

SOP 6a.1 Microdialysis Surgery

Preparation

Animal	<ul style="list-style-type: none"> • Single house surgery animals one day prior surgery in to MiDi cages
Metacam	<ul style="list-style-type: none"> • Prepare injection volume <ul style="list-style-type: none"> ◦ 12 µl Metacam + 1 ml MilliQ for 0.1 ml / 20 g mouse weight • Prepare drinking volume <ul style="list-style-type: none"> ◦ 3 drops (ca. 80 µl) per 100 ml tap water
Anaesthetic	<ul style="list-style-type: none"> • Load isoflurane into the oxygen anaesthetic system • Check that all valves are closed • Set isoflurane contribution to 5% • Open valve of large oxygen bottle • Set oxygen outflow to 0.4 l/min
Surgery tools	<ul style="list-style-type: none"> • Add ethanol to hold box of surgery tools • Place scalpel and tweezers in ethanol! don't leave tools in ethanol ! • Check razor batteries
MiDi system	<ul style="list-style-type: none"> • Insert MiDi dummy into cannula <ul style="list-style-type: none"> ◦ Widen opening of cannula with dummy ◦ Mount dummy & cannula into bracket of the stereotaxic device • Place screws into ethanol

Surgery

Anaesthesia	<ul style="list-style-type: none"> • Place mouse in isoflurane cage -> observe breathing • Remove mouse when breathing slow and no body movement • Weigh mouse
Head fixation	<ul style="list-style-type: none"> • Place respiration mask over mouse -> ensure slow and steady respiration • Fix head via ears, first left then right, control correct position with index & middle finger • Place heat plate under mouse • Open snout with tweezers and fix teeth on metal bar
Exposing skull	<ul style="list-style-type: none"> • Shave head on top of skull between ears • Apply Betadine solution in spiral motion (outward) for sterilization • Cut open skin on top of skull with scalpel in a straight line, exposing skull
Screw holes	<ul style="list-style-type: none"> • Clean & sterilize skull with EtOH to increase visibility of Bregma and improve adhesion of superglue & cement • Drill three screw holes, one in each quarter of the brain, apart from the one for the MiDi probe <ul style="list-style-type: none"> ◦ Carefully drill only through skull, not deeper and always straight ◦ Use syringe tip to break through the meninges between skull and brain
MiDi probe guide cannula	<ul style="list-style-type: none"> • Carefully manoeuvre the tip of the cannula directly on top of Bregma via micromanipulators • Subtract the target coordinates from the actual Bregma position • Move the cannula onto the new target position, mark the spot with a blue fine marker pen <ul style="list-style-type: none"> ◦ Lift the cannula (with the manipulators) and drill a straight hole



through the marked spot

Screw & probe insertion	<ul style="list-style-type: none"> Carefully screw in the anchor screws into the drilled screw holes - !! Without touching the MiDi cannula !! !! Screws should enter skull as little as possible and not touch the brain !! Carefully lower MiDi cannula just above the drilled hole Slowly lower cannula through the drilled whole, carefully observing if cannula touches any part of the skull, in which case cannula needs to be lifted again and hole adjusted to actual cannula width Once cannula passes through hole safely, lower cannula to the point where the tip just about enters the skull hole Use z-coordinates to lower cannula in correct position 																				
Cementing & Tether	<ul style="list-style-type: none"> Apply 1 drop of superglue around cannula and screws, without moving any of the objects Mix cement in small tray (for ICV: 1.5 spoons + 4 drops, MiDi: 2 spoons + 5 drops) Carefully apply cement, covering all screws and around cannula, ideally forming a cone/pyramid, without touching the face mask or the cannula holder MiDi: while cement is still mouldable: add tether just in front of MiDi cannula in 45° angle 																				
Injection & glue	<ul style="list-style-type: none"> Inject (s.c.) mouse with 100 µl of metacam solution Once cement dried, lift cannula holder (with the manipulators), while securing cement with tweezers Remove ear-holder and mouth piece ICV: insert dummy into cannula Add a thin layer of superglue onto the cement (reduces crumbling of cement) !! careful not to apply glue onto eyes !! Return animal to cage onto a fresh tissue and add a few oat flakes 																				
Common coordinates	<table border="1"> <thead> <tr> <th>Area</th> <th>X: (Bregma)±</th> <th>Y: (Bregma) ±</th> <th>Z: (top of skull) ±</th> </tr> </thead> <tbody> <tr> <td>ICV for lateral ventricle (right hemisphere)</td> <td>-0.025 cm</td> <td>-0.1 cm</td> <td>-0.15 cm</td> </tr> <tr> <td>CA1, dorsal hippocampus (right hemisphere)</td> <td>-0.2 cm</td> <td>-0.15 cm</td> <td>-0.2 cm</td> </tr> <tr> <td>PFC, right hemisphere</td> <td>+0.17 cm</td> <td>-0.05 cm</td> <td>-0.15 cm</td> </tr> <tr> <td>M1, right hemisphere</td> <td>+0.17 cm</td> <td>-0.17 cm</td> <td>-0.09 cm</td> </tr> </tbody> </table>	Area	X: (Bregma)±	Y: (Bregma) ±	Z: (top of skull) ±	ICV for lateral ventricle (right hemisphere)	-0.025 cm	-0.1 cm	-0.15 cm	CA1, dorsal hippocampus (right hemisphere)	-0.2 cm	-0.15 cm	-0.2 cm	PFC, right hemisphere	+0.17 cm	-0.05 cm	-0.15 cm	M1, right hemisphere	+0.17 cm	-0.17 cm	-0.09 cm
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Equipment:

- 70% Ethanol, spray bottle and fluid for disinfection of hands (gloves) and tools
- Dental cement: KETAC CEM EASEMIX Clinic Pack 3 x 30g Powder 3 x 12ml Liquid, ESP-56906; supplier AU: Halas Dental Limited
- Superglue
- Anchor screws, length: 2mm, cat. No 4002002 (100 pcs per pack), Microbiotech/se AB, Sweden

