

## LC SURGICAL & PHYSIOLOGICAL RECORDING PROCEDURE

### ❖ Preparations

#### ➤ Hardware

- Place absorbent drape under stereotax and surgical space
- Position stimulator & connect input & output, check manual stimulator
- Position head-stage & amplifier
- Turn on & position heating pad
- Confirm light sources are available and working
- Check hydraulic drive function

#### ➤ Computers

- Turn on recording, stereotax, & pulseOx computers
- PulseOx: set reasonable alarms:
  - Pulse Distention: no alarms
  - HR: 200-400
  - O2: 95-70
  - Breath Rate: no alarms
  - No anesthesia comparison alarms
- Stereotax: confirm both arms are reading value changes in Leica atlas program
  - If needed, connect microscope camera and open window in Infinity analyze
- Initialize Brainware
  - Check zBus for connection & v80 of drivers
  - Create data folder and files for the experiment on storage drive
  - Open Brainware
    - ◆ Open relevant channels:
      - Neural Data – Amp A – Pins 7-10

Name	Chan. #'s	Add. #'s	Filtering	Blanking	Gain	Trigger	File Type
Raw	1 & 2	3 & 4	None	None	1000	n/a	dam
Un-Blanked Spikes	5 & 6	7 & 8	300-3000	None	10000	500	src
Blanked LFP		9, 10, 11, & 12	5-300	Yes	?	n/a	dam
Blanked Spikes	13 & 14	15 & 16	300-3000	Yes	10000	500	src
Stim. Waveform	31	n/a	n/a	n/a	1	n/a	dam
Blanking Trigger	32	n/a	n/a	n/a	1	n/a	dam

#### ➤ “Bio” Data – Amp B – Pin 2-5

Name	Chan. #'s	Filtering	Blanking	Gain	Trigger	File Type
Bio	17, 18, 19, 20	None	None	?	n/a	dam
cAP	21, 22, 23, 24	300-3000	None	?	?	dam

- ◆ Check channel gains, trigger levels, and file types
- ◆ Load schedule file to scheduler & stim search to stimulus grid
- ◆ Run batch to set save path and allow to run for >5 sweeps
  - Confirm file creation in correct data folder.
  - Stop batch and delete files
- ◆ Set scheduler to Run #1 and run schedule.
  - Confirm file creation in correct data folder
  - Stop scheduler & batch, delete files
  - Reset scheduler to Run #1 in preparation for recording first site
- Instruments
  - Open autoclaved instruments and position them on the left
  - Position surgical retractors
  - Prepare cautery
  - Open weck-cells
  - Dural pick created and ready
  - Silicone oil, ink, H2O2
  - Scalpel blade on handle
  - Check & position suction
  - Install positioning probe on right stereotax arm
    - Upper clamp is for the positioning probe
    - Lower clamp is for the hydraulic drive
- Injectables
  - SR, DR, LM, Glyco, Dopram, Saline
- Cuff
  - Obtain & inspect cuff
- Electrodes
  - 2 pair with impedances ~1.5 MΩ
- Weigh & Confirm Subject ID
  - Enter subject demographics in recording app
- ❖ Initial Anesthesia Induction
  - If vitals are stable, shave head and neck.
  - Instrument rat with temperature probe for feedback controlled warming pad
  - Instrument rat with pulse oximeter
  - Administer glycol, suction mouth, extract tongue
- ❖ Implant VNS cuff & Confirm H-B Reflex
  - Position rat in right lateral recumbent/supine
    - If not already completed, shave head and neck.
  - Inject local anesthetic (LM) subcutaneously over anticipated incision.
  - Make incision with scalpel and blunt dissect fascia to expose the parotid gland.
- ❖ Obtain surgical access to the LC
  - Place silk suture loosely in mouth around lower
    - This will be used to articulate the jaw and identify the Me5

- Position rat in stereotax
  - Position symmetrically in ear bars 1<sup>st</sup> (left bar starting at 5 → 1.21)
  - Position upper incisors around the bite bar and adjust height of bar to approximately level the skull
  - Tighten snout restraint bar
- Clamp suture from mouth with hemostats so it can be used to articulate the jaw
- Inject local anesthetic (LM) subcutaneously along midline of skull
- Make incision along midline of scalp using scalpel
- Bluntly dissect scalp from skull to expose bregma and lambda as well as area posterior to lambda
  - Use H2O2 if needed with cotton applicator to delineate suture lines
- Confirm skull between bregma and lambda is level with positioning probe
  - If not level, loosen snout restraint bar and adjust bite bar height to obtain level
  - Retighten snout restraint bar & recheck level
  - Repeat as need until vertical position of bregma and lambda are within 0.1 mm.
- Locate position to drill
  - Dry the posterior skull with a cotton swap
  - Use the positioning probe and ink to mark a spot **1.1 mm lateral and 3.6 mm caudal to lambda** on the skull
- Drill hole
  - Using a small drill bit, create a pilot hole at the marked position on the skull through most but not all of the skull
  - Follow pilot hole with larger drill bit through the skull
  - If bleeding occurs, use cotton swab to achieve hemostasis
  - Remove bone shards and make sure hole is free from obstructions which would impede electrode penetration.
  - Cut through and remove dura
  - After drilling hole and achieving hemostasis place oil over exposed brain
  - Remove positioning probe

#### ❖ Electrophysiological Recording

- Attach mounting clamp to left stereotax arm and mount hydraulic drive
- Grounding & Reference
  - Install grounding needle electrode (26 ga) subcutaneously behind left ear
  - Install grounding clamps to needle electrode, microdrive, snout clamp spring, hemostats
- Install and connect electrodes
  - Red→ on left to white
  - Blue→ on right to black
- Make electrophysiological recordings
  - Zero electrodes at surface
  - Lower electrodes to depth and look for evocable activity
    - ~2000 lots of activity
    - ~4000 pop-through burst of activity
    - ~4500 lots of activity
    - ~5500 still lots of activity
    - ~5750 quieter but still firing
  - LC was reported as 5.5-6.5 mm from dural surface with this approach

❖ Wrap-Up

- Euthanize animal if needed
- Soak, wash, rinse, & lubricate instruments
- Put used surgical towels in hamper for laundering
- Dispose of biohazard trash
- Cleanup
  - Wipe down surfaces
  - Put away reusable injectables
- Turn off suction
  - Dispose of suction tip if soiled
- Charge devices
- Backup data
- Put away instruments when dry



## LC SURGICAL & PHYSIOLOGICAL MATERIALS



### ❖ Consumables

- Scalpel blade
- Suture
- Ground/Reference needle
- H2O2
- Weck-Cells
- Absorbent drape
- Cotton swabs
- Surgical gloves
- Cuff

### ❖ Confirm Ready/Available:

- Suction w/ tip
- Position marking probe w/ ink
- Silicone oil in untipped syringe
- Autoclaved surgical instruments
- Clipboard
- Electrodes