**Shape

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Description automatically generatedLC Surgical & Physiological   
Recording Procedure**

* Preparations
  + Hardware
    - Place absorbent drape under sterotax 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 1st (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

**Shape

Description automatically generated with medium confidenceLogo, company name

Description automatically generatedLC 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