A check list for monitoring stimulated dopamine release in anesthetized animals

1. Animals (keep them uniform in terms of genetics, weight and shape of the skull, and use appropriate brain atlas)

2. Testing of the stereotaxic frame (performed once)

   2.1. Squareness of the apparatus (including the most critical verification of the vertical axis move).

      2.1.1. Verification of the proper vertical movement of the stim electrode:

         2.1.1.1. Fix a straight rod (diameter of the stim electrode holding body, the length of the stim electrode) in the stereotaxic arm, using the same holder that will be used for a stim electrode.

         2.1.1.2. Position it above the plate with a hole fixed between the ear-bars (the hole diameter should be 15-20% larger than the rod diameter).

         2.1.1.3. Move the rod downward through the hole; check that the rod still moves at the centre of the hole.

         2.1.1.4. The same procedure may be used for straightening the stim electrode before implantation.

   2.2. Zeroing the apparatus in a medial-lateral (ML) direction:

      2.2.1. Join the tips of the conically shaped ear-bars exactly in the middle line (LM=0).

      2.2.2. Take a straight rod with a conical sharp tip with a diameter of the rod similar to the diameter of the stim electrode body. Fix it in the stereotaxic arm.

      2.2.3. Move it downward to the point where the ear-bars tips touch each other.

      2.2.4. Take and use the LM reading as the reference point. Your real stim electrode, when fixed in the stereotaxic arm and positioned at the reference point, must go to the same point above the ear-bar tips.

3. Fixation of the skull in the stereotaxic frame.

      3.1.1. Fix the skull between the ear-bars – first, right ear (ear-bar is tightened at the position), after that the left ear, by moving another bar into the ear and tightening that bar. Note that proper positioning of the conical ear-bars (for acute experiments in rats) will leads to penetration of the timpanic membrane, often associated with pock sound.

3.1.2. Ensure that the ear-bar tips are positioned symmetrically relative to the middle line. In the future, if the animals in use are the same size, you will not need to adjust the right ear-bar at all.

3.1.3. Verify that the skull moves strictly vertically up and down around the ear-bars axis.

3.1.4. Fix the nose so that it does not move (sway the nose up and down during tightening of the nose clamp).

4. Trepanation.

4.1.1. Expose the skull. Remember, that the skull should most of the time be wet except for a few minutes during mark-up and before dipping the electrodes. The latter helps you find the reference plan (if the skull is the reference level) for calculation of the depth of the electrodes.

4.1.2. Put the straight sharp tip rod in the electrode holder and verify that first the lambda and the bregma, and secondly, two symmetrical points 3 mm from the middle line are on the same level.

4.1.3. Alternatively, use an Invilog bubble level probe for levelling the skull.

4.1.4. Mark the skull with a sharp soft pencil showing the coordinates for the working and stim electrodes. The mark for the stim electrode is a line about 3 mm perpendicular to the midline suture on the level of the lateral hypothalamus (for MFB stimulation).

4.1.5. Drill the skull at the marked places.

4.1.6. If you have any doubt about the position of the skull in the frame after the drilling, measure the horizontality of the skull surface again. Use the Invilog bubble level probe for fast and precision verification.

4.1.7. Penetrate the dura at the working electrode trepanation hole with a needle. Stop bleeding.

4.1.8. Penetrate the dura along the trepanation line for the stim electrode. Cut the dura with pointed knife. Stop the bleeding.

5. Electrode implantation.

5.1. Put the reference electrode on the skull.

5.2. Take a new CF electrode (second use of the working electrode is permitted only for special conditions e.g. the same day experiment; the CF electrode kept wet between use) and mechanical treatment (for 32 um CF) with the following calibration and **determination of the time-response**).

5.3. Insert the working electrode in place. Slow down the dipping 0.5 mm above the destination site.

5.3.1. Ensure that background current via the CF electrode is the same in shape and amplitude as it is during the calibration of this or other electrodes.

5.4. Position the arm with straightened stim electrode (see para. 2.1.1) at the coordinates of the midline. Move the tip of the stim electrode closer to the skull (**do not touch the skull with the tip**). Ensure that the tip is above the midline.

5.5. Pull back the stim electrode and move it above the stim site (above the knife cut in the dura; the AP location should be precise, or, otherwise the electrode will bend).

5.6. Start stimulation when the tip of the electrode is 7.0 mm (for rats) below the skull. The position of the tip can be lower after several successful surgeries. Use 1 s 50 Hz stimulation. Protrude the electrode 0.3 mm, wait 1 min and stimulate again.

5.7. The lower the current that produces dopamine release, the better you placed the tip of the stim electrode. While behavioural reaction for the rewarding MFB stimulation may start from 30-50 µA in freely moving animals, 150-250 µA current (1 ms pulse) is required to evoke measurable dopamine release in anaesthetized animals. Sometimes, in a critical experiment, the current on the stim electrode can be increased to 500-600 µA. However, this current will damage the tissue around the tip.