KOPF CARRIER

September 2019

Kopf Carrier #96

PUBLISHED BY DAVID KOPF INSTRUMENTS ■ TUJUNGA, CALIFORNIA

A customizable procedure for angled stereotaxic implantation and microinjection in the rodent brain

Chelsea L. Faber; Miles E. Matsen; Thomas H. Meek, PhD;
Jordan E. Krull; Gregory J. Morton, PhD
Department of Medicine, University of Washington, Seattle, WA.
Correspondence: kasperc@uw.edu

Introduction

Stereotaxic targeting of the rodent brain, a staple procedure in the modern neuroscience lab, can be complex and challenging. Targeting structures near the sagittal midline is often problematic given their position below the superior sagittal sinus, puncturing of which may elicit excessive bleeding. When targeting deep brain structures along the midline (e.g. mediobasal hypothalamus), an added challenge of avoiding the third ventricle is present. Moreover, increasingly sophisticated neuroscience techniques (e.g. optogenetics, fiber photometry) require the implantation of significant hardware to the brain, and space constraints are a common problem to stereotaxic placement.

Here we present a customizable protocol for targeting brain structures using an angled stereotaxic approach. As a representative example, we use stereotaxic coordinates for targeting the mouse hypothalamic arcuate nucleus, however, this protocol is suitable for either mouse or rat, and can be modified for diverse experimental approaches and to target other brain regions.

Before beginning, note that successful targeting requires multiple iterations of validation (e.g. using dye, fluorescent indicator) to account for animal-to-animal differences in assigning bregma, leveling, etc. It is therefore recommended that each surgeon refine his/her own technique across multiple trial animals to improve individual precision.

Finally, note that this procedure is based on the use of the Kopf Model 1900 stereotaxic system, which allows rotation of the head holder apparatus. The use of other models (e.g. Model 1430) is not suitable for this technique.

Equipment

Kopf Model 1900 Stereotaxic Alignment System including:
- Model 1940 Micro Manipulator
- Model 1915 Centering Scope*
- Model 1905 Alignment Indicator
- Model 1900-51 Center Height Gauge
- Model 1923-B Mouse Gas Anesthesia Head Holder
- Model 1922 60 degree Non-Rupture Ear Bars

* Use of the centering scope greatly improves targeting precision. However, assigning bregma and aligning the center of rotation can be achieved with the use of a cannula/electrode tip if your lab does not have the centering scope. Please contact the authors for suggestions on this modified procedure.

I. Calculate Angled Coordinates

1. On a coronal diagram of the brain, draw a right triangle such that the hypotenuse of the triangle passes through your target region. An example is shown in Figure 1, wherein we target the arcuate nucleus (ARC) at a 10° angle from the coronal midline.

    a. It is recommended not to exceed a coronal rotation angle of 15°; although the apparatus can rotate up to 30°, the screw of the Anterior-Posterior Shift knob often prohibits rotation beyond 15° (see Figure 2).

    b. Note that the position of the axis of rotation denoted in Figure 1 is arbitrary, and can be altered to suit your target region. While this may seem counterintuitive, later steps in the surgical procedure will adjust the head position in the Z axis such that this arbitrary point lines up with the stereotaxic center of rotation. See **Section V** for details on aligning the center of rotation.

2. Once you have established your desired angle, **a**, and the estimated length of side **B**, use basic trigonometry to calculate the length of side **A**. This length will be important for aligning the center of rotation properly.

    a. In the example in Figure 1, we wanted to target the ARC at a degree of 10 from center. Based on the gridlines from the reference atlas, we estimated the length of side B to be 7.576mm. Using this information, we can calculate the length of side A:

$$\tan(10°) = \left(\frac{A}{B}\right) = \frac{A}{7.576mm}$$

$$A = \tan(10°) * 7.576 = 1.336mm$$

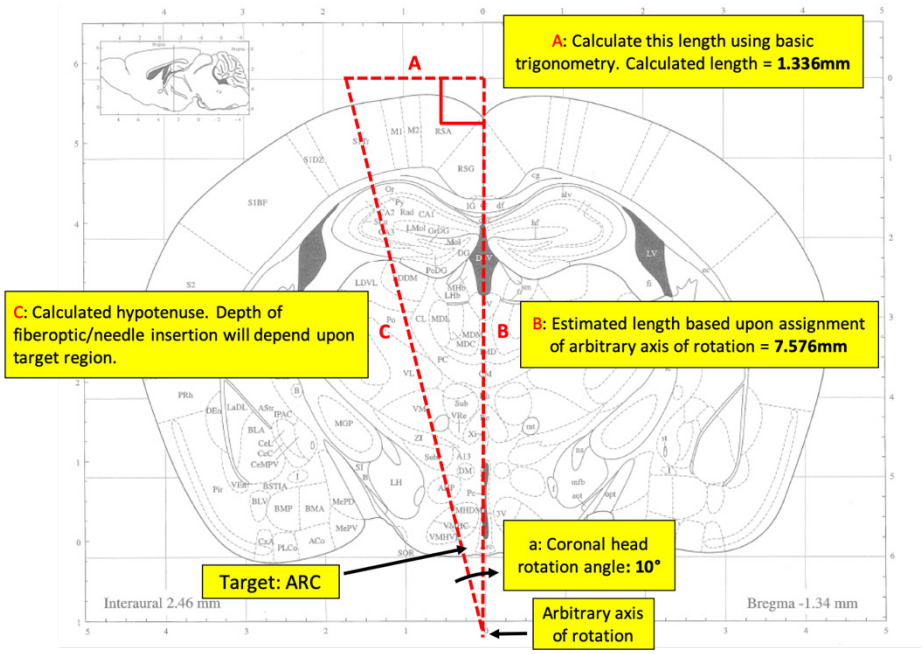

Figure 1. Calculating angled coordinates targeting the arcuate nucleus (angles and line segments not drawn to scale).

In this example, 1.336mm indicates the distance from the midline where the cannula/needle tip will enter the brain, when the head is rotated 10°.

II. Set-Up

1. Have all surgical materials, supplies and equipment sterilized prior to performing surgery.
2. Before anesthetizing animal, ensure stereotaxic frame and manipulator have been properly swept in and calibrated (see Kopf Manual for full protocol).
3. Since we will be rotating the animal's head about the ear bars in the interaural plane, before beginning we want to ensure that the ear bars themselves are in alignment with the head holder's axes of rotation. To do this, first place the Center Height Gauge into the socket of the head holder base.
4. Next place the Centering Scope in the tool holder. Sight down the scope and use the knobs on the micromanipulator to align the crosshairs in focus on the gauge. At this stage, the scope is being positioned into the focal plane of the head holder axis of rotation.
5. Next place the ear bars into the holders and center them such that the indicator lines on both sides read 0.
6. Taking care not to adjust the Z position of the centering scope, use the Medial-Lateral and Anterior-Posterior knobs on the head holder (Figure 2) to line up the ear bars so that they are centered

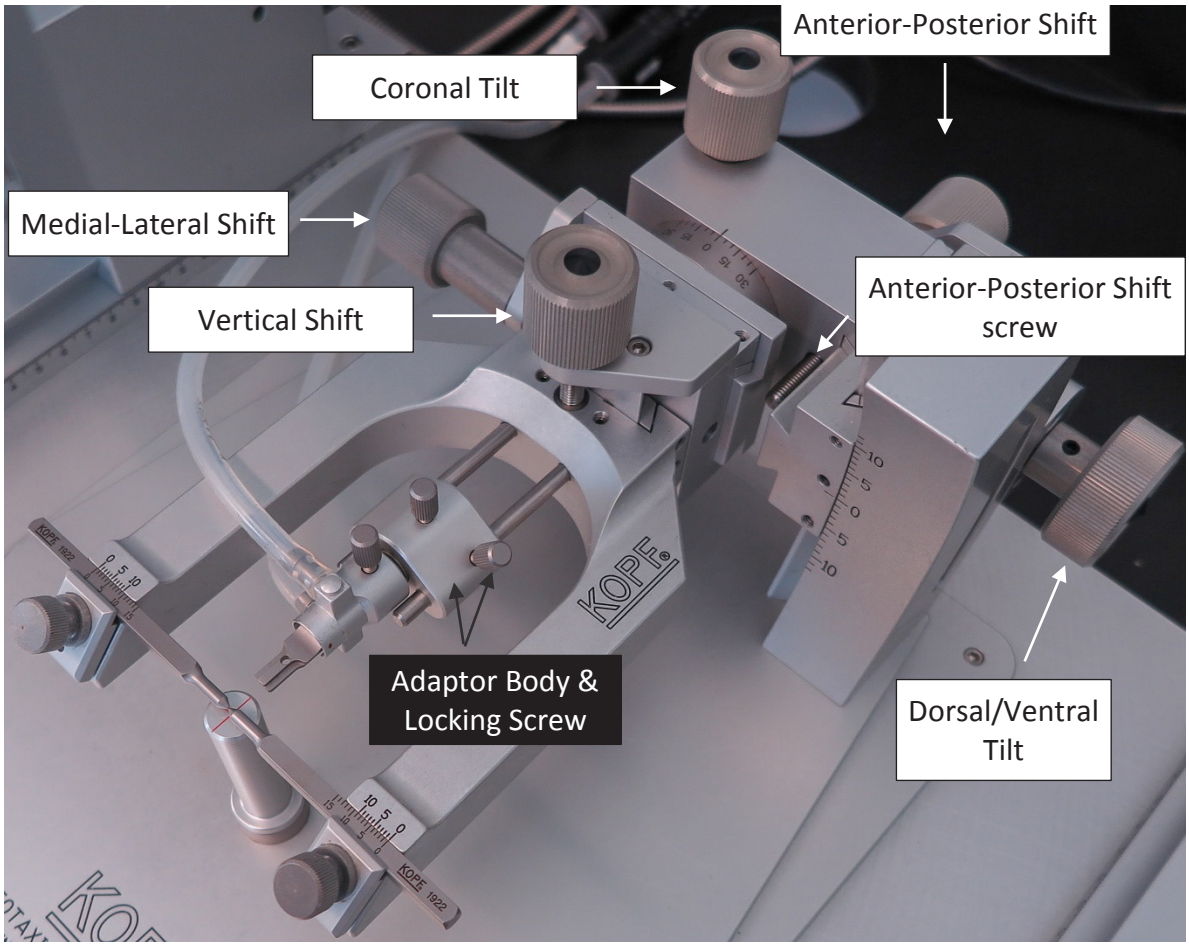

Figure 2. Adjustment knobs for the head holder apparatus.

in X and Y axes just above the crosshair of the centering height gauge (Figure 3A).

7. Now we need to align the ear bar position in the Z axis. To do this, remove the ear bars from the holder to allow removal of the centering height gauge. Replace the ear bars and again center them at 0.

8. Sighting down the scope, use the Vertical Shift knob to lower the ear bars until they come into focus. To confirm the ear bars are aligned properly, use the Coronal Tilt knob to rotate the ear bars while sighting down the scope. Continue to adjust the Z axis with the Vertical Shift knob until the crosshairs remain centered – when properly calibrated the crosshairs on the scope should remain centered throughout coronal tilt (Figure 3B and 3C).

9. The stereotax is now calibrated and ready. **Do NOT make any further adjustments to the head holder knobs.**

III. Anesthesia

1. Record mouse's pre-operative body weight.

2. Perform anesthesia (e.g. ketamine/xylazine injection or continuous isoflurane) and provide analgesia according to your approved laboratory IACUC protocols.

3. Prepare animal for stereotaxic surgery and shave the scalp from just behind

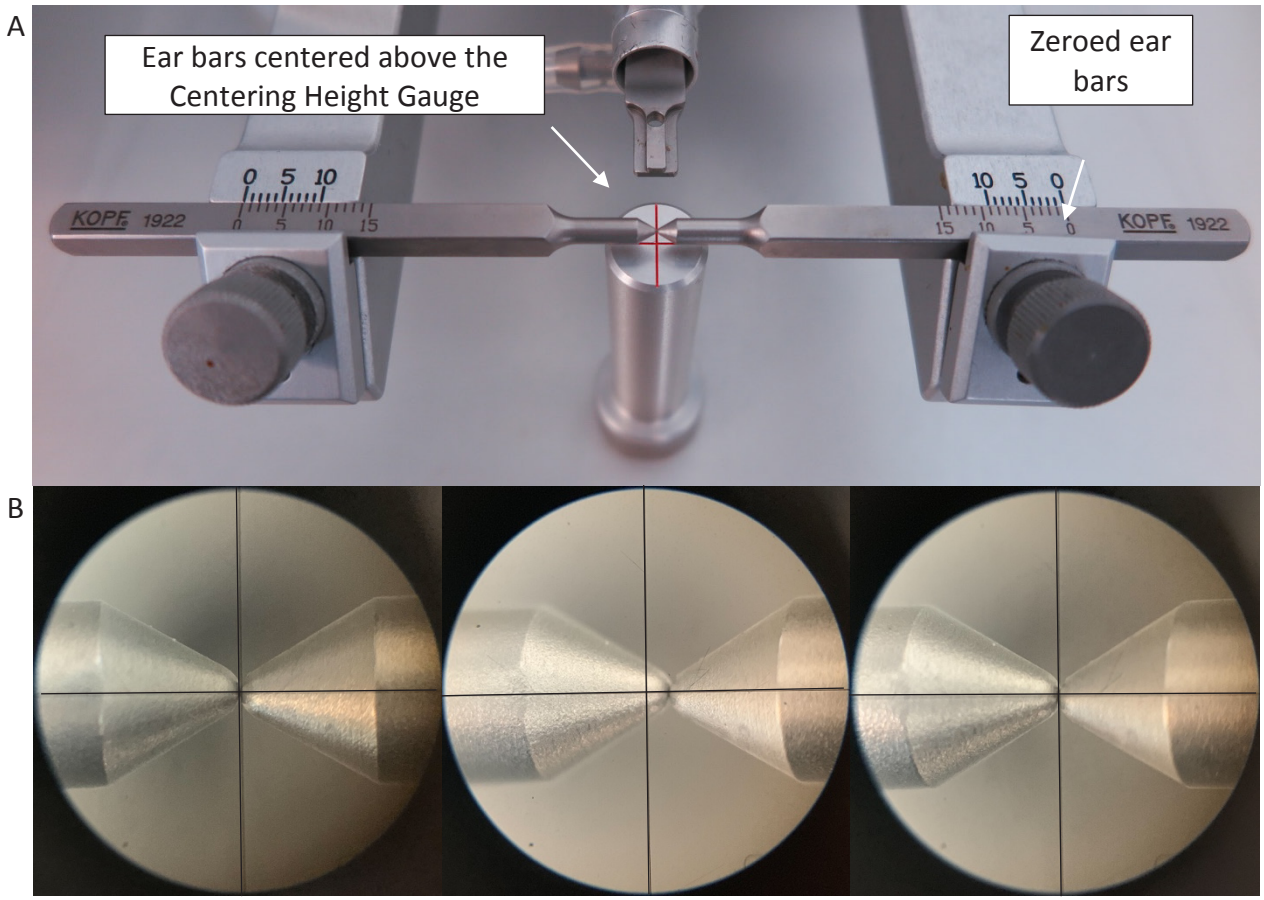

Figure 3. Aligning the head holder center of rotation. A) Positioning the ear bars. B) Sighting down the scope at 0° level coronal rotation (left panel), 15° rotation before adjusting the Vertical Shift and the center is misaligned (middle panel), and at 15° after adjusting the Vertical Shift to align the center of rotation (right panel).

the ears to just behind the eyes with a hair clipper.

4. Apply eye ointment.

IV. Surgical Procedure.

1. Confirm appropriate depth of anesthesia, indicated by lack of response to toe pinch with regular, deep respirations. Take care to continuously monitor the animal throughout the procedure, and provide thermal support as appropriate.

2. Place the head into the head holder by placing the upper incisors into the gap in the bite/palate bar, making sure that the tongue is below the bite/palate bar. It may be helpful to use non-sterile forceps to gently open the jaw during positioning.

    a. If using isoflurane anesthesia with the Model 1923-B head holder, position gas mask/nose cone snugly over nose and tighten the lock screw on top of the cone.

3. Next, secure the head in the ear bars by gently inserting the ear bars into the external auditory meatus. **Take care that the ear bars are symmetrically placed. This step is critical to ensure the head is stable and centered for rotation.** For an adult mouse, the ear bar indicator lines should both be between 3 and 4 when positioned correctly.

    a. If using the Model 1923-B head holder adaptor, a useful strategy is to gently move the adaptor body fore and aft using the locking screw on the right hand side while the head is locked in place in the ear bars – if the head moves when the adaptor body moves, the animal is not secure. Loosen one ear bar at a time while gently shifting the adaptor body fore and aft, until the ear bar settles under the zygomatic arch and the head no longer moves. Tighten the ear bars and the locking screw.

4. Sterilize the shaved incision area with 3 alternating scrubs of betadine and alcohol swabs.

5. Expose the skull by making an incision along the sagittal midline of the scalp.

6. Gently scrape the surface of the skull to remove any fascia and expose the sutures. Clean with ethanol and cotton-tipped applicator.

7. Place the Centering Scope into the holder and center the crosshairs on bregma (Figure 4A).

    a. Note that the suture lines do not always cleanly intersect at bregma, and often demonstrate a sharp caudal dive, as in Figure 4. The true bregma location may therefore be difficult to determine, and care should be taken to develop a consistent assignment strategy, and to validate targeting across multiple animals.

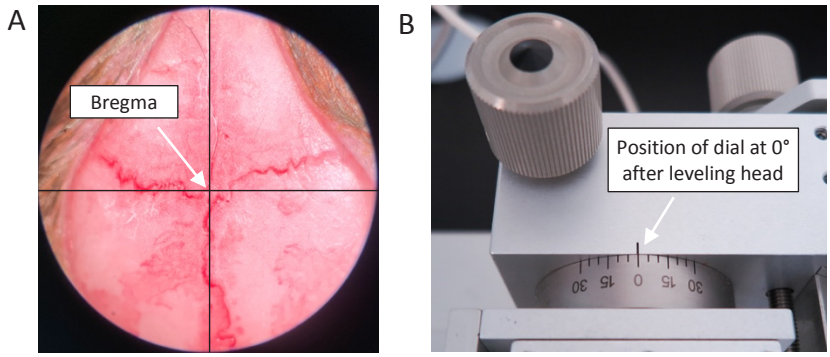

Figure 4. Leveling the skull and assigning bregma. A) Representative image indicating typical bregma placement. B) Noting the coronal rotation position.

8. Once you have approximated bregma, zero the digital read-out (DRO) on the micromanipulator.

9. Now, move the crosshairs caudally to lambda. Note the bregma-lambda (B-L) distance.

    a. The B-L distance is variable between different rodent strains and is highly dependent upon the consistency of the surgeon in assigning bregma.

    b. For an adult mouse, average B-L distance is 4.21mm. If B-L distance is significantly less or greater than 4.21mm, make any necessary adjustments to your assigned bregma to get within a reasonable distance from 4.21mm.

10. Replace the centering scope with the Alignment Indicator. First, level in the sagittal plane (nose-up or down) by placing the probes on lambda and bregma and adjusting the dorsal tilt knob of the head holder until level.

11. If you made any changes to the dorsal tilt, use the Centering Scope to reassign bregma.

12. Now, use the Alignment Indicator to level in the coronal plane, using the coronal tilt knob.

    a. It is good practice to level at multiple points on the rostral/caudal axis to account for any surface deformations in the skull.

13. If you made any changes to the coronal tilt, again use the Centering Scope to reassign bregma. **Make sure to note the position on the dial of the coronal tilt knob – this is your 0° rotation position (Figure 4B)**.

14. Double check levelness of skull in both directions with the Alignment Indicator before proceeding.

V. Centering Axis of Rotation

1. Place the Centering Scope into the holder and move the micromanipulator to your calculated coordinate from part I.

    a. Per the example in Figure 1, the coordinates targeting the ARC are: A/P: -1.4, R/L: [1.336] at 0° coronal rotation, [0.00] at 10° coronal rotation, D/V: -5.85. **Remember that the R/L coordinate corresponds to the length of side A from part I**.

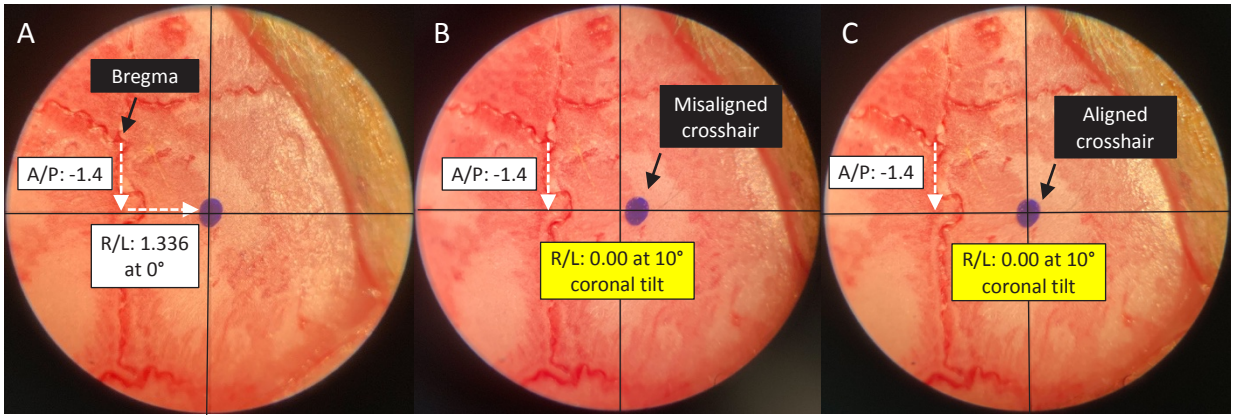

Figure 5. Aligning the animal head with the central axes of rotation. A) Drawing a reference mark before alignment. B) Image of unaligned axis of rotation. C) Properly aligned axis of rotation, after adjusting the Vertical-Shift and reassigning bregma.

2. Using a fine-tipped marker, draw a mark at this coordinate (Figure 5A). This represents where your needle/cannula should enter the brain **once rotated.**

3. Return the micromanipulator to the coronal midline (R/L: 0.00). Use the Coronal Tilt knob on the head holder apparatus to rotate the head to your specified angle (Figure 5B).

    a. If the crosshairs already line up with the mark, you are ready to proceed with your microinjection/cannula placement.

    b. If the crosshairs of the centering scope do not line up with the mark, your axis of rotation is not aligned properly. To adjust this, turn the Vertical-Shift knob up or down to shift the head in the Z axis until the crosshairs line up as close as possible to the mark.

        i. Note that it may not line up perfectly at this stage, as shifting the head in the Z axis will affect the position of bregma.

4. Rotate the head back to the 0° rotation position with the Coronal Tilt knob and reassign bregma using the Centering Scope.

5. Repeat steps 3 and 4 until you consistently hit the mark when the head is rotated (Figure 5C).

6. At this stage, you are ready to proceed with your microinjection or cannula/electrode placement per your lab's approved IACUC protocol, followed by routine postoperative care.**

** If using the centering scope to assign bregma and your injection coordinate, the coordinates when switching to the needle/cannula holder will be different. Therefore, it is recommended either to i) reassign bregma when switching to a new tool holder, or ii) use the Kopf Stereotaxic Drill (Model 1911) to drill at the coordinate defined with the centering scope, which is precisely calibrated with the centering scope to maintain common centerpoint position. If using the Stereotaxic Drill, simply move your cannula/needle to the center of the burr hole and zero the DRO in the Z axis when touching the dura mater.