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Protocol extension

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# Opto-juxtacellular interrogation of neural circuits in freely moving mice

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# Supplementary Information

## Opto-juxtacellular interrogation of neural circuits in freely-moving mice

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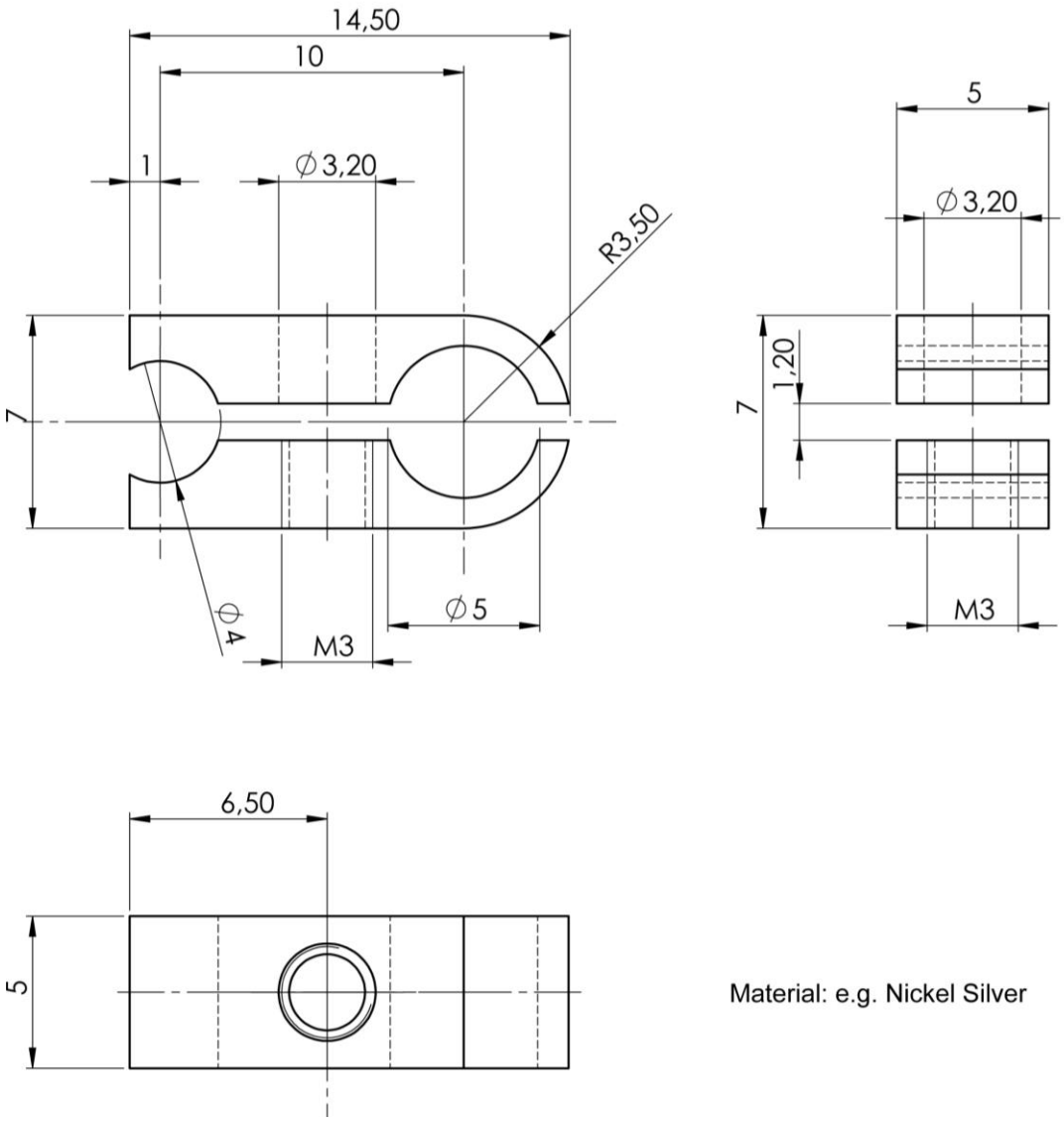
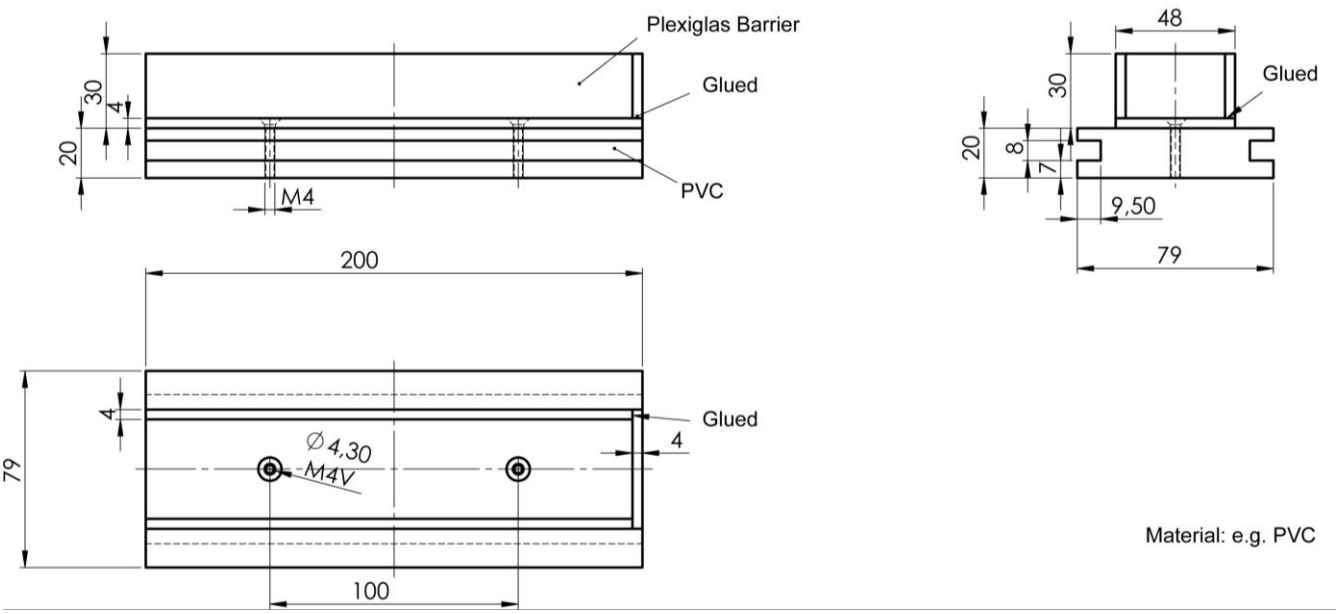
## SUPPLEMENTARY FIGURES

### Supplementary Figure 1



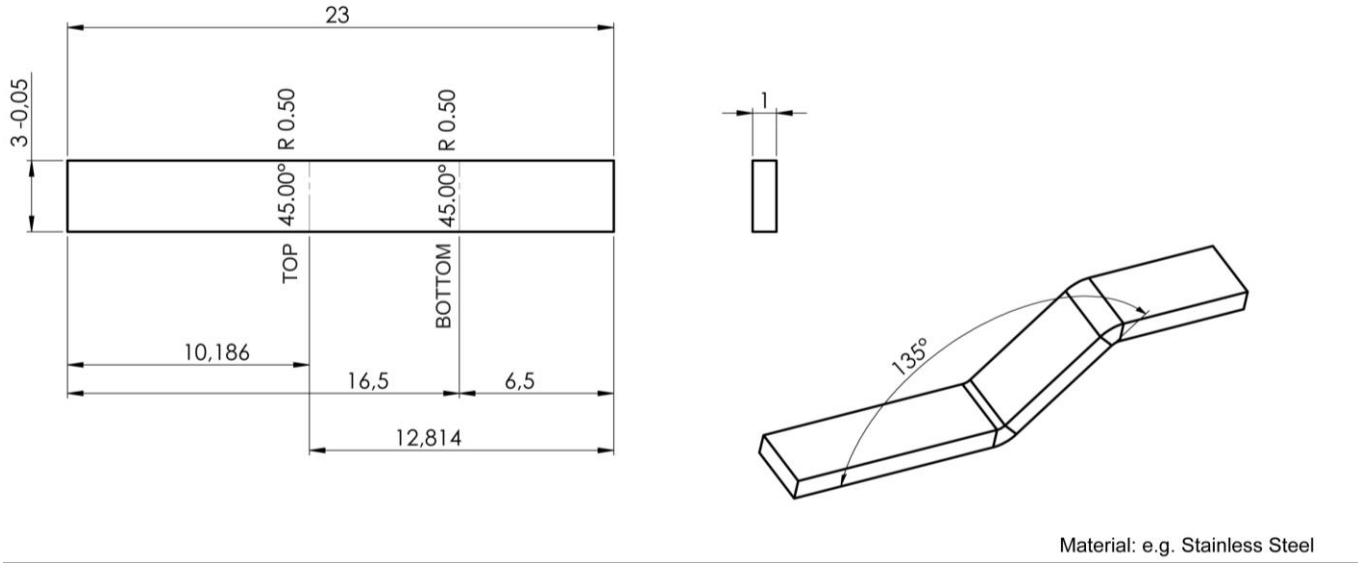

**Supplementary Figure 1. Technical drawing of the Adaptor Piece** (see 'Equipment Setup').

**Supplementary Figure 2**



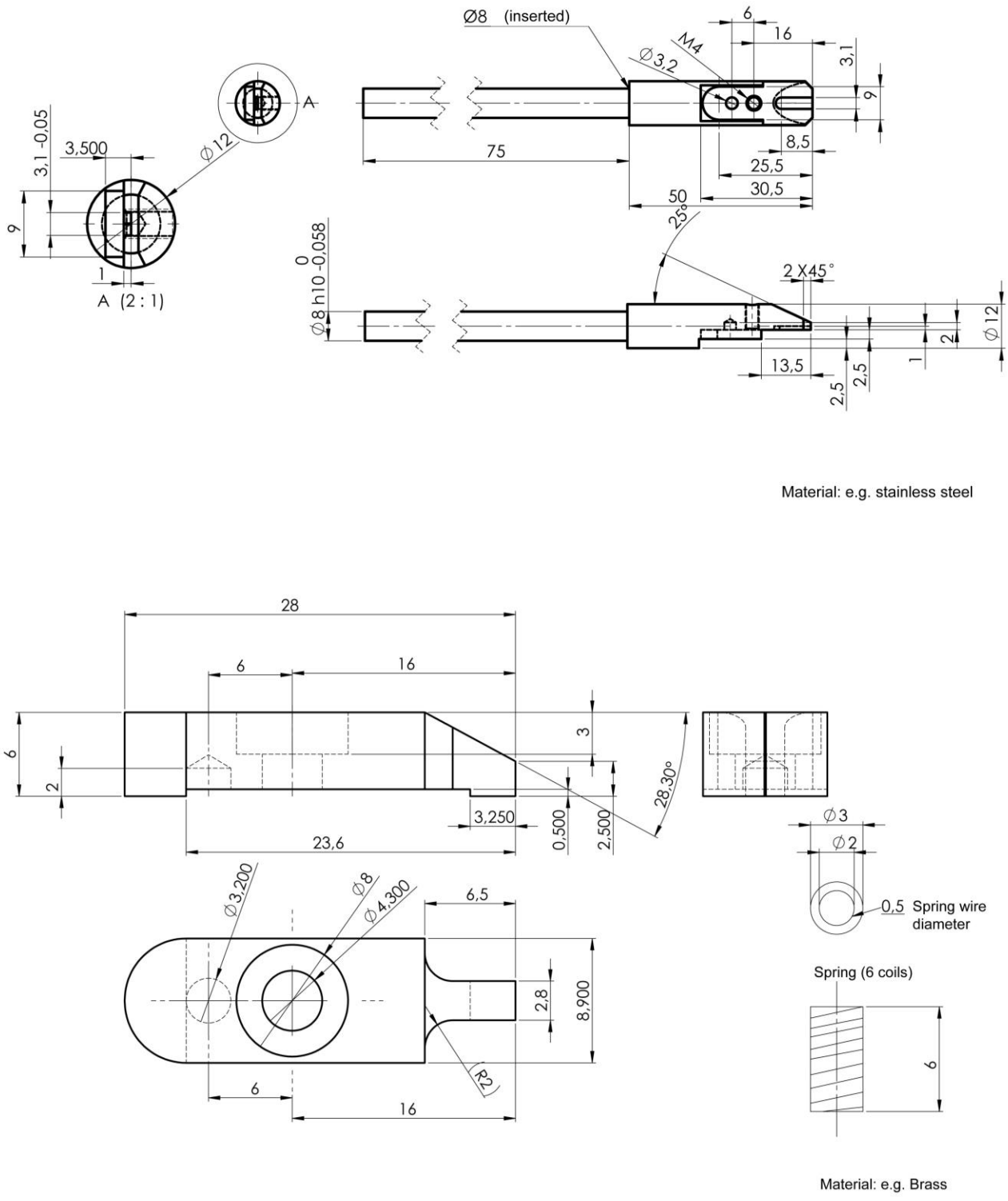

**Supplementary Figure 2. Technical drawing of the Mouse Box** (see 'Equipment Setup').

**Supplementary Figure 3**



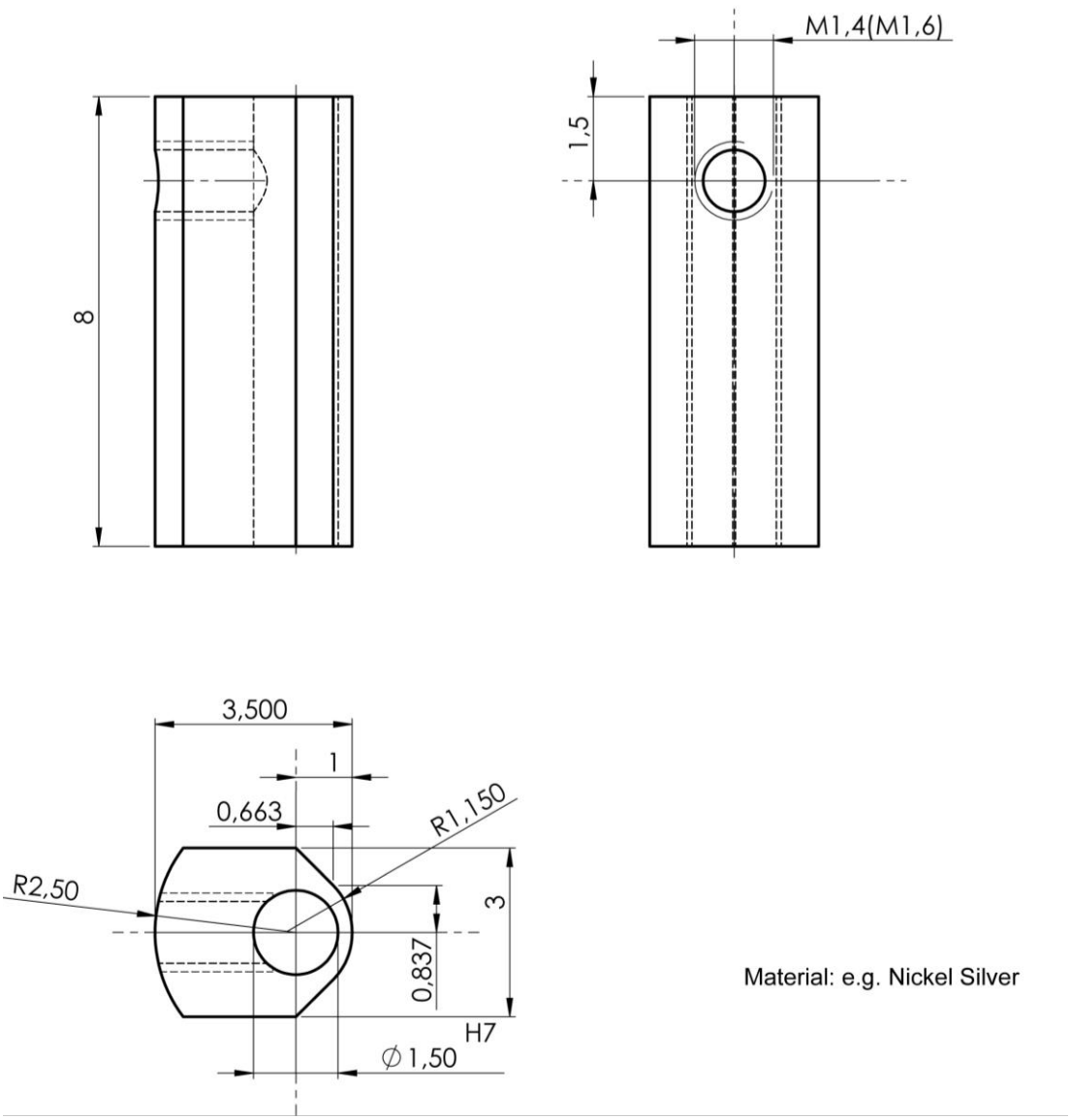

**Supplementary Figure 3. Technical drawing of the Head-Post** (see 'Equipment Setup').

**Supplementary Figure 4**



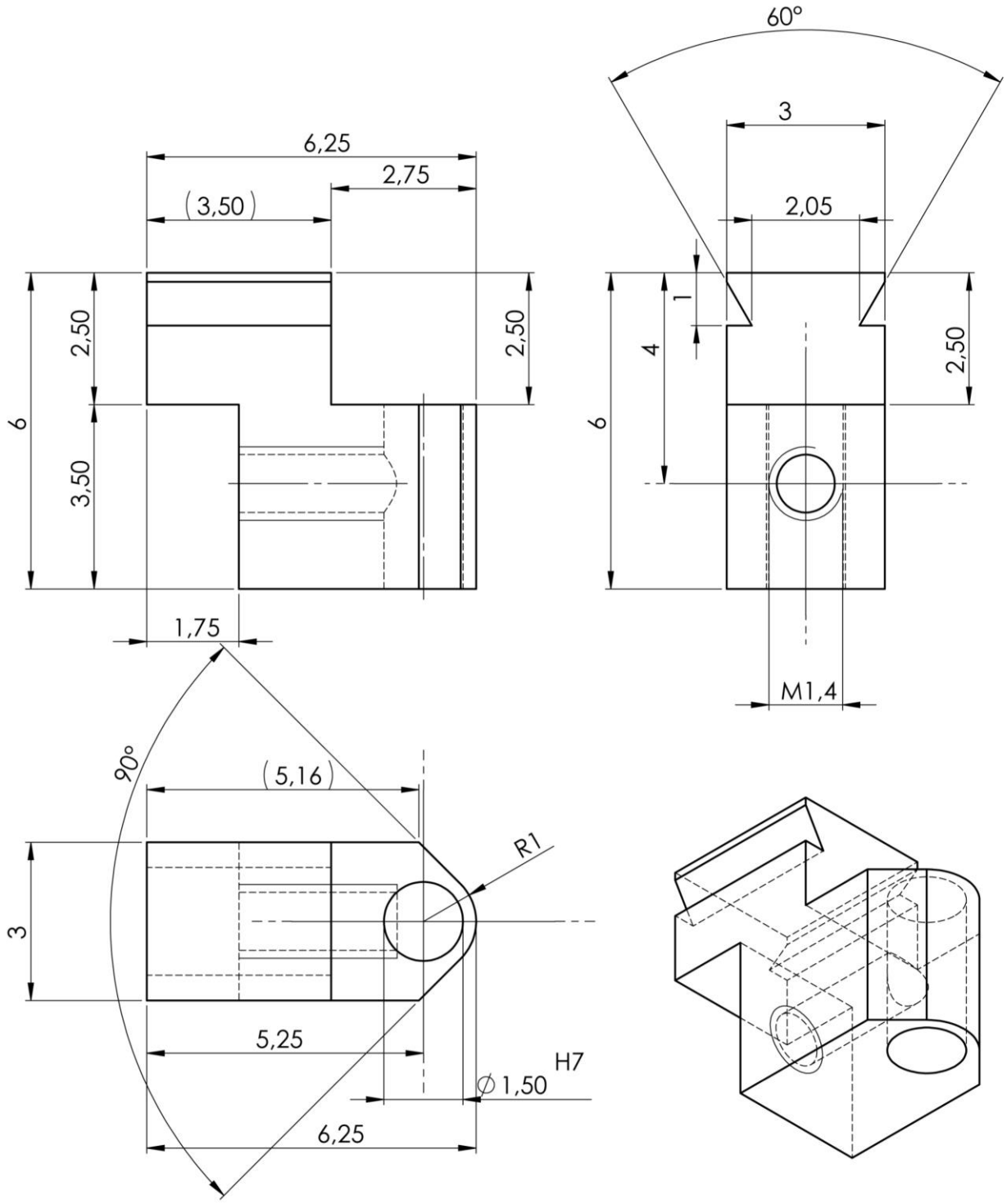

**Supplementary Figure 4. Technical drawing of the Head-Post Holder** (Top, Part_1; bottom, Part_2) (see 'Equipment Setup').

**Supplementary Figure 5**

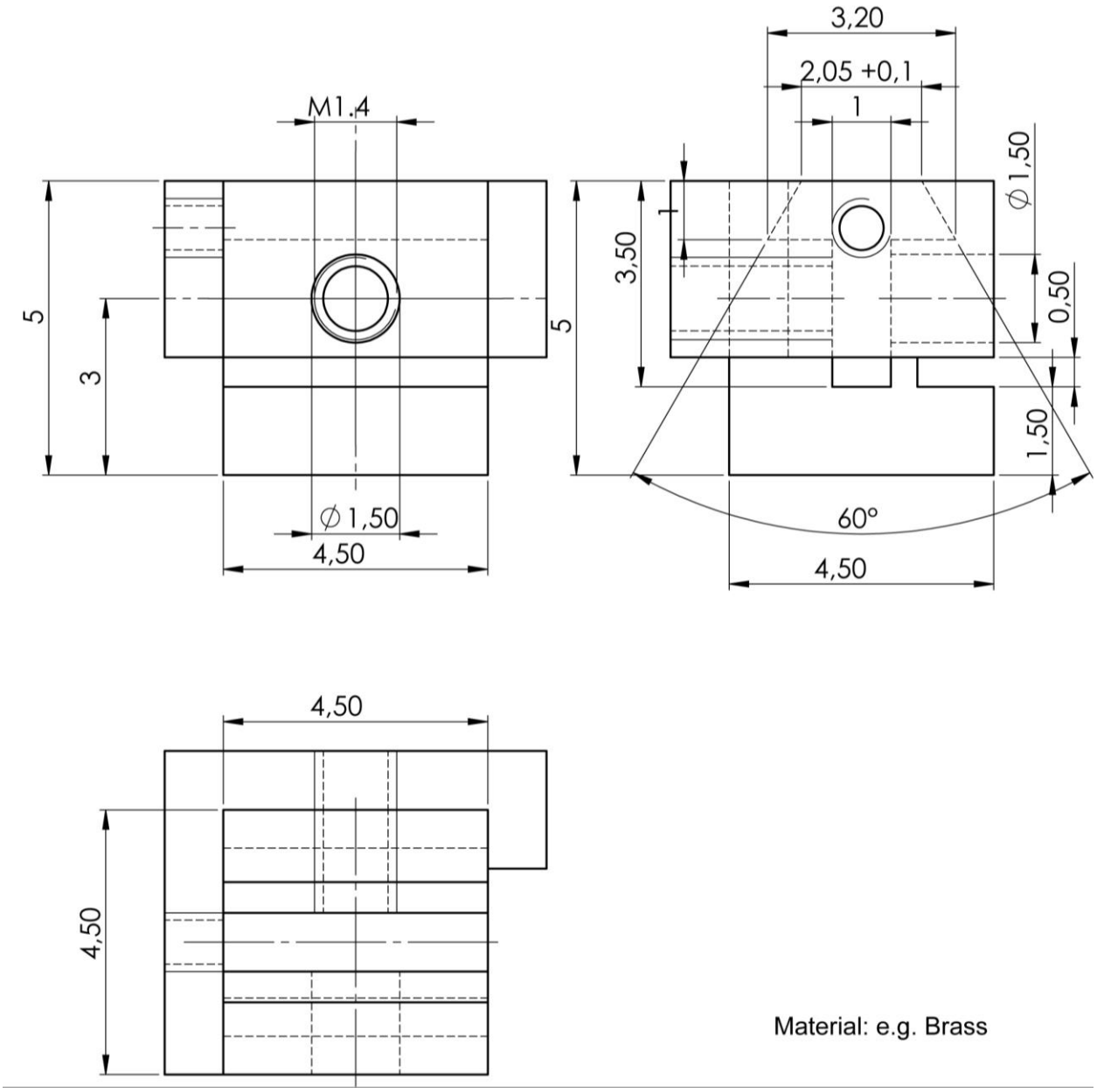



**Supplementary Figure 5. Technical drawing of the Manipulator Base** (see ‘Equipment Setup’).

**Supplementary Figure 6**




Material: e.g. Brass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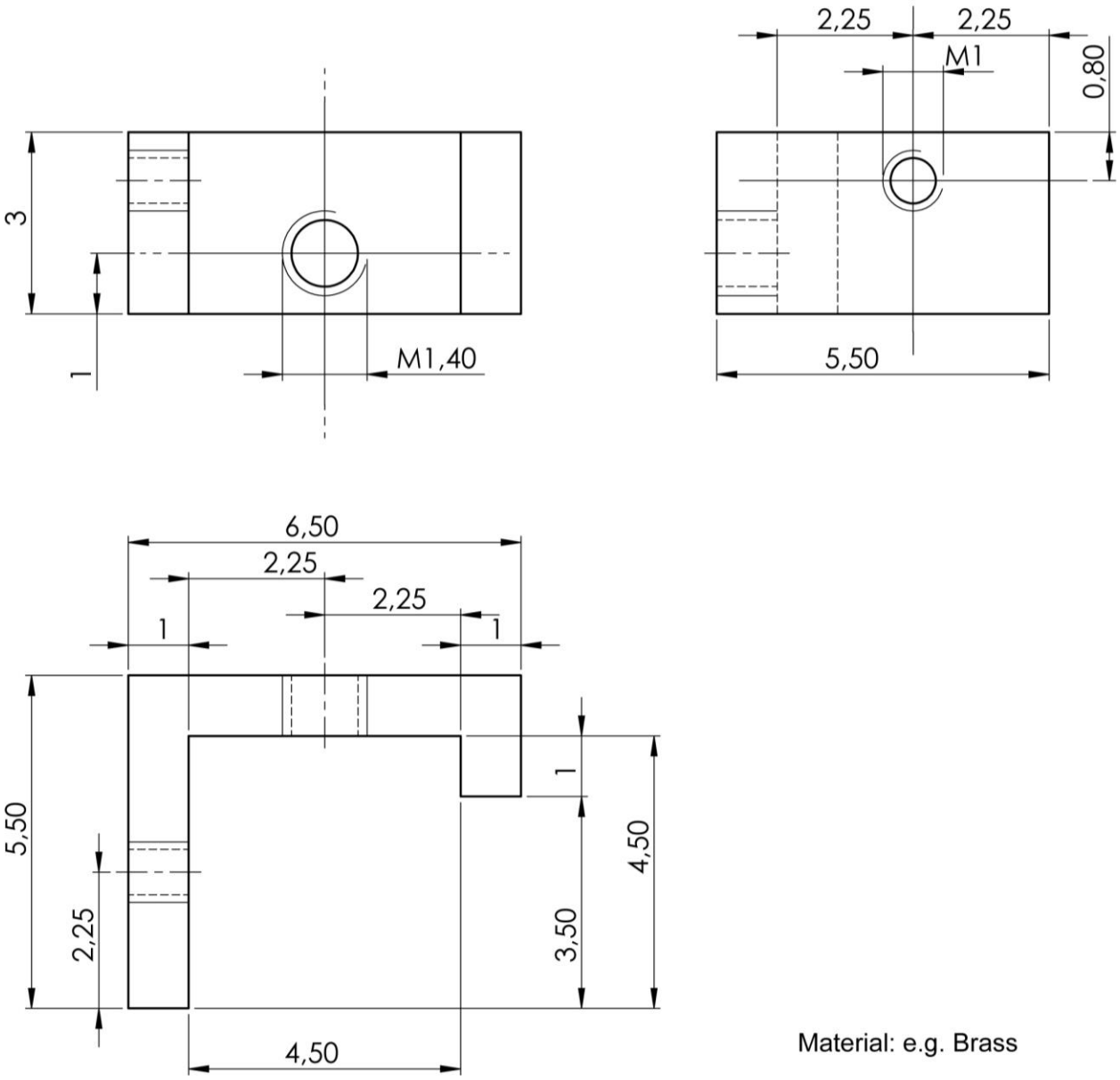
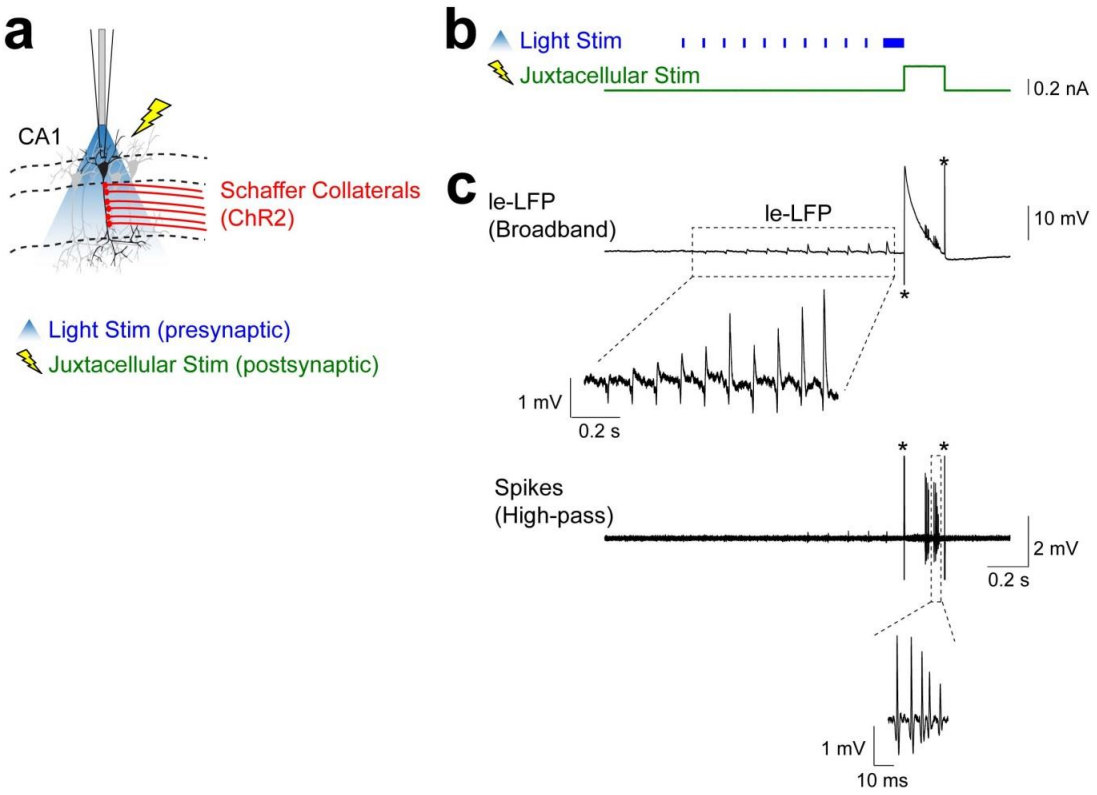
Material: e.g. Brass



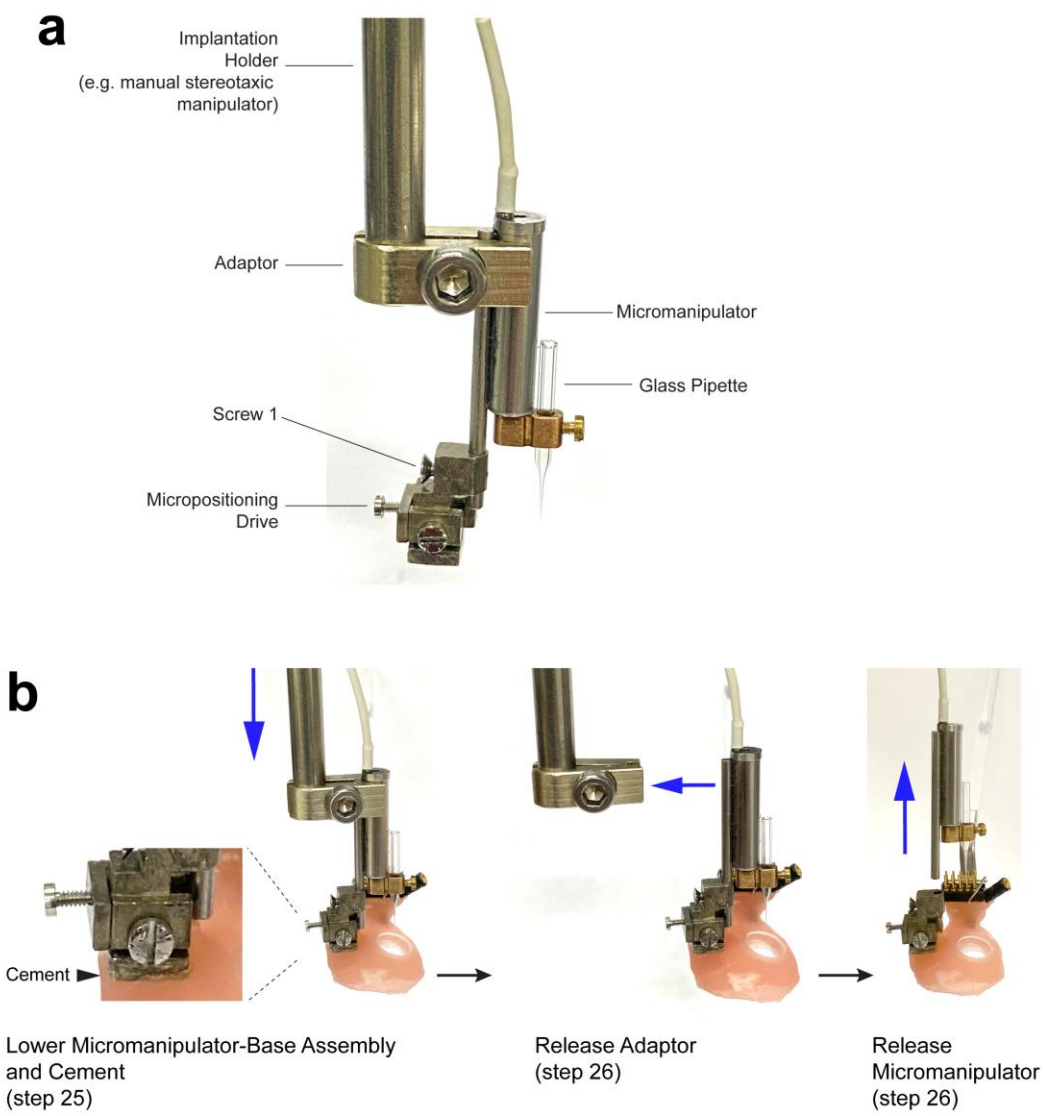

**Supplementary Figure 6. Technical drawing of the Micropositioning Drive** (parts 1 to 3) (see ‘Equipment Setup’).

**Supplementary Figure 7**



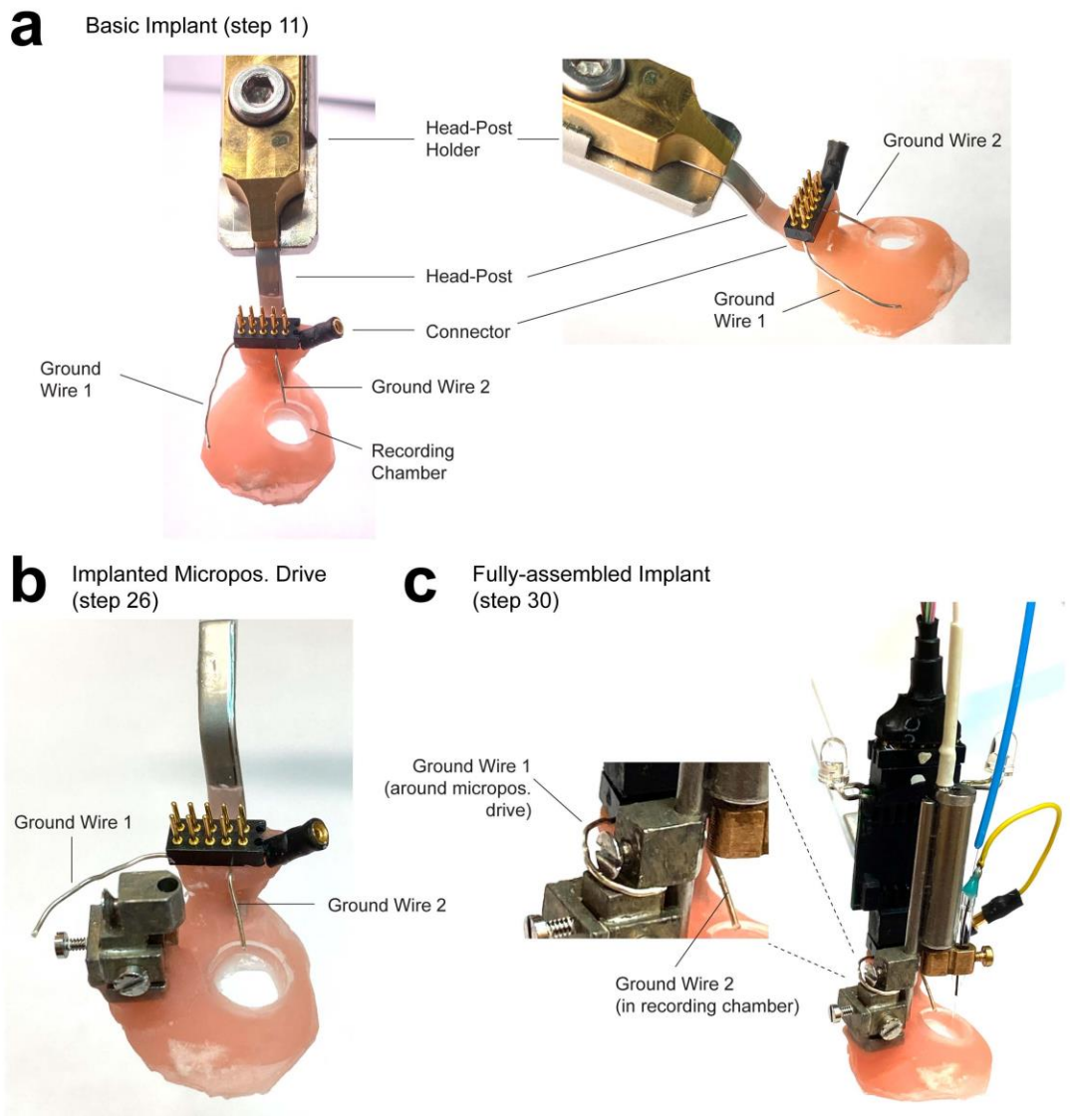

**Supplementary Figure 7. Pre-Post pairing via optojuxtacelluar stimulation.**
**(a)** Schematic representation of the experimental configuration of the pairing protocol. ChR2 is expressed in CA3 pyramidal neurons, thereby labelling Schaffer Collateral inputs to the CA1. An optojuxtacellular recording is obtained from a CA1 pyramidal neuron. Presynaptic stimulation can be achieved by photo-activation of ChR2-positive Schaffer Collaterals, and paired to postsynaptic activation via juxtacellular single-cell stimulation (see ref.[1-3]).
**(b)** Representative stimulation protocol showing repetitive light-activation (blue) of Schaffer Collateral inputs (e.g. 5 ms pulses, ~5 mW power at the fiber tip), used to induce synaptic facilitation (see (c) below). The last light-pulse (100 ms) is then paired to postsynaptic current injection into the CA1 pyramidal neuron (200 ms current pulse, typically 5-10 nA).
**(c)** Representative juxtacellular voltage trace from a CA1 pyramidal neuron during the pre-post pairing protocol (as shown in (b)). Top, broad-band voltage trace, showing the facilitation of the le-LFP response to light activation of Schaffer collaterals (high-magnification inset). Note the positive polarity of the le-LFP response, which reflects the current source within the recording site (pyramidal layer) coupled to the ChR2-mediated current sink in the stratum radiatum (see (a)). Bottom, high-pass filtered trace showing the spiking activity of the recorded CA1 neuron in response to juxtacellular current injection. The high-magnification inset shows the occurrence of burst-firing, in response to current stimulation.

**Supplementary Figure 8**



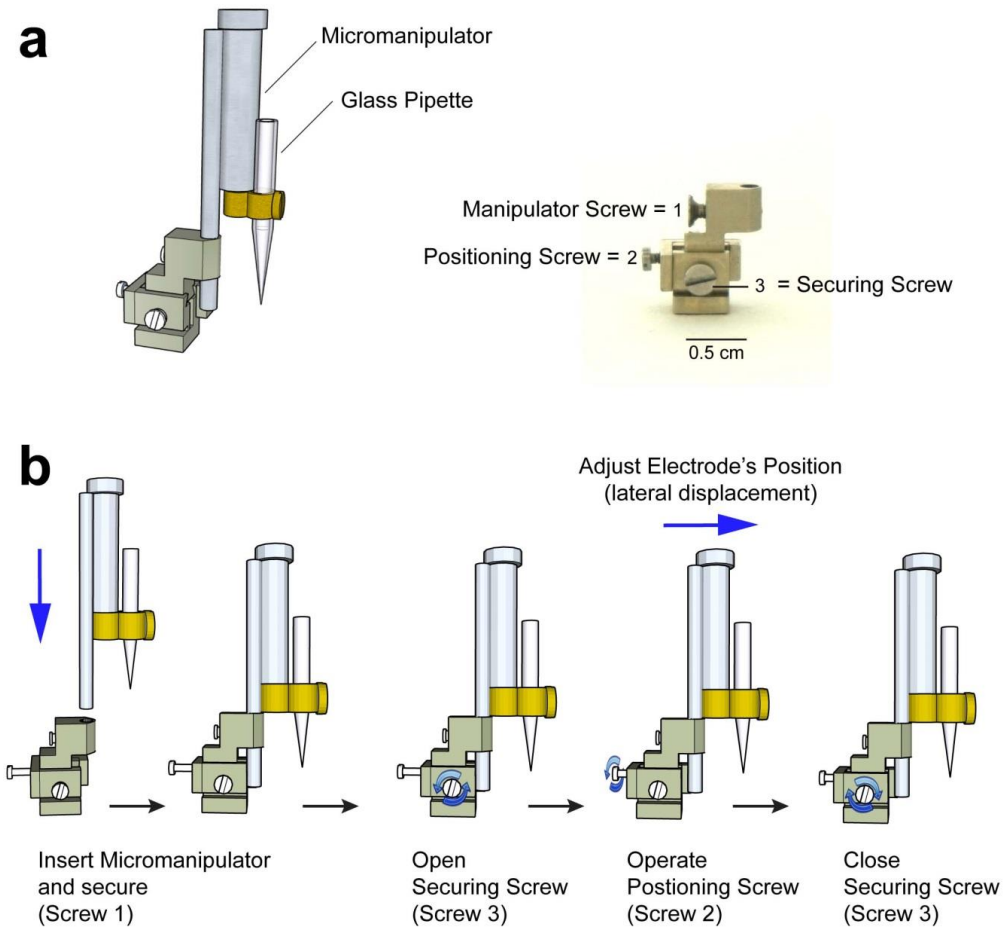

**Supplementary Figure 8. Implantation of the micropositioning drive.**
(**a**) Picture showing the micromanipulator-base assembly for implantation of the micropositioning drive. A sham pipette is inserted in the micromanipulator. The latter is then mounted on the micropositioning drive. The micromanipulator-base assembly is then secured to a manual stereotaxic manipulator by means of an adaptor. These components are indicated in the picture.
(**b**) Pictures showing details of the micropositioning drive implantation (steps 25 and 26). From left to right: the micromanipulator-base assembly (as shown in (a)) is advanced to the desired position by means of a manual stereotaxic manipulator, and the base of the micropositioning drive is cemented onto the basic implant ('Lower Micromanipulator-base Assembly and Cement', step 25). After the cement is hardened, the micromanipulator-base assembly is released by unscrewing the adaptor piece ('Release Adaptor', step 26). Last, the micromanipulator is released from the micropositioning drive by unscrewing the Manipulator Screw (Screw 1) ('Release Micromanipulator', Step 26).

**Supplementary Figure 9**




**Supplementary Figure 9. Implant assembly and ground wiring.**
(**a**) Front view (left) and side view (right) of the basic implant secured onto the head-post holder. The two ground wires (#1 and #2) are indicated.
(**b**) Image showing the two ground wires after cementing the micropositioning drive.
(**c**) Image showing the placement of the two ground wires in the fully-assembled implant. Ground wire 1 is placed around the micropositioning drive, while ground wire 2 is placed within the recording chamber. During recording, the chamber is filled with agarose solution (step 32). An agarose bridge can be used for improving the electrical contact between the ground wire #1 and the micropositioning drive (see Troubleshooting table).

**Supplementary Figure 10**




**Supplementary Figure 10. Operation of the micropositioning drive**
(**a**) Left, 3D schematic representation of the micropositioning drive/micromanipulator assembly for obtaining juxtacellular recordings in freely moving mice. Right, side view of the micropositioning drive. The three screws for the operation of the micropositioning drive are indicated. Adapted from ref.[4]
(**b**) Steps for operating the micropositioning drive. From left to right: first, insert the micromanipulator in the dedicated hole of the drive and secure it by operating Screw 1. Then, loosen the Securing Screw (Screw 3), which enables the lateral adjustment of the pipette position by means of the Positioning Screw (Screw 2). Once the desired position is reached, tighten the Securing Screw (Screw 3).

## References


1 Diamantaki, M. et al. Manipulating Hippocampal Place Cell Activity by Single-Cell Stimulation in Freely Moving Mice. Cell Rep 23, 32-38 (2018). https://doi.org:10.1016/j.celrep.2018.03.031

2 Coletta, S., Frey, M., Nasr, K., Preston-Ferrer, P. & Burgalossi, A. Testing the Efficacy of Single-Cell Stimulation in Biasing Presubicular Head Direction Activity. J Neurosci 38, 3287-3302 (2018). https://doi.org:10.1523/JNEUROSCI.1814-17.2018

3 Diamantaki, M., Frey, M., Preston-Ferrer, P. & Burgalossi, A. Priming Spatial Activity by Single-Cell Stimulation in the Dentate Gyrus of Freely Moving Rats. Curr Biol 26, 536-541 (2016). https://doi.org:10.1016/j.cub.2015.12.053

4 Ding, L. et al. Juxtacellular opto-tagging of hippocampal CA1 neurons in freely moving mice. Elife 11 (2022). https://doi.org:10.7554/eLife.71720