Title:

Global Mapping of Mouse CSF Flow via HEAP-METRIC

Phase-contrast MRI

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1 Abstract

2 Roles of Cerebrospinal fluid (CSF) in brain waste clearance and
3 homeostasis has been increasingly recognized, thus measuring its flow
4 dynamics could provide important information about its function and
5 perturbation. While phase-contrast MRI can be used for non-invasive flow
6 mapping, so far its mapping of low velocity flow (such as mouse brain CSF)
7 is not possible. Here we developed a novel generalized Hadamard
8 encoding based multi-band acceleration scheme dubbed HEAP-METRIC
9 (Hadamard Encoding Approach of Multi-band Excitation for short TR
10 Imaging accelerating), and with significantly increased SNR per time,
11 HEAP-METRIC phase-contrast MRI achieved fast and accurate mapping
12 of slow ($\sim 10^2$ micron/s) flow. Utilizing this novel method, we revealed a
13 heterogeneous global pattern of CSF flow in the awake mouse brain with
14 a averaged flow of ~ 200 micron/s, and further found isoflurane anesthesia
15 reduced CSF flow that was accompanied by reduction of glymphatic
16 function. Therefore, we developed the novel HEAP-METRIC phase-
17 contrast MRI for mapping low velocity flow, and demonstrated its
18 capability for global mapping of mouse CSF flow and its potential
19 alterations related to various physiological or pathological conditions.

20

1 **Introduction**

2 Cerebrospinal fluid (CSF) has been increasingly recognized as an
 3 important component in brain waste clearance and homeostasis. Produced
 4 by choroid plexus in ventricles, CSF is traditionally believed to flow from
 5 the ventricular system into the subarachnoid space and exit via cranial and
 6 spinal nerves and arachnoid granulations¹. Recent proposed glymphatic
 7 system theory² further extends the role of CSF, as it flows through the brain
 8 parenchyma along the paravascular space and exchanges with interstitial
 9 fluid (ISF). The (dys)function of glymphatic system has been implicated in
 10 various physiological and pathological processes, such as sleep, aging and
 11 neurodegenerative diseases^{3,4}.

12 As an integral part of brain's waste clearance system, the CSF flow
 13 dynamics might play an important role in this process. Studies found that
 14 ageing related reduction of the CSF flow velocity and its association with
 15 cognitive deficits^{5,6}. Moreover, the CSF flow is positively correlated with
 16 the CSF protein level, suggesting its role in protein clearance from the
 17 brain⁷.

18 However, the CSF flow measurement in rodents has been difficult:
 19 invasive methods would most likely perturb the flow, while non-invasive
 20 methods such as conventional phase-contrast MRI (PC-MRI) cannot map
 21 the presumably low velocity CSF flow in the rodent brain. Phase contrast
 22 MR pulse sequence is widely used for flow velocity quantification (e.g.,

1 blood or CSF flow in human). However, human CSF flow is on the order
 2 of $\sim 10^4 \mu\text{m/s}$ ^{8,9} and with much small brain size in rodents, CSF flow in
 3 rodents would likely be several orders of magnitude lower. Such extremely
 4 slow flow cannot be readily mapped using conventional PC-MRI: firstly,
 5 low SNR phase maps are heavily contaminated by Rician noise and thus
 6 create large bias errors in velocity images¹⁰⁻¹²; Secondly, such slow flow
 7 requires high amplitude of toggling gradients in PC-MRI, which leads to
 8 high eddy current field induced phase errors¹³⁻¹⁵. Two factors combined,
 9 mapping low velocity flow such as CSF in rodents has not been achieved.

10 Therefore, we developed a novel PC-MRI method based on a
 11 generalized Hadamard multi-band (MB) acceleration approach dubbed
 12 HEAP-METRIC (Hadamard Encoding Approach of Multi-band Excitation
 13 for short TR Imaging accelerating) PC-MRI, which can readily achieve a
 14 MB band of ~ 20 . We applied this novel method to achieve global mapping
 15 of mouse ventricular CSF flow for the first time, and found a spatially
 16 heterogeneous CSF flow pattern with an averaged flow around $216 \mu\text{m/s}$
 17 in the awake mouse brain. Importantly, we found a reduction of CSF flow
 18 in the anesthetized condition compared to the awake condition, further
 19 highlighting HEAP-METRIC PC-MRI's ability to detect physiological or
 20 pathological alterations of CSF flow in rodents. HEAP-METRIC PC-MRI
 21 can be openly accessed ([https://github.com/ZhifengLiangLab/HEAP-](https://github.com/ZhifengLiangLab/HEAP-METRIC)
 22 METRIC).

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2 **Results**

3 **Design of HEAP-METRIC PC-MRI and its validation for low velocity** 4 **mapping**

5 SNR may be significantly improved by Hadamard multi-band (MB)
6 approach in multi-slice fast imaging with short repetition time (TR), as it
7 enables multiple signal averages without extra scanning time. Previous
8 Hadamard MB Encoding methods¹⁶⁻¹⁸ used phase modulated
9 Radiofrequency (RF) waveforms to simultaneously excite and acquire
10 multi-slice signals, thus accelerating the imaging acquisition. In the current
11 work, we developed a generalized Hadamard encoding scheme using
12 complex Hadamard matrix (see Methods) which is dubbed HEAP-
13 METRIC (Hadamard Encoding Approach of Multi-band Excitation for
14 short TR Imaging accelerating), allowing MB factor to be any arbitrary
15 number. Fig. 1 illustrates the approach we developed (see Methods for
16 details): with Hadamard encoding phase modulation a series of RF
17 waveforms $W_n(t)$ were designed (Fig. 1a); and the MB excitation profiles
18 $M_n(\omega)$ were shown in Fig. 1b, with each band signal phase encoded in
19 complex domain based on Hadamard matrix. The above generalized
20 Hadamard encoding was applied in mouse CSF mapping (Fig. 1c). After
21 Hadamard decoding reconstruction (Fig. 1d), phase unwrapping, eddy
22 current field shimming (Fig. 1e) and combining three directional results

1 yielded quantitative CSF velocity maps (Fig. 1f).

2 The substantial SNR gain from HEAP-METRIC acceleration is
3 critical for mapping low velocity flow (Fig. 2). Without any acceleration,
4 conventional PC-MRI could not yield reasonable CSF flow in mouse brain
5 (Fig. 2, single band and low MB factors). Higher MB factors, enabled by
6 HEAP-METRIC, clearly lead to increasingly smaller bias error reduction
7 in velocity maps (Fig. 2a, b). Importantly, our HEAP-METRIC PC-MRI
8 was validated using a slow flow phantom (Fig. 3), as the mapping results
9 showed very high correlation with set averaged flow velocities ranging
10 from 100-600 $\mu\text{m/s}$. Therefore, HEAP-METRIC PC-MRI clearly
11 demonstrated fast and accurate multi-slice mapping of low velocity flow,
12 and can be openly accessed ([https://github.com/ZhifengLiangLab/HEAP-](https://github.com/ZhifengLiangLab/HEAP-METRIC)
13 METRIC).

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15 **Global mapping of mouse ventricular CSF flow utilizing HEAP-** 16 **METRIC PC-MRI**

17 Equipped with this novel method and combined with our previously
18 established awake mouse MRI method^{19,20}, we systematically
19 characterized ventricular CSF flow at the whole brain level in the awake
20 mice for the first time, and further revealed the impact of anesthesia on
21 CSF flow. With pilot data (Fig. 2) and considering the mouse brain size,
22 we used MB factor of 18 to achieve global CSF mapping (Fig. 4). High

1 resolution (0.08×0.08mm in plane resolution and 36 0.4mm slices) PC-
 2 MRI was completed in 21.8 mins with MB factor of 18. Without HEAP-
 3 METRIC acceleration, conventional single band acquisition would require
 4 388.8 mins and be clearly infeasible. Globally, CSF flowed at very low
 5 velocity (average velocity 216.89 $\mu\text{m/s}$, Fig. 4e) in the awake condition
 6 and exhibited spatial heterogeneity (Fig. 4b, e). To the best of our
 7 knowledge, this is the first report of CSF flow mapping in the mouse brain.
 8 Not surprisingly, CSF flowed at higher speeds in narrow space such as
 9 cerebral aqueduct compared to much larger lateral ventricle (Fig. 4b, e). In
 10 addition to the velocity, we observed clear directions of CSF flow in
 11 various structures including lateral ventricle, third ventricle and cerebral
 12 aqueduct (Fig. 5), which generally agrees with the current understanding
 13 of CSF flow directions. For example, the direction of CSF flow in the
 14 lateral ventricle was mainly dorsal-ventral, while in the third ventricle and
 15 cerebral aqueduct, it was mainly anterior-posterior (slice direction).

17 **Isoflurane anesthesia reduced CSF velocity and glymphatic function**

18 Importantly, HEAP-METRIC PC-MRI revealed significant
 19 reduction of CSF velocity under isoflurane anesthesia (Fig. 4c, e), which
 20 has not been reported before. Such reduction was not spatially uniform, as
 21 it was more pronounced in third and fourth ventricles. Furthermore, we
 22 found significant correlation between heart rate and averaged CSF velocity

(Fig. 4f, g), suggesting a possible physiological basis for anesthesia induced CSF velocity reduction. Interestingly, the same isoflurane anesthesia was found to reduce the glymphatic function (as measured by dynamic contrast enhanced MRI, DCE-MRI) compared to the awake condition (Fig. 6), suggesting a potential link between macroscopic ventricular CSF flow and microscopic CSF-ISF flow in brain parenchyma.

Discussion

In summary, we developed HEAP-METRIC phase-contrast MRI method, which allows non-invasive and global mapping of low-velocity CSF flow in the mouse brain for the first time. Utilizing this novel technique, we revealed anesthesia induced reduction of CSF flow velocity, compared to the awake state.

To accelerate PC-MRI acquisition, we developed HEAP-METRIC acceleration approach using Fourier basis vectors constructed complex orthogonal matrices to achieve arbitrary number simultaneous slice scanning. This generalized Hadamard encoding scheme achieved $\sqrt{N}$ SNR per time improvement, which is critical for global mapping of slow CSF flow in the mouse brain. For previous Hadamard MB encoding method the simultaneously slice number was limited to 2 or 4k (k is an integer) which is mathematically a necessary but not sufficient condition for the construction of a real Hadamard matrix. Our HEAP-METRIC

1 approach uses complex orthogonal matrix to achieve a general form of
 2 Hadamard encoding, which is supported by the fact that MRI signal is
 3 complex. Thus, any arbitrary number of slices for Hadamard encoding
 4 acquisition can be achieved. In practical applications, the maximum MB
 5 factor is mainly limited by two hardware factors: minimum dwell time of
 6 RF transmitter and maximum output power of RF amplifier. Under those
 7 two constrains, we estimate maximum MB factor can reach ~ 100 in our
 8 Bruker scanner for a regular mouse experiment under the conditions: 1000
 9 Watts power of RF amplifier, 3 ms RF duration and 15° flip angle.
 10 Furthermore, this new HEAP-METRIC scheme may be extended to other
 11 short TR MRI applications, including TOF sequence for vascular imaging,
 12 FLASH or SSFP sequence for multi-slice cardiac imaging, and EPI
 13 sequence for fMRI, in all of which SNR per time may be increased
 14 substantially.

15 The current multi-band PC-MRI is developed for mapping of low
 16 velocity ($10^2 \mu\text{m/s}$) flow, including but not limited to mouse CSF flow. CSF
 17 plays an important role in the homeostasis and waste clearance in the
 18 brain^{21,22}. In addition to its mechanical cushion function, CSF also
 19 communicates with ISF and maintains the extracellular environment
 20 homeostasis²³, and CSF dynamics perturbation induces cerebral pathologic
 21 changes, such as hydrocephalus²⁴. Thus, CSF flow dynamics may serve as
 22 biomarker for those pathological conditions²⁵. In the past decades, various

1 methods were developed to measure the production and absorption of CSF
 2 in animal models²⁶⁻²⁸. These approaches, while being highly valuable, are
 3 invasive. The nature of the CSF circulation dictates that invasive
 4 measurement would likely perturb its dynamics. Therefore, noninvasive
 5 methods such as MRI may be complimentary to existing invasive methods.
 6 While PC-MRI has been widely used in human for (mostly local) CSF flow
 7 measurement²⁹⁻³¹, global mapping of much lower velocity CSF in rodent is
 8 only achieved in the current study with our newly developed HEAP-
 9 METRIC scheme (Fig. 1).

10 With mouse being the predominant animal model in neuroscience
 11 research, the current method paves ways for dissecting roles of various
 12 genetic, physiological or pathological factors on CSF flow dynamics, and
 13 may have further implications on the glymphatic system. It is known that
 14 CSF flow is pulsatile and is dependent upon cerebral arterial pulsation³²,
 15 which is considered as the main driver of glymphatic system^{33,34}. Therefore,
 16 we speculate there might be underlying relationship between CSF flow and
 17 glymphatic function. The current study already provided a good example,
 18 showing isoflurane anesthesia both significantly reduced CSF velocity and
 19 glymphatic function (Fig. 6), compared to the awake state. The exact
 20 relationship between CSF flow and glymphatic function will require
 21 further examination, possibly utilizing the current HEAP-METRIC PC
 22 MRI method.

In conclusion, we developed the novel HEAP-METRIC phase-contrast MRI technique, which enables non-invasive and global mapping of mouse CSF flow velocity. Utilizing this novel technique, we further revealed isoflurane anesthesia induced CSF flow reduction, compared to the awake condition. The technique can be potentially applied in mapping other low velocity flow, and the HEAP-METRIC scheme can also be extended in other MR techniques to significantly boost SNR per time.

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Figure 1. HEAP-METRIC PC-MRI for mouse CSF velocity mapping.

a, HEAP-METRIC RF waveforms using MB factor 6 as an example ($W_1(t)$ - $W_6(t)$). Each waveform was plotted as a time dependent 3D complex pulse with a projection on complex plane at its upper right corner. Note in actual experiment MB factor 18 was used. b, excitation profiles $M_1(\omega)$ - $M_6(\omega)$ of $W_1(t)$ - $W_6(t)$ calculated by Bloch equations. Each profile was plotted as a frequency dependent 3D complex curve with a projection on a complex plane at its upper right corner. Note that each band of each profile rotated on the complex plan by a certain phase angle according to Hadamard matrix. c, illustration of the designed six-band HEAP-METRIC RF pulses applied on a PC 2D sequence for mouse CSF velocity mapping. d, reconstruction of HEAP-METRIC data. The k-space data were Hadamard decoded and Fourier transformed to obtain magnitude and phase images. Note images reconstructed without Hadamard decoding were also displayed in lower left as a comparison but not used. e, eddy current field shimming. Left column, raw phase contrast images of three velocity encoding directions (slice, phase and read) and manually drawn mask on static tissues. Middle column, three spherical maps of eddy current field estimated by 2nd-order spatial polynomial fitting on the static field. Right column, phase images after phase correction including unwrapping and shimming. f, final quantitative CSF velocity maps after combination of three directional results.

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Figure 2. Reduction of bias errors in velocity mapping with SNR improvement. a, reconstructed quantitative velocity maps from conventional single band to MB factors from 2-20 showed notable reduction of bias errors with increasing MB factors. Red outlines, manually drawn CSF ventricle ROI. b, averaged ventricular velocity and SNR (based on CSF ROI in a) exhibited decreasing and increasing trends with increasing MB factors, respectively, which clearly showed high SNR provided by HEAP-METRIC scheme was critical for accurate measurement of low velocity CSF flow in mouse brain.

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1 **Figure 3. Phantom experiment validates HEAP-METRIC PC-MRI**
2 **velocity mapping.** a, set up of slow flow phantom. b, HEAP-METRIC
3 velocity mapping of flow phantom with set averaged flow velocity from
4 100-600 $\mu\text{m/s}$. c and d, high correlation between the measured velocity and
5 the set phantom velocity in the left and right tube, respectively.

Figure 4. Global mapping of mouse ventricular CSF flow in awake condition and its reduction by isoflurane anesthesia. a, illustration of atlas-based cerebral ventricular definition. b and c, cerebral ventricular CSF velocity mapping in the awake (b, n=6) and isoflurane anesthesia (c, n=7) conditions. Numbers under each slice denoted relative distance from bregma. d, illustration of 3D reconstructed cerebral ventricles. e, reduction of CSF flow by isoflurane anesthesia at global and individual ventricle level. global ventricles: $p = 0.0406$, third ventricle: $p = 0.0184$, fourth ventricle: $p = 0.01$. f, reduction of heart rate in isoflurane anesthesia compared to the awake condition. ****, $p < 0.0001$. g, significant correlation between heart and global CSF velocity.

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Figure 5. Representative CSF flow directions revealed by HEAP-METRIC mapping. a and b, representative anatomical MRI slices showing lateral and third ventricles, and cerebral aqueduct. c and d, representative color vector maps in the same slices showing directional CSF flow. Green, red and blue color represent vertical (dorsal-ventral), horizontal (medial-lateral) and slice (anterior-posterior) direction, respectively.

Figure 6. Isoflurane anesthesia reduced the glymphatic function compared to the awake condition using DCE-MRI. (a to d) Representative T1-weighted MRI images in the awake condition before (a), 32min (b), 60min (c) and 120min (d) after infusing Gd-DTPA. (e to h) Representative T1-weighted MRI images in the isoflurane anesthetized condition. i, average time signal curves (TSC) of Gd-DTPA induced signal change from whole brain. *, $p < 0.05$. Isoflurane, $n = 5$ animals; Awake, $n = 6$ animals.

Methods

1. Imaging Theory

1.1 Hadamard Encoding Approach of Multi-band Excitation for short TR Imaging aCcelerating (HEAP-METRIC)

A multi-band excitation RF waveform can be expressed by a sum of individual RF pulses with different on-resonance frequencies:

$$W(t) = A(t) \cdot \sum_{m=1}^N e^{i\omega_m t} \quad [1]$$

Where $W(t)$ denotes time dependent multi-band RF waveform; $A(t)$ denotes a standard single band selective excitation pulse waveform (e.g., a sinc or hyperbolic secant); N is number of simultaneous bands (or slices); m is the index of band and ω_m is the on-resonance frequency of m^{th} band. With Hadamard Encoding phase modulation, a set of N RF waveforms are generated, in which case, Eq. [1] becomes:

$$W_n(t) = A(t) \cdot \sum_{m=1}^N e^{i(\omega_m t + \varphi_{nm})} \quad [2]$$

Where n denotes the index of HEAP-METRIC RF pulse; φ_{nm} denotes the Hadamard encoding phase of m^{th} band of n^{th} RF waveform. With elements $e^{i\varphi_{nm}}$, an $N \times N$ Hadamard Matrix is constructed to be:

$$H = \{e^{i\varphi_{nm}}\} = \begin{bmatrix} e^{i\varphi_{11}} & \dots & e^{i\varphi_{1N}} \\ \vdots & \ddots & \vdots \\ e^{i\varphi_{N1}} & \dots & e^{i\varphi_{NN}} \end{bmatrix} \quad n, m \in [1, N] \quad [3]$$

H is orthogonal and satisfies Eq. [4]:

$$HH^* = NI \quad [4]$$

Where I is the identity matrix; H^* is the conjugate transpose of H ; note that $H^* = H^T$ when H is real. Previous work¹⁻⁴ all used real Hadamard matrix with all ± 1 entries (equivalent to $\varphi_{nm} = 0^\circ$ or 180° either) thus N is limited to 2 or $4k$ (k is an integer). Here we generalize Hadamard matrix to be complex thus N can be any arbitrary integer. Fourier basis vectors are used to construct our new Hadamard matrix $H = \{e^{i\frac{nm2\pi}{N}}\}$.

The MR signal excited by the n^{th} HEAP-METRIC RF pulse is then formulated as:

$$S_n = \sum_{m=1}^N X_m \cdot e^{i\varphi_{nm}} \quad [5]$$

which can also be simplified as Eq. [6]:

$$S = HX \quad [6]$$

Because the H is orthogonal then each individual band signal X_m can be obtained by Eq. [7]:

$$X_m = \sum_{n=1}^N S_n \cdot e^{-i\varphi_{nm}} \quad [7]$$

which again can be simplified as Eq. [8]

$$X = H^*S \quad [8]$$

For practical MRI applications, Hadamard decoding by Eq. [7] or [8] can be done either before or after Fourier transform of K space data. N separated scans are required to obtain all N bands images. Comparing with

1 single band RF approach acquiring signal slice by slice, HEAP-METRIC
2 provides N times signal averages with N simultaneous slices with identical
3 acquisition time. Therefore, HEAP-METRIC accelerates image
4 acquisition by a factor of N, thus increases the SNR per time by a factor
5 $\sqrt{N}$.

6

7 1.2 Quantitative CSF Velocity Mapping

8 The aforementioned HEAP-METRIC method was incorporated into
9 conventional PC-MRI, to substantially speed up acquisition. For in vivo
10 imaging we designed a group of 18 Hadamard encoded eighteen-bands MB
11 RF waveforms and each RF waveform consists of 10000 phase modulated
12 complex points. A Hanning windowed three-lobes-sinc function was used
13 as the base RF waveform $A(t)$.

14 All excited bands of MB RF pulses were designed to be equally
15 spaced. Adjacent bands were gapped by one band distance thus it needs
16 twice slice-selection (2 transmitting frequencies) to complete a whole brain
17 scan. 36 slices data were finally reconstructed in total. Our HEAP-
18 METRIC PC pulse sequence was implemented on Bruker ParaVision 6.0.1
19 system. The specific looping structure of a running sequence is as:

1 McMaster-Carr, Douglasville, GA) and a 10 mL syringe (BD, Franklin
2 Lakes, NJ) were integrated into a semi-closed loop fluid flow system using
3 a syringe pump (Model 33, Harvard Apparatus, Holliston, MA). Artificial
4 cerebrospinal fluid (aCSF) was pumped at a control flow rate during MR
5 imaging. The imaging part of the holder was filled with 1% agarose gel
6 and the silicone tubing was placed in the agarose (Fig. S2). This flow
7 phantom system was tested outside the magnet by weighing the aCSF
8 delivered at the set flow rate which resulted in errors less than 10%.

9 During the phantom experiment, the flow rate was sequentially set to
10 0.314 $\mu\text{l/s}$, 0.628 $\mu\text{l/s}$, 0.942 $\mu\text{l/s}$, 1.256 $\mu\text{l/s}$, 1.57 $\mu\text{l/s}$, 1.884 $\mu\text{l/s}$,
11 respectively corresponding to 100 $\mu\text{m/s}$, 200 $\mu\text{m/s}$, 300 $\mu\text{m/s}$, 400 $\mu\text{m/s}$,
12 500 $\mu\text{m/s}$, 600 $\mu\text{m/s}$. The imaging parameters were: TR/TE = 30/9.1 ms,
13 Flip angle = 10° , receiver bandwidth = 100 KHz, FOV = $16 \times 16 \text{ mm}^2$,
14 matrix size = 180×200 , slice thickness = 0.4 mm, VENC value = 0.15 cm/s,
15 pulse duration = 1 ms.

16

17 3. Animal HEAP-METRIC PC-MRI Experiment

18 Male adult C57BL/6 mice were used in the current study (14 weeks
19 of age, weighted between 25 and 30 g) with food and water ad libitum.
20 Overall, six mice were imaged during isoflurane anesthesia and seven mice
21 were imaged in the awake condition. All animal experiments were
22 approved by the Animal Care and Use Committee of the Center for

1 Excellence in Brain Science and Intelligence Technology, Chinese
2 Academy of Sciences, Shanghai, China.

3 For awake imaging group, awake mouse preparation and imaging
4 setup was done according to our previous study^{9,10}. Briefly, a head holder
5 was implanted above the animal's skull for head fixation during imaging.
6 After recovery from the implantation surgery, mice were acclimated to the
7 MRI environment with head fixation and recorded imaging noises.

8 For anesthetized imaging group, mice were initially induced with 5%
9 isoflurane and endotracheally intubated. During imaging, mice were
10 ventilated and anesthesia was maintained at 1.3% isoflurane delivered by
11 a mixture of oxygen and air (20%:80%).

12 All imaging data were obtained on a Bruker 9.4T scanner. Scanning
13 parameters were optimized as: TR/TE = 30/9.1 ms, Flip angle = 10°,
14 receiver bandwidth = 100 KHz, FOV = 16×16 mm², matrix size = 200×
15 200, slice thickness = 0.4 mm, VENC value = 0.15 cm/s, pulse duration =
16 1 ms. Total scanning time was 21.8 minutes.

17

18 4. Animal DCE-MRI Experiment

19 For the study of the glymphatic system, we applied the dynamic
20 contrast enhanced MRI (DCE-MRI) to observe the function of
21 glymphatic system in different conditions. All the included mice referred
22 to the above, and totally five mice were imaged during isoflurane

1 anesthesia and six mice were imaged in the awake condition.

2 For all imaging groups, mouse preparation and imaging setup were
3 done according to the previous studies^{11,12}. Briefly, anesthetized mice
4 were placed in three-point stereotaxic apparatus. A custom-made
5 borosilicate capillary (tip diameter of approximately 100-150 μm)
6 attached to PE-10 tubing filled with aCSF was inserted into the cisterna
7 magna as described previously¹³. Afterwards, a head holder was
8 implanted above the animal's skull for head fixation during imaging.
9 After recovery from the implantation surgery, awake mice were
10 acclimated to the MRI environment with head fixation and recorded
11 imaging noises. For the anesthetized imaging group, mice were intubated
12 and ventilated as above during imaging.

13 All imaging data were obtained on a Bruker 9.4T scanner. T1-
14 weighted 3D-FLASH images were acquired every 4 min. The first three
15 images were baseline images. The paramagnetic contrast agent
16 gadopentetic acid (Gd-DTPA, 938Da) (Byer, German) was infused at the
17 beginning of the fourth scan. The contrast (Gd-DTPA : aCSF = 1 : 40)
18 was infused at a rate of 0.8 $\mu\text{l}/\text{min}$ for a total volume of 10 μl . 40 scans
19 were acquired continuously for at least 160 min for each study.

20

21 5. Data Analysis

22 All data were processed using custom scripts in MATLAB 2018b

1 (MathWorks, Natick, MA), SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>)
2 and ITK-SNAP (<http://www.itksnap.org/>).

3 First, MR images were normalized to the mouse brain template
4 (https://www.nitrc.org/projects/tpm_mouse) using nonlinear Symmetric
5 Normalization (SyN) algorithm in ANTs. Then we applied the deformation
6 matrix to transform the corresponding flow velocity images into the
7 template space. For the MR image segmentation, a probabilistic atlas
8 (https://www.nitrc.org/projects/tpm_mouse) was aligned with the MRI
9 image and used as a spatial prior for segmentation. Then the MR images
10 were segmented into 5 tissue classes (gray matter, white matter, CSF, tissue
11 outside the brain, and air) using SPM.

12 To compare global CSF velocities between the awake and
13 anesthetized states, the CSF mask was created by thresholding CSF tissue
14 probability map with probability > 0.9 and with further minor manual
15 correction. The modified CSF mask was applied in the corresponding
16 velocity image to calculate the mean CSF velocity. Allen atlas
17 (<https://atlas.brain-map.org/>) was utilized to further extract mouse
18 ventricles (lateral ventricle, third ventricle, fourth ventricle and cerebral
19 aqueduct). The above ventricle specific masks were used to obtain mean
20 CSF velocities of each individual ventricle.

21 For the DCE-MRI data, all images were converted to .nifti format,
22 corrected for head motion, and spatially smoothed. Then the percentage of

1 signal changes from the baseline were calculated in each voxel, as
2 described previously¹². For each mouse, the time signal curve (TSC) of the
3 whole brain was calculated using MATLAB.

4 All statistical analysis was performed using GraphPad Prism 8.0
5 (<https://www.graphpad.com/>) or MATLAB 2018b. For comparisons
6 between awake and anesthetized groups, unpaired Student's t-tests were
7 performed. For the phantom MRI experiment and the physiological
8 parameter's analysis, the linear least squares regression was used for
9 calculation of correlations between the measured velocity (or global CSF
10 velocity) and the set phantom velocity (or heart rate).

11

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