# Recording and stimulating using ECoG array with PEEK film backing under Ketamine/Xylazine anaesthesia:

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**Ketamine/Xylazine specific instructions:**

Table

Description automatically generated**Note: A tick is 0.01ml, or one mark on the insulin syringe we use.**

On the top you can see dosage and concentrations of drugs we use. The units in the table are in terms of 'ticks' in our insulin syringes so e.g. 2 ticks = 0.02 ml. In practice it is hard to dose anything finer than 0.5 tick. If the mouse is v light (~20g), we’d give it 2 ket + 1 xyl ticks. If heavier (~25-30g), it can get 2.5 ket + 1.5 xyl. I’d err on the side of caution because it is easy to overdose them and then the experiment is over. The mouse will be anaesthetized very fast if it is straight after isoflurane induction or may take several minutes if done from awake. Expect the mouse to wake up after an hour after first dose. Check for responses every 10 minutes. If you get a response, readminister 1 tick of ket and see if this is sufficient.

**Experiment Goal:** **Determine whether ECoG is feasible measurement solution**

1. Can I record visual evoked potentials from an LED stimulus?
2. Investigate stim/recording temporal interference paradigm with impedance metrics.

**Experiment Pre- Prep of E-phys system:**

1. Turn on the oxygen tank for the experiment area to 15psi.
2. Turn on the heat mat, turn on the lights.
3. Check the isoflurane level and fill if needed.
4. Turn on heat mat. Turn on gas canister, but not yet the motor or power up.
5. Have injectable saline syringe ready.

**Experiment:**

1. Move the mouse from the surgery table to the experiment table.
2. Fit headplate into neurotar.
3. Mouse is already anesthetized via IP Ket/Xyl injection at the surgery table. Keep an eye on the timing.
4. Toe pinch.
5. Cover mouse with warming cover.
6. Turn on the oxygen 1.0, in readiness to use isoflurane should I need a back-up anaesthetic. Turn on gas canister but not motor.

**Question 1:** **Can I induce and record a visual evoked potential?**

(Do this before applying any TI signal – as this could cause shocks to the brain). 5-minute dark adaptation for the mouse. Then run vep\_test.py. Do this in darkness, with very bright white LED, positioned anterior to adjust for mouse eyes moving backwards under anaesthesia.

Run for 60 seconds. Gain on preamp = 1000.

1. Light removal test. VEP test at frequency = 1, 2, 4Hz with and without light. Use something to block the LED.

Check:

**Are the eyes open?**

**Were they well covered with eye lubricant?**

**NOTE: UNPLUG THE LED cable SO IT DOESN’T RUN DURING THE TI TESTS, however I still want the USB to be connected as this is how my marker channel works.**

**Question 2: Can I do TI stimulation and recording using ECoG? Use fg filter. The impedance adapter should be placed after the fg filter.**

**Reference TI Data(assuming a 2mm distance between stim electrodes):**

|  |
| --- |
| 0.03mA->0.5Hz  0.07mA 10Hz/100Hz  0.25mA ->2kHz  0.4mA 0>5khz  **(data from Pat, based on awake mice, Note: Xiaoqi uses higher amplitudes)**  **Note: 0.1mA is used to evoke forepaw MEP in CELL TI paper.**  **Tennant paper on forelimb MEP uses about 30 microamps, i.e. 0.03mA, and doesn’t go above 60 microamps.**  **Start with V out = 0.1V and measure current it using gradient descent.** |

**Run gradient descent to find 0.1mA or less. What is the impedance?**

Run ti\_test.py with a low amplitude and determine the impedance. i.e. 0.1V

Apply 2000Hz, 20001Hz.

1. What amplitude creates a TI induced signal? (use either gradient descent, or and determine an amplitude that evokes a response)
2. Artefact test: Is the amplitude at the measurement electrode at 1Hz, larger than the amplitude at the stimulation electrodes? (To show this, I will need very low mixing at the output of the filter attached to the function generator).

Remove all the filters. Voltage out should be set low, but visible. These are the last tests on the mouse.

1. Do carrier frequency ramp test. – this will need post processing
2. Do difference frequency ramp test. – this will need post processing.
3. Do transfer function test.

**Clean Up and Power Down:**

- put mouse into nosecone if still under ket/xyl to ensure it gets a good high dose of anaesthetic before it is terminated.

- turn isoflurane up to 3 for 2 minutes, put decapitation scissors on surgical bench.

**- Turn off the amplifier.**

- unplug the stimulation electrodes.

- Unscrew mouse from neurotar and remove to surgical bench.

- kill the mouse in 2 ways. Neck break with simultaneous head bar removal, decapitation scissors.

- remove the head bar from the mouse.

- place mouse in yellow bag.

- turn off isoflurane on experiment rig.

- Turn off the oxygen cylinder for experiment rig, letting it flow out of anaesthetic apparatus.

- Once empty, turn down PSI to 0, and then turn off oxygen on anaesthetic apparatus.

- Turn off all hardware on experiment rig.

- Lock computer.

- Put the head bar in the acetone.

- wash all surgical instruments with anti-bacterial scrub, dry on towels alongside plastic containers.

- tidy up all used items.

- Turn off all equipment on surgical bench and oxygen machine.

- Write down new weights on gas canisters on surgical bench and experiment area.

- turn off power board.

- cover area with plastic sheet.

- replace cover on faraday cage.

- put mouse in freezer in the other experiment room.

- Do a final check all systems are off.

- leave and turn off lights.

- update the internal lab spreadsheet and A-tunes to report termination of mouse with cage number.

**Desired Outcomes:**

1. Proves the ECoG works for both stimulation and recording. This will enable me to get back to Eric Glowacki with a new design for an ECoG with stim(2mm apart), smaller electrode surface area, and a larger Parylene base so that the ECoG itself covers the craniotomy. (this is an unblocker, as then I could do recovery ECoG experiments providing I sort out a good ecog sterilization method)
2. Proves TI isn’t confounded by artefacts in function generator sor measurement system?
3. Shows TI trends with the impedance.