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A BRAIN BLOCKER/SLICER FOR THE STEREOTAXIC PLANE OF *THE RAT BRAIN IN STEREOTAXIC COORDINATES*

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Dr. Watson received his M.D. in 1964 and his PhD. in 1974. He taught first at the University of Western Australia and then at the University of New South Wales. In 1982, he look a position with the Health Department of Western Australia, became Director of Health Promotion Services in 1985, and in 1989 was made Director of Disease Control. Dr. Paxinos received his degree in psychology from McGill University in 1972. Following a postdoc at Yale University under Professor Flynn, he went to the University of New South Wales where he is now an associate professor. He has edited the two volume work The Rat Nervous System (Academic Press, Sydney, 1985) and is now constructing an alias of the human brain in collaboration with Prof. Istvan Tork.

THE EVOLUTION OF THE STEREOTAXIC TECHNIQUE

Prior to the 1950's accurate stereotaxic procedures were encumbered by the unavailability of precise stereotaxic instruments and stereotaxic maps. Empirically derived coordinates of structures could substitute for the lack of an accurate atlas, but the results were then compromised by the lack of an accurate stereotaxic instrument. The first breakthrough came with the advent of precision stereotaxic frames and placement devices such as the Kopf small animal stereotaxic frame. This development was followed by the appearance of more comprehensive atlases such as the Konig and Klippel (19863) and the Pellegrino and Cushman (1967) which had a degree of accuracy that went some way in satisfying the detail and precision required by researchers in the mid 1960s.

However, the requirements for detail and precision have greatly increased in recent decades to the point that, for instance, nearly no researcher targets the general area of the hypothalamus, but instead they target one of the roughly 60 subnuclei in which the hypothalamus has been parcelled.

In early 1980 when we commenced the construction of our atlas, we made an in-principle decision that the main feature of our work would be satisfactory slcrcotaxic accuracy. We spent one year and cut 115 brains before we obtained three brains which we thought were cut with sufficient stereotaxic accuracy. Throughout our work we used the Kopf small animal stereotaxic frame.

We used fresh tissue to avoid the differential shrinkage and distortion caused by fixation. The atlas coordinates for some central nuclei ((he trochlear and the bed nucleus of the anterior commissure for example) vary by less than 0.2 mm in the coronal, sagittal and horizontal planes. For structures located further from the stereotaxic reference points, the atlas coordinates may differ by as much as 0.5 mm. Even if rats of different strains and sex are used, the coordinates will be substantially accurate if the weight is close to that on which the atlas is based (290g).

But this is not the end of the story; the evolution of quality stereotaxic procedure is not complete without a reliable method for ensuring that brain histological preparations are aligned in the same plane as the atlas used. We describe here a blocker for ensuring accurate sectioning of the rat brain so that histological sections can be routinely cut in a plane approximating that of *The Rat Brain in Stereotaxic Coordinates.*

BLOCKING THE BRAIN PRIOR TO HISTOLOGICAL SECTIONING

The preparation of histological sections that correspond to one of the cardinal planes of the stereotaxic atlas is a process that is not related to the success or failure of stereotaxic placement in the brain, but it is nonetheless vital for satisfactory study of brain anatomy relevant to a particular experiment. If sections are cut in a plane that docs not correspond to the atlas, interpretation of the anatomy is often difficult and time consuming and the resulting sections arc not suitable for illustration in journal articles.

A poorly blocked brain can result in sections that arc not only asymmetrical but which are tilted at 20-30 degrees to the cardinal plane. If the resulting sections are tilted in this way the researcher may have to consult as many as four atlas plates to identify the relevant nuclei. The difficulty is

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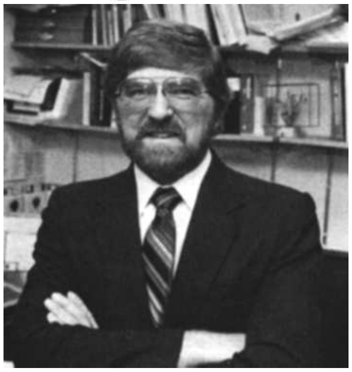

Editor's Column

The Carrier is a newsletter of the David Kopf Instrument Company intended to provide its readers with articles describing techniques and instrumentation which have been developed by the authors of those articles for specific purposes and uses. In addition, the Carrier will occasionally feature articles of general interest and significance, such as the article which was published some issues ago giving an overview of the Kopf plant and how the stcreotaxic instruments are made. In coming issues, we will have articles on the history of the stereotaxic technique and instrument. In line with these coming articles, please note that the UCLA Brain Research Institute, through Drs. Louise Marshall and Horace W. Ma-goun, has loaned the second version of the original Hor-slcy-Clarke steroetaxic instrument to the Kopf Company and it will be on display at the Kopf Booth at the upcoming Society for Neuroscience Meeting. Please plan on stopping at the booth to see this interesting and historic instrument, which David Kopf Instruments has completely refurbished. Since the articles featured in the Carrier are written by scientist colleagues about techniques and instruments they have developed, the instruments described are generally not commercially available from David Kopf Instruments or other sources. If they are available, it is usually from the author of the article, although the authors try to give sufficient detail in the article so that the reader could have a copy built. We will, in the future, try to include information on where that author can be contacted if the reader wishes to discuss the technique or instrument described, or feel free to contact me at the address and phone below for such information. However, in general, the Kopf Company docs not sell instruments described in the Carrier, although as noted in this issue, the brain blocker described here will be available from Kopf Instalments. Again, the primary purpose of the Carrier is to provide a means of supplying information on techniques, instruments and issues of general interest which would not be available elsewhere. We encourage anyone who has a technique, instrument modification or instrument which you think you would like to share with the scientific community, to consider writing a Carrier article. If you have an idea for such an article, please contact me about it so that your colleagues can benefit from your experience.

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compounded when the researcher works with material with little definition such as low levels of receptor binding or low levels of reactivity.

Blocking of the brain into coronal slices is commonly carried out by placing the brain on its dorsal or ventral surface (usually the former) and attempting to slice the brain at right angles to the imagined horizontal plane. An experienced worker with a practiced eye can often make these slices accurately enough to ensure that resulting histologi-cal sections correspond satisfactorily to the plane of the atlas being used. However, the penalty for misjudging the plane of slicing is significant; the resulting sections may be anything from 5 to 30 degrees away from the desired plane of section.

Accurate blocking can be achieved by reinserting the head in the sterotaxic frame and lowering a wide blade aligned with the coronal plane of the atlas. This is a cumbersome procedure which involves the removal of much of the skull surface and the jagged skull edges make it difficult to make a complete cut across the brain. It is difficult to cut serial 1 mm thick sections with this method.

DEVELOPMENT OF THE BLOCKER FOR THE RAT BRAIN IN STEREOTAXIC COORDINATES

The solution to the problem of blocking errors is a fairly obvious one - the use of blocking device that ensures that the slices of brain tissue are cut in one of the atlas planes before the tissue slice is mounted on the microtome chuck but after the brain has been removed from the skull. Such blocking devices have been manufactured in an ad hoc way in many laboratories over the years, usually fabricated from wood or metal with slots to guide a slicing blade. A better design for such a blocker made in perspcx was pioneered by Jacobowitz (1974).

However, the usefulness of a blocking device is proportional to the extent with which it matches the plane of section of the atlas being used. Because of this, we have developed a rat brain blocker to correspond with the planes of the Paxinos and Watson atlas (see Figure 1).

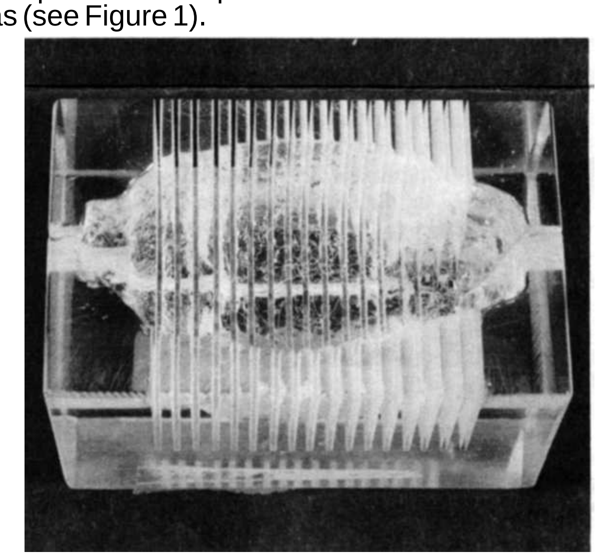

Figure 1. The Brain Blocker

Our device consists of a rectangular block of clear per-spex in which a deep mold of the rat brain has been impressed so that the brain can be laid on its dorsal surface inside the blocker (Figure 2). The impression of the brain was made with molding rubber and the cast was filled with acrylic XE80. It is not possible to cast acrylic on acrylic so a plasticine impression of the acrylic shape was taken. Molding rubber was used to fill the plasticine impression to produce a rubber brain shape which could finally be used to make the acrylic mold in which the brain is the lie. The mold was oriented by trial and error so that the horizontal axis of the brain corresponds to the horizontal plane of the Paxinos and Watson atlas. A series of 16 coronal slots (slicing guides) were cut across the mold at 1 mm intervals with a precision slitting saw. One slot was cut in the sagittal plane 1mm from the midline on die right.

USING THE PAXINOS/WATSON BRAIN BLOCKER

The blocker can be used with fresh or fixed specimens. We have tested the blocker and have found that it can produce well oriented sections that are symmetrical and reasonably approximate the planes of the atlas. The blocker is designed to accommodate the brains of large male rats (up to 350 or 450g) so smaller brains will not fit flush with the walls of die blocker (Figure 2). We have not found this to be a significant disadvantage when smaller brains are used. It does mean, however, that care has to be taken to align the brains of smaller animals in the blocker.

When the brain is lying flat in the blocker, a single sided razor blade or scalpel is drawn across the pair of slots at the desired level to slice the brain (Figure 3). It is, of course, more difficult to slice fresh tissue than fixed tissue so more care must be taken and it is advantageous to pre-chill the blocker and the fresh brain prior to sectioning. The blocker can be packed in wet ice.

After blocking, the brain needs to be placed on a chuck with its surface parallel to the knife. For cryotomes with no zero stage tilt position, this can be achieved by freezing some mounting medium direcdy on to the chuck and cutting dirough the mounting medium to create an aligned surface on which to position the block of tissue.

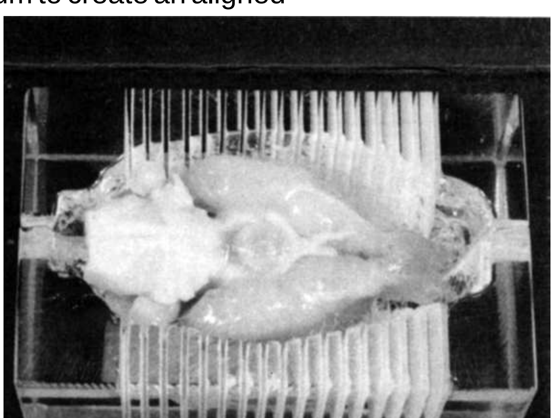

Figure 2. A brain positioned in the blocker

AVAILABLE FROM KOPF

Although the principles of manufacture of the perspex blocking device are simple, we found it necessary to make a series of prototypes before we were satisfied with the accuracy of orientation of the mold in relation to the slots that guide the slicing blade.

David Kopf Instruments now markets this blocker which is designed to match the plane of The Rat Brain in Stereo-toxic Coordinates. We hope that this blocker will make the information in our atlas more accessible to researchers who will now be able to match their sections more direcdy to our atlas plates.

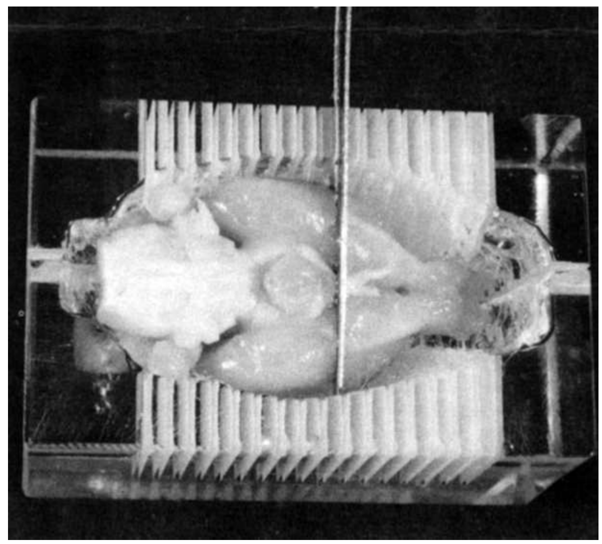

Figure 3. A razor blade slice being made using the blocker

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