

*This file accompanied the compact disc inserted in an envelope on the back cover of the book.*

for *Brain Maps: Structure of the Rat Brain (3rd edition)*

## **CD-ROM User's Guide**

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b. *Brain Maps, Computer Graphics Files 3.0 /COMPLETE*

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a. Rat CNS flatmap 3.0

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B. Abbreviation Lists (Microsoft® *Word*)

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2. *Developmental Brain Maps* (Alvarez-Bolado & Swanson 1996)

C. Archival material (Adobe® *Illustrator* files)

1. *Brain Maps I*, shaded drawings (Swanson 1992, for book)

2. *Brain Maps, Computer Graphics Files 1.0* (Swanson 1993, from floppy discs)

3. *Brain Maps II, Computer Graphics Files 2.0/Map only* (Swanson 1998-1999, from CD-ROM)

D. *User's Guide* for CD-ROM (Adobe® *Acrobat*)

## **CD-ROM User's Guide**

### **I. System requirements**

A computer with Adobe® *Illustrator 10* and Microsoft® *Word* installed and running efficiently is required. A free tryout version of *Illustrator 10* can be downloaded from the Adobe Website.

### **II. Overview**

This CD-ROM contains a variety of *Interactive Brain Maps*—computer graphics files that can be used for many purposes such as learning about the structural organization of the brain, mapping experimental results, constructing 3-D models, and creating databases of spatial neuroanatomical information. Because the files are based on vector graphics they can be modified easily by users, are almost infinitely scalable, and have a relatively small file size.

The heart of this material is an *Interactive Brain Maps* atlas. Atlas Levels 1-73 in section VII of the book were derived from these computer graphics files. Two versions of the *Interactive Brain Maps* atlas are provided. One version is *complete*, and features bilateral maps, a Nissl-stained section on one side, a list of abbreviations, two sets of coordinates—stereotaxic and physical, and a simple yellow mask. Because these features are placed in different transparent overlays or layers, they can be viewed in any combination and order desired by users. The other, simplified, version is designed for *mapping* experimental results, and only contains the bilateral drawings of the brain, along with an alignment box for databases and 3-D modeling.

Layers are an extremely important feature of the *Interactive Brain Maps*. Layers are perfectly aligned transparent overlays, and they can be viewed in any combination and

rearranged in any order. This powerful feature allows one to separate information (for example, the results of different experiments, or different features of the atlas such as abbreviations, cell groups, and fiber tracts) in useful, creative ways, and then view or hide, lock or unlock, and delete or add individual layers. As one works with these complex drawings, the value of layers becomes increasingly obvious, especially when taking advantage of the ability to move graphical elements between layers, and to duplicate information in more than one layer.

#### **IV. Using downloadable Adobe *Illustrator*® files**

Microsoft® *PowerPoint* has introduced most scientists to the potential usefulness of computer graphics. However, in this day and age the creation, manipulation, and publication of neuroanatomical information requires a working knowledge of more powerful graphics software. The downloadable files provided here were created with Adobe® *Illustrator 10* and Adobe® *Photoshop 7*. Essentially identical versions of these applications are available for the PC and Mac. Adobe® *Photoshop* is designed primarily to work with bitmapped (rasterized) images such as videophotos or scanned images and films, whereas Adobe® *Illustrator* is designed to create vector-based drawings, which may be combined with placed bitmapped art (for example, from *Photoshop*) for the construction of illustrations for *PowerPoint* presentations, printing, 35 mm slides, and publication. There are several other popular computer graphics applications—including Macromedia® *FreeHand 10*, Deneba® *Canvas 8*, and CorelDRAW® 11—and Adobe® *Illustrator* files usually can be opened and worked on in them (contact the manufacturer if procedures are not obvious).

*The Adobe® **Illustrator tutorial** is an essential and enjoyable prerequisite for those not comfortable with the program.*

**Always work with copies of the files.** It is essential to keep at least one copy of the original, unaltered files.

#### ***A. Tips on making illustrations***

The general philosophy of using computer graphics applications for producing neuroanatomical illustrations is outlined in section IVB3 of the book. The following discussion is based on using Adobe® *Illustrator 10*, although the general principles apply to other popular applications.

It is necessary at the outset to learn how to use the *layers manager or palette*. Layers are like a perfectly aligned stack of transparent overlays. Their stacking order can be changed at any time, they can be viewed in any combination by hiding individual layers, and any combination of layers can be chosen to print. One obvious use would be to place the results of a tract tracing experiment in a layer over an atlas template, or the results of two tracing experiments in two layers over an atlas template (with the results of each experiment in a different color; see files in A4). It is important to remember that opaque objects in a layer “block out” or obscure all objects in layers below them in the stacking order, just as in a stack of transparent overlays. This must be considered when changing the stacking order of layers. It is also useful to know that objects can be moved from one layer to another simply by selecting the object (or objects) and moving the resulting dot in the layers palette to the desired layer. Similarly, an object(s) in one layer can be duplicated (select, copy, paste in front) and then moved to any desired layer, so the same object appears in two layers.

*Drawing* in a layer is, of course, a basic operation or skill that is usually the most frustrating part of learning a computer graphics application. Like pen and ink drawing, it requires practice,

and can always be improved. Curved lines in *Illustrator* are based on creating Bezier curves, which are smooth and mathematically defined (explaining why they are essentially infinitely scalable without loss of resolution).

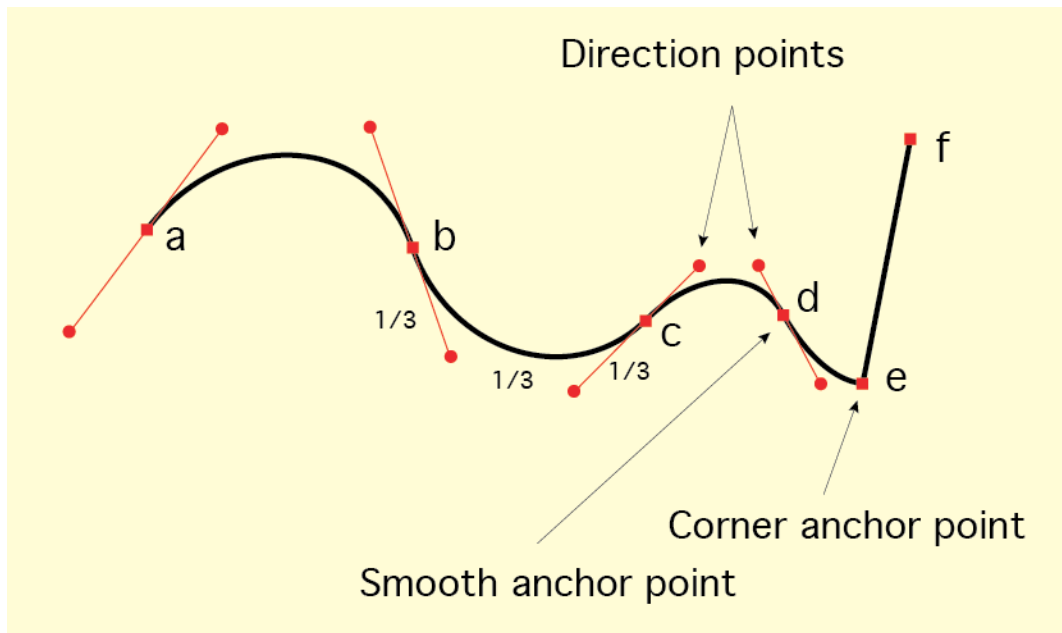


Figure 1. Anatomy of a path drawn with the pen tool.

To learn how to use the *pen tool* for drawing, first read the *Illustrator User's Guide*. Basically, curved lines (paths) are drawn by creating a series of *smooth anchor points*, each with a *direction line* on either side, and a *direction point* on the end of each direction line (Figure 1). These points and lines are used to edit in a very precise way paths that have already been drawn. Several rules help produce the most esthetically pleasing drawings. (1) Use the minimum number of anchor points consistent with the desired line. This requires practice, and planning ahead while drawing. It is essential that one does not try to draw too fast; think deliberately about what you are drawing. It is instructive to see how *Illustrator* draws a circle. To do this, use the circle

tool, and then select it to see the anchor points, direction lines, and direction points. Note only four anchor points, and equally spaced direction points. In general, use fewer anchor points on less curved regions of a path. (2) Keep facing direction points for adjacent anchor points on the same side of a path. And (3), try to follow the “thirds rule”: in the space between two anchor points, each direction line, and the distance between the two direction points, should be approximately equal (that is, each should be about a third of the distance). This is perfectly shown in the way *Illustrator* draws a circle.

Paths can also contain *corner anchor points*. The path in Figure 1 was drawn with six anchor points, four of them smooth (a-d) and two of them corner (e, f). Corner anchor points are created by clicking (rather than clicking and dragging) and are used to create angles in a path (use the rectangle tool to create a rectangle or square and note that it consists of only four corner anchor points).

Learn how to use all of the tools in the *toolbox palette*. They are used for editing paths, as well as for scaling, magnifying, rotating, reflecting, and skewing; for placing and modifying text; and so on. Also learn how to change line thickness and color, and how to create dashed lines.

The *pencil tool* is useful for drawing irregular lines (such as PHAL-labeled axons), although you will note that even these paths are actually Bezier curves that *Illustrator* creates for you, and may thus be edited like paths drawn with the pen tool. Although it takes essentially no practice to use the pen tool, it is also not appropriate to use when drawing smooth paths.

Symbols (circles, squares, and so on) from the *Symbol font* are usually best to use for items showing the location of labeled neurons and so on. Although they require more memory than symbols created with *Illustrator* paths, they maintain their relative positions when maps are rescaled.

For most routine drawing, it is helpful to *show two Adobe® Illustrator 10 palettes along the top of the Window*: one in the upper left with the *Layers, Character, and Paragraph* subpalettes, and the other in the upper right with the *Stroke, Color, and Swatches* subpalettes.

### ***B. Permission to use the illustrations***

While these files have broad applications, their use in published figures should acknowledge that they are based on *Brain Maps: Structure of the Rat Brain*, third revised edition (Swanson, 2004). No other permission is required. [Note added in 2015: This material is now open access and simply licensed under a Creative Commons Attribution-NonCommercial 4.0 International License; basically, for noncommercial use only, and please cite source of illustration].

### ***C. Interactive Brain Maps (Adobe® Illustrator 10 files)***

#### *1. Adult rat brain atlas*

There are three files or sets of files dealing with the Atlas. One file is called *Atlas levels (sagittal)*, and it is a midsagittal view of the rat brain showing the location of the 73 Atlas Levels (Fig. 10 in the book). The two sets of files contain the individual atlas levels themselves. *Brain Maps, CGF 3.0/COMPLETE (Brain Maps, Computer Graphics Files 3.0/COMPLETE)* is the third edition of *Brain Maps, Computer Graphics Files* (Elsevier, Amsterdam, 1993; included in archival material, below) and is a truly interactive atlas, with bilateral maps, accompanying photomicrographs of the Nissl-stained sections, a list of abbreviations, two coordinate systems, and a simple yellow mask; and *Brain Maps, CGF 3.0/Map only*, which contains only bilateral atlas maps for creating neuroanatomical data overlays. The drawings are all at the same scale, are in register, and are designed to fit on a piece of US letter size (8.5 x 11 in.) paper when printed.



Individual atlas levels in *Brain Maps, CGF 3.0/COMPLETE* are easy to use when opened in Adobe® *Illustrator 10*, with the *Layers* palette showing and extended to display all nine layers (after opening a file, select *Fit in Window* in the *View* menu). Simply hide and show different combinations of layers. When printing, remember that you have the option to print or not to print each layer. It is also simple to move different components of the atlas drawing into different layers. This is easy because different components are *grouped*, and can be selected with the *selection tool* (black arrow, upper left). Create a new layer and move the selection to it. The *atlas* drawing layer has the following groups, which must be stacked in the following order of layers (from bottom to top): (1) cell group outlines, (2) fiber tracts (shaded or *filled*) and on top of them fiber tract subdivisions (lines or *paths*), (3) brain outlines and ventricles, and (4) abbreviations.

The *yellow mask* layer just below the *atlas* layer is useful when one atlas level is placed partly over another in a composite illustration because the mask blocks out the part of the bottom atlas level that lies directly underneath the top atlas level (Figure 2). The mask is a *compound path*, so that the ventricles are like holes in the tissue section that are not masked. Of course the fill color of the mask can be changed to anything desired, including most often white for publication.

Individual atlas levels in *Brain Maps, CGF 3.0/Map only* are much simpler; they consist only of the atlas layer of *Brain Maps, CGF 3.0/COMPLETE* (and thus have no mask), as well as an alignment box layer. They are very convenient for straightforward mapping of neuroanatomical data in a new overlying layer or layers.

*Cropping* part of an atlas level in *Brain Maps, CGF 3.0/Map only* for a final illustration is relatively straightforward. The easiest way to start involves creating a rectangle of the appropriate size and location, *locking* it; and then using the *scissors tool* to cut lines in the atlas

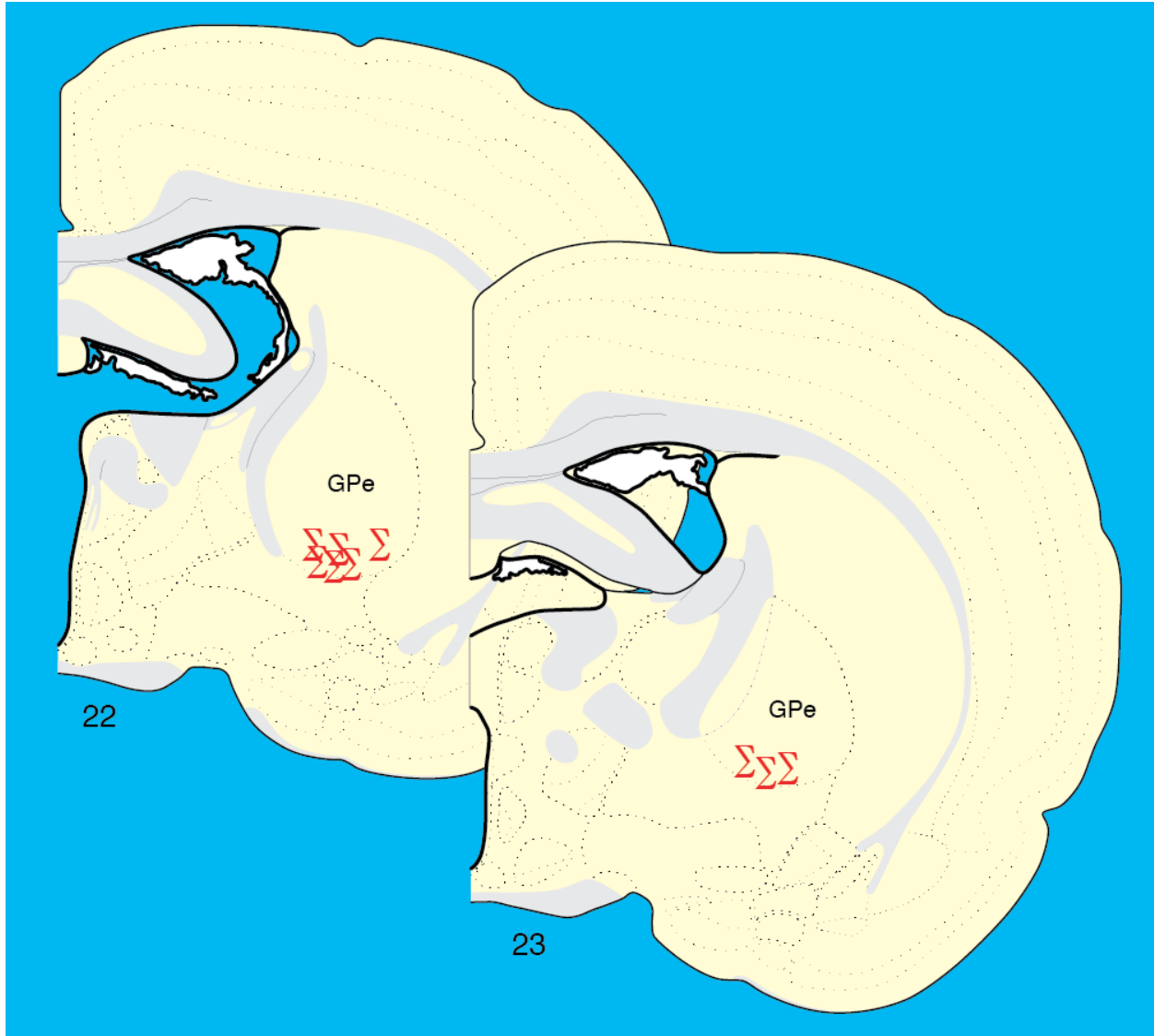


Figure 2. Use of mask (yellow) in stacking atlas levels.

drawing along the border of the rectangle. Then select lines and text outside the rectangle and delete. The only problem arises when certain oddly-shaped filled objects (fiber tracts) are cut in such a way that the fill pattern is disrupted. When this happens, some redrawing (usually forming a closed path at the corner of the locked rectangle) is necessary (Figure 3); this is especially easy if the fiber tracts are placed in a separate layer for editing, and then returned to the atlas layer

(between cell groups and brain outlines/ventricles groups; the stacking order of selected objects, such as fiber tracts, can be altered from the *Arrange* submenu in the *Object* menu).

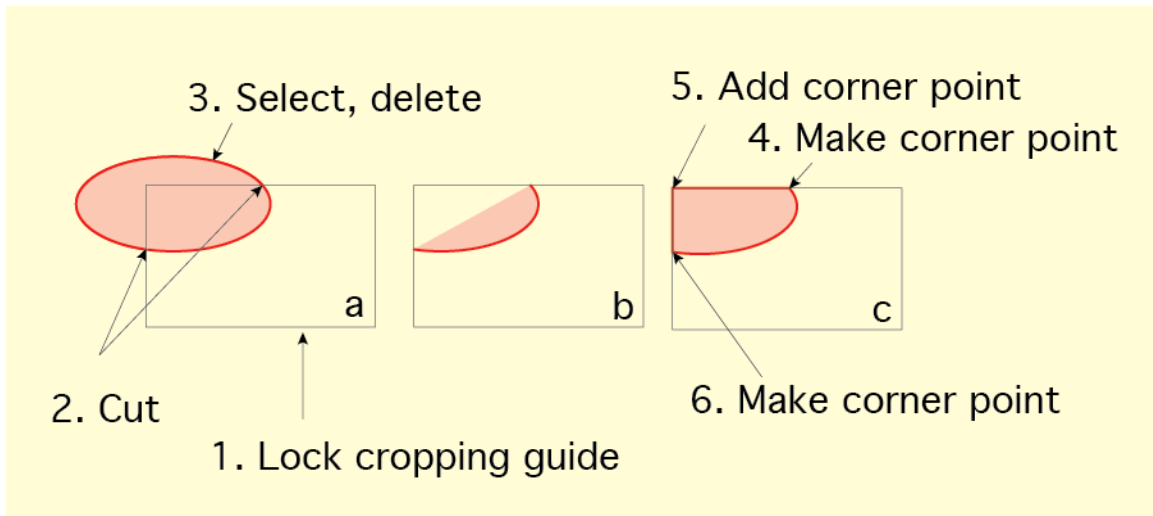


Figure 3. Method for cropping a filled object.

Note that all atlas levels in both *Brain Maps, CGF 3.0/COMPLETE* and *Brain Maps, CGF 3.0/Map only* have a layer called *box for database/3D model*. This layer contains a bounding blue rectangle, which is a *database fiducial*. It has two purposes. First, it is perfectly in register along the *z*-axis of the brain for all 73 Atlas Levels. Taken together, they can be thought of in three dimensions as forming the sides of a rectangular box around the brain. And second, the blue rectangle should be copied to data layers that are used in neuroanatomical graphical databases; it assures perfect alignment of the data layers (in the absence of the atlas layer, for example)—hence the name “database fiducial” (see section IVC in the book and section 4b below).

## 2. Flatmaps

The purpose of these design aids is to provide standardized templates for illustrating all neural systems in a uniform way (see section V in the book, as well as the frontispiece and poster, which were created with the Rat CNS flatmap). *Rat CNS flatmap 3.0* is an updated, computer graphics version of Figure 7 in the first edition of *Brain Maps: Structure of the Rat Brain* (Elsevier, Amsterdam, 1992), which did not contain pathways. Open this file in Adobe® *Illustrator 10*, show the *Layers* palette and all of its contents, and then begin near the bottom of the *Layers* palette to show and hide the various layers to see what they contain (general contents are indicated by the name in the *Layers* palette).

The *Rat CNS flatmap 3.0* file contains most of the major known pathways in the rat, placed in a standardized position. This version of pathway placement over the flatmap should be considered provisional—as a “work in progress” that will undoubtedly benefit from refinements in the future.

To create a circuit diagram, view the *SIMPLE OUTLINE (for pathways)* layer, and hide all the others. View a layer containing pathways of interest. Select, copy, and paste in front, then move duplicated pathways to a *NEW DRAWING* layer. Fine tune the ends of pathways with the pen tool, show cell groups of interest (e.g., by a circle created with the symbols font), arranging the circuit in an aesthetic way (choose appropriate line thicknesses, colors, and so on). Repeat this process until you have all of the pathways necessary for the circuit diagram, and refine the overall design. Labels are provided in the *footprint abbreviations* layer. Near the end, copy your *NEW DRAWING* layer(s) and an appropriate outline layer to a new file (lock all other layers and *select all*), where components may be placed in layers as desired, and the diagram can be printed from a much smaller file.

The approximate location of atlas sections through the cerebral cortex is shown in relation to the *Rat CNS flatmap 3.0* in a file called *Cortex sections on flatmap* (this drawing is 250% larger than the flatmap itself), as well as in a layer of the *Rat CNS flatmap 3.0* file. The idea here is to provide a qualitative way to map the distribution of neuroanatomical data onto a flattened representation of the cerebral cortex. Variations in the contour pattern are a reflection of curvature on the surface of the intact cerebral cortex. Each line in the contour map is derived from the cortical surface of a particular Atlas Level (as indicated).

A flatmap of the rat hippocampal formation (*Unfolded hippocampal formation*) is also provided. Its shape is not derived from the *Rat CNS flatmap 3.0*, but rather from the surface of a three-dimensional model of the hippocampal formation (Swanson et al. 1978). The original map has been modified in light of more recent data (Petrovich et al. 2001) and understanding. As with the above files, different types of information are in different layers.

Finally, a simple *Human CNS flatmap 1.0* file is provided for comparison with the rat. This file was used to produce Figure 2, and the accompanying poster, in Swanson (1995). The major feature of interest here is a flatmap of Brodmann's areas in the cerebral cortex.

### *3. Determining section angles*

It is very useful for mapping to have a good estimate of how the plane of section of an experimental brain compares with that of the standard atlas plane of section (see Figure 7 in the book). The solution presented here is graphical. Assuming that the midline of the experimental sections is set to vertical, there are two degrees of freedom: a dorsoventral difference in plane of section, and a mediolateral difference in plane of section. The former is seen as a difference in the sagittal view, whereas the latter is seen in a dorsal (used here) or ventral view of the brain.

The basic principle is to determine how particular pairs of fiducial points differ between the atlas and an experimental brain.

The two files presented here are designed to determine plane of section for structures located in or near the interbrain. For other parts of the brain, the user should determine appropriate fiducials (see section IVB2 in the book, and the file *Sagittal & dorsal brain views*).

*Interbrain section plane (practical example)*. This file is a 150% enlargement of the interbrain region from the file *Sagittal & dorsal brain views* (which displays the same fiducial points). It contains sagittal and dorsal views of the interbrain region of the atlas brain (which was embedded in celloidin, and thus shrank considerably; see section IIIA in the book) with the series of Atlas Levels superimposed. It also contains sagittal and dorsal views of a brain prepared by frozen sectioning (the atlas brain was scaled to approximate the coordinates in Paxinos and Watson, 1986; see section IIIA in the book).

The basic principle is to align a regularly spaced Nissl-stained series of sections from the experimental brain (frozen sections) on the frozen-sectioned brain outlines here, basing the alignment on the position of fiducial points. A series of lines representing the experimental series is constructed to scale (using the rulers), and then rotated relative to the standard series, based on the position of the fiducials. This is a graphical solution that does not require mathematical calculations, although the angles can of course be determined.

One practical strategy to determine the plane of the experimental brain is first to determine how many sections from the experimental series fit between two widely-spaced fiducials, such as the rostral tip of the suprachiasmatic nucleus (SCH) and the caudal end of the mammillary recess (V3mr). This number of sections is fitted between the two fiducials (e.g., by moving, rotating, and scaling the *sample frozen section series* provided in the Layers palette) in the drawing of the

*MIDSAGITTAL VIEW*. Second, to determine the “dorsoventral” error in plane of section, observe which section in the experimental series passes through a dorsal fiducial (e.g., the caudal anterior commissure, ac), and which section passes through a fiducial more or less ventral to the first (e.g., the rostral SCH). Rotate the group of experimental sections to reproduce these relationships. If necessary, rescale the set of experimental sections so that the correct number of sections still stretches between the rostral and caudal fiducials determined in the first step. One now sees how the experimental series is oriented relative to the atlas sections. And third, to determine the “mediolateral” error in plane of section, repeat the above process using the drawing of the *DORSAL VIEW*, with appropriate fiducials. Obviously, error will be reduced by using fiducials that are as far apart as practical (see section IVB in the book).

#### 4. Database prototype

A web-based browser for neuroanatomical data mapped onto *Brain Maps*, CGF 3.0 is under development (see Dashti et al. 1997, 2001). The two files in this folder show how data layers from this database could be used in the layer manager of Adobe® *Illustrator*. The basic idea is discussed in section IVC of the book. Note that file *Sample data layer (Level 19)* contains only the data itself, and a blue bounding rectangle, the database fiducial, which is found in every atlas level (in the layer called *box for database/3D model*). It can be copied, pasted in front, and moved to any other (data) layer.

#### **D. Other downloadable files**

Several other useful file sets are provided on the CD-ROM. First, lists of abbreviations are provided for this edition of *Brain Maps*, and for our atlas of the developing rat brain. Together,

they constitute a systematic, internally consistent nomenclature (with abbreviations) for all stages of rat brain development, and the adult brain.

And second, three folders are provided in the *Archival material* section. One folder contains the files of *Brain Maps, Computer Graphics Files 1.0* (Elsevier, Amsterdam, 1993) which were originally supplied on four floppy discs; the second folder contains the original files used to print the shaded drawings in *Brain Maps: Structure of the Rat Brain* (Elsevier, Amsterdam, 1992), and the third folder contains the *Computer Graphics Files 2.0/Map only* files, supplied originally on CD-ROM.

**These files must not be confused or mixed with *Brain Maps, Computer Graphics Files 3.0* supplied above.** They are provided for historical reasons, so that interested readers can compare changes in the atlas from the first to the second to the third edition (for example, a considerable amount of data was published using the first two editions as templates).