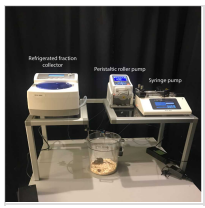

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Push-pull microdialysis sampling protocol

Christiana.bjorkli [1]

[1]The Norwegian university of Science and Technology

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Sandvig lab

Christiana.bjorkli

Our modified push-pull microdialysis method was first validated *ex vivo* with human CSF samples, and then *in vivo* in in an AD mouse model, permiting assessment of dynamic changes of CSF Aβ and tau and allowing for better translational understanding of CSF biomarkers. At the beginning of our experiments, all microdialysis probes were perfused with artificial CSF with 4 % bovine serum albumin (BSA) at a flow rate of 60mL/min in order to fill the entire system with the perfusate. A 7-day equilibration period was always allowed before starting *in vivo* CSF sampling.

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Chemicals and solutions

1. Artificial cerebrospinal fluid (aCSF)
2. 30% Bovine serum albumin (BSA) solution (Sigma-Aldrich)
3. 70% ethanol
4. Distilled H_2O

Equipment and accessories for microdialysis

1. Microdialysis probes
2. Microdialysis syringe pump
3. Microsyringe for syringe pump
4. Peristaltic pump
5. Refrigerated fraction collector
6. Sample collection vials for refrigerated fraction collector with caps and labels
7. FEP tubing
8. Peristaltic tubing with stoppers
9. Tubing adaptors
10. Cage for freely moving mice
11. One-time use filter and syringe

Habituation of animals

1 Habituate mice to human handling and restraining for one week prior to microdialysis sampling.

Setting up the system

2 Set a refrigerated fraction collector (CMA 470) to **6 °C** for the storage of collected CSF in polypropylene plastic vials.

3 Place fluorinated ethylene propylene (FEP) peristaltic tubing (CMA Microdialysis AB, Kista, Sweden) inside each plastic vial for collection and connected to the cassette of the peristaltic roller pump (Reglo ICC Digital).

4 Connect peristaltic FEP tubing to the outlet side of the microdialysis probes (β-irrigated 2 mDa microdialysis probe; CMA 7;CMA Microdialysis AB, Kista, Sweden) with a polyethersulfone 2 mm membrane with tubing adapters bathed in 75% ethanol.

5 Connect FEP tubing (CMA Microdialysis AB, Kista, Sweden) to each microsyringe. Then connect FEP tubing to the inlet part of the microdialysis probes.

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6 Prepare transparent cages with 1.5 cm of bedding, filled water bottles, and treats.

7 Use artificial CSF (CMA Microdialysis AB, Kista, Sweden) that consists of NaCl (147 mmol/l), KCl (2.7 mmol/l), $CaCl_2$ (1.2 mmol/l) and $MgCl_2$ (0.85 mmol/l). Mix the artificial CSF with 4 % BSA (30 % in saline; Sigma-Aldrich, Saint-Louis, MO, USA) to increase osmotic pressure of the perfusate to allow for better recovery of proteins. Filter the solution after adding BSA. Make this solution fresh prior to each sampling session. Load artificial CSF inside a gastight microsyringe (CMA Microdialysis AB, Kista, Sweden), and place the microsyringe into the syringe pump (CMA 4004).

8 Calculate the 'dead volume' of the FEP outlet tubing (1.2mL/100mm). Use 100 cm of FEP outlet tubing, and thereby the first **12 µL** of CSF sampled from each animal should be discarded.

9 Run the syringe and peristaltic pump at **60 µL** /min to fill the entire system with artificial CSF + BSA solution.

In vivo Microdialysis

10 Restrain mice in order to remove the dummy probe of the guide cannula and replace it with the microdialysis probe. Prior to inserting the microdialysis probes into the guide cannula, condition the probe in 75% ethanol for better recovery of analytes.

11 Set the flow rate on the syringe and peristaltic pump to **0.2 µL** /min for **01:12:00** (1h 12m).

After the experiment

12 At the conclusion of microdialyte sampling, centrifuge **100 rpm** vials of **60 µL** CSF and keep at **-80 °C** until further analyses.

13 Discard all tubing after the experiment.

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