

Example TD Drive Implant Surgery – Preparation and Surgical Protocol

This protocol provides an example for implantation of the TD drive. The procedures presented are approved by approved by the Central Commissie Dierproeven (CCD). The listed brands are the ones used in our facility (Radboud University, Netherlands). When replacing them with local alternatives, please double check the correct concentrations and usage.

NOTE: The following craniotomy coordinates were used in the study as described in the paper. Furthermore, HPC and RSC craniotomies can be connected to form one larger shaped craniotomy to facilitate the placement of both wire bundle.

- PFC: +1 ML, +3.5 AP is midpoint of craniotomy; upper left corner 0.5ML, 4.5AP (for 1x1 mm craniotomy); wire bundle protrudes 3.5mm from bottom of tube
- HPC: upper left corner is -3.8 AP and -2.5 ML; wire bundle is flushed inside the tube
- RSC: upper left corner is -5.8 AP and 1.0 ML; wire bundle protrudes 1.5mm from bottom of tube

Protocol:

1. Pre-surgery animal procedures (1-2 days in advance)
 - 1.1. Single-house the animal in preparation of single housing post-surgery
 - 1.2. Provide the rat with high protein wet food mixed with Brinta (a wheat-based instant porridge powder)
 - 1.3. One day pre-surgery, apply a drop of oral antibiotic (Baytril 5mg/kg bottle) to an O-shaped chocolate flavored breakfast cereal (Weeto) and feed it to the rat
2. Preparation of the surgery room (1 day in advance)
 - 2.1. Autoclave surgery tools; once the process is done, freeze the sterilized Metabond ceramic plate in -20°C.
 - 2.2. Check Isoflurane level and refill machine if needed
 - 2.3. Prepare Carprofen (Rimadyl, concentration 5 mg/ml) by mixing 1ml carprofen + 9 ml sterile NaCl. Dosage for injection: 0.1ml per 100gr. animal weight
 - 2.4. Prepare a mixture of 4 mg/kg lidocaine and 1 mg/kg bupivacaine in 0.9% NaCl physiological serum. For this mix 2ml NaCl + 2ml lidocaine (10mg/kg bottle) + 1ml bupivacaine (5mg/kg bottle). Dosage for injection: 0.1 ml per 100gr. animal weight.
 - 2.5. Prepare 2x large syringes with NaCl (5ml) and leave overnight in the fridge (used in case of larger bleeding)
 - 2.6. Sterilize the following items overnight in 70% alcohol: Drill bits (0.8, 0.9 and >1.0), M1x3 screws, EEG/ground wire and hooks + weights
 - 2.7. Get high-top cages with water bottles and food bowls for housing the implanted rats
3. Pre-surgery preparation (same day)
 - 3.1. Clean the whole surgical area (stereotax, table, chair) with ethanol
 - 3.2. Cover the table with surgical linings
 - 3.3. Switch on heating pad
 - 3.4. Take bags with surgical tools etc. from the autoclave
 - 3.5. Weigh the rat
 - 3.6. Pull up syringes with medication according to the weight of the rat and 2 syringes with 2ml NaCl. Place all syringes in the incubator at 37°C.
4. Surgery
 - 4.1. Switch on gas anesthesia to fill the induction chamber (O2 at a flow of 1.25l/min, N2O at a flow of 0.5l/min, 5% isoflurane)
 - 4.2. Place rat in induction chamber
 - 4.3. When the rat is immobilized, redirect gas anesthesia flow from induction chamber to stereotaxic mask and set isoflurane level to 2%.

- 4.4. Place the rat into the stereotax and attach nose cone
- 4.5. Apply lidocaine cream to the ear bars and fix the head of rat in stereotax using ear bars. Make sure that the bars are placed symmetrically in order to have the head of the rat centered in the stereotaxic holder
- 4.6. Connect heart rate monitor to the foot of the rat and insert the rectal temperature probe with the use of some vaseline
- 4.7. Inject rimadyl subcutaneous
- 4.8. Cut out an eye-sized piece from the aluminum wrapping of a scalpel blade that will act as "shades" for additional eye protection
- 4.9. Apply eye cream (Ophthosan) and cover eyes with protective "shades"
- 4.10. Shave head of the rat and apply povidone-iodine (Betadine) to clean the shaved area
- 4.11. Injection 2mL saline subcutaneous
- 4.12. Injection bupi/lido mix into the skin overlying the incision area
- 4.13. Change to sterile gloves and surgical gown
- 4.14. Using a scalpel, make an incision in the skin on the top of the head, parallel to the midline of the skull
- 4.15. Use hooks+small fishing weights to pull the skin to the side and expose the skull
- 4.16. Clean the skull using a Q-tip with saline
- 4.17. Using a dry Q-tip partially detach the muscles on the side of the skull by gently pushing the muscle downwards
- 4.18. Locate bregma and lambda, and measure the deviation between them on the dorsoventral axis. If there is a difference, adjust the placement of the head by lifting or lowering the mouth piece on the stereotax
- 4.19. Measure craniotomy coordinates (see above) and mark them with the surgical marker
- 4.20. Mark EEG screw electrodes site
- 4.21. Check if ear bars need to be adjusted (i.e., tightened)
- 4.22. Using the 0.8 or 0.9 drill bit, drill holes at the previously marked screw hole positions including a hole on each side of the skull at the place where the muscle was detached
- 4.23. Attach EEG, ground and anchor screws (approx. 3 full turns)
- 4.24. Prepare metabond and apply a small layer around the all screws to stabilize them and create a surface for the dental cement to hold on to
- 4.25. Using the >1.0 drill bit, drill craniotomies at previously marked locations
- 4.26. Apply a small amount of sterile saline to the exposed brain to keep it moist
- 4.27. Remove the dura using a fine needle with bent tip
- 4.28. Place the TD drive in the holder attached to the stereotactic arm
- 4.29. Position the TD drive above the skull, so that the ends of the guide tubes align with the craniotomies. Be careful and keep an eye on the PFC and RSC wire bundles which stick out from their respective guide tubes
- 4.30. Slowly lower the drive until the PFC and RSC wire bundle completely inside the brain and the HPC guide tubes are right above brain surface
- 4.31. Apply a small amount of Vaseline around the guide cannulas and inside the open parts of the craniotomies to protect them from dental cement.
- 4.32. Apply a first layer of dental cement around the vaseline covered guide cannulas, lower drive parts and screws in order to stabilize the drive and anchor it to the skull
- 4.33. Connect the EEG/Ground wires on the TD drive with the wires connected to the implanted EEG/Ground screws
- 4.34. Bundle the EEG/Ground wires together with a colored string and wrap the bundle as close as possible to the drive body
- 4.35. Apply a second layer of dental cement in order to cover the bundle of EEG/Ground wires
- 4.36. Carefully remove the TD drive holder from the stereotactic arm
- 4.37. Attach the two side panels to the drive

- 4.38. Apply the last layer of dental cement up to the bottom part of the side panels of the drive, in order to completely anchor the drive on the skull. Be careful that the skin does not get covered by the dental cement and make sure that the dental cement near the skin is smooth.
- 4.39. Apply sutures when needed and clean the wound around the implant using a Q-tip with saline.
- 4.40. Turn the moveable HPC wire bundles 6 full turns down and place protective cap on top of the drive
- 4.41. Injection of 2ml NaCl subcutaneous
- 4.42. Turn of the gas anesthesia and remove animal from stereotaxic holder
- 5. Post-operative care
 - 5.1. Weigh the rat. Place rat in a clean cage lined with surgery drape inside the incubator while the rat is recovering from anesthesia. Once the rat is awake and moving around place rat into homecage.
 - 5.2. Provide high protein food mixed with Brinta in small bowl, this makes it easier for the rat to eat in the homecage.
 - 5.3. Monitor the rat until it is freely moving around the cage, eats and drinks.
 - 5.4. Fill in all surgery-related information in the digital logging system of the animal facility.
 - 5.5. 24-72hrs post-surgery, inject carprofen (0.1ml per 100gr. Animal) s.c. and up to 3 days post-surgery give the rat a Weeto with Baytril.
 - 5.6. First week post-surgery: weigh rat daily and provided with high protein wet food mixed with Brinta.