III. Preparation and Use of the Atlas

A. Histology and Map Production

An ideal atlas would present the brain at high resolution in an undistorted way—as it might be viewed in the living animal. Unfortunately, this is not possible for a number of technical reasons, as well as the fact that the shape of the CNS (particularly the lower brainstem and spinal cord) changes with head and body movements (fig. 3). Thus, the production of an atlas involves a series of compromises that are important to consider because they influence the accuracy of the resulting maps (also see Kruger et al. 1995).

The most difficult technical issue in preparing a brain atlas is the production of tissue sections that are as uniform and undistorted as possible. There are two common ways to produce tissue sections: the brain may be frozen and cut, or it may be embedded in one material or another and cut at room temperature. Frozen sections are very popular today for histochemical procedures because they can be produced rapidly, there is relatively little tissue shrinkage, and the tissue is not subjected to the harsh physicochemical treatments usually required for embedment. There are, however, several reasons why the present atlas was not based on this approach.

First, with currently available technology the rat brain must be cut into two or more blocks for sectioning; thus, an uninterrupted series of sections from the same brain cannot be obtained. Second, each frozen section is distorted in a different, uncontrollable way (sometimes indicated by a bubble, fold, or tear in the tissue) when mounted on a glass slide, whether the sections are transferred from the knife of a cryostat or are mounted with a brush from an aqueous solution.

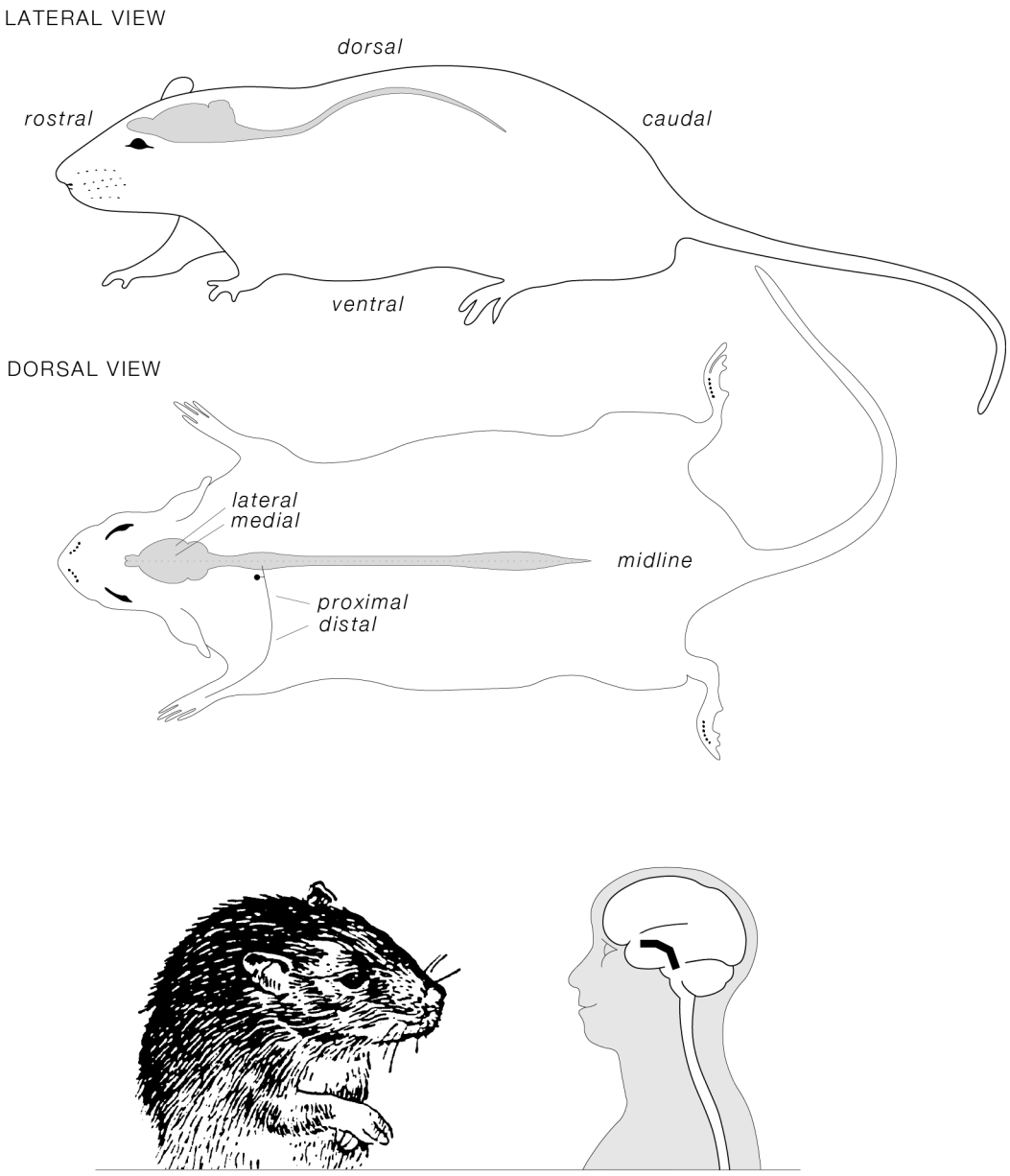

Fig. 3. Fundamental terms used to describe positional information in the embryonic and adult (top two drawings) CNS of fish and quadrupeds. As indicated below (right), human terminology is complicated by an approximately right angle bend in the axis of the brain, due to our typical upright posture. This is not to say, however, that the rat CNS (lower left) cannot undergo changes in its axis during the course of normal behavior. (Rat drawing from Barnett 1963).

This problem may be greatly reduced as tape-transfer methods for cryostat sections are refined (Ornstein 1986). Third, small, unconnected pieces of the brain may be lost in the mounting process (for example, from the cerebellum or caudal cerebral hemispheres), particularly when

sections are mounted from an aqueous solution. And fourth, it is exceedingly difficult to obtain long, uninterrupted series of sections in a cryostat.

Two materials have long been used to embed the brain: paraffin and celloidin. The major advantages of this approach are that serial sections through the entire brain are much easier to obtain, various separate pieces of the tissue section are typically held firmly in place, and (relative to frozen sections) there is less uncontrollable distortion due to the mounting procedure. The major disadvantage of this approach for atlas production is that the brain as a whole shrinks due to osmotic influences during the embedment procedure.

When all of these considerations were weighed, it was concluded that an embedded brain should be used for the atlas. Paraffin was eliminated because a perfect brain was never obtained (after many attempts to refine the method); the paraffin embedment procedure requires rather harsh conditions (heat, in particular) for the tissue, which hardens to a very different extent in different regions, the olfactory bulbs and cerebellum providing the extremes. Thus, for example, when excellent sections through the bulbs were obtained, the cerebellum was corrugated, and so on. This left the celloidin method, which is now commonly used for the preparation of Golgi material.

The Brain. The brain of a 315 gram adult male Sprague-Dawley rat was finally chosen to use for the atlas. First, the animal was deeply anesthetized with chloral hydrate (1 ml of a 3.5% solution/100 grams of body weight) and was perfused transcardially (through the ascending aorta), after clamping the descending aorta. A brief saline rinse to remove most of the blood was followed by 300 ml of a 4% paraformaldehyde solution in potassium phosphate-buffered saline at pH 7.5. The head was removed and placed in the same fixative overnight before the brain was

removed carefully, and then the dura mater removed carefully from the brain. The brain was then fixed in the paraformaldehyde solution for an additional 10 days.

The brain was embedded in celloidin (specifically, low viscosity nitrocellulose), essentially as described by Morest and Morest (1966), and sectioned in the frontal (transverse) plane with a sliding microtome. The brain was oriented as close to the vertical (longitudinal) plane as possible using the region of the superior sagittal sinus (the longitudinal cerebral fissure) and the base of the brain as guides. Cutting the rat brain in this orientation provides sections that are approximately transverse to the longitudinal axis of the central nervous system (fig. 3).

Every section through the brain was collected, stained, and mounted. The first 133 sections (through the olfactory bulbs) were 30 μm thick, whereas the last 423 sections (to the transitional region between the medulla and first cervical segment of the spinal cord) were 40 μm thick. Relatively thick sections were used because cytoarchitectonic boundaries are easier to determine when more cells are present. Finally, the sections were stained with thionin, and covered with DPX. Weights were placed on the coverslips as the DPX dried, to help flatten the sections.

It is important to consider what types of distortion were produced by the embedment, cutting, and mounting procedures, and exactly what plane of section was obtained. As mentioned above, there is very little shrinkage associated with the preparation of frozen sections from brains perfused and fixed as our atlas brain; therefore, the linear dimensions of our sections were compared with the sections presented in the atlas of Paxinos and Watson (1986), which was based on frozen sections. Measurements at a number of levels indicate that the mediolateral width in our brain is about 28% shorter than those of Paxinos and Watson, whereas the dorsoventral height in our brain is about 38% shorter than those in Paxinos and Watson; and finally, the rostrocaudal length of our brain is about 21% shorter than in their brains.

A number of factors may contribute to the differential "shrinkage" along each of the three axes; the following are some of the more obvious. First, due to inhomogeneities (for example, in the location of fiber tracts, ventricles, and large masses of gray matter), the tissue may well not shrink uniformly. Second, compression from the cutting procedure itself may contribute to the fact that the dorsoventral axis is proportionately shorter than the mediolateral axis (the tissue was cut from dorsal to ventral, rather than from lateral to medial). And third, our brain was from a Sprague-Dawley rat, whereas the brains used by Paxinos and Watson (1986) were from Wistar rats; strain differences in the shape of the brain have been documented (for example, see Table 1 in Paxinos and Watson 1986; Kruger et al. 1995).

A second obvious distortion in our brain is the expanded state of the ventricular system. It is in fact not yet possible to determine the exact conformation of the ventricular system *in vivo*, but it is clear from the literature (see McFarland et al. 1969; Westergaard 1969; Paxinos and Watson 1986; also see fig. 8) that it is expanded in the current atlas, undoubtedly due in large measure to tissue shrinkage during embedment, although perfusion may have contributed to it. This problem is reflected in the dorsal separation between the alveus and corpus callosum/external capsule (in a region occupied by the lateral ventricle during embryogenesis) seen in the photomicrographs accompanying Levels 32-41 (Swanson 1992a and current CD-ROM). And finally, the position of the pituitary was estimated (see fig. 77 in Paxinos and Watson 1986) because it changed considerably following removal of the dural sheath.

The plane of section was examined carefully. In the first place, it corresponds rather well to the frontal plane of Paxinos and Watson (1986). However, when the same structure was examined on both sides of the brain, it was consistently found that the atlas brain was not sectioned perpendicular to the long axis; instead there was an error of about 4° (fig. 4).

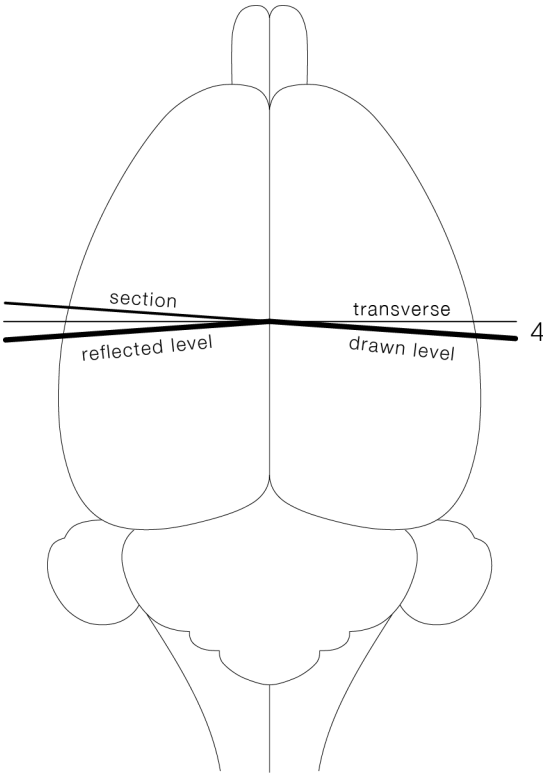

Fig. 4. The brain atlas was not cut perpendicular to the longitudinal axis; there was an angle of about 4° between the transverse plane and the sections themselves.

Photomicrographs. Experience has shown that high contrast and resolution are necessary to appreciate best low-power photomicrographs of Nissl-stained sections. To this end, the slides were placed in an Omega enlarger with a point-light source, and an image of the section was projected onto a 4X5 inch sheet of Kodak Kodalith Ortho (2556) film. The film was developed in Kodak Kodalith fine line developer and was printed with a Durst enlarger and Schneider Kreuzanch Componon-S lens (*f* / 150 mm) on 11X14 inch sheets of Kodak Kodabrome II RC paper, contrast grade F5.

The Drawings. The original maps (Swanson 1992a) were drawn with Adobe Illustrator 3.0 on a Macintosh II*fx* computer with a 19" SuperMac color monitor. For this, a copy of the photomicrograph was scanned at 72 dpi with a Hewlett-Packard ScanJet scanner and used as a template for tracing the major features of the section. A microscope was placed next to the computer, and all features of the section, as well as those on adjacent sections, were carefully examined before they were drawn. The drawings were printed with an Agfa Compugraphic ProSet 9800 imagesetter with Emerald RIP at 1200 dpi. The current files are manipulated in Adobe Illustrator 7 (available for both the Macintosh and PC).

It is important to point out that the drawings are not faithful renderings of the sections in one major respect: a straight vertical line was used for the midline so that drawings may be reflected

to produce pseudobilateral maps of the brain. However, the midline of the sections themselves is never perfectly straight, due to small distortions that accompany mounting. For this reason, detailed comparisons between midline regions in a photomicrograph and its corresponding map reveal slight differences. In addition, the artificial separation between the alveus and corpus callosum referred to above (seen in the photomicrographs accompanying Levels 32-41 on the CD-ROM) was eliminated in the drawings.

In a general sense, the original maps were designed to show regions of gray matter in gray, and regions of white matter in white. Obvious regions of dense Nissl-staining were indicated with darker shades of gray, although this was done in a very qualitative way with only three shades of gray. The maps presented here were redesigned for optimum display of mapped data. Therefore, gray matter is shown as white (or light yellow), and white matter is shown in light gray—which tends to emphasize major divisions of the gray matter.

Level Selection. Short of illustrating every section through the brain (which is somewhat impractical because an average of about 40 hours was spent on each drawing), a reasonable number of levels must be chosen that nevertheless adequately shows all of the various parts. Because the brain is a very heterogeneous structure, some levels require fewer sections than others for adequate illustration. Thus, a regularly-spaced series of sections was not chosen; for example, it is not necessary to illustrate a 1-in-4 series of sections through the entire length of the olfactory bulb.

Instead, sections were chosen that best illustrate the largest number of features, and more closely-spaced sections were used in regions containing smaller structures. One exception to this approach was necessary at about the level of the red nucleus because a very large scratch appeared in about 10 consecutive sections. This was produced by an unsuspected particle on the

cutting edge of the microtome knife and rendered the sections unsuitable for photography. A somewhat smaller scratch also occurred at the level of the anterior commissure.

In the end, 73 out of 556 sections through the brain (70 out of 423, excluding the olfactory bulbs) were chosen for illustration. This density was sufficient to illustrate virtually every structure in at least two (and usually at least three) Levels. Of course, all of the intervening sections were examined as the drawings were prepared.

B. Coordinate Systems: Stereotaxic Surgery and Databases

In descriptive neuroanatomy, it is customary to define location by referring to named structures such as fiber tracts, cell groups, or parts of the ventricular system. This is like referring to countries and highway systems in geographical maps. However, there are several applications in neuroanatomy where reference is made to location with respect to *x*, *y*, and *z* values in a three-dimensional coordinate system (Cartesian thus far), much as longitude, latitude, and altitude are used in geographical maps (though not typically in a Cartesian system). The most common use today of brain coordinate systems is in stereotaxic surgery, although they will play an increasingly important role in constructing and navigating through computer graphics models of the brain, and in searching neuroanatomical computer graphics databases (because coordinates are free of nomenclature biases or confusion).

Two sets of coordinates are associated with the maps presented here. One is a set of *physical coordinates* related directly to the tissue sections themselves, and they are supplied with the computer graphics files. The other set of coordinates, which are printed in section VII (and provided in the computer graphics files as well), is a calculated set of *stereotaxic coordinates*

derived from the atlas of Paxinos and Watson (1986). The derivation of these two coordinate systems is described in the preceding section.

In the physical coordinate system, the *z* axis begins (= 0) at the rostral tip of the olfactory bulb, the *y* axis begins along an imaginary line that corresponds approximately to a surface that the brain is lying upon, and the *x* axis begins at the midline. This system is currently being used for 3-D computer graphics modeling and for database construction because we have not yet determined the actual, *in situ* shape of a 315 gram male Sprague-Dawley rat brain.

In the Paxinos and Watson stereotaxic scheme a flat skull position was achieved when the incisor bar was 3.3 mm below horizontal zero, and z = 0 at bregma, y = 0 along a line approximately parallel to the dorsal surface of the skull, and x = 0 at the midline. Despite these differences, it was found that our atlas brain and those used by Paxinos and Watson were cut in virtually the same transverse plane, at least through the forebrain and rostral midbrain.

As discussed at length by Kruger et al. (1995) there are inherent limitations to the accuracy of stereotaxic surgery in rats and other species, no matter what atlas is used. Some obvious sources of error include differences in animal body weight, sex, and strain; interanimal variability in skull and brain anatomy; and less than perfect orientation of the stereotaxically placed electrode, cannula, or pipette. Therefore, experience has taught that while many atlases provide good starting points for stereotaxic placement, actual coordinates need to be refined in successive experiments; it is very difficult to hit exactly the same point in different brains, even using the same coordinates; and placements must always be confirmed histologically.

Coordinates also may be used to indicate locations in the brain, as an alternative to structural names. This has the advantage of avoiding problems with nomenclature, but at the same time has the very severe limitation of imparting very little information when used alone, because the

numbers themselves have little mnemonic value for most workers. Nevertheless, a mathematical description of brain space is essential for quantitative, computer aided determinations of lengths, areas, and volumes; for the accurate construction of 3-D computer graphics models; and for sophisticated queries of graphical databases, which require spatial indexing (assigning coordinates to all points) of all maps (Dashti et al. 1996).

In addition to the rat brain atlases already mentioned (Paxinos and Watson 1986; Kruger et al. 1995) the reader may also wish to consult those by DeGroot (1959), König and Klippel (1963), Wünscher et al. (1965), Albe-Fessard et al. (1966), and Pellegrino et al. (1979).

C. Changes to the Original Atlas

Three general types of change have been made to the first edition of the atlas. First, new architectonic information has been incorporated. Second, these changes are reflected in the annotated nomenclature tables, whose general organization has been improved. And third, the accuracy of the dorsoventral and rostrocaudal alignment of the atlas sections has been improved, and the schematic midsagittal drawing used to indicate section location (figs. 1 and 10) has been redrawn based on the new alignment of the atlas drawings themselves. The third type of change has resulted from preliminary work on 3-D computer graphics models, and also includes modification of the outlines for Levels 29, 39, and 72, and other minor changes.

The following structures have been added or modified significantly: amygdalar capsule, bed nucleus of the stria medullaris, external capsule, lateral septal nucleus, lateral tegmental nucleus, nucleus incertus, periaqueductal gray (including commissural and precommissural nuclei),

parapyramidal nucleus, nucleus reuniens, lateral agranular retrosplenial area, septohippocampal nucleus, trigeminal and vestibular ganglia, and tuberal nucleus.