Accelerated clearing and molecular labeling of biological tissues using magnetohydrodynamic force

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Supplementary Information:

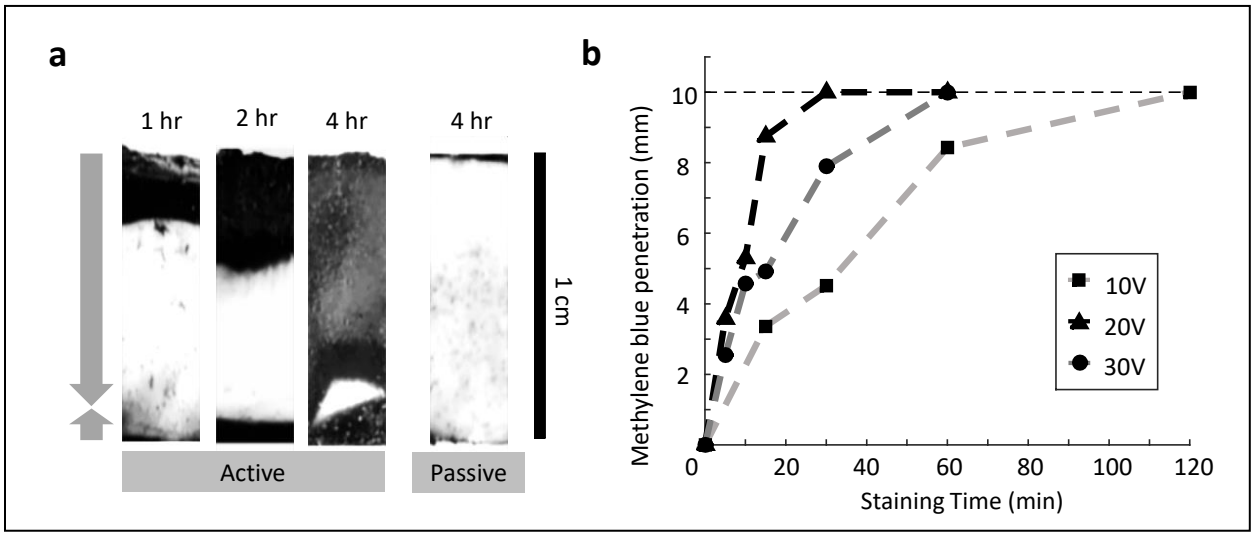

Figure S1: A) Penetration of methylene blue into a 1 cm^3 cube of homogeneous brain tissue as a result of MHD force over 1, 2, and 4 hours (N = 1). The fourth image shows a comparative 4-hour stain without MHD force. The arrows on the left-hand side of the images demonstrate the direction of the MHD force with respect to the tissue. The length of the arrows demonstrates the proportion of time when the MHD force was aimed in the direction indicated by each arrow. B) shows the comparative staining of methylene blue into agarose cubes as a result of various strengths of electrical force conjugated to MHD force. The distance the methylene blue penetrated into the agarose cubes is measured against the amount of time stained with 10, 20, or 30V conjugated to a constant magnetic field.

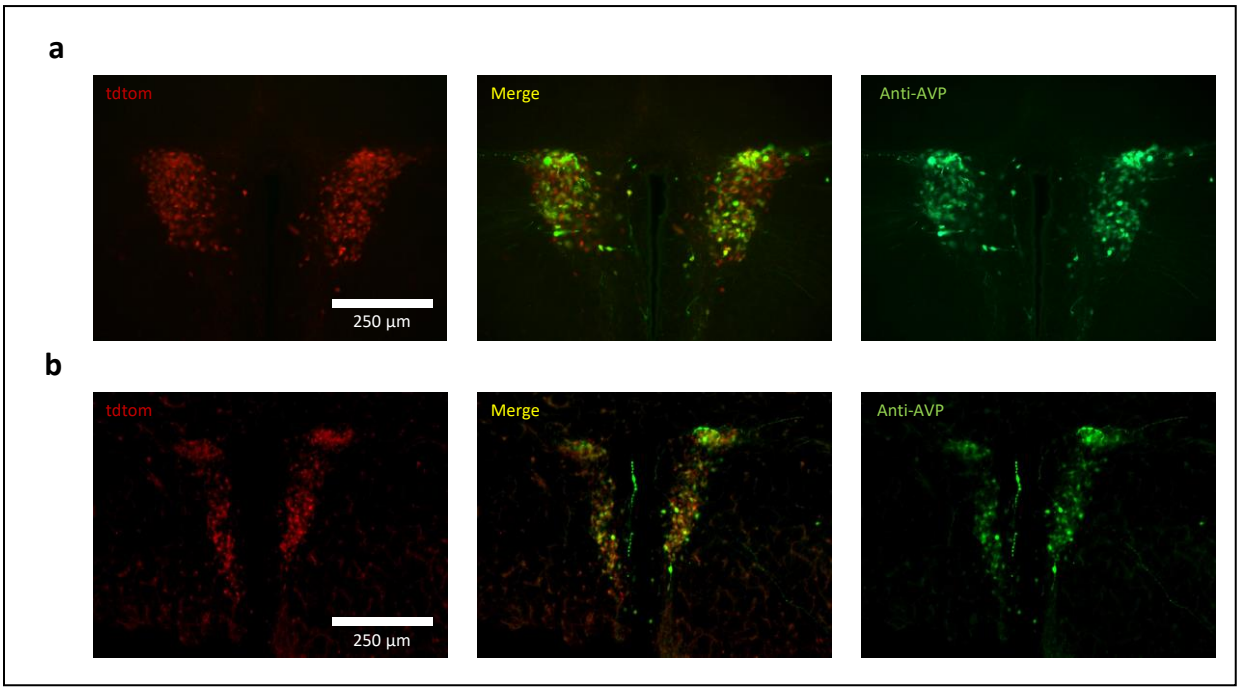

Figure S2: Traditional antibody labeling of a 100 µm, PFA-fixed slice from a mouse that expresses tdtomato under control of the AVP promoter (red; AVP-cre X rosa26-lsl-tdTomato) with α-vassopressin antibody (green) using a pH 7.0, PBS-based buffer A) or the pH 9.5 electrophoresis buffer used in MHD-accelerated labeling. In both A) and B) endogenous fluorescence is on the left, antibody fluorescence is on the right and a merge of the two images is in the center.

Technique	Time to clear Full mouse brain (hr)	Antibody Penetration over time (mm/hr)	Level of Difficulty	Cost
MHD-accelerated Clearing	12 - 48	0.15	Low	$
CLARITY (Chung, et al., 2013)	120 – 216	0.0074	High	$$
Stochastic Electrotransport (Kim, SY, et al., 2015)	72	0.20	Very High	$$
ACT-PRESTO (Lee, et al., 2016)	6	0.040	High	$$$
SCALEs (Hama, et al., 2015)	72	0.066	Medium	$
uDISCO (Pan, et al., 2016)	198	0.0046 - 0.010	Low	$
CUBIC/ CUBIC-HistoVIsion (Susaki, et al., 2015; Susaki, et al., 2020)	72 – 168	0.007 - 0.060	Medium	$
Adipo-Clear (Chi, et al., 2016)	24 – 48	0.042 - 0.050	Medium	$
SWITCH (Murray, et al., 2015)	168 – 672	0.083	Low	$

Supplementary Table 1: A direct comparison of multiple popular clearing techniques, based on literature, that shows the reported time it takes to clear an intact mouse brain, the relative antibody penetration into the tissue over a single hour, the degree of difficulty to setup and use the technique, and the amount of money it costs to implement the technique effectively. Degree of difficulty is a subjective measure of the amount and complexity of steps and solutions required to implement each technique and the level of expertise required construct devices for required for the technique and use these devices to clear mouse tissue. Level of difficulty ranges from easy (easy setup and/or requiring very few easy steps) to very hard (intricate setup that requires a high level of specialized expertise and/or requires many difficult steps) Cost to implement the technique is displayed as less than $1000 ($), less than $10,000 ($$), and over $10,000 ($$$).

Solutions

- Hydrogel monomer solution:
    - 4% acrylamide (Sigma Aldrich; A3553)
    - 0.05% bis acrylamide (Bio Rad; 1610142)
    - 0.25% Initiator (Fisher Scientific; NC0632395)
    - 0.01 M PBS (Fisher Scientific; BP2944100)

- Clearing Solution:
    - 4% sodium dodecyl sulfate
    - 200 mM Boric Acid
    - pH to 8.5 with 0.1 M NaOH

- Electrophoresis Buffer:
    - 0.1 M Borate Buffer
    - 0.1% Triton X-100 (Fisher Scientific; 85111)
    - pH to 9.5 with 0.1 M LiOH

- Optiview Imaging Solution (RI: 1.45):
    - Sodium Diatrizoate: 0.173 M (Sigma Alrdrich; S4506);\
    - Meglumine Diatrizoate: 0.816 M (Sigma Aldrich; M5266)
    - Diatrizoic Acid 0.816 M (Chem Impex; 24150)
    - pH 8 EDTA: 0.00005 M
    - Tween-20: $9 \times 10^{-7}\mathrm{M}$ (Sigma Aldrich; P1379)

MHD-accelerated tissue clearing protocol

1. Euthanize and perfuse the animal with PBS followed by 4% PFA.
2. Postfix in 4% PFA for 24 hours at 4˚ C.
3. Incubate sample of PFA-perfused tissue in *hydrogel monomer solution* for 24 hours at 4˚ C.
4. Remove oxygen from the sample using three rounds of pulling a vacuum and replacing the gas surrounding the hydrogel and sample with pure nitrogen gas.
5. Polymerize sample by placing in a water bath at 37 ˚ C for 2 – 3 hours.
6. Remove excess hydrogel from the surface of the sample.
7. Place in sample in 40 mL clearing solution (4% SDS solution in 200 mM Boric Acid) at 37 ˚ C for 24-48 hours.
8. Place sample in a clearing basket and transfer to the central channel of the MHD-accelerated clearing device (submerge in a five-liter bath of clearing solution*).
9. Apply 0.3 to 0.5 Amps across the tissue at 36.7 ˚ C (we find 0.3 Amps for 12 hours to be an effective starting point).

*clearing solution can be reused many times until the pH drops below 8.2

Refractive Index-Matching

1. Wash tissue sample in 0.01 PBS at 37 ˚ C overnight or for 8 to 12 hours
2. Incubate tissue in 30 mL Optiview for 24 hours. (At this stage the tissue sample should be barely visible in the solution)

MHD-accelerated antibody labeling protocol

Tissue: Prepare cleared tissue as above. The antibody labeling procedure also removes lipids, though not as quickly as the protocol above.

Preparation

1. Cut dialysis (6-8 kDa Spectra Por1) tubing to 2.25" + size of tissue sample (along the longest axis)
2. Equilibrate dialysis tubing in ddH_2O for at least 30 min at RT in bath of electrophoresis buffer
3. Fill dialysis tubing and one-liter chamber with *Electrophoresis Buffer*
4. Place tissue in the center of the dialysis tubing
5. Submerge dialysis tubing with tissue inside in one-liter chamber filled with Electrophoresis Buffer
6. Prepare a concentrated 300 µL solution of antibody in electrophoresis buffer
    a. 1:30 antibody, 1% Heparin
7. Load antibody solution into a syringe

Device setup

8. Submerge the torus-shaped tube in electrophoresis buffer making sure to flush air from tube.
9. Attach each end of the dialysis tubing, with the tissue inside, to the dialysis tubing adapters.
    a. Use nylon screws to tighten the end of the barbs on the dialysis tubing adapters.
10. Ensure that the entire tubing system is water-tight with no leaks and no air bubbles (this is critical as leaks will allow the antibody to leave the staining device).

Active antibody incubation:

11. Place the device, with the tube intact, over a waterproofed N52 neodymium magnet and affix the electrode array over the device to create a channel that is held in place, with an electrode on either side of the tissue, by the attraction between the top and bottom magnets
12. Submerge the intact device in electrophoresis buffer in the one-liter chamber
13. Use the inputs to the torus-shaped tube to flush the concentrated antibody solution in the syringe into the intact torus and dialysis tube system
14. Ensure that there are no bubbles or leaks in the system and that the tissue is positioned in the center of the dialysis tubing at the intersection of the magnetic and electric fields
15. Provide power to the electrode array by activating the power supply at 30-60 VDC and 0.2 to 0.3 Amps
16. periodically add electrophoresis buffer into the system to maintain the inflated dialysis tube.

Active wash:

17. Flush the antibody solution out of the system and refill with pure electrophoresis solution.
    a. Save the antibody solution as it can be reused.
18. Repeat the 'active antibody incubation' protocol without additional antibody for an additional 6 to 12 hours

Antibodies used:

Zebrafish:

Primary: Mouse IgG anti-Acetylated Tubulin antibody (Sigma-Aldrich)
Secondary: Goat IgG anti-mouse Alexa 647 nm (ThermoFisher)

Nudibranch:

Primary: Rabbit anti-5-HT (Immunostar)
Secondary: Goat anti-rabbit Alexa 488 nm (ThermoFisher)

Mouse:

Primary 1: Rabbit anti-oxytocin antibody (Immunostar)
Primary 2: Rabbit anti-AVP antibody (AbCam)
Secondary 1: Goat IgG anti-rabbit Alexa 647 nm (Jackson ImmunoResearch)
Secondary 2: Goat IgG anti-rabbit Alexa 488 nm (ThermoFisher)

Materials used to build clearing device:

0.25 mm diameter 99.9% platinum wire (Sigma-Aldrich; 349402)
1” x 2” x 1” N52 Neodymium magnet (Applied magnets; NB057-6-N52)
3D printed device (Printed in polyamide using an EOS Formiga P110 3D Printer)

Materials used to build antibody labeling device:

1” x 2” x 0.5” N52 Neodymium Magnet (Applied magnets; NB057-6-N52)
0.5” cubed N52 Neodymium Magnet (Applied magnets; NB022-N52)
0.25 mm diameter 99.9% platinum wire (Sigma-Aldrich; 349402)
0.25” diameter Spectra Por 1 6-8 kDa dialysis tubing (Spectra Por; 132645)
1/8” inner diameter vinyl tubing (ThermoFisher: S504591)
1/4” to 1/8” male-to-male tubing adapters (Cole-Parmer; UX-45501-20)
Nylon bolts (Mcmaster-Carr; 93939A734)
25-gauge winged infusion needle and IV (Fisher Scientific; 22-289913)