

## FINE TUNING STEREOTAXIC SURGERY

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A common problem encountered by investigators utilizing stereotaxic placement of electrodes, micropipettes or syringe needles into the central nervous system of laboratory animals is the variability in target location as a function of animal size, strain, maturation, etc. As a first approximation of controlling for this sometimes large discrepancy in target location, the body weight of the experimental animal may be assumed to be an indicator of the ultimate target site based upon its relation to visible or inferred landmarks such as lamda, bregma, the interaural line and the upper incisor bar. Unfortunately, this method is still quite inexact, as the above mentioned animal differences can all contribute to the inaccuracies encountered in stereotaxic work.

A few years ago Wishaw et al. (1977) presented a method for improving the accuracy of localizing electrode placement within l-specific brain structures based not upon the body weight of their laboratory rats, but instead dependent upon the angle formed between the interaural line (IAL) and the upper incisor bar (UIB). Using the stereotaxic coordinate system employed by Pellegrino and Cushman (1967), they determined that an angle of 8° 29' above the horizontal should be maintained, as measured between the IAL and the UIB (See figure 2A). Pellegrino and Cushman's 1967 atlas utilized male hooded rats of the Royal Victoria Hospital strain weighing between 280 and 320 grams. Wishaw et al. presented a method for extending the usable weight range of animals from 161 to 782 grams, employing a simple regression formula relating the distance between the IAL and bregma, to the body weight of their animals.

A simple and inexpensive addition to the standard David Kopf instrument Company series 900 rat stereotaxic headholder markedly facilitates proper alignment of any size rat at this angle. A small section of a plastic metric ruler is glued to the flat plate which supports the UIB and nose clamp. In addition, one of the ear bar verniers is removed from its mounting and glued onto the stationary mainframe of the stereotaxic unit (See figure 1). Proper placement of the section of plastic ruler and the vernier can be accomplished with the aid of the electrode carrier itself. A stiff, straight wire or insect pin is installed into the electrode carrier, and the carrier is zeroed in the anterior-posterior plane at the center of the ear bars, the interaural line. The electrode carrier is then moved in the anterior direction a distance of 30 mm. The most anterior edge of the UIB is then positioned at a point just underneath the insect pin, and the sliding portion of the UIB/nose clamp support is tightened onto the mainframe using the knurled screw. This sets the UIB reference point exactly 30 mm anterior to the IAL. A section of a plastic metric ruler (e.g., a piece containing calibrations from 1.5 to 6.5 cm) is then glued onto the sliding portion of the UIB/nose clamp support using silicone glue or cyanoacrylate glue. At the point directly apposed to the 3 cm mark on the ruler, the zero point of the vernier scale is aligned and glued to the stationary mainframe of the stereotaxic unit.

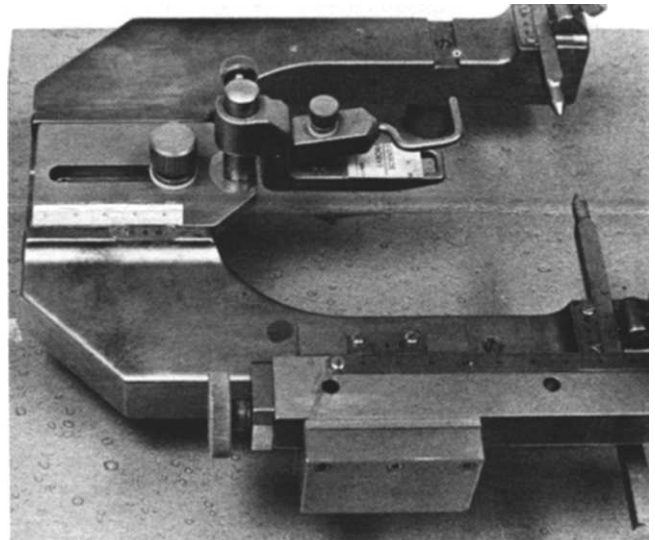


FIGURE 1: Photograph of the stereotaxic headholder with the two additions detailed in the text.

When the rat is firmly mounted into the headholder, the distance from the IAL to the UIB (with the UIB set a 0 mm vertical position) can be read directly from the millimeter scale and vernier, accurate to 0.1 mm. Knowing this distance, one may easily calculate the vertical elevation of the incisor bar necessary to maintain the skull at the appropriate angle of 8° 29' above the horizontal, using the formula:  
vertical elevation = IAL to UIB distance X sine 8° 29' = IAL to UIB distance X 0.1475

The sliding support holding the UIB/nose clamp is then loosened and moved slightly posterior, and the vertical elevation of the UIB is set. The sliding support is then gently moved in the anterior direction until the animal's head is snug, and the knurled screw is tightened.

The look-up table provided in Table I facilitates finding the appropriate vertical elevation of the UIB once the IAL-UIB distance is determined from the plastic ruler and vernier.

An additional problem may arise when approaching targets which lie far anterior to the IAL. Unfortunately, the hole in the UIB into which the incisors fit is rather wide in this instrument, and the head of the animal may be slightly angled to one side if the incisors do not lie in the exact center of the UIB. By placing another section of the plastic millimeter scale directly in front of, and attached to, the UIB, it is quite simple to sight along the plastic scale and properly align the animal's incisors in the direct center (medial-lateral direction) of the UIB (See figure 1).

By combining these two simple additions to the standard stereotaxic headholder, we have been able to position highly reliable and accurate lesions and iontophoretic deposits within specific targets of the rat brain, with a minimum of inter-animal variability.

(Continued on page 2)

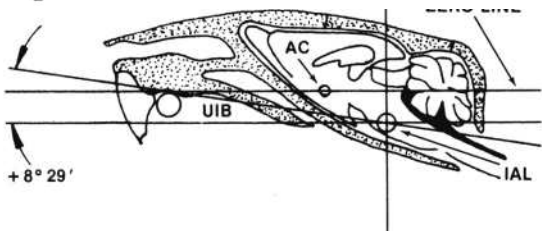


FIGURE 2A

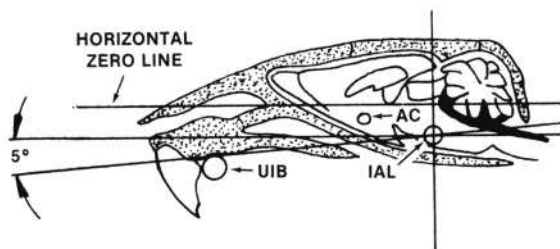


FIGURE 2B

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**FIGURE 2A)** Schematic representation of a midsagittal section through the rat skull showing location of the brain with respect to (he calvarium and external landmarks, as used in the stereotaxic atlas of Pellegrino and Cushman (1967). Note that the angle formed between the upper incisor bar (UIB) and interaural line (IAL) is  $8^{\circ} 29'$ . This corresponds to a UIB elevation of 5 mm in an animal with the IAL-UIB distance about 34 mm, a typical value for a 300 gram rat. 2B) Similar representation showing the orientation of the rat brain as used in the stereotaxic atlas of Konig and Klippel (1963).

**TABLE I.** Vertical coordinates required to position the upper incisor bar at the proper orientation with respect to the interaural line when using two different stereotaxic atlases. The atlas of Pellegrino and Cushman requires that the upper incisor bar be maintained at an angle of  $8^{\circ} 29'$  above the horizontal; the atlas of Konig and Klippel uses an angle of  $5^{\circ}$  below the horizontal.

| Interaural line to upper incisor bar distance | Pellegrino and Cushman vertical elevation (above horizontal) | Konig and Klippel vertical elevation (below horizontal) |
|---|--|---|
| 25 mm   | 3.69 mm  | -2.18 mm  |
| 26  | 3.84   | -2.27   |
| 27  | 3.98   | -2.35   |
| 28  | 4.13   | -2.44   |
| 29  | 4.28   | -2.53   |
| 30  | 4.43   | -2.61   |
| 31  | 4.57   | -2.70   |
| 32  | 4.72   | -2.79   |
| 33  | 4.87   | -2.88   |
| 34  | 5.02   | -2.96   |
| 35  | 5.16   | -3.05   |
| 36  | 5.31   | -3.14   |
| 37  | 5.46   | -3.22   |
| 38  | 5.61   | -3.31   |
| 39  | 5.75   | -3.40   |

## ALTERNATE COORDINATE SYSTEM

The simple additions mentioned above can, of course, be equally well applied to orienting the animal's head in the stereotaxic instrument to comply with the atlas of Konig and Klippel (1963). Konig and Klippel's atlas used 150 gram female rats of the Wistar BR 46 strain. They defined the horizontal zero plane as being situated 4.9 mm above the interaural line, and situated their animals in the stereotaxic device such that the upper edge of the UIB was set 2.4 mm below the interaural line, provided that the IAL-UIB distance was 28.6 mm (See figure 2B). In this instance, the angle maintained between the upper incisor bar and the interaural line is about  $5^{\circ}$  below the horizontal, and a similar look-up table can be used for this coordinate system (See table I). A more permanent method for maintaining the angle of  $5^{\circ}$  below the horizontal was given by Pieri and Tschirky (1974), which required machining a block of metal with a  $5^{\circ}$  inclined slope, and bolting this metal block to the stereotaxic instrument.

Note:

The stereotaxic atlas of Pellegrino and Cushman (1967) has recently been reissued in a second edition (Pellegrino et al., 1979), which offers the advantage of extending the caudal extent of the original atlas to include the entire brainstem or plotter readout.

## References:

- De Groot, J. The Rat Forebrain in Stereotaxic Coordinates. Verh. K. Ned. Akad. Wet. B. Natuurkd. 2: 1-40, 1959
- Konig, J.F.R. and R.A. Klippel. The Rat Brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins, Baltimore, 1963
- Pellegrino, L.J. and A.J. Cushman. A Stereotaxic Atlas of the Rat Brain. Plenum Press, New York, 1967
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- Pieri, L. and H. Tschirky. Device for Stereotaxic Positioning of rats according to the atlas of Konig and Klippel. Physiol. Behav., 12: 675-677, 1974
- Wishaw, I.Q., J.D.D. Cioe, N. Previsich and B. Kolb. The variability of the interaural line vs the stability of bregma in rat stereotaxic surgery. Physiol. Behav., 19: 719-722, 1977

A factory installation of this modification will be available

## NEW PRODUCTS STEREOTAXIC DRILL PRESS.

David Kopf Instruments is now offering a new stereotaxic drill system. Our model #1463 consists of a 0-14000 RPM Foredom motor with an 84 cm flexible shaft, and variable speed foot switch. Features include a duplex spring drive connection for extra flexibility in the handpiece where precision control is required. Replacement springs are available and can be installed by the user. The handpiece has a maximum diameter of 14 mm and a tip diameter of 4.7 mm. A #1465 holder is available and allows the use of this handpiece with any DKI manipulator. The 1467 hand-piece has a geared chuck and will accept shank sizes up to 4 mm. The 1467 is 2.5 cm in diameter and 13 cm long. The 1469 holder will mount this handpiece to any DKI manipulator. Available as 115v60HZ or 230v 50 HZ.

The above forms an inexpensive carrier mounted system for placing holes in the skull in precise stereotaxic coordinates for electrodes, cannulae or micro-injecting. The price of this system with handpieces and holders is about one-half of what a triple arm system would cost. Either handpiece may be used freehand.



FIGURE 3

**RFG4A**

**RESEARCH RF LESION GENERATOR SYSTEM**

Radio frequency lesioning plus continuous tip temperature monitoring assure reproducible localized lesions. Precise temperature control enables one to produce temporary lesions. The thermocouple permits an electrode tip diameter as minute as 0.25 mm, ideal for smaller animals. A TCZ calibrated electrode, mounted in a 1279 electrode holder, will attach to any OKI manipulator. Electrodes with larger tips are available as a special order.

Features include: phantom load to test generator output, 1 RPM clock timer, voltage and current meters (current scale is compressed to allow a 25 Ma mid-scale reading), precise temperature control, external stimulator, and output jack for charting current. Frequency is 100 KHz, maximum output 4 watts, size 12 3/4 wide x 7 3/4 deep x 7 1/2 high. Available as 115v 60 HZ or 230v 50 HZ. TCZ calibrated thermocouple electrode has a 0.25 mm tip diameter, tip length is 0.5 mm, and stepped down shaft length 100 mm. Write for complete specifications, prices, and availability. See figure 3.

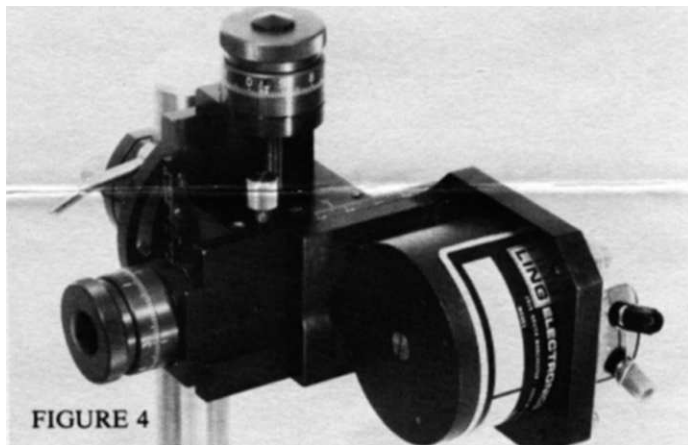


FIGURE 4

**MANIPULATOR FOR HEAVY OBJECTS**

Figure 4 illustrates a special manipulator for accurate micro-positioning of heavy objects. The wide dovetail slides and heavy-duty clamp furnish large bearing surfaces and permit rigid support of heavy objects. Backlash free screws drive the dovetail slides and have 1.0 mm travel per revolution. 3 cm diameter dials are provided with calibrations representing 10 microns per division. Travel is 3 cm in two planes. The manipulator, as shown, mounts on and may be adjusted or rotated around the 2.5 cm post. Angle positioning is also provided. This manipulator can be made to hold various large heavy objects. Write for a quotation to suit your needs.

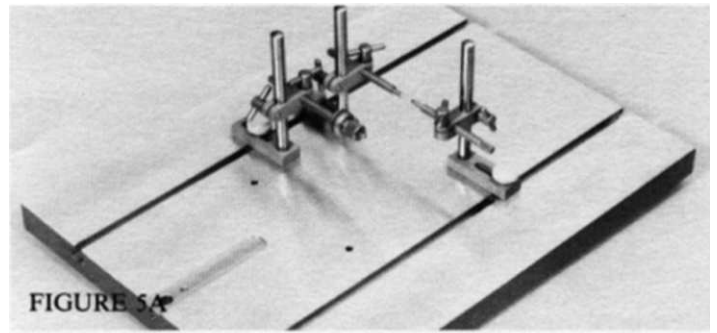


FIGURE 5A

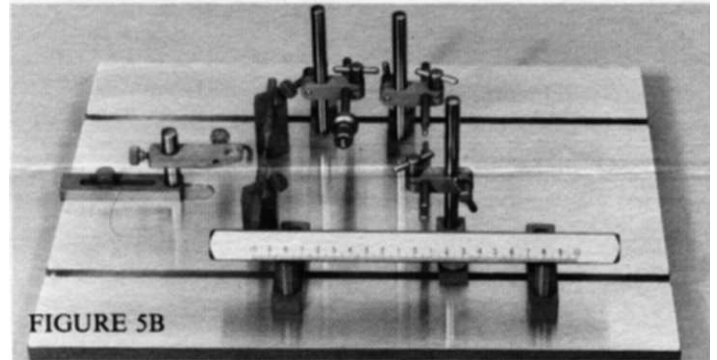


FIGURE 5B

**THE VERSATILE 981 RAT SPINAL SYSTEM**

FIGURE 5A) shows the standard 981.

FIGURE 5B) shows the 981 with optional 922 calibrated AP bar and brackets, the 921 head holder with ear bars. Also available (not shown) are 987 V notch spikes and 988 retractors.

The rat spinal plate #980B is precision machined with two tee slots for the mounting of accessories. The top is left natural to assure precise location of components. Attached to the base plate via the tee slots are three #982A Adjustable Base Mounts with post and clamp. These base mounts are held with thumb screws and tee nuts, no wrenches are necessary. The #986A Vertebrae Clamp and #985A Hip Spikes are attached to the base mount clamps.

Additional accessories are the #987 V Notch Clamp and #988 Retractors. They may be attached to the spinal unit with the #982A Base Mounts and Clamps. #992 Calibrated A-P Bar and Brackets (A-P bars are available in sizes to fit all of our carriers). Two head holders, the #931 Rat Surgical Head Holder with a vertical post and clamp, and the #921 Head Holder which consists of a tooth bar with adjustable vertical post and a set of calibrated rat ear bars.

The design of the Rat Spinal Unit assures ample lateral and vertical travel of all components. The tee slots allow rapid attachment and removal of various accessories.

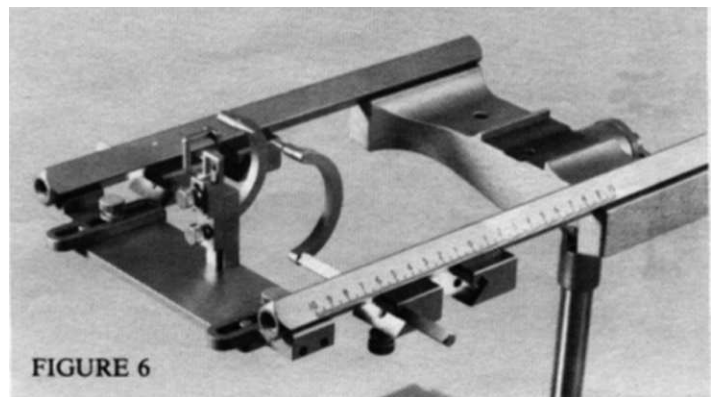
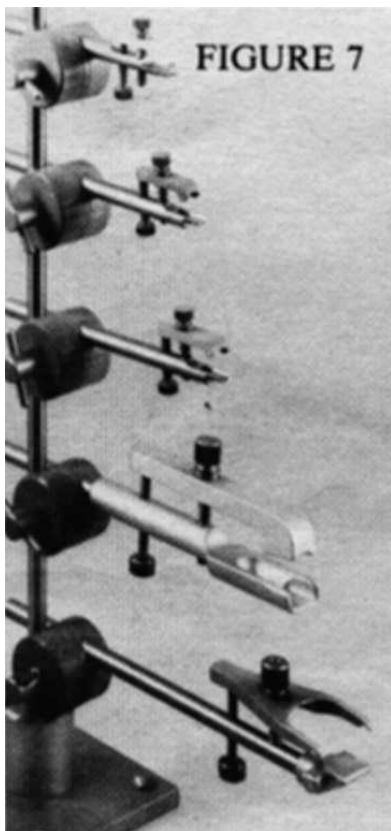


FIGURE 6

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## **SMALL ANIMAL OPTICAL INVESTIGATION UNIT 876**

Small animal optical investigation unit (Figure 6) may be used with mice, rats, hamsters, guinea pigs, and chinchilla. Features include curved earbars and narrow nose clamp to minimize visual obstruction. Tooth bar is adjustable  $\pm 5$  mm. The 876 optical investigation unit may be factory installed on the 1430, 1530 and 1730 stereotaxic frames.



### **SURGICAL HEAD HOLDERS**

We now have a series of five surgical head holders. Starting from the top: Figure 7 mouse, rat, chinchilla, rabbit, and cat. Any of these may be used with the 310 universal stand.

### **A REMINDER OF OTHER RECENT PRODUCTS**

120 Scouten Adjustable Wire Knife. Versatile, efficient, and easy to use. This wire knife can be used as an alternative or supplement to electrolytic or chemical lesioning. The knife holder will extend the knife blade a precise distance via a screw drive. Rotational cuts may also be made. Write for detailed brochure.

### **922 MOUSE ADAPTOR**

Now available is the #992 Mouse Adaptor. This adaptor does not require ear bars and may be attached to any David Kopf stereotaxic frame. This adaptor will minimize breathing problems associated with ear bar insertion.

858, 958, and 1758 Trocar Ear Bars are available for all DKI stereotaxic frames. The ear bars may be used on cats, monkeys, and dogs.

Please write for more detailed information, prices and delivery on any of the new or recent products.