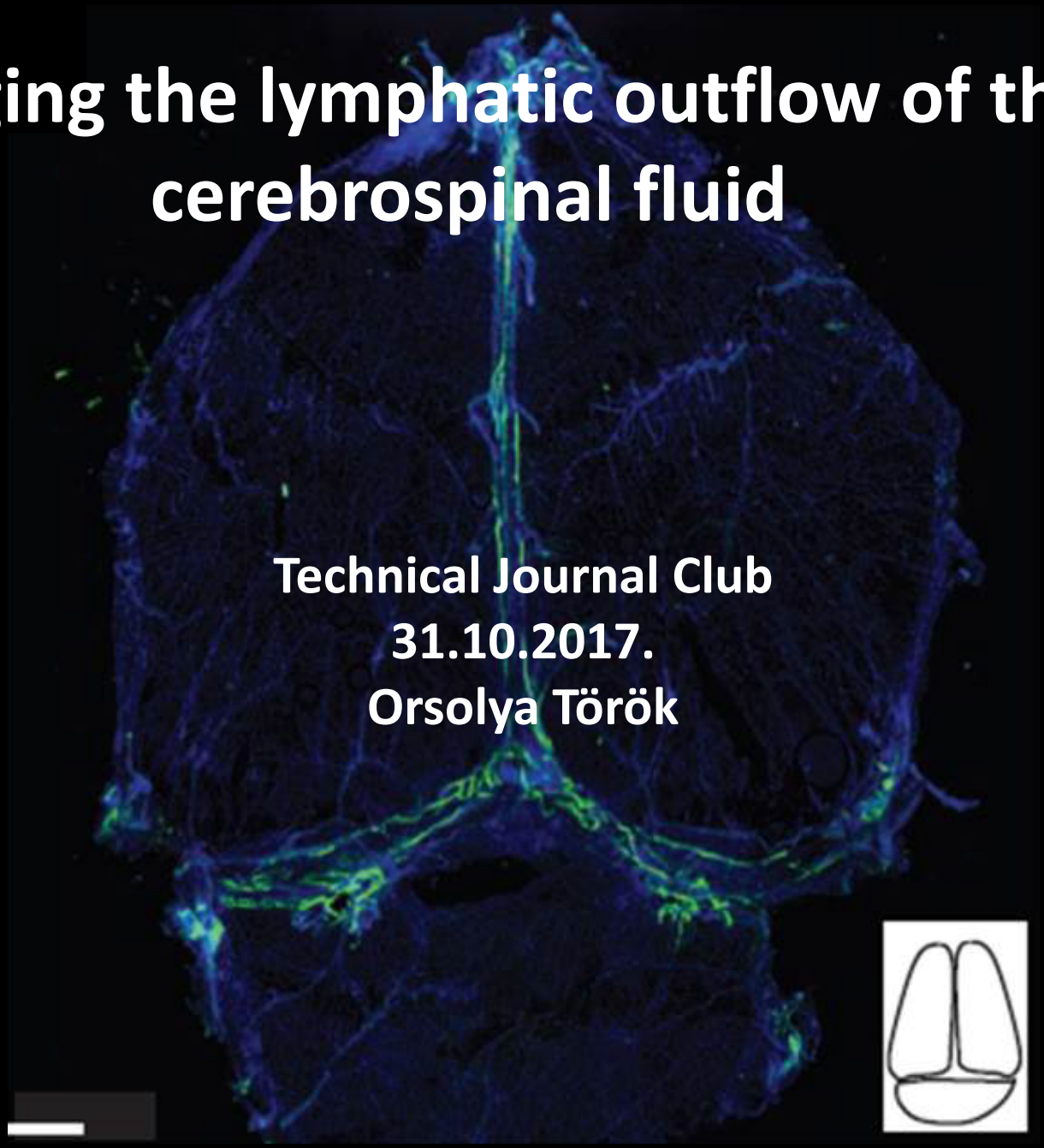
Imaging the lymphatic outflow of the cerebrospinal fluid



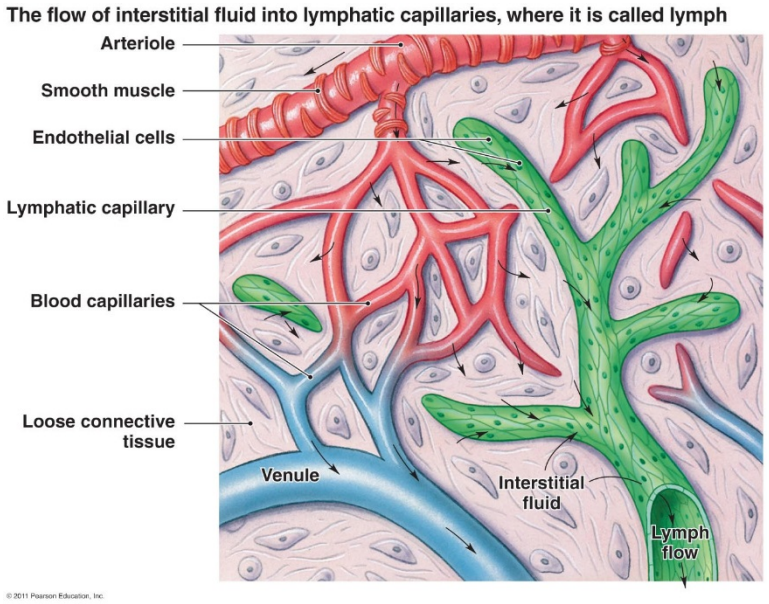
Technical Journal Club
31.10.2017.
Orsolya Török

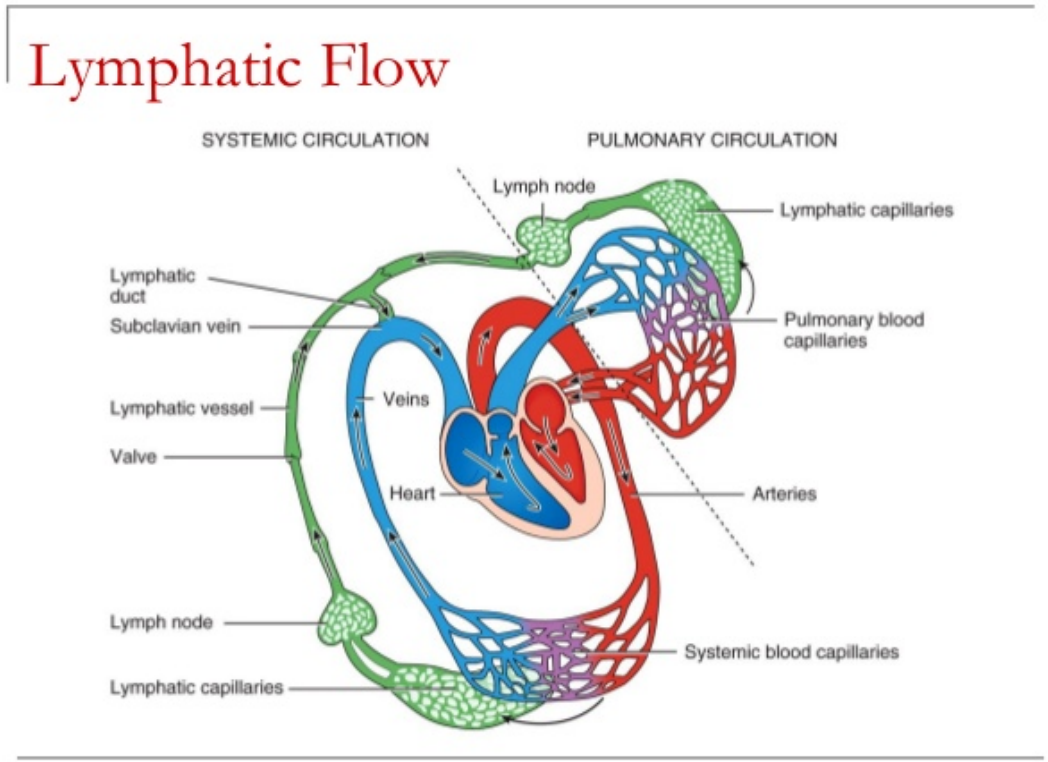
Image: Structural and functional features of central nervous system lymphatic vessels, Louveau et al., Nature, 2015.

Background – lymphatic flow in the periphery

Function of the lymphatic system:

- transports clean fluid back to the blood
- drains excess fluid back from tissues
- removes debris
- transports fat from the digestive system
- defence system for the body



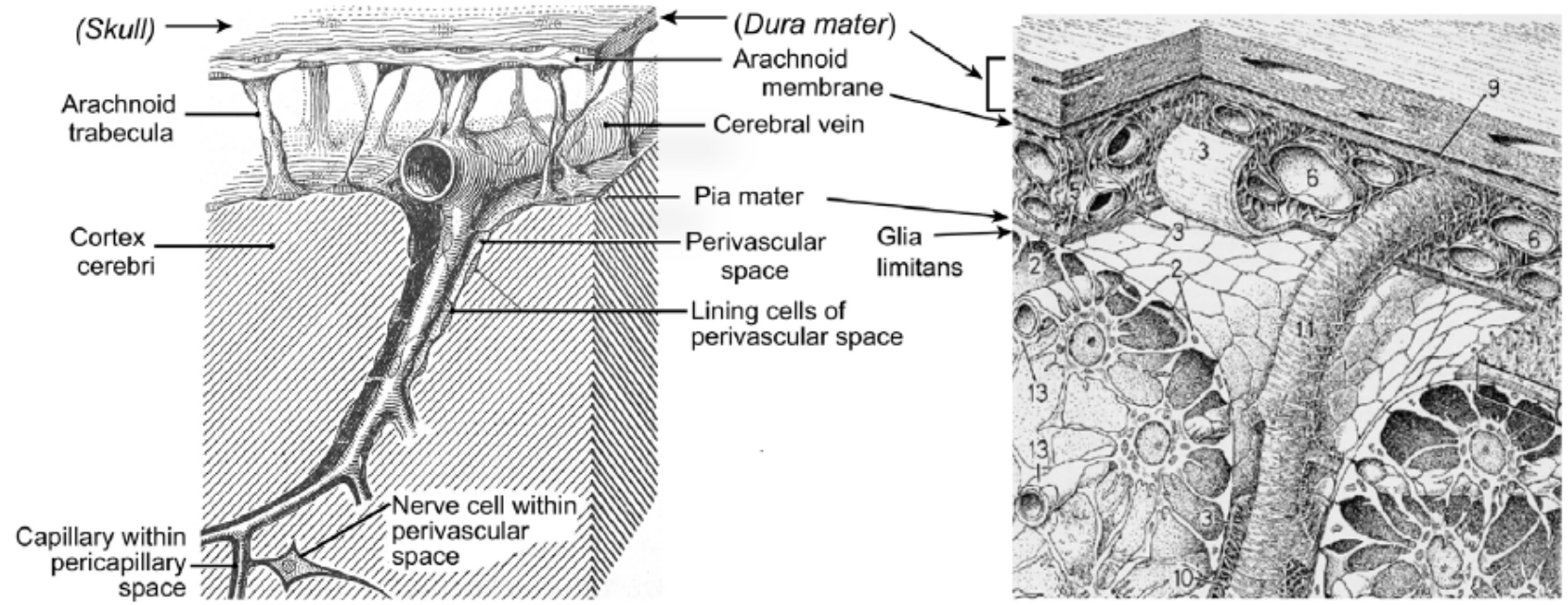


Background – is there a `lymphatic flow` in the CNS?

The cerebrospinal fluid (CSF) is transported from the subarachnoid space (SAS) along the perivascular arterial spaces (PAS or Virchow-Robin space) of penetrating arteries.

Interstitial fluid of the CNS drains via perivascular channels into the CSF, allowing perivascular macrophages and other antigen presenting cells (APCs) to sample CNS antigens.

Soluble antigens placed the cerebral ventricles induces detectable antibody-secreting cells in the deep cervical lymph nodes (DCLNs). – The CSF acts as functional equivalent of lymph.[1]



1. R. M. Ransohoff, B. Engelhardt, Nat.Rev.immun., 2012 Sep;12(9):623-35

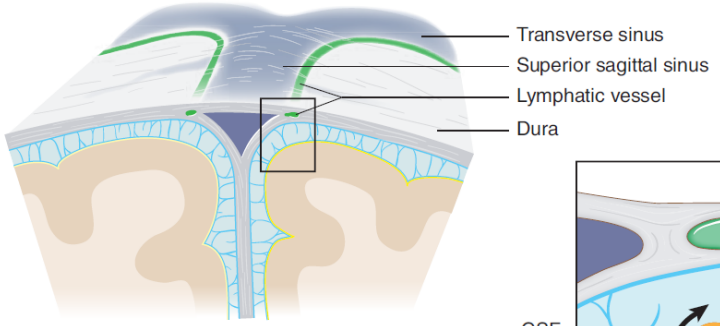
Image: Coles et al., Progress in Neurobiology 156. (2017) 107-148

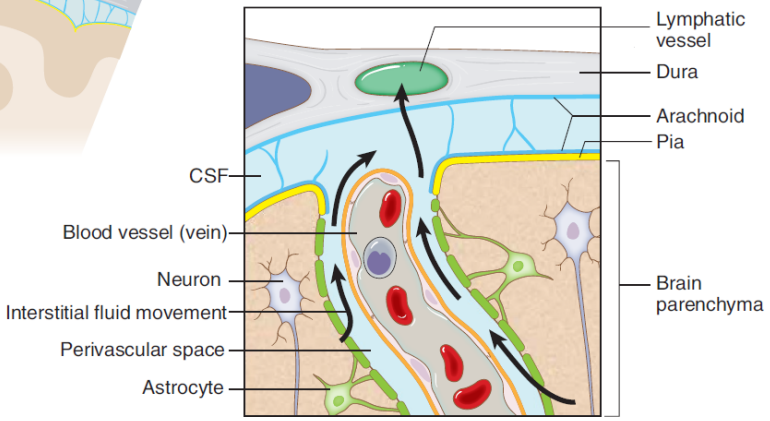
Background – is there a `lymphatic flow` in the CNS?

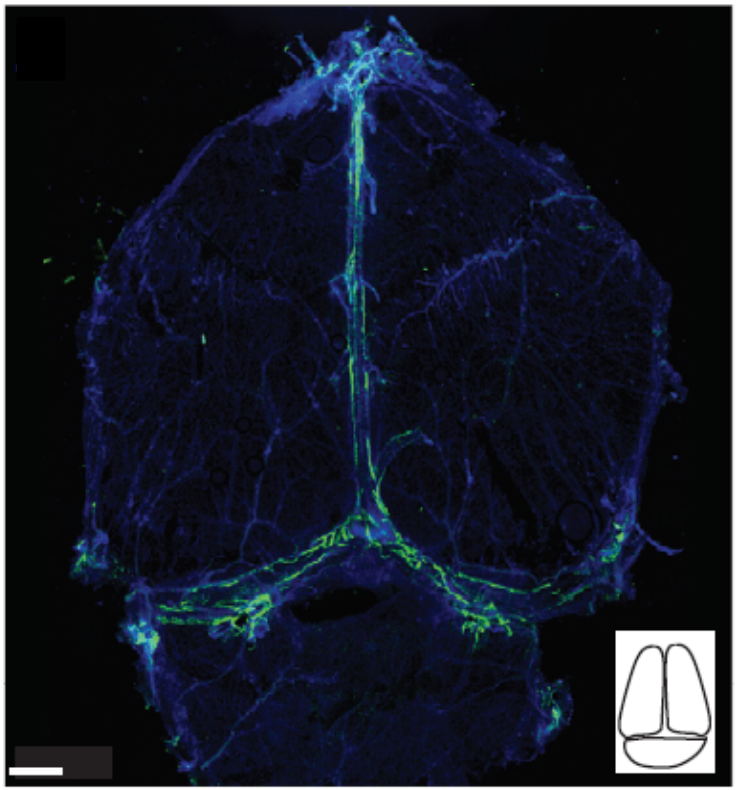
First described by Paolo Mascagni Italian anatomist in the 19th century.[2]

The existence of a network of true lymphatic vessels within the mammalian dura mater has been described.[3]

Alternate conduit for drainage of immune cells and CSF via the cribriform plate into the ethmoid region and DCLNs.[4]







Images: Louveau et al., Nature, 2015., Jul 16;523(7560):337-41

2. Mascagni and Bellini, 1816.
3. Louveau et al., Nature, 2015., Jul 16;523(7560):337-41
4. Weller et al., 2009., Acta Neuropathologica 117:1–14.

Imaging blood vascular system vs. lymphatic vascular system

	Blood vessel imaging	Lymphatic vessel imaging
Delivery of contrast agents	Routinely	Invasive for clinical research; Nearly impossible for basic science investigations
Functional flow imaging	Scattering of red blood cells routinely (Doppler-ultrasound)	Acellular lymph escapes interrogation
Molecular imaging	Under development	Molecular targets for lymphangiogenesis and lymphatic remodelling not fully developed

Lymphatic imaging technologies

1. In situ imaging is possible with:
   - staining with blue-dye (Evans blue dye)
   - immunohistological stainings: ***VEGFR-3***, prospero-related homeobox-1 (***PROX-1***), ***podoplanin*** or lymphatic vessel endothelial hyaluronan recep
2. In vivo imaging:
   - direct lymphangiography
   - indirect lymphangiography
     1. Lymphoscintigraphy
     2. MR lymphangiography
     3. Fluorescent microlyphang
     4. Near-infrared fluorescenc

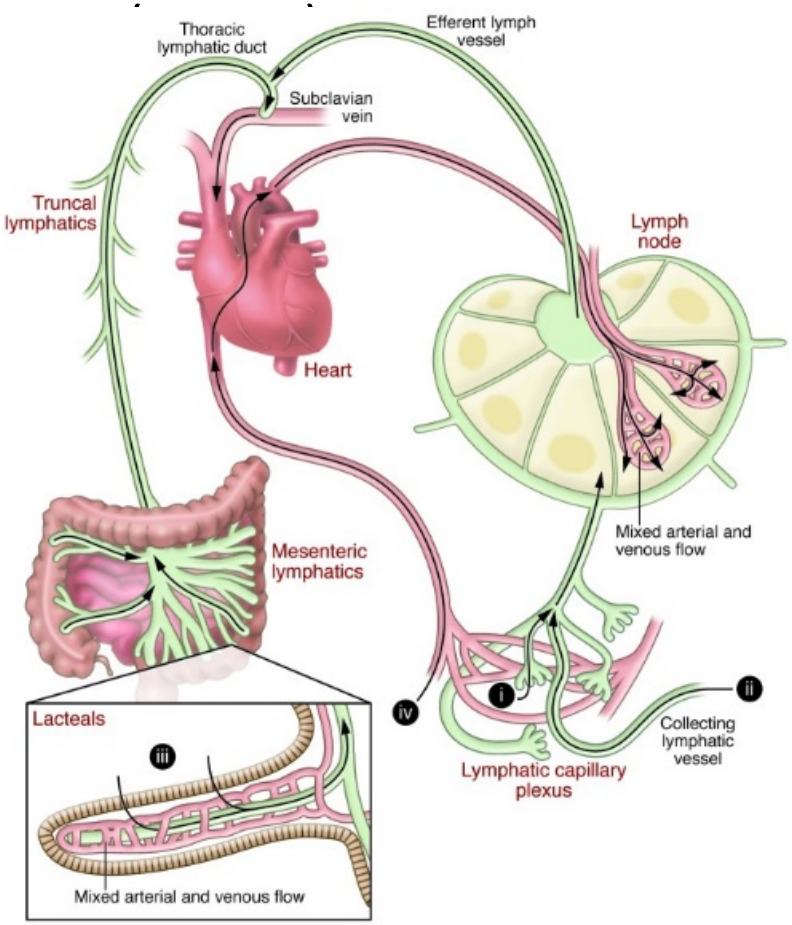
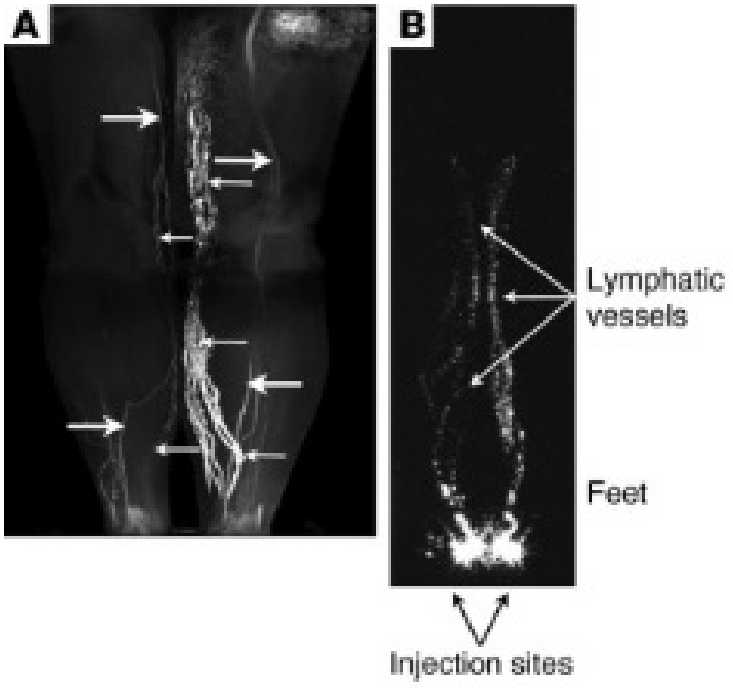


Image: E. M. Sevick-Muraca, S. Kwon, J. Clin. Invet., 2014 Mar;124(3):905-14

In vivo indirect lymphangiography

1. **Lymphoscintigraphy:**
   - routine method used in clinics to assess lymphatic function and structure,
   - intradermal injection of radiocolloid, imaging with gamma-camera
   - low temporal and spatial resolution
   - not suitable for small animal models
2. **MR lymphangiography:**
   - gadolinium-based contrast agents injected into the intradermal and subcutaneous spaces
   - provides a better resolution than lymphoscintigraphy
   - the development of new MR contrast agents enabled the localisation of LNs and peripherial lymph drainage pathways



Images: E. M. Sevick-Muraca, S. Kwon, J. Clin. Invet., 2014 Mar;124(3):905-14

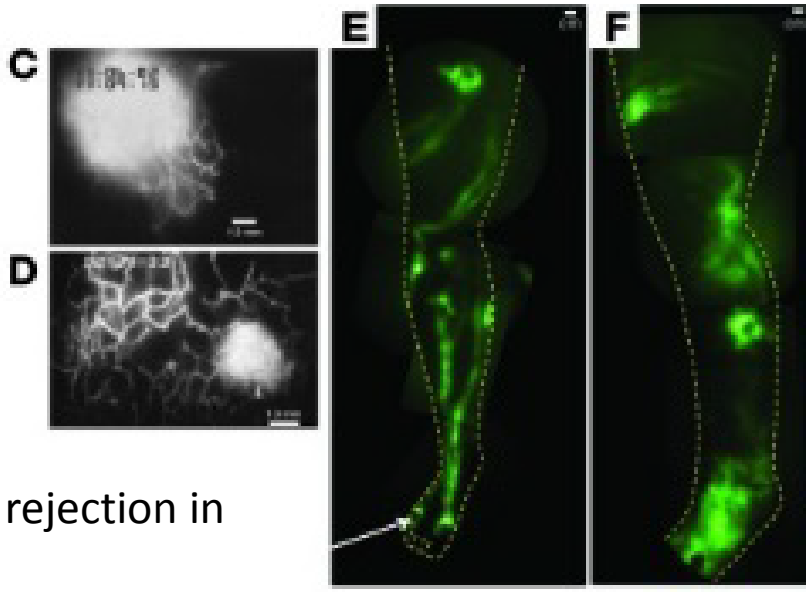
In vivo indirect lymphangiography

3. Fluorescent microlyphangiography (FML):

- first fluorescent technique used to non-invasively image the lymphatics
- ***FITC-dextran*** injected intradermal, imaged with video fluorescence microscopy techniques: limited resolution (100-150 µm of tissue depth)
- ***indocyanine green (ICG):*** enhances the tissue penetration (200 µm of tissue depth)

4. Near-infrared fluorescence (NIRF):

- greater penetration depth (up to 3-4 cm tissue depth)
- but lower resolution
- ***ICG*** injected
- CCD cameras used
- inital lymphatics, collecting and conducting lymphatics, and draining LNs can be visualized
- clinical application: to detect sentinel-lymph nodes preoperatively or intraoperatively
- basic research studies: to assess tissue rejection in hind limb transplantation studies
- dynamic function of lymphatics



Images: E. M. Sevick-Muraca, S. Kwon, J. Clin. Invet., 2014 Mar;124(3):905-14

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Research paper

Fluorescence imaging of lymphatic outflow of cerebrospinal fluid in mice

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Goal: **in vivo investigation of the routes of CSF outflow and a non-invasive interrigation of the dynamics of the CSF outflow**

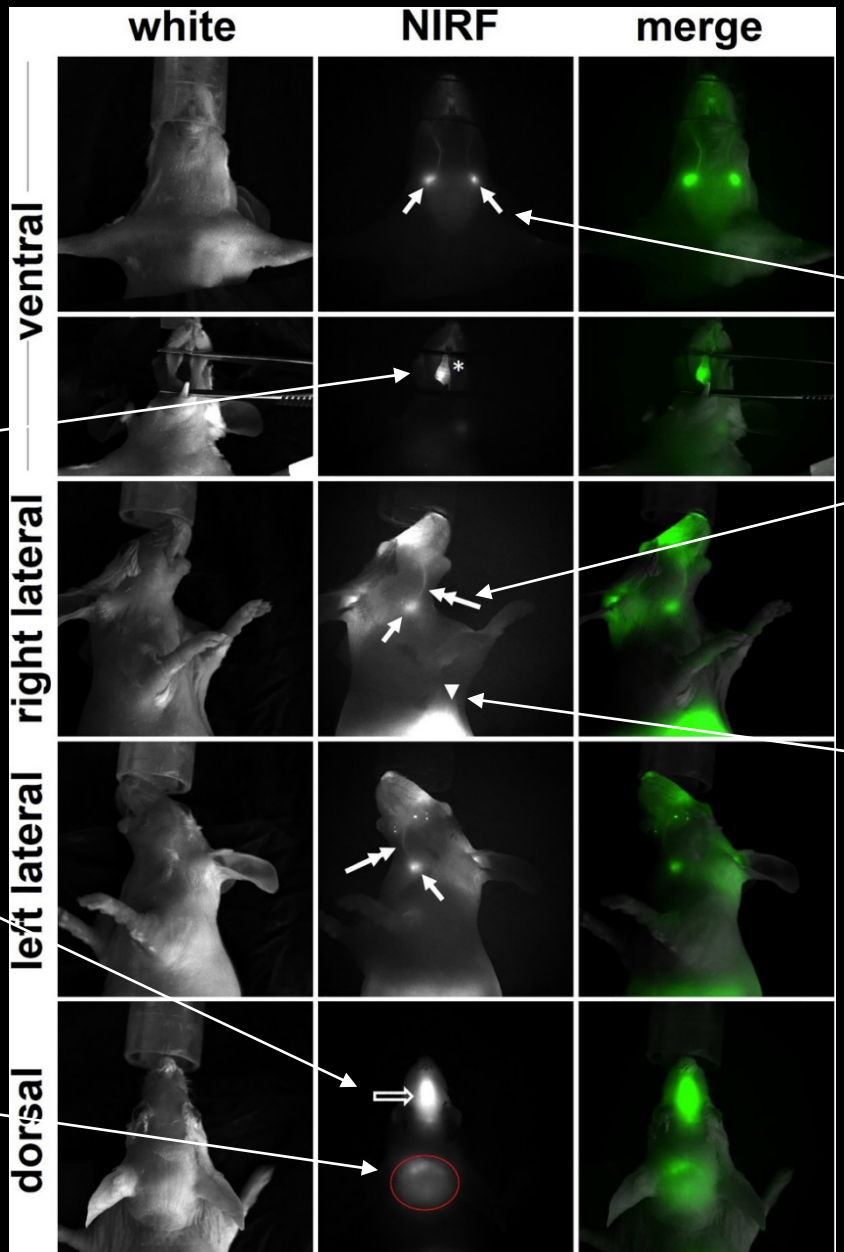
Intrathecal injection of fluorescent dyes in the lumbar region of the spinal cord

Intrathecal injection: - in anaethesia after tail flick response between L5-L6 vertebrae

- 0,33 µl/g (10 µl in a 30 g mouse) of 645 µM indocyanine green (***ICG***)

Imaging: - 30 min postinjection with dynamic whole-body near-infrared fluorescent imaging (***NIRFI***)

Intrathecal injection of fluorescent dyes in the lumbar region of the spinal cord



MLNs

Upper hard palate

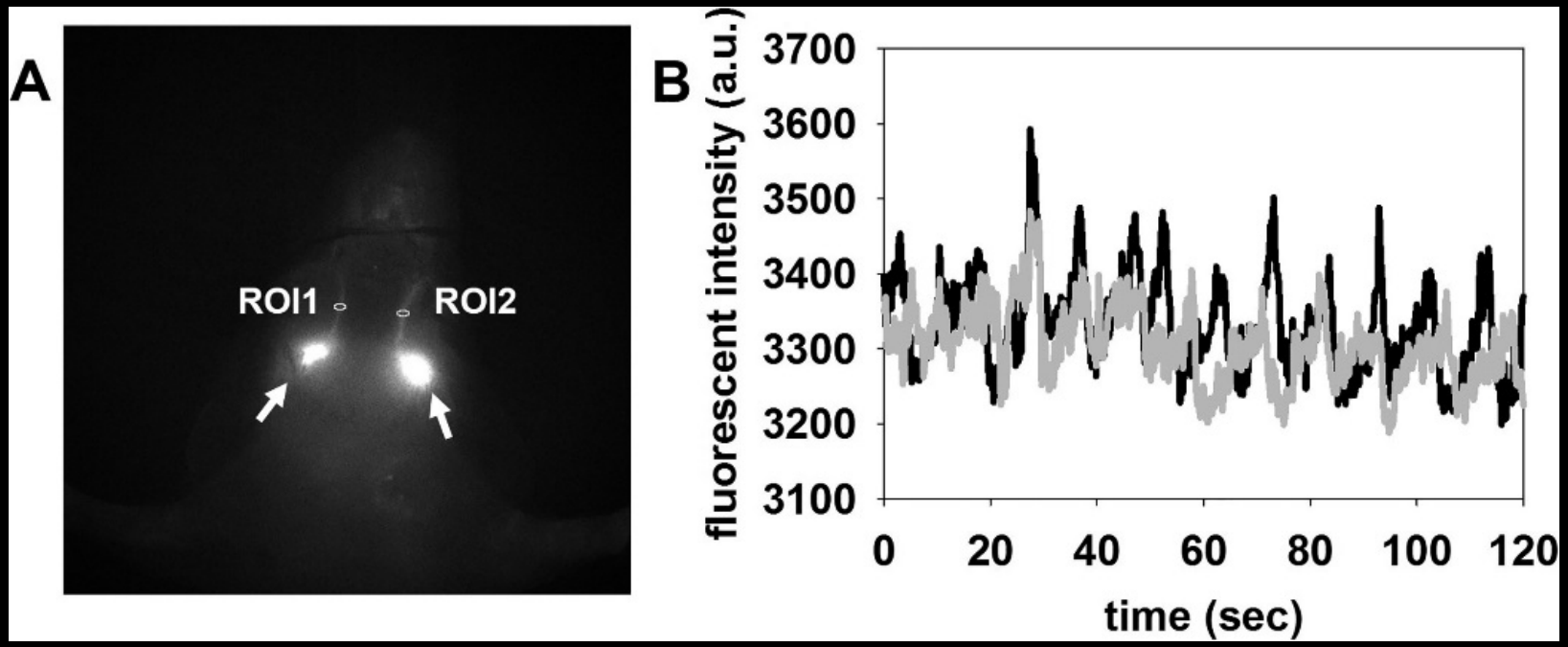
Lymphatic vessels

liver

Endoturbinates

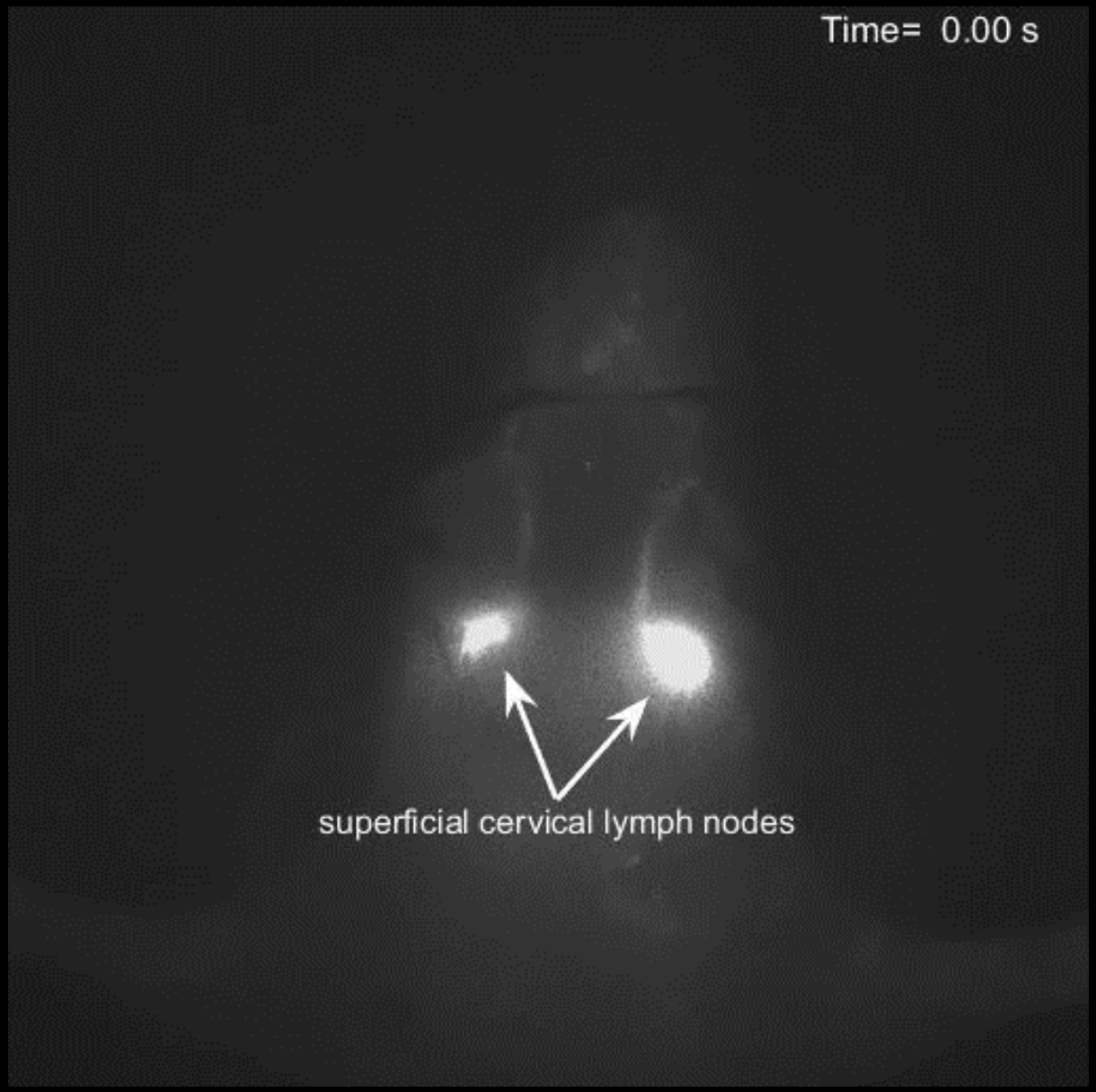
Cisterna magna & Supracerebellar cistern

Propulsive lympahtic function in peripherial lymphatic vessels to MLNs

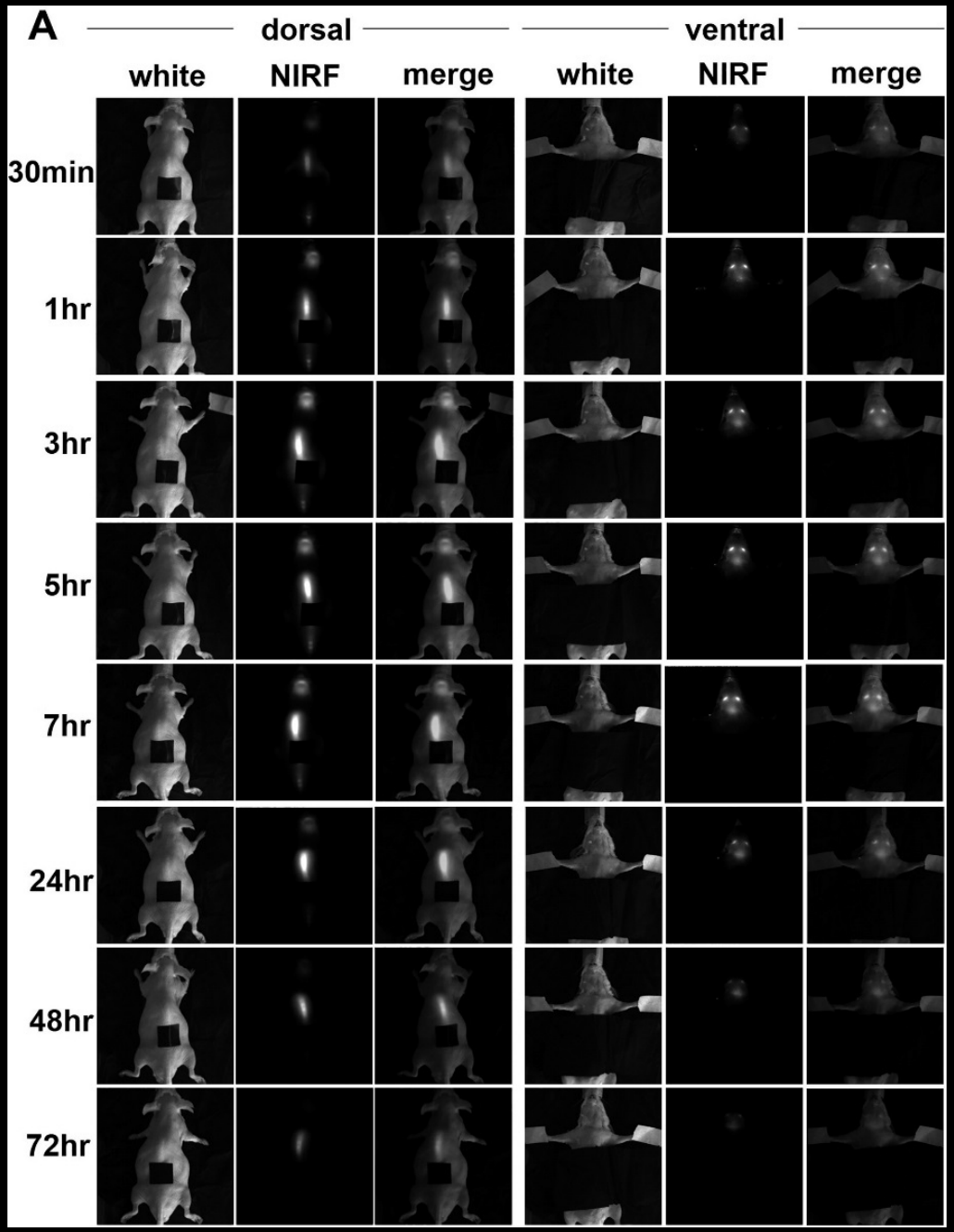


- Representative image taken 30 mins after intrathecal injection of ICG
- Fluorescent intensity measured in 2 ROI selected in the peripherial vessels
- Average contraction frequency of the vessels draining to the MLNs was:
  5,2 ± 0,5/min (n=5)

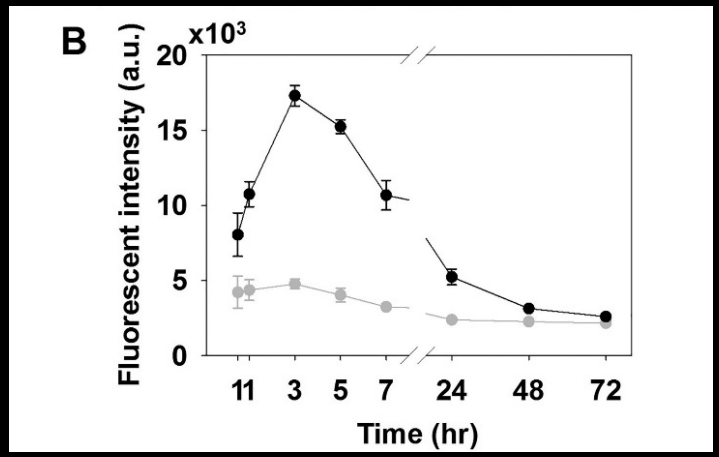
Propulsive lympahtic function in peripherial lymphatic vessels to MLNs



ICG distribution along the neuraxis over time

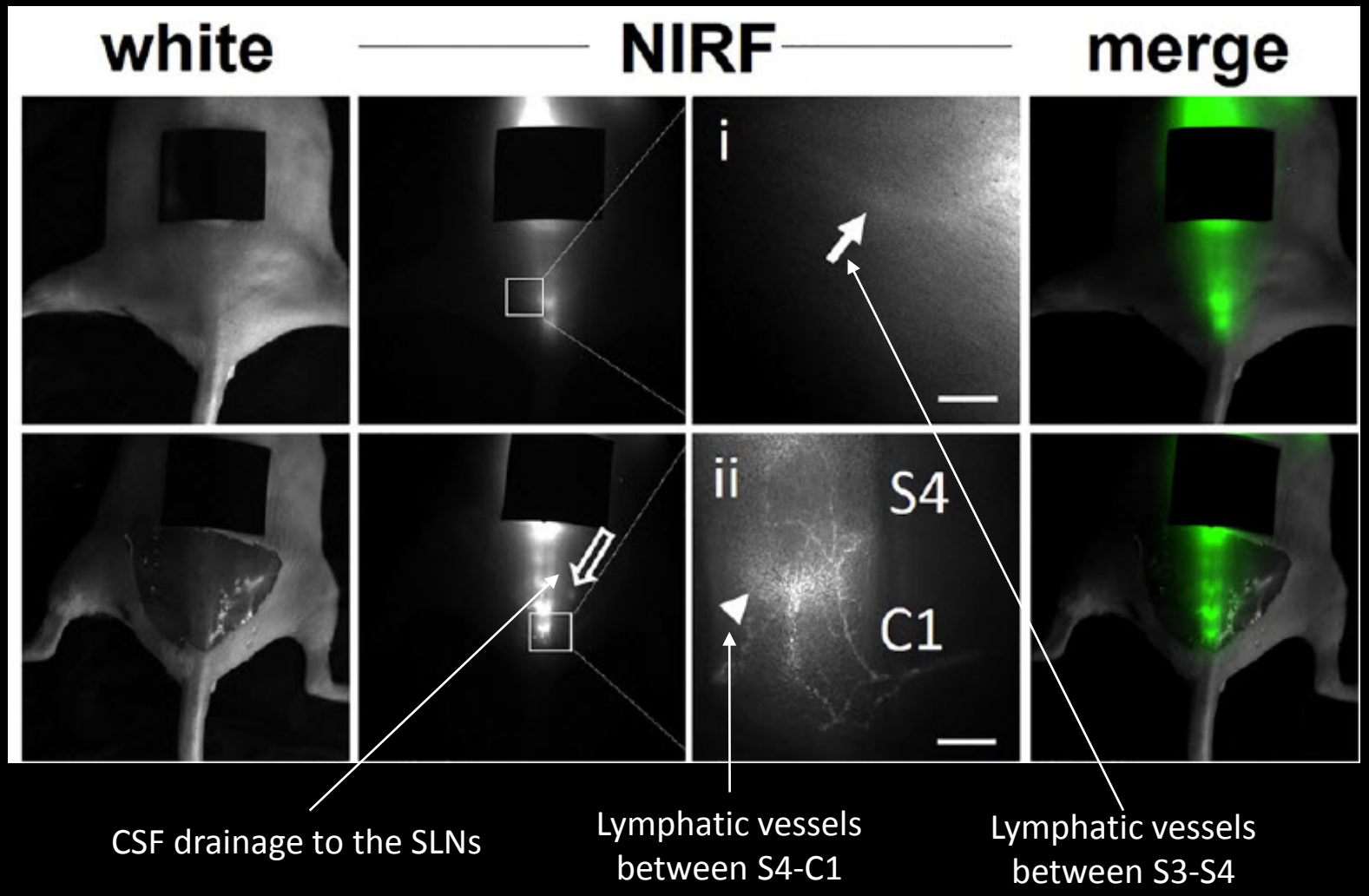


- Both rostral and caudal diffusion of the fluorescent signal
- Cisterna magna: 30 min p.i.i., peak: 3 hours p.i.i., cessation: 72 hours p.i.i.
- Between S4 and C1: 30 min p.i.i.
- Fluorescent intensities in the cisterna magna were significatnly higher than in the MLNs



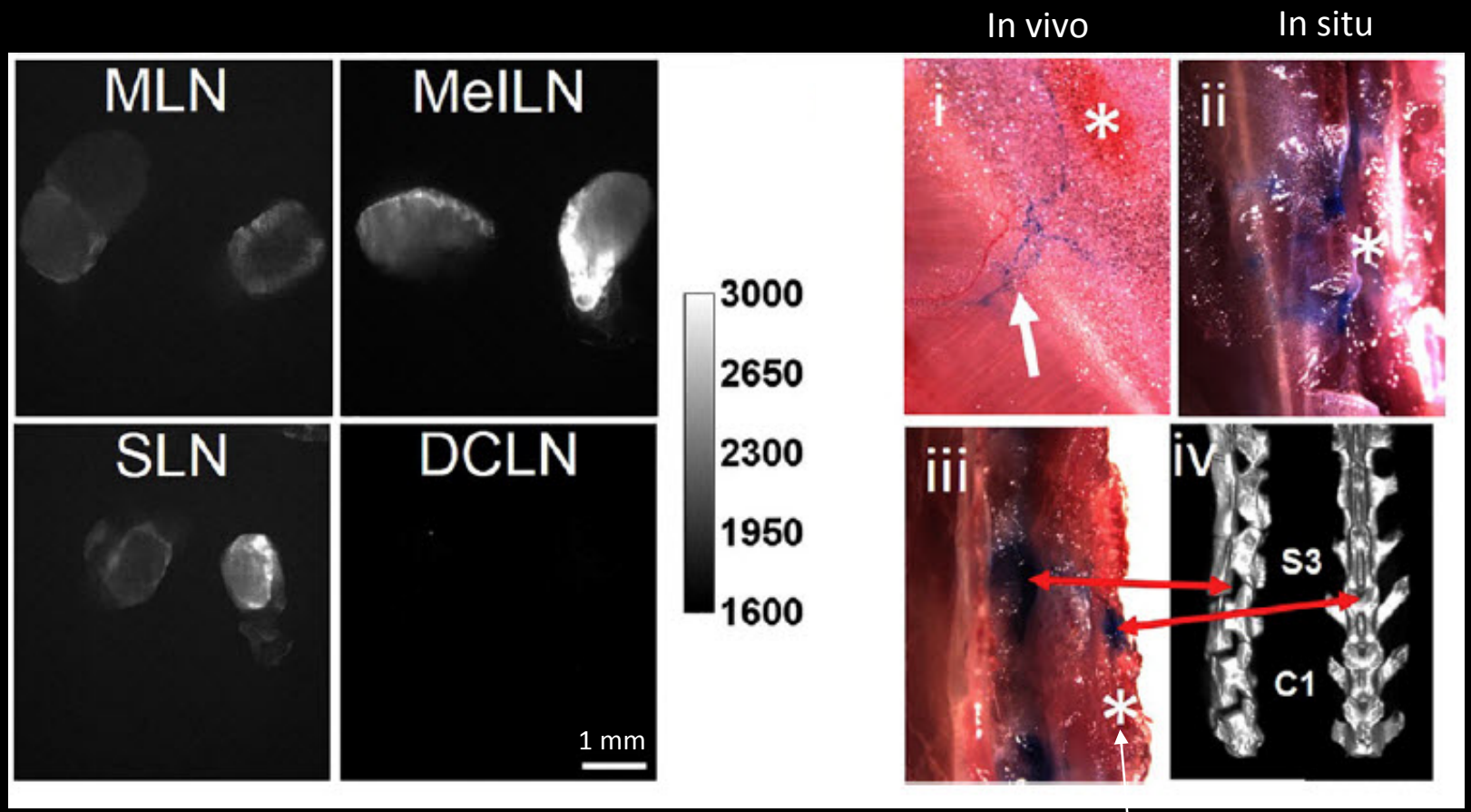
Lymphatic drainage to the sciatic lymph nodes (SLNs) upon injection of higher volumes

Intrathecal injection: 0,6 µl/g bolus of ***ICG***



Lymphatic drainage to the sciatic lymph nodes (SLNs) upon injection of higher volumes

Intrathecal injection: 0,6 µl/g bolus of ***ICG*** + 15 µl of Evans-blue dye ***(EBD)***

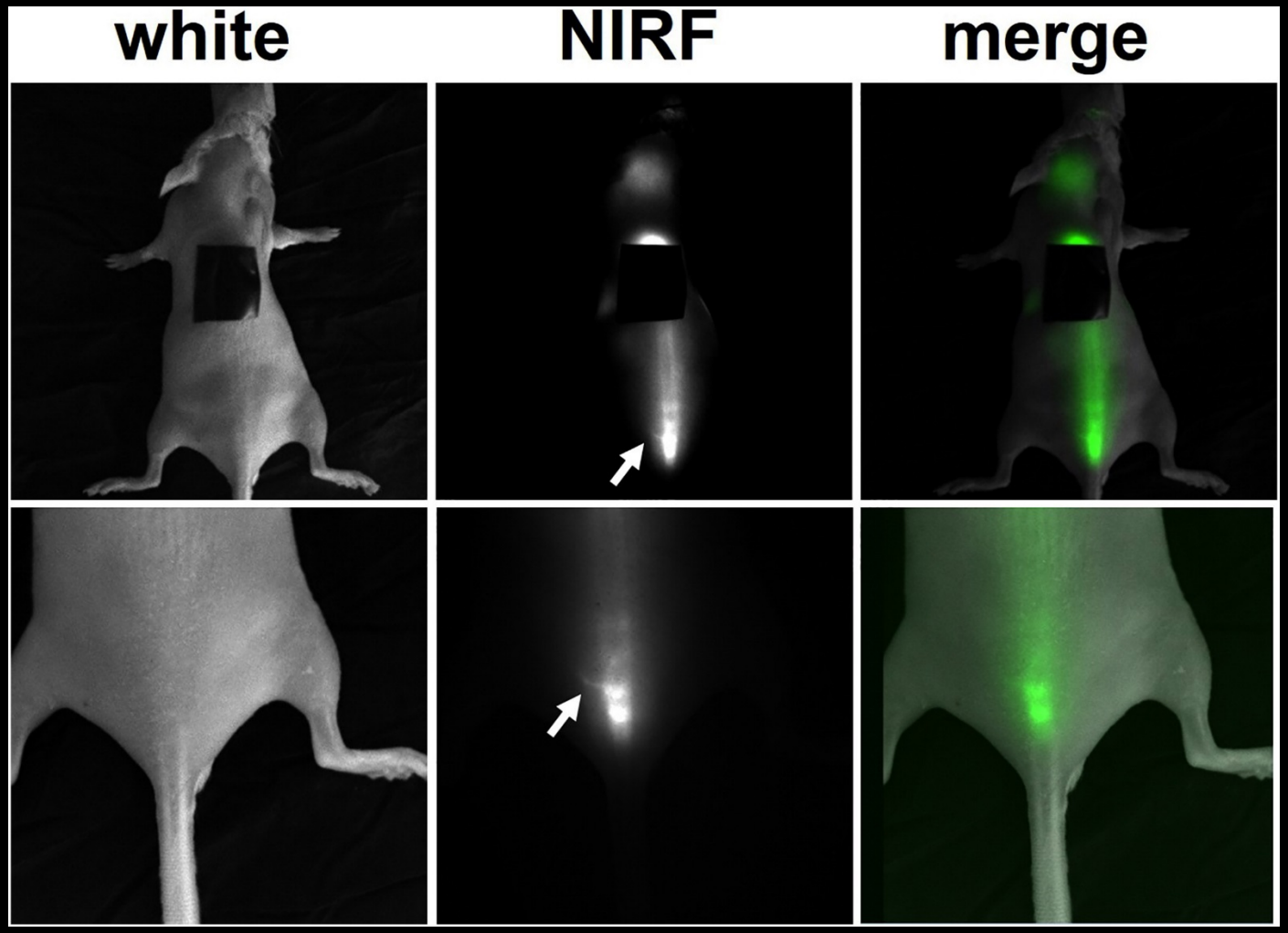


MNL: mandibular LN, MeIL: medial iliac LN, SLN: sciatic LN, DCLN: deep cervical LN

Lymphatic drainage to the sciatic lymph nodes (SLNs) upon injection of higher volumes

Intrathecal injection: 0,6 µl/g bolus of ***ICG*** + 15 µl of Evans-blue dye ***(EBD)***

Location of injection: T10-T11 vertebrae



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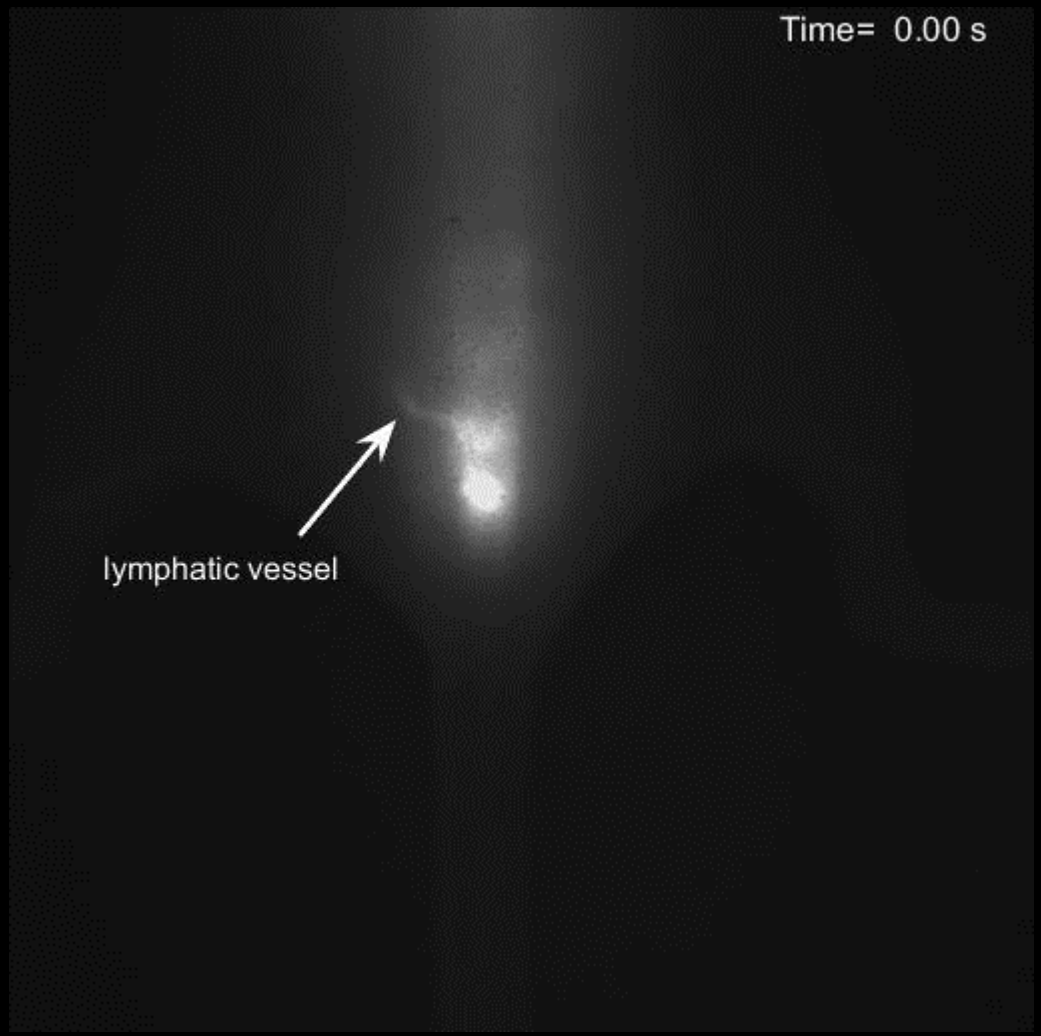
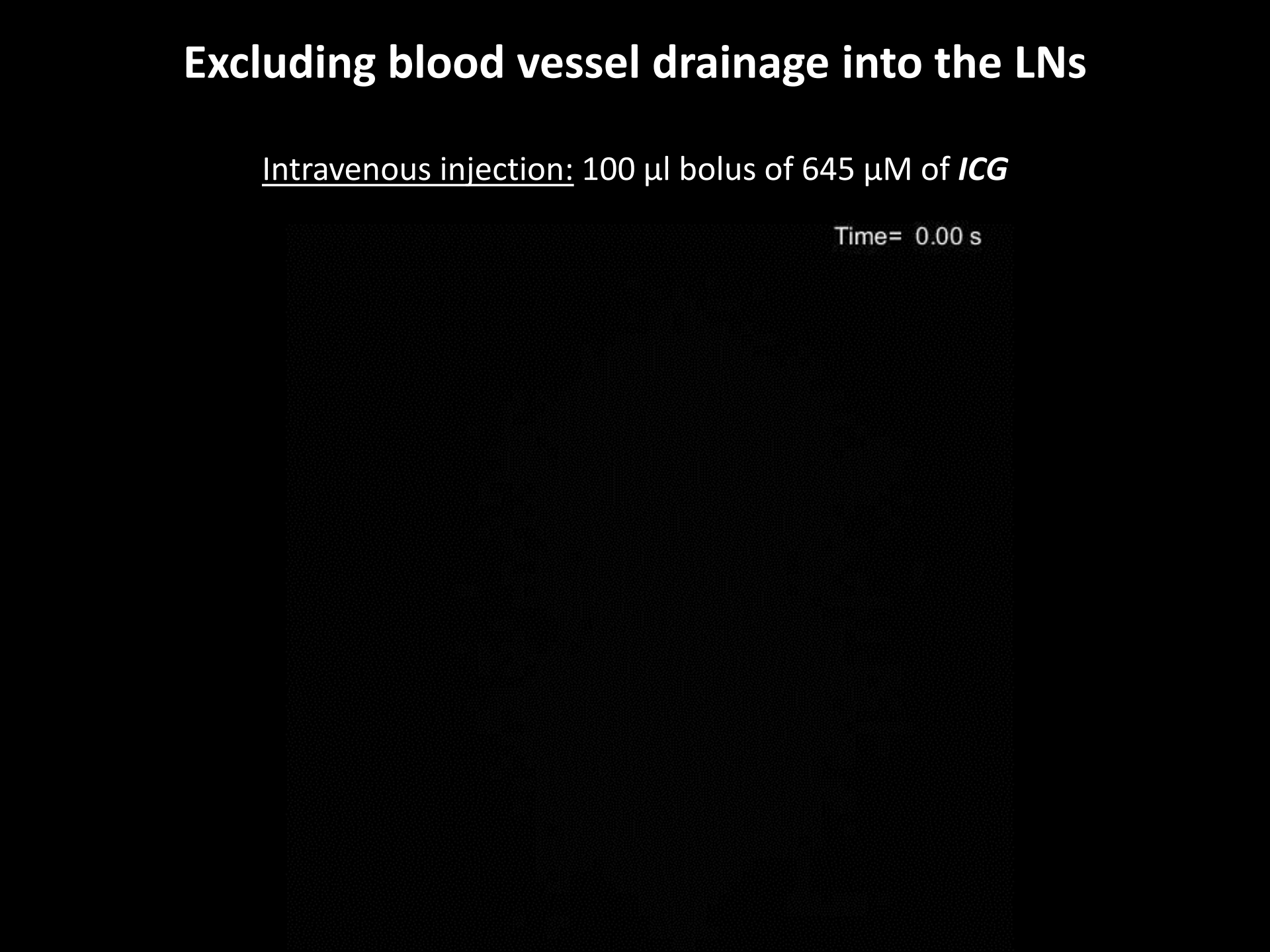


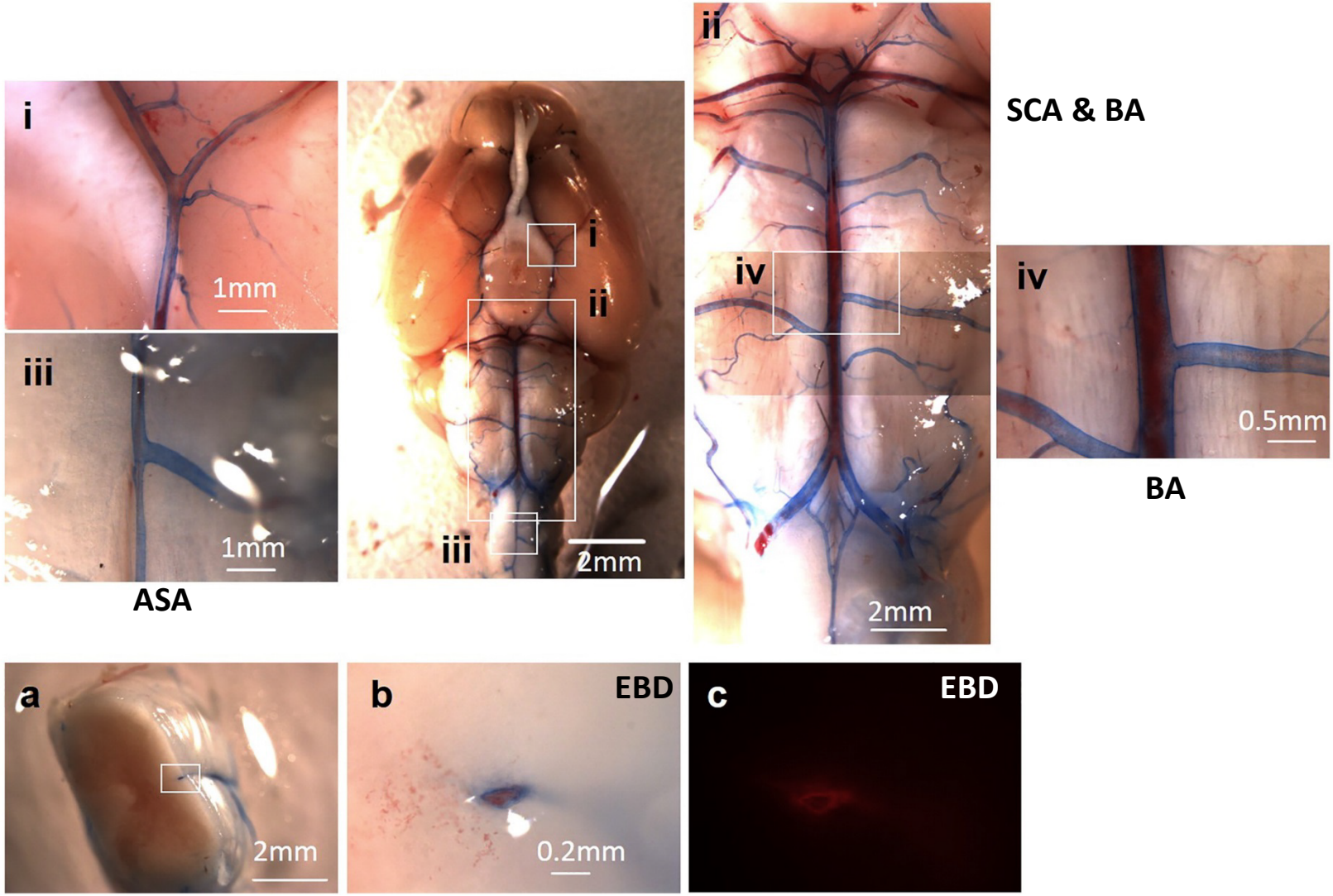
Excluding blood vessel drainage into the LNs

Intravenous injection: 100 µl bolus of 645 µM of ***ICG***



Examining the tracer distibution in the brain

Intrathecal injection: 0,33 µl/g of **EBD**; 30 min p.i.i.



MCA: middle cerebral artery, ACA: anterior cerebral artery, ASA: anterior spinal artery, SCA: superior cerebellar artery, BA: basilar artery

Conclusions

Improvements: - provided direct imaging in vivo of spinal CSF outflow

- confirmed that under physiological pressures the CSF is preferentially absorbed via the cribriform plate
- under higher CSF pressure drainage into LNs in the lumbosacral region occurs
- model for imaging pathophysiological changes in e.g. hydrocephalus

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SHORT REPORT

Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI

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[1]Translational Neuroradiology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, United States; [2]Hematopathology Section, Laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, United States; [3]Center for Brain Immunology and Glia, Department of Neuroscience, School of Medicine, University of Virginia, Charlottesville, United States; [4]Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, United States

In vivo imaging of meningeal lymphatic vessels in human and nonhuman primates

MRI imaging sequences used:

1. T2-weighted fluid-attenuation inversion recovery (T2-FLAIR) pulse sequence
   - detecting lesions in the parenchyma; sensitive to gadolinium-based contrast agents
2. `Black blood`sequence: vascular wall thickness; atherosclerotic plaques
3. T1-weighted Magnetization Prepared Rapid Acquisition of Gradient Echoes (MPRAGE) sequence: structural brain imaging

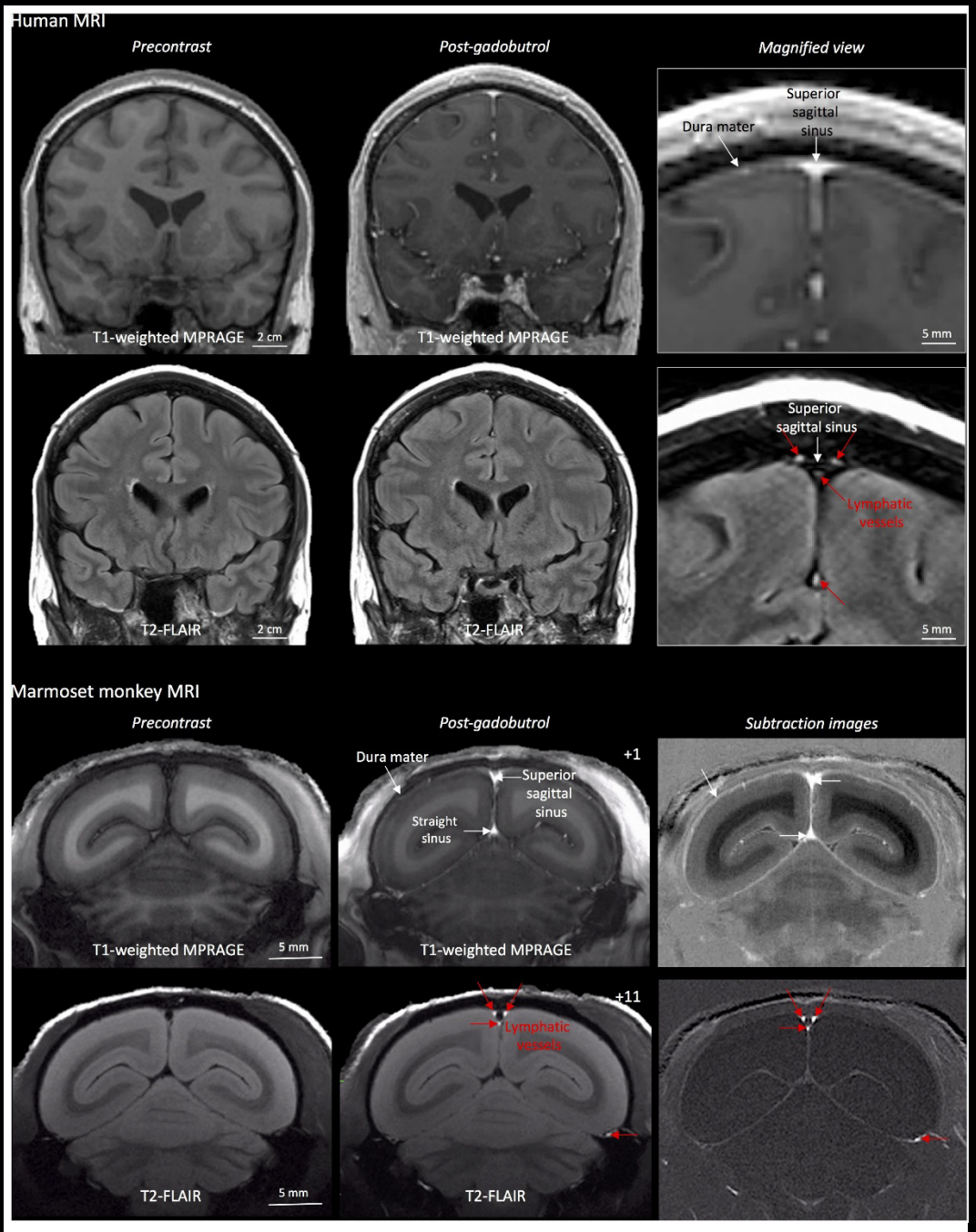
MRI contrast agents used:

1. Gadobutrol: extravasate across permeable capillary endothelial barrier
2. Gadofosveset: blood-pool contrast agent

MRI visualization of dural lymphatic vessels with gadobutrol

Human

Marmoset

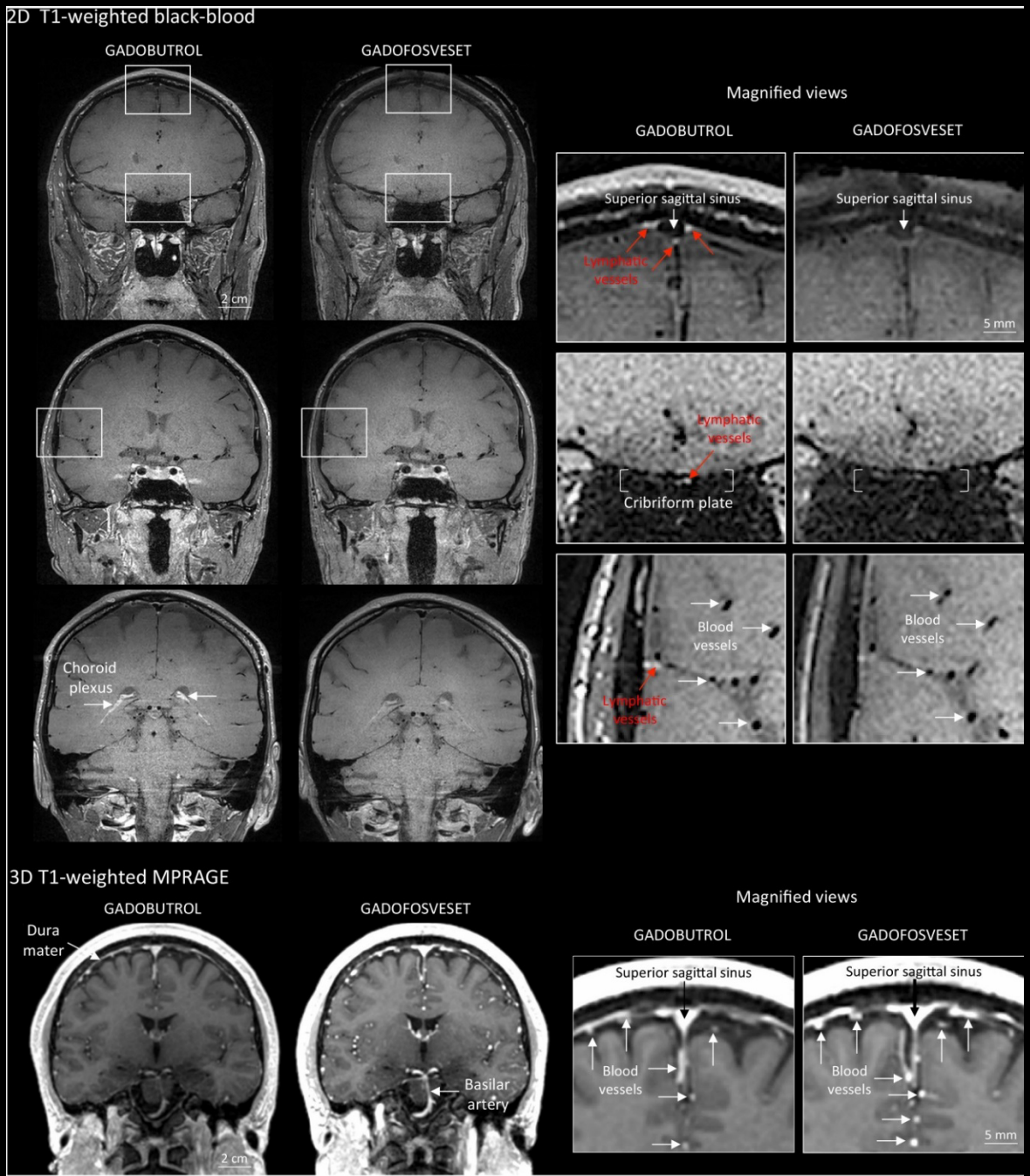


n=5

n=3

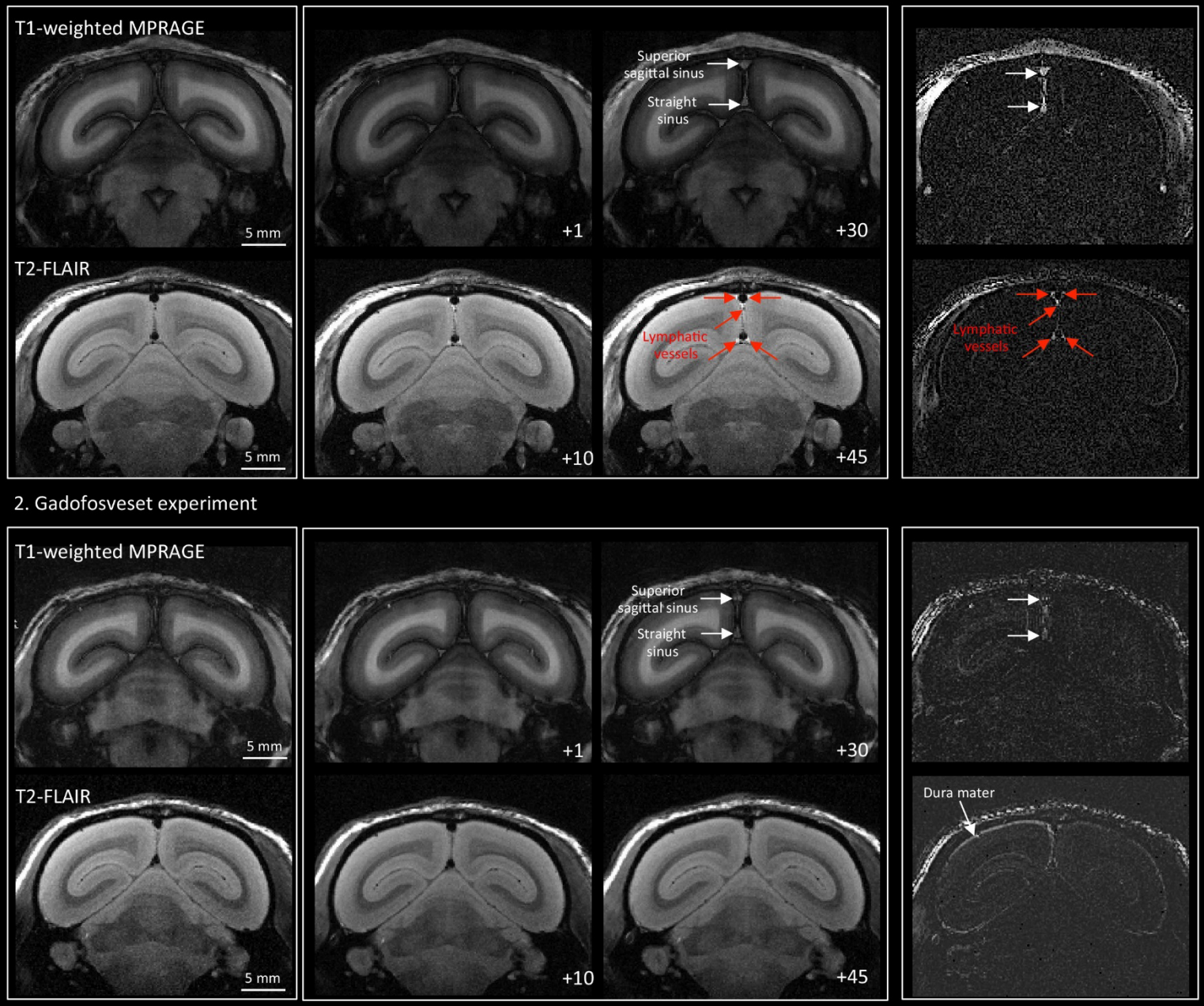
MRI visualization of dural lymphatic vessels – gadobutrol vs. gadofosveset

Human

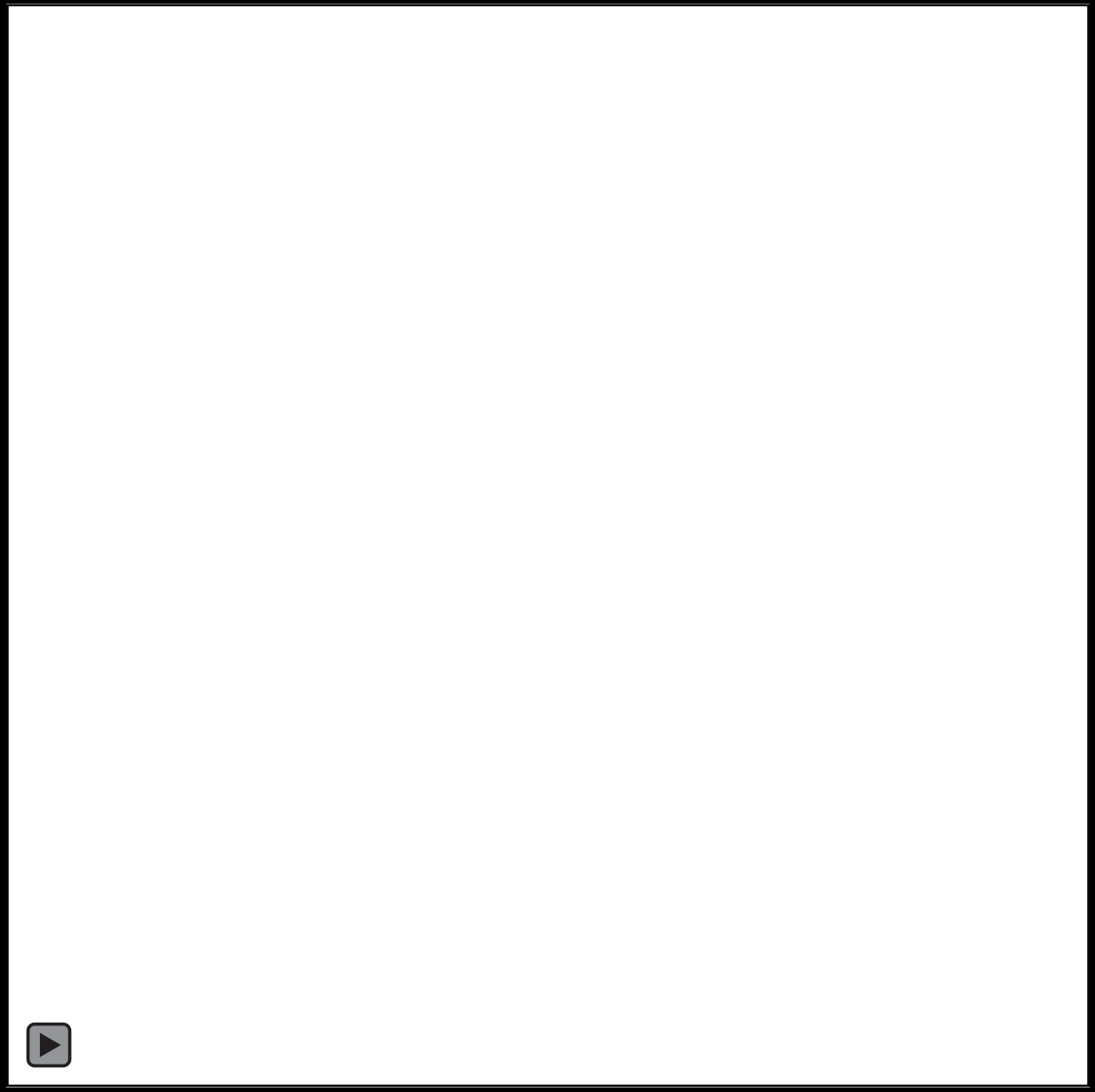


MRI visualization of dural lymphatic vessels – gadobutrol vs. gadofosveset

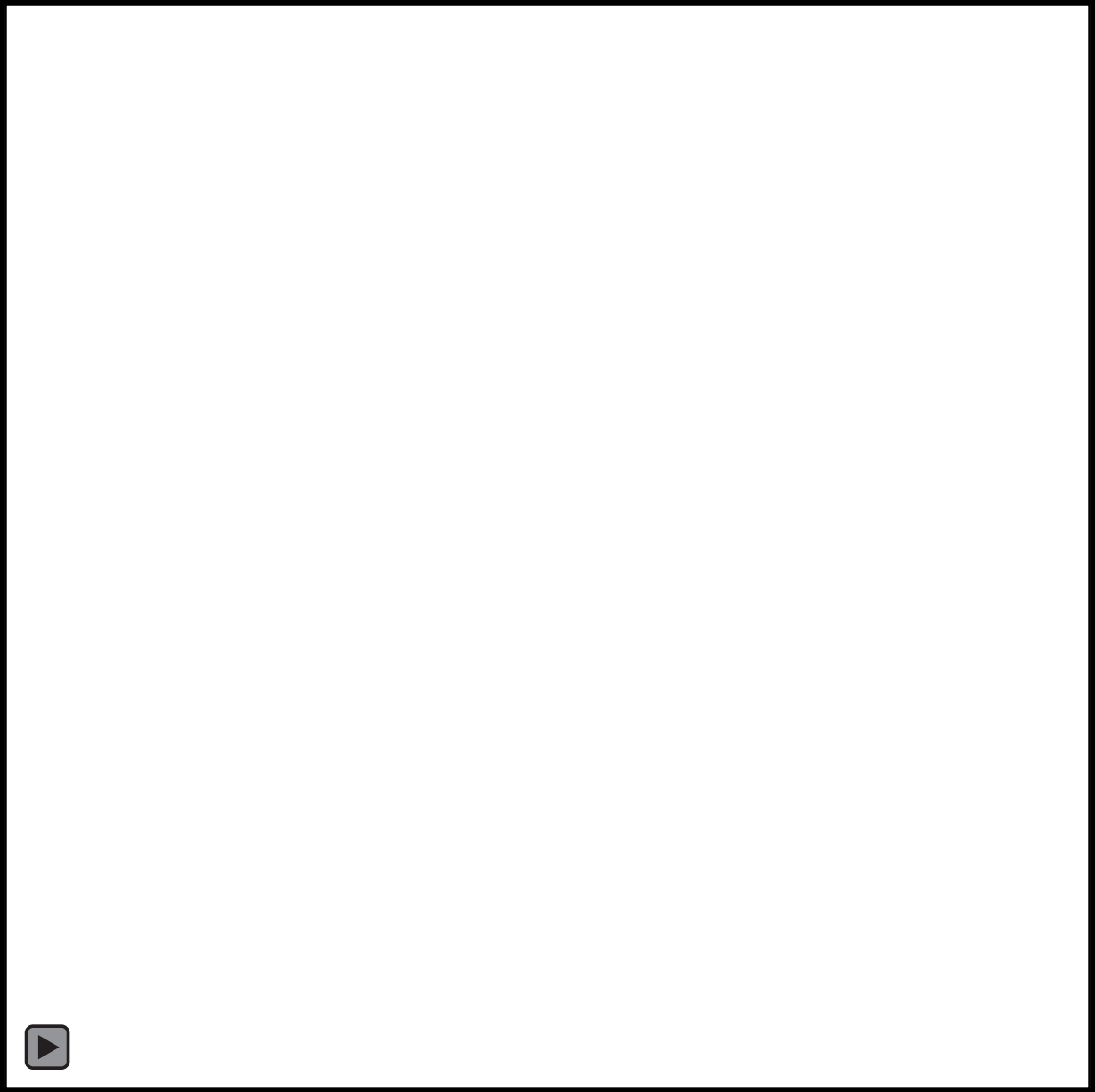
Marmoset



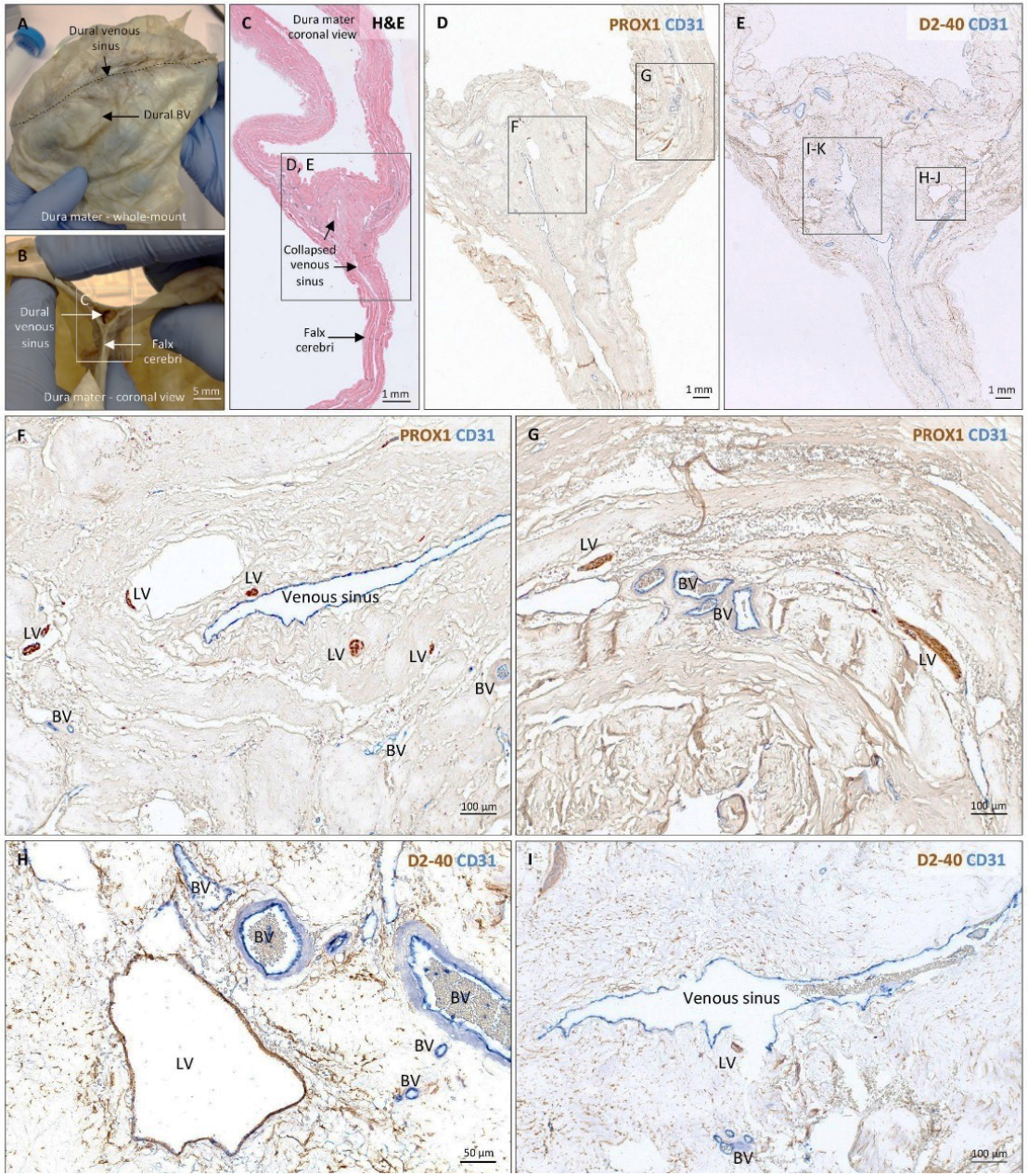
3D rendering of of subtraction MRI images - human



3D rendering of of subtraction MRI images - marmoset



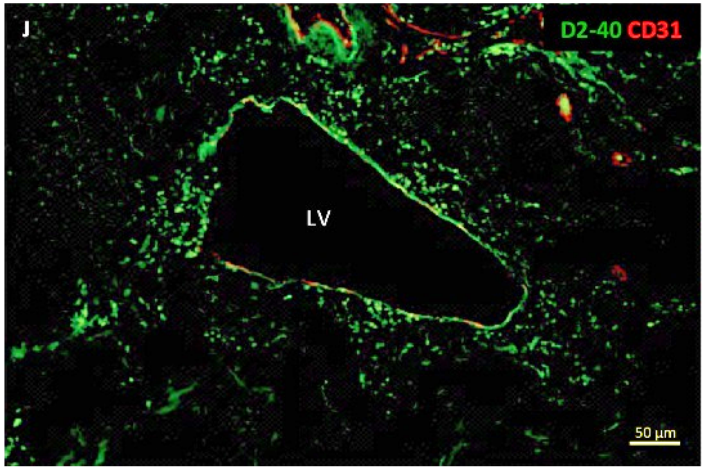
Histology of human dural lymphatic vessels

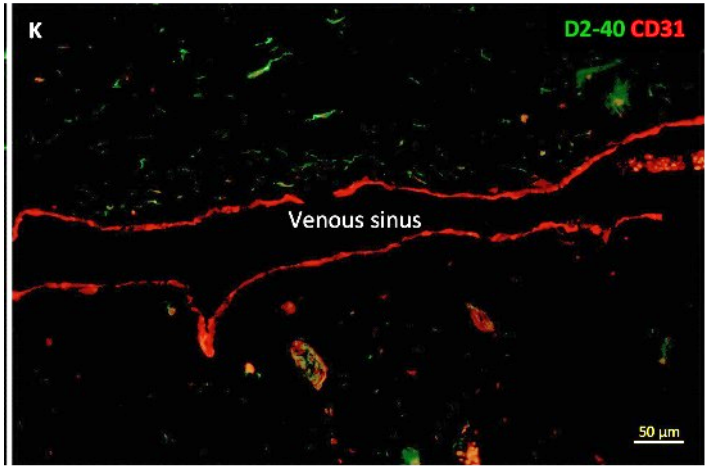


Selective double immunostaining:

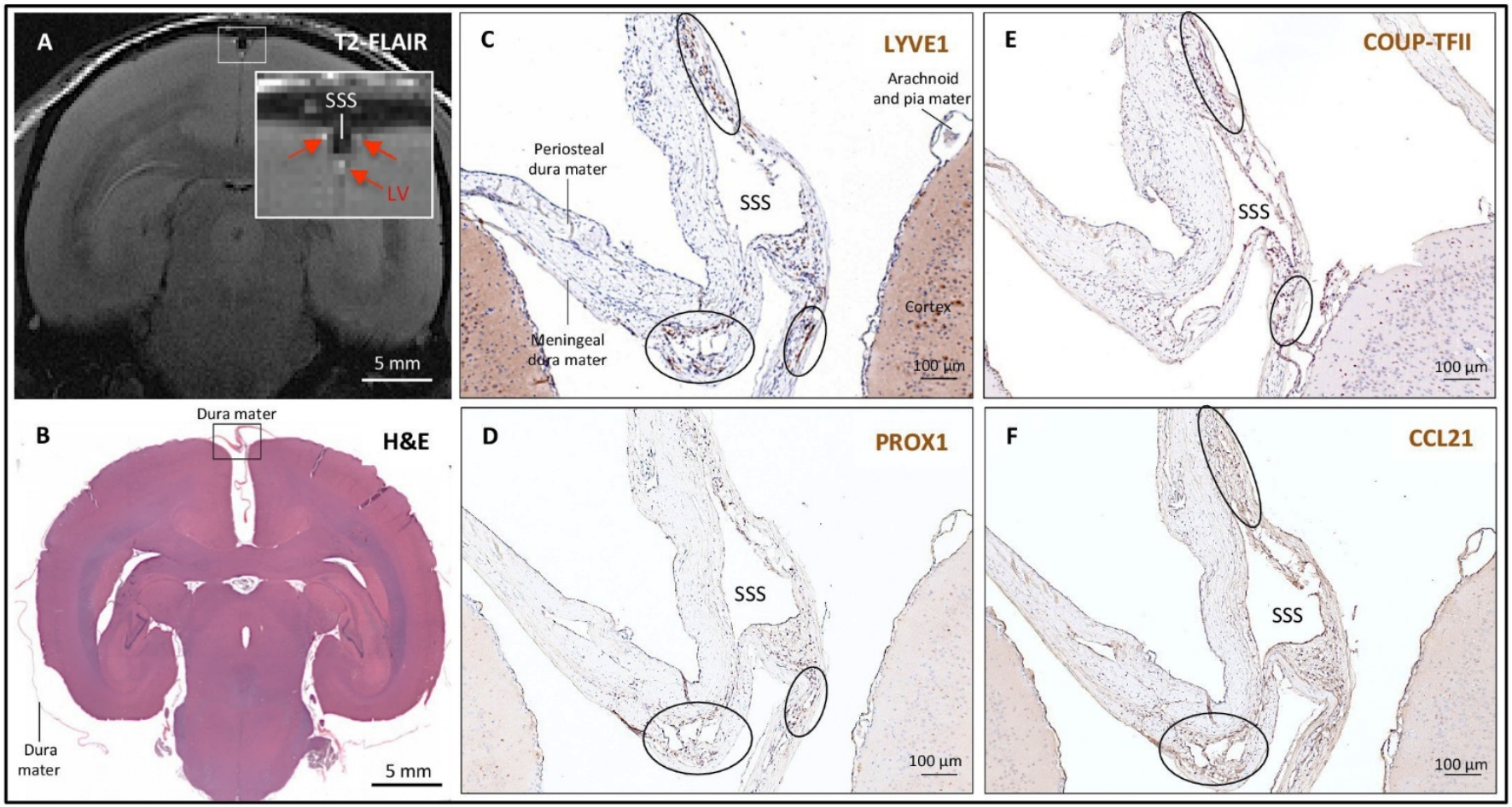
- D2-40 podoplanin/CD-31
- PROX-1/CD-31

Identified 93 human dural lymphatics



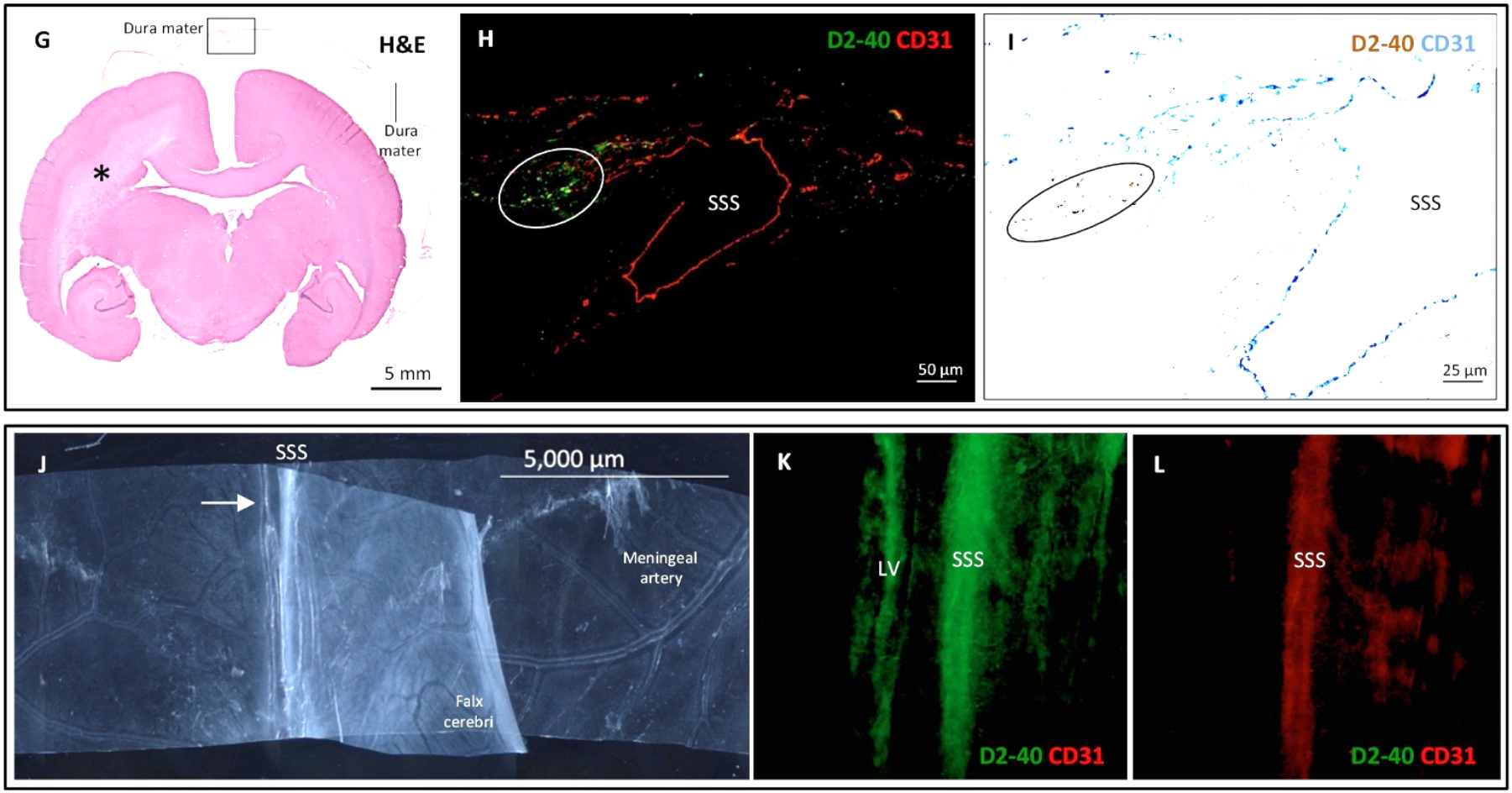


Histology of marmoset dural lymphatic vessels



MRI-histology correlation in a 4,4 years old marmoset

Histology of marmoset dural lymphatic vessels



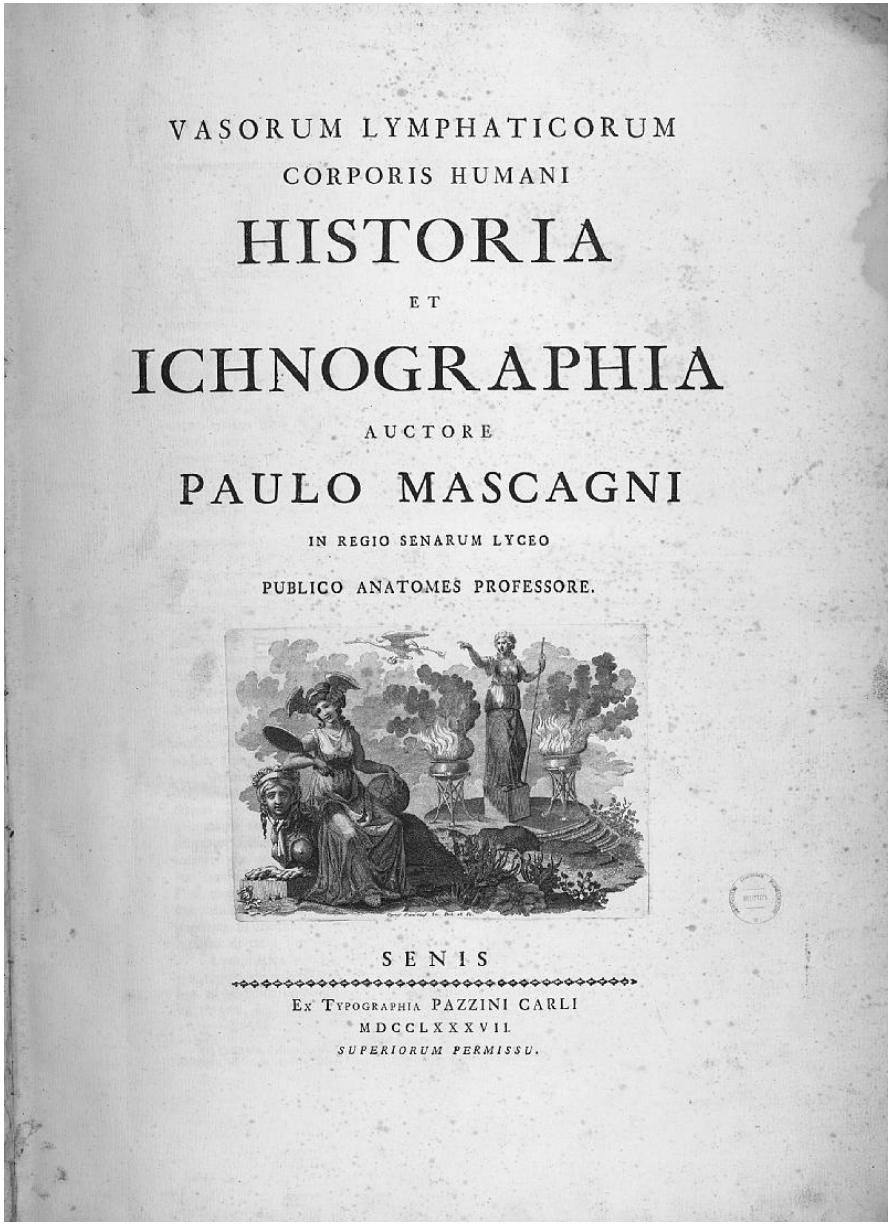
MRI-histology correlation in a 10,3 and 3,7 years old marmoset

Conclusions

- <u>Advantages:</u> - demonstrate the existence of lymphatic vessels confirming Paolo Mascagni's observations from the 19th century
  - in vivo imaging of dural lymphatic vessels

- <u>Limitations:</u> - no functional information on immune cell, CSF or other substance drainage
  - no assessment of link with the glyphatic system or lymph nodes

Thank you for your attention!



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PAULO MASCAGNI
IN REGIO SENARUM LYCEO
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SENIS
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