Chronic Procedure

1. Check list to have ready the day before the surgery to Autoclave. Do not autoclave plastic!

    - 3 good forceps (for removal of dura etc.)
    - 3 junk forceps
    - Forceps, screw specialized.
    - Scissors
    - 5 Skin hemostats + 1 fine hemostat to put in EMG wires.
    - 2 Bone scrapers
    - Sterile gel foam
    - Cotton swaps
    - Scalpel blades and holder
    - 2 Razor blades
    - Drill bits, size 1/4 and 34.
    - Screws, at least 6 pieces.
    - Tap and screw driver.
    - Suture, size?
    - Air drill chuk.
    - Ear bars.
    - Cotton tipped applicators (comes in autoclaveble bags, mark turns from pink to gray when autoclaved).
    - 10 Small aluminum trays.
    - Mouth piece
    - ACSF
    - Atropin solution, 1:10 stock concentration.

2. Things to have ready (Non–autoclaveble)

- EMG–wires (Coated Tungsten 0.002" /0.0040") with 25 gauge needles in 70 % C_2H_5OH. Coating off 1-2 mm for the EMG wires and 4-6 mm for the EMG–reference wires.
- Silver ground wire in 25–gauge needle
- Alcohol, C_2H_5OH, 70 % and 100 %.
- Anestesia, Ketamine and Xylazine.
- Lidocaine and Neosporine
- Glucose solution
- 2 Pin connectors.
- Dental cement.
- Locktite super glue, 493.
- Solder, fine.
- Soldering iron, fine.
- Solder liquid.
- Fine pair of pliers.
- Fine pair of cutters.
- Thermal blanket and regulating unit.
- Heating base for post surgery.
- Shaver
- Mineral oil, for eyes.
- Oxygen mask and supply
- Small teflon tube to mount the EMG wires onto the bone – usually in left top drawer on the blue tray.
- Stimulation unit, wires and Remote device.
- ACSF pipettes
- New cage top, drinking tap and post-op papers.
- Sterile gloves. What is your size? 7 1/2 is medium.
- Sutures to close the wound.
- Good music (CDs).

3. Procedure for the Chronic surgery

- Before the surgery, clean up all surfaces on the surgery table top with alcohol, especially the area around the rat head. Also, clean surfaces where you may be going to touch with your fingers, e.g. the microscope knobs and eye pieces, chair top, micro–minipulator knobs, ear bar knobs, fume screen, soldering iron handle, steel cart handle, stimulator box and stimulator remote control.
- Turn on the HEPA-filter.
- Determine the mass of the rat.
- Get the initial anestizia dosage ready. Ketamine: $\frac{50ml}{1000grat}$, Xylazine: $\frac{15mg}{1000grat}$ in seperate syringes.
- Knock out the rat with halothane gas in glass container. Do not leave her in there for more than one minute! There is a limit of oxygen in there and it may cause brain damage to have her in there for longer time.
- Take her out from the glass container and quickly inject the anestetics. Let her recover on a tissue in the sink. Ideally, she should wake up with in 30 secs and then get unconsious again after 5-10 min, as the Ketamine/Xylazine starts to work.
- Shave off hair on top of her head. Watch the whiskers.
- Put her in the ear bars, thermo regulator, adjust mouth piece and level the head.
- Put a drop of mineral oil on each eye to keep then moist. Repeat this later in the surgery if nessesary.
- Give her initial dosage of Atropin ($\frac{0.005mg}{100grat}$) 1:10 dilution i.e. $\frac{0.13ml}{100gRat}$ and supplements every hour, $\frac{0.03ml}{100grat}$.
- Give her the oxygen mask and turn on the the hydrated oxygen.
- Now the sterile surgery starts. Have all tools in 70 % alcohol, wear sterile gloves and watch were you have your hands. If you are out of sterile gloves use non–sterile gloves and wash them in alcohol.
- Open up the skin on top of head by cutting with a sterile scalpel slowly in anterior–posterior direction. Be carefull not to get any

hair in, but in case it happens just take it out with a sterile cotten tipped applicator.

- Scrape off the connective tissue with the scalpel or a bone scraper and polish with a Q–tip and rub with Q–tip. Let it dry. If bleeding absorb with a sterile Q-tip.
- When dry, carefully put on the special low viscous lock–tite superglue (493) on the bone. This to get a better grip onto the bone and all the pores. Make sure it does not come on the tissue. Let dry for 5-10 min.

4. Mounting screws.

   - Before putting in the EMG–wires mount two screws to hold the tube where all the EMG–wires are going to go through. Put the screws symmetrically, 4 mm apart anterior to the eyes.
   - Start slowly with the 1/4 size drill, untill almost through. Optional to change to the 34 size drill. This will widen the hole and make it more circular, but be careful not to make it too wide, then the screw woun't stay in.
   - Turn the tap in the hole until it get's a grip – then make 2 complete turns or less. Do not turn it too much then it will widen the hole so the screw woun't stay in, and the screw itself can threat itself to some extend.
   - Put the screw in and turn it two complete turns, when it has caught the threat. Coat with Locktite 430.

5. Ground wire: Wrap an uncoated silver wire (e.g. 0.005") around both screws, but leave space for the plastic tube.

6. Putting in EMG–wires.

   - The pair of tungsten wires has been put in the 25–gauge needles and been soaked in alcohol overnight.
   - Slowly loosen the skin in front of the eyes by pushing a hemostat tip down opening and closing, all the way below the whisker pad.

- Put the needle in the hemostat and guide it through the hole, turn it forward through the muscles in the pad till it's 3/4 way through to the nose, then take it through the skin. Pull back the wires so they are in the pad but slightly seperated.
- The EMG–reference wires are put in on top of the nose under the skin. Just push the needle through in posterior–anterior direction under the skin till the very tip of the nose.
- Gather all the wires through the small plastic tube (about 2 mm in length), and mount the tube between the two screws with some drops of dental cement.

7. Putting in stimulation electrodes.

   - Drill very carefully where you think the right spot for stimulation is with the 1/4 size drill. When almost through peel off the bone with fine foreceps. You should be able to see the blood vessels, through the dura. Now, carefully make a cut in the dura for the electrode to go through.
   - Lower the electrode with the micromanipulator about 1000–1200 μm.
   - Test the electrode location by stimulating. If there is no response try turning up the current slightly. If there is still no response drill another whole and repeat the procedure.
   - If this is now the right spot, dry the bone with Q-tips and air–drill and fix the electrode with a couple of drops of dental cement and let dry for 10 min.
   - When dry (check with foreceps) detach the electrode holder and mount the eledtrode fully with dental cement. Don't get any dental cement on the tissue. Repeat on the other side. If possible mount a screw in the bone close by with the previous procedure and anker it with dental cement. In the end you should have around a total of 6 screws in to make the whole probe stable. Remember to wrap the ground wire around each screw, but if you forget one or to, it is not the end of the world.

8. Connect the 10–pin connector by holding it with the crocodile clip–setup and solder each wire to the pins using the fine solder and soldering iron. Before soldering put in a small drop of soldering liquid in the connector with a pair of foreceps and with help from capilary action. DO NOT OVERHEAT! and be careful not to drop any solder into the rat's eyes. Same goes for the dental cement.

9. In the EMG setup pin 9 has to be ground and pin 10 has to be EMG reference. If more than one EMG reference, solder them together in the pin 10.

10. connect the stimulation or EEG–recording electrode in a seperate connector bus than the EMG–wires.

11. When all the wires are connected mount the connector or connectors with dental cement to the existing pile of cement. Build it up so all wires are covered and the connector is well mounted, the rat may try to get it off. Make sure you leave no dental cement on the skin or the tissue.

12. Close the remaining hole by sewing together the skin with sterile sutures. Size 00 is the best. You should cut off some of the redundant skin.

13. Release her from the stereotaxic frame and spread a mixture of lidocaine and neosporine on the wound. Mix it for example in an aluminum tray.

14. Put her back in the cage on the heating pad. Change the cage top to the special chronic top and the bend drinking tab.

15. Fill out paper work, put sticker on and clean up the surgery room.

16. Turn off the HEPA filter, Oxygen, thermo–regulator, soldering iron and microscope. Do not leave the surgerical tools in the ultra–sound bath overnight.