SOP 6b.1 Microdialysis sample collection

Setup preparation	• Replace Medicam drinking water with normal water 2 days before sampling
	• Attach swivel arm to the cage
	• Attach collection tube holder
	• Attach tether holder & tether to swivel
	• Cut the tube pieces (with scalpel – NOT SCISSORS!) in the appropriate length and number
	  o Per mouse/cage:
	  ▪ 1x 1 m for syringe-swivel
	  ▪ 1x 18 cm for swivel to probe (inflow)
	  ▪ 1x 20 cm for probe to swivel (outflow)
	  ▪ 1x 12 cm for swivel to collection tube
	  ▪ Tube timing at 1 µl/min: 10 cm per min
	• Fill syringe(s) with distilled H2O and place in pump
	• Start pump at 2 µl/min and attach the tubes in the following order, confirming the flow before each new piece: 1m piece to syringe, attach swivel, attach inflow, connect to outflow, outflow to swivel, collection tube

MiDi probe insertion (**2-person task**)	• Place probe in 1.5 ml tube containing aCSF for 10 min • Change syringe content to aCSF, change pump speed to 1 µl/min • Attach in- and outflow tubes to probe and confirm that all collection tubes have a steady and equal flow • Gather tether adapter tube and fine tweezers • Pick up mouse and hold (around the neck, gently prevent head movement), close to the cage door • Remove dummy from probe-holder and replace with probe o Carefully guide the probe in the holder, if membrane gets damaged during this step, take a new probe • Attach tether with adapter tube to the tether holder • Return mouse to cage
Sample collection setup	• Fill collection tube holder with crushed ice and place 0.5 ml eppendorf in the holder, with the collection tube attached to gather the dialysate during recovery o Depending on the targets of interest, it might be necessary to pre-load the eppendorf tube with relevant protective substance (e.g. HCl to prevent dopamine oxidation) • Fill appropriate canister with liquid nitrogen for later sample storage
Baseline	• The first 10 min baseline sample can be collected 2.5 hours after probe insertion and in a completely undisturbed environment
Intervention	• Load separate set of Hamilton syringes with treatment solution and position on pump rack • Detach tubing from aCSF syringes and attach to treatment surying • Place treatment syringes in the pump adjust the pump to new syringe size • Reverse procedure once treatment is over
Sample collection	• Outflow tubing ends in 0.5 ml eppendorf tube, which is placed on ice • Depending on flow speed and desired sample volume, replace the eppendorf tube in fix intervals, snap-freezing the previous one • Always note any differences between expected and actual volume, since this will affect the normalisation steps during sample analysis
K^+	• Same procedure as for treatment intervention, but new syringe contains aCSF

stimulation	with 50 mM K+
Post collection procedure	• Detach animal from tube system (recover probe if desired) • Switch syringe content to distilled water and connect all tubes in such a way, that dH2O is flushing and cleaning the entire system • After 1h, pump air in to the system for another 1h • Detach all the tubing and allow the system to thoroughly dry • Store swivels in boxes with rice

Equipment:

- Collection tube holder (e.g. small cup, large enough for some crushed ice, with hinge for tubes)
- Crushed ice
- Liquid nitrogen
- Artificial cerebrospinal fluid (aCSF)

Compound	Our recipe: 1L milliQ
NaCl (119mM)	6.954 g
KCl (2.5mM)	186.375 mg
MgSO4 (1.3mM)	156.52 mg
CaCl (2.5mM)	277.475 mg
NaH2PO4 (1mM)	119.98 mg
NaHCO3 (26.2mM)	2.2011 g
Glucose (11 mM)	1.9818 g
(BSA 1.5%)	(1.5 g)
pH	7.4

- Filter before use and store at +4C for <6month