II. On Mapping the Brain

A. What is a Map?

> *The very best map-reader has to suffer some shocks when he comes face to face with reality.*
>
> —*Josephine Tey*

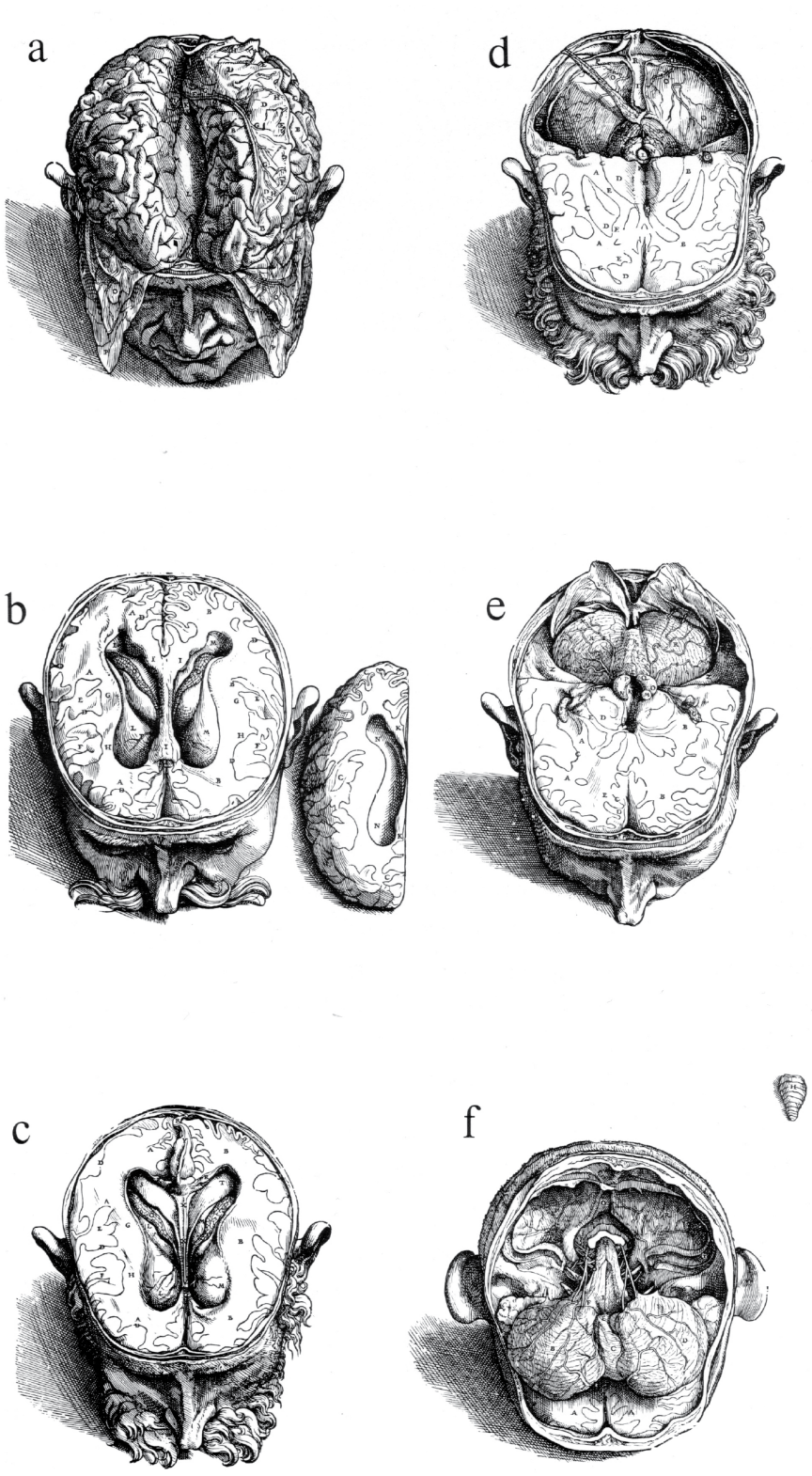
Maps are so familiar that we seldom pause to reflect on the assumptions that underlie their production. However, it is useful to bear in mind from the outset the principle advantages and limitations of maps, which are usually thought of as representations of some part of the environment on a flat surface (see Robinson and Petchenik 1976). The most important feature of this concept is the word *representation*, because it emphasizes the fact that a map is by definition an abstraction, often from three dimensions to two.

The main advantages of maps are obvious: three-dimensional objects can be represented in two dimensions for publication; large or small objects can be scaled to convenient sizes; and, perhaps most importantly, the essential features of a complex object can be represented in a simplified, abstract way. The most obvious disadvantages of maps are that they cannot by their very nature reproduce all of the details contained in the mapped object, and that they can impart false or misleading information if errors are made during the process of abstraction. In the end, the usefulness of a map is a function of its accuracy, clarity, and ability to display a particular type of information.

Text fig. 1. This medieval view of brain structure and function was published in 1537 by the well-known professor of mathematics and medicine at Marburg, Johann Eichmann, also referred to as Dryander (from Clarke and Dewhurst 1972).

The classical problem in cartography—displaying the surface of the earth (which is an approximate sphere) on a sheet of paper—is well-known. The problem of mapping a highly irregular object like the brain is much more difficult, in part because, unlike conventional cartography, the interior of the object must also be represented (like a geological representation of the concentric strata forming the earth), and in part because the interior features are irregular (not concentric).

This is certainly not the place to review the history of attempts to map the nervous system. However, it is of interest that the first known brain maps were drawn in the middle ages as very abstract, two-dimensional views of the head to show the location of the brain ventricles, along with what was assumed to be their critical functions in mental life (text fig. 1; see Clarke and Dewhurst 1972). The first great medical book of the Renaissance, Vesalius' *De Humani Corporis Fabrica* (1543), contains a brilliant series of three-dimensional drawings or maps (his plates 66-72) that together constitute an atlas of the human brain, which may be viewed as slices are progressively removed from dorsal to ventral (text fig. 2). The style developed by Vesalius has of course been refined over

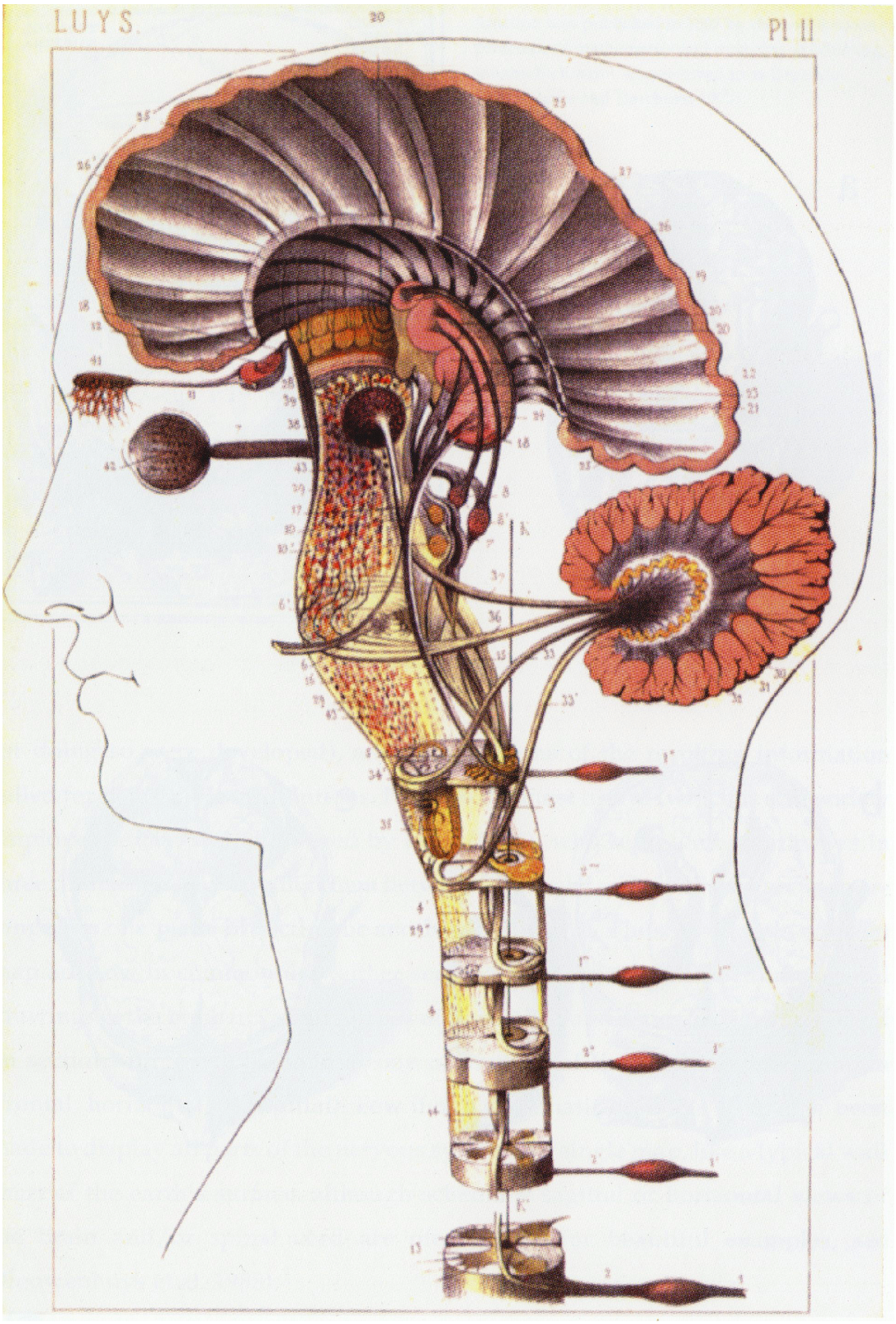


Text fig. 2. This series of woodcuts was assembled from the first modern book in biology (De humani corporis fabrica libri septum), written by Andreas Vesalius and published by J. Oporinus at Basel in 1543. It constitutes the first realistic views—as well as the first atlas—of brain structure.

the years, but it remains the cornerstone for displaying the gross anatomical distribution of gray and white matter, and the major features that can be discerned within and around them.

By the 19th century, neuroanatomists had become preoccupied with the tracing of fiber tracts between various parts of the gray matter (as sophisticated methods for doing so were developed), and presentations of the resulting information called for new graphical solutions. Perhaps the most useful (which is still widely employed today) was pioneered by Luys (1865), who represented pathways in three dimensions by drawing them between a series of widely spaced sections arranged in one plane of section or another (text fig. 3). Thus, two kinds of brain map are now in common use (either separately or together): three-dimensional drawings of the brain in various stages of dissection, and series of drawings based on sections through the brain in one or another of the three standard planes (frontal, horizontal, or sagittal). Few if any systematic attempts have ever been made to display all parts of the nervous system in a single map, like a typical wall chart of the earth's surface, although schematic sagittal or horizontal views of the brain and/or spinal cord are often used (for beautiful examples, see Nieuwenhuys et al. 1988).

This brings us to the sets of maps presented here. Since a map is a representation or abstraction, there are multiple ways of transforming an object like the brain from one coordinate system to another, depending on the intended use of the map. The atlas of the adult rat brain presented here was designed with three major uses in mind. First, it is intended to summarize current views on the gross morphology of the rat brain, including boundaries of the major cell groups (whether nuclear or laminated) and the location of major fiber tracts. Second, it may be used as a template for presenting neuroanatomical data in a standardized format. And third, it



Text fig. 3. This drawing by Luys (1865) was the first attempt to combine cross-sections with long pathways in the central nervous system, a style that is now widely used to illustrate the three-dimensional organization of neuronal circuitry.

may be used as a starting point for the construction of three-dimensional computer graphics models of the brain.

Another set of maps was designed to present in a schematic way the major stages of nervous system development common to mammals, and in fact rather similar throughout the vertebrate series as a whole. These maps are useful because they serve to clarify the basic design principles of the nervous system, starting at the neural plate stage, and progressing through the formation of the neural tube with its three, and then five, primary brain vesicles. The developmental approach is also useful for those unfamiliar with what are commonly regarded as the basic subdivisions of the nervous system, and how their names have emerged from continuously evolving embryological and other concepts.

And finally, a single map of the rat central nervous system containing virtually all of the cell groups outlined in the adult atlas has been derived from the embryological atlas. This highly schematic map is essentially a "best guess" fate map of the rat neural plate, which is a flat, spoon-shaped epithelium that is only one-cell thick (pseudostratified). As more experimental information becomes available, and such "world maps" are refined, they may come to serve the same general purpose as an atlas of the brain, except in two dimensions. Thus, a global map of the nervous system could be used to summarize neuroanatomical data and to construct models of its circuitry.

These and other brain maps should ultimately allow us to illustrate the architecture of the brain and to understand its basic design principles more clearly.

B. The Rat Brain

Much more is known, and is being learned, about the structure and chemistry of the brain in the rat than in any other animal. Many of the reasons for the popularity of the rat in neurobiological and behavioral work have been summarized in a delightful book written by S.A. Barnett (1963), but economy and small size are important factors, along with the fact that these animals have a relatively smooth cerebral cortical mantle, as opposed to the highly convoluted mantle found in many larger species. Two major disadvantages associated with the use of rats come readily to mind. First, the organization of the rat brain is obviously not identical to that of the human brain. Therefore, the clinical relevance of neuroanatomical information obtained in the rat should be confirmed in human material, which often may not be possible for ethical reasons; and conversely, certain important problems like the neurobiology of language may be difficult if not impossible to study in the rat. And second, the types of genetic analyses that can be carried out in mice will not be possible anytime soon in rats for practical reasons. On the other hand, the mouse brain is often too small for critical analysis with available experimental neuroanatomical techniques.

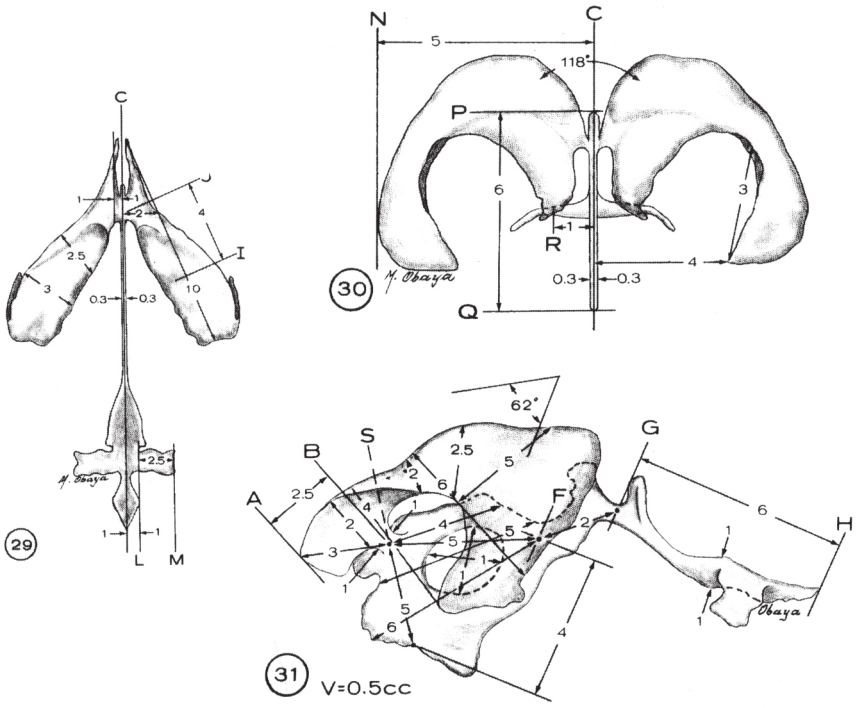
The general organization of the adult rat nervous system, as well as the parts of the body that it innervates, has been summarized thoroughly by Greene (1968) and by Hebel and Stromberg (1986), and the work of Donaldson (1924) contains a wealth of information about the changing size of various organs and major subdivisions of the central nervous system during the course of development

While our primary concern in this book is with the disposition of the major cell groups and fiber systems in the rat brain, certain other features should be mentioned for the sake of completeness. To begin with, the central nervous system of a 315 g adult male rat (the size and

sex of the rat used for our atlas) weighs on the order of 2.7 grams, with the brain contributing about 2.0 grams and the spinal cord about 0.7 grams (Donaldson 1924). Furthermore, the central nervous system is completely surrounded by connective tissue sheaths (*the meninges*), contains a fluid-filled central cavity (*the ventricular system*), and has a rich *blood supply*. The general principles of *cerebrospinal fluid* (CSF) production by the *choroid plexuses*, and its flow through the ventricular system and *subarachnoid space*, as well as the flow of blood through the central nervous system, are similar in all mammals, and are reviewed in most textbooks of human neuroanatomy (for good accounts see Carpenter and Sutin 1983, and Williams et al. 1989). The central nervous system does not, of course, have a true *lymphatic system*; instead, the function of this system is generally thought to be subserved by the cerebrospinal fluid.

There are certain specializations or differences associated with these structures or systems in the rat itself. Nothing remarkable about *the meninges* in the rat has been reported; their general disposition is described by Zeman and Innes (1963), Greene (1968), and Hebel and Stromberg (1986). The shape of the *ventricular system* has been described in detail by McFarland et al. (1969), Westergaard (1969), and Jarvis and Andrew (1988); according to McFarland et al. (1969), it contains approximately 0.5 ml of cerebrospinal fluid in the adult, although the accuracy of this measurement is difficult to assess (text fig. 4). The *vascular system* of the rat central nervous system has not been the subject of detailed, systematic investigation. For general accounts of the major arteries and veins supplying the rat central nervous system, as well as the general distribution of capillaries, see Craigie (1920), Zeman and Innes (1963), Brown (1966), Greene (1968), and Hebel and Stromberg (1986). An introductory guide to more detailed accounts of particular regions would include the following: spinal cord (Tokioka 1973;

Tveten 1976); brainstem and cerebellum (Craigie 1933; Moffat 1957); diencephalon and pituitary



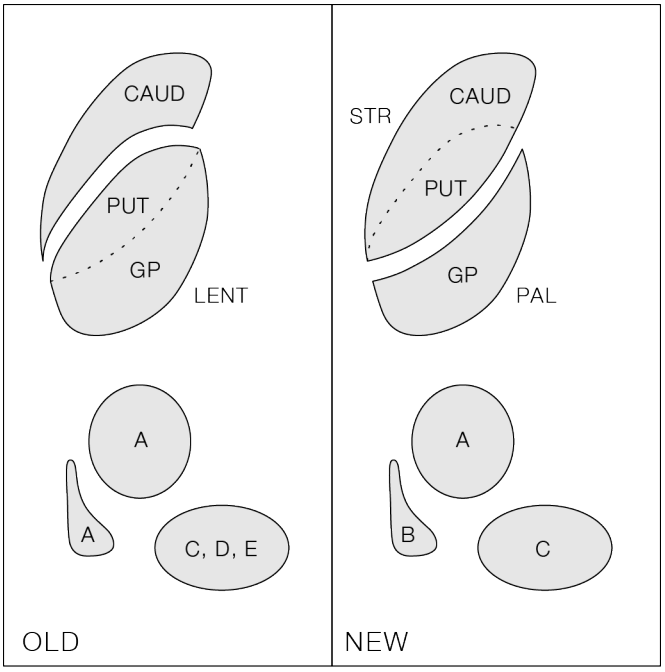
Text fig. 4. The ventricular system of the rat brain as viewed from the top (upper drawing), from the front (middle drawing), and from the side (lower drawing); measurements are in mm (from McFarland et al. 1969).

(Ambach and Palkovits 1979); septum (Ambach et al. 1975); amygdala (Merksz et al. 1978); and cerebral cortex (Craigie 1921, 1932; Eayrs 1954).

C. On Nomenclature

The vocabulary used to describe the structure of the brain has evolved over the course of more than 2,300 years, since the first great body of work on comparative anatomy by Aristotle. However, this nomenclature is still changing rather quickly and has not in practice been standardized, unlike that for most other parts of the body (*Nomina Anatomica* 1983; Staubesand

and Steel 1988; O'Rahilly 1989). There are many reasons for this, but fundamentally, many parts of the brain have been identified and delineated on the basis of considerably less than complete information, and as new methods are developed and more work is done, our understanding of its basic components has changed, and will continue to change. In fact, a very good argument can be made for the position that attempts to enforce a standardized nomenclature for the brain are counterproductive because they inhibit work designed to understand the true structure of the brain, and encourage the perpetuation of current misunderstandings. In short, it is essential that neuroanatomical nomenclature remain flexible. Having said this, it is equally important to emphasize that not all data and names are equally valid.



Text fig. 5. Schematic view of how neuroanatomical nomenclature may change over time; see text for details. Abbreviations: A, B, C, D, and E, hypothetical names for indicated cell groups; CAUD, caudate nucleus; GP, globus pallidus; LENT, lenticular nucleus; PAL, pallidum; PUT, putamen; STR, striatum.

Before describing how the nomenclature used here was selected, it is important to consider in more detail the nature of certain problems associated with the ultimate development of a standardized nomenclature for the nervous system (text fig. 5). Five types of problem come readily to mind. First, there is the problem of synonyms: virtually every structure in the brain has been referred to in a number of different ways at one time or another. In theory, the solution to this problem is trivial, but in practice the scholarship needed to establish thorough, accurate lists of synonyms is a difficult exercise and has rarely been practiced (for example, see Billings-Gagliardi et al. 1974;

Valverde 1977). Second, the same name has been used for entirely different structures. Third, the boundaries of a particular structure are often placed differently by different workers, or differently by the same worker in different papers; and similarly, the subdivision of a particular structure often varies. For example, more than 20 different lamination schemes for the primary visual area of the cerebral cortex were reviewed by Billings-Gagliardi et al. (1974). One obvious consequence of changing the boundaries of one structure is that the boundaries of neighboring structures must also change in a corresponding way. Another likely consequence is that the properties of regions enclosed by different borders are not identical. Fourth, two very different structures may be incorrectly grouped together under one name. A well-known example is the now obsolete "lenticular nucleus", a term introduced by Burdach (1819-26). This "nucleus" includes the globus pallidus and putamen, and it is now clear on architectonic and connectional grounds that the putamen and caudate nucleus form part of one structure, the striatum, whereas the globus pallidus is a major component of the pallidum (see Tables A and C). The term "lenticular nucleus" does refer to a gross anatomical feature of the human brain, but it is rapidly and rightly going out of use. And fifth, new structures may be discovered, and this, of course, necessitates a redefinition of all neighboring structures as well.

From a very practical standpoint, it is easy to appreciate why these problems retard progress in neuroanatomy, and why they will actually become more serious as workers rely increasingly on key words in computer-assisted literature searches. However, they also raise two important questions: first, how are structures identified, and borders drawn in the brain; and second, how should names for structures be chosen?

The identification of structures—and for now we shall limit the discussion to discrete cell groups—is a process that has evolved greatly over the years, but at the present time most

workers agree that a combination of architectonic and connectional criteria must be taken into account. A cell group may be laminated or not (in which case it is usually referred to as a *nucleus*), and almost always contains more than one type of neuron, in addition to glial cells. What defines a cell group, or one of its subdivisions, is a relatively homogeneous distribution pattern of cell types, or the distribution of these cell types in a recognizable gradient.

The problem faced by neuroanatomists is to define what constitutes a cell type. Again, there is general agreement that neurons with the same pattern of axonal projections and the same set of inputs form a cell type (see Cajal 1909-11), although it is possible that in the future certain biochemical features may need to be added; for example, the differential expression of neurotransmitters or receptors for neurotransmitters or hormones may serve to distinguish subsets of anatomically-defined cell types. For technical reasons, it is often very difficult to establish the existence and complete distribution of neuronal cell types.

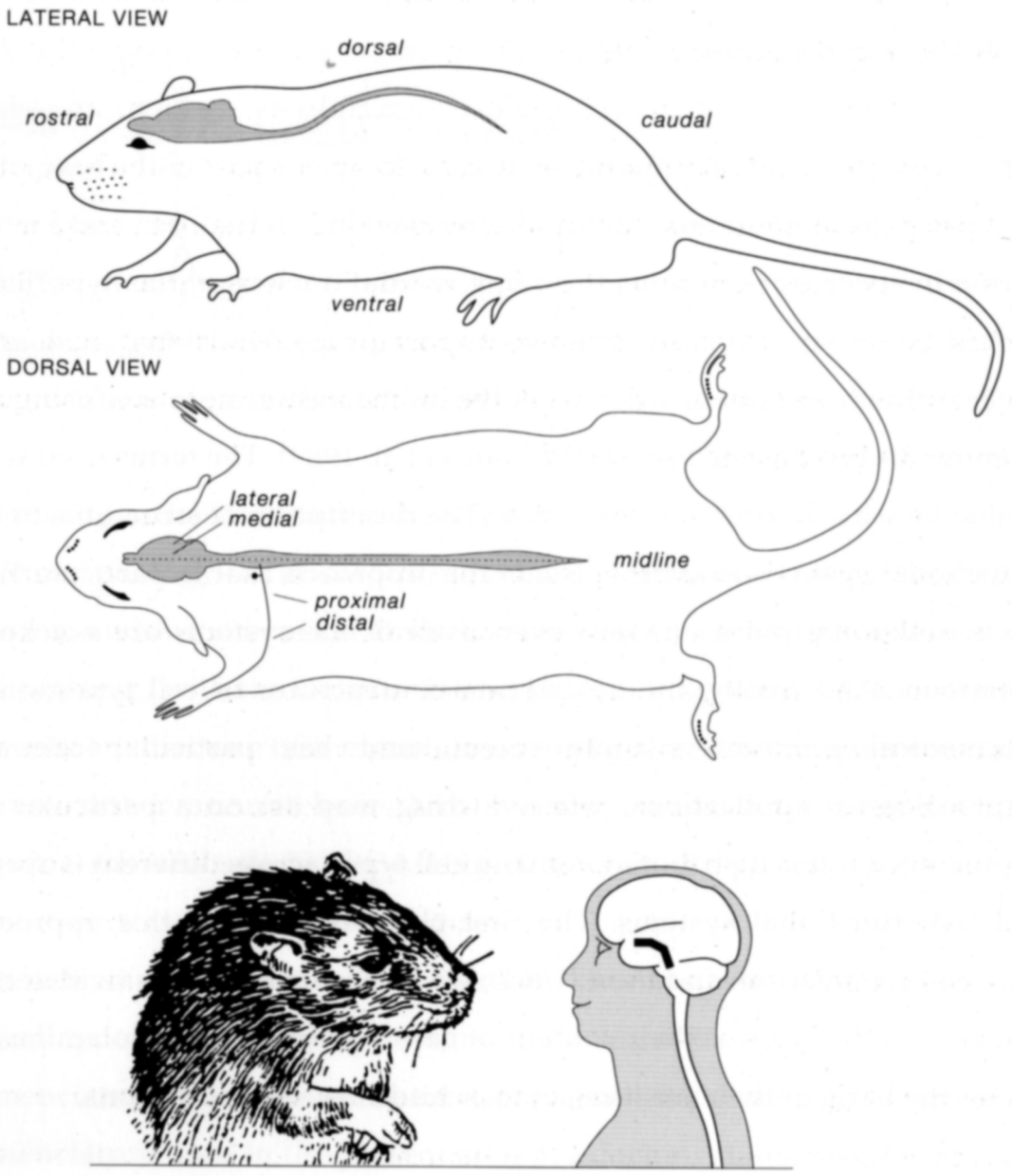
The second question that needs to be dealt with is: how should names for structures be chosen? The overriding principle to bear in mind here is that neuroanatomical nomenclature is part of a language that is meant to convey spatial (and often functional) information; therefore, it seems obvious that nomenclature should be as simple, descriptive, unambiguous, and applicable across species as possible. With over a thousand named structures in the brain, it is little wonder that occasional, well-intentioned attempts to name regions of the brain in an "objective" way with numbers or letters have virtually always failed because they convey little or no positional, structural, or functional information, which is important for mnemonic purposes.

In dealing with all of the problems associated with developing the most useful nomenclature, it might seem best just to apply the criteria of simplicity, descriptiveness, clarity, and generality mentioned above. However, there is a strong belief among taxonomists that

historical precedence should also play a major role in determining what name is used. In practice, this criterion is also frequently surrounded by controversy: should precedence be granted to the first illustration, the first mention in the text, the first name, or the first really adequate description?

The nomenclature adopted here was guided by the principles that the best names are simple, descriptive, unambiguous, and applicable across species, and that when several synonyms are in common use, that with historical precedence should be favored. However, the field is so complex and requires so many subjective judgments that no two professional neuroanatomists agree on all or even most aspects of nomenclature and parcellation. And it must be admitted that historical precedence alone is not necessarily a useful criterion; logic must ultimately prevail. The best example of this is the development of today's widely accepted nomenclature for the thalamus (Berman and Jones 1982): so many nuclei are involved that it is now parcellated successively into divisions, groups, nuclei, and subnuclei (and then cell types within subnuclei). The important point here is that consensus has emerged in naming many thalamic nuclei: word order is determined by the parcellation order just referred to. For example, the small-celled part of the posterior nucleus within the ventral group of the dorsal division is known as the *ventral posterolateral nucleus, parvicellular part*; a functional synonym (which is more convenient) is the *thalamic gustatory nucleus*. Wherever possible, we have tried to apply the above word order rule to the nomenclature adopted here, and to do so in English. In addition, we have sometimes resorted to functional synonyms, particularly in the cerebral cortex, where they are rather widely used.

In closing this section, it may be useful to point out that the structures of the brain may be described systematically in three ways, in terms of topography, systems, and neurochemistry. In



Text fig. 6. Fundamental terms used to describe positional information in the embryonic and adult (top two drawings) central nervous system of fish and quadrupeds. As indicated below, human terminology is complicated by a right angle bend in the axis of the brain, due to our typical upright posture; this is not to say, however, that the rat central nervous system cannot undergo changes in its axis during the course of normal behavior. (Rat drawing from Barnett 1963).

topographical descriptions, which are the least informative, structures are merely related to the major part of the brain in which they reside, be it forebrain, midbrain, hindbrain, and so on, and/or to their position in space (for example, the ventromedial nucleus of the hypothalamus). It would be convenient if a common set of terms for describing locations in the vertebrate nervous system were to evolve; in the meantime, a confusing array of synonyms and so on is in use (see Williams et al. 1989). The terms used wherever possible here are outlined in text fig. 6. The description of structures in relation to functional systems is another venerable approach that is particularly useful from a functional point of view, even if all of the systems are not known or understood. And finally, the description of structures or cell types strictly on histochemical grounds is quite recent, and has particular relevance to pharmacological applications, where a drug may act on a particular type of receptor, even if it is distributed in many cell types across different (superficially unrelated) functional systems. The first clear example of this approach was provided by Dahlström and Fuxe (1964), who classified neurons (with letters and numbers) on the basis of their content of catecholamines or indolamines, rather than on the basis of their position, cytoarchitecture, or connections.

D. How to Use this Atlas

> *It is not sufficient to search, ferret out, take notes, become familiar with things and publish what you have been able to unearth in your delvings, but you must first know your material thoroughly and then exercise selection in reducing it and displaying it in a definite and clear manner.*
>
> *—Bernhard Siegfried Albinus*

As already mentioned, the major purpose of the atlas presented here is to summarize what is currently known about the location of major cell groups and fiber tracts in the rat brain, in a series of maps that may also be used as templates for the presentation of other neuroanatomical data.

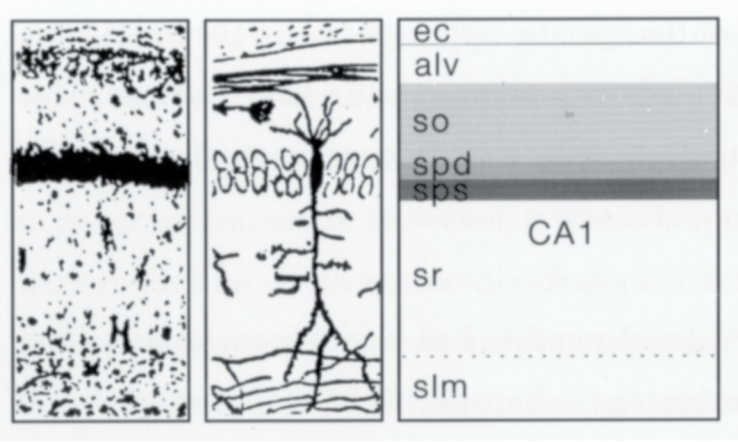
The maps are based on what can be observed in the simplest, most reliable neuroanatomical preparations, which require no exotic reagents: tissue sections stained with the Nissl method and viewed under the microscope with brightfield and darkfield illumination (e.g., Wolf 1971; Appendix A). The Nissl method relies on the use of basic dyes that stain nucleic acids; under brightfield illumination, the preparations reveal what is referred to as the cytoarchitecture of the brain, whereas darkfield illumination reveals the myeloarchitecture of the brain.

Cytoarchitecture. Because neurons are secretory cells with a well-developed rough endoplasmic reticulum, the Nissl method is commonly used to determine the general size, shape,

staining intensity, and distribution (location and packing density) of neuronal cell bodies in tissue sections: the field of cytoarchitectonics. When used alone, this approach is of very limited value because it does not reveal dendritic morphology or the disposition of axons. However, when the results of connectional and histochemical studies, as well as those dealing with neuronal morphology (e.g., with the Golgi or intracellular filling methods), are correlated with the cytoarchitecture of a region, it is usually possible to recognize specific cell groups rather accurately in Nissl-stained preparations alone (for good discussion, see Lorente de Nó 1934, his p. 166).

Nevertheless, in practice, too little is often known about the distribution of specific cell types to allow the unambiguous placing of borders. This is why maps and nomenclature must evolve. The major reasons for choosing the boundaries and nomenclature used in the present atlas are documented in Section V.

Two additional considerations may be of interest. First, how were the boundaries of cell groups drawn? Since they are based on cytoarchitectonic material, they are drawn around groups of cell bodies, without regard to the distribution of their dendrites, which cannot be determined in this material. For example, in the hippocampus, the pyramidal cell layer is outlined, even though this region contains only cell bodies, not apical and basal dendrites (which reside in other layers; text fig. 7). And second, it must be emphasized that while the photomicrographs accompanying the atlas maps are useful for gaining an impression of the most obvious cytoarchitectonic features of the brain, the resolution is much too low to see why many boundaries in the accompanying maps were drawn. In fact, experience shows that photographs at any magnification almost never convey as much information as can be observed directly under the microscope.



Text fig. 7. left: photomicrograph of a Nissl-stained section through hippocampal field CA1; middle: drawing of a Golgi-impregnated pyramidal cell (h) in this same region, from Cajal (1909/1911); right: how this region is represented in drawings for the atlas (e.g., see Level 35). See List of Abbreviations.

Myeloarchitecture. There are, of course, a number of specialized stains for myelineated and unmyelineated fiber systems (axons), but it is convenient that the principal myelineated fiber systems can be identified readily in Nissl-stained preparations that are viewed with darkfield illumination. Like cytoarchitectonics, myeloarchitectonics is of little value when used alone. However, when the results of experimental pathway tracing methods are correlated with the myeloarchitecture of a region, it is possible to observe in the latter material the approximate location of specific fiber systems (i.e., fibers with a known origin and destination).

It is an unfortunate fact that most of the specific fiber systems in the brain have indefinite borders and are intermingled with other specific fiber systems. This design feature complicates the interpretation of many experimental manipulations of the brain.

Mapping Experimental Results. A novel peptide has been characterized from brain homogenates. Where it is synthesized, what specific fiber systems utilize it as a neurotransmitter, and what is its functional significance? Problems like this can be approached neuroanatomically. *In situ* hybridization and immunohistochemistry can be used to localize cell bodies that synthesize the peptide (and its mRNA), and immunohistochemistry combined with axonal transport methods can be used to plot the course and destination of their axons.

Two problems arise. First, how does one describe the location of stained cell bodies and fiber systems? The most informative way is with reference to previously delineated and named structures in the brain (Tables A through D). As obvious as this may sound, it is remarkable how often it is not done carefully in practice. This is important in relation to the second problem mentioned above: what do the results mean? The initial approach to this problem is also obvious: knowing the precise location of immunostained neurons and associated fiber systems, it may be possible to correlate them with a known cell type, and thus take advantage of a great deal of previous neurobiological research. For example, if large neurons between the granular and molecular layers of the cerebellum were stained, it would seem likely that the peptide is expressed in Purkinje cells, and the search for its functional role would be greatly facilitated.

From a practical standpoint, it is always better to counterstain neuroanatomical preparations of any kind with the Nissl method, or if this is not possible, to prepare an adjacent Nissl-stained series of sections; this allows for a more accurate description of the results in relation to the previous literature. However, it is important for those not familiar with neuroanatomical research to realize that detailed information about the exact location of all borders associated with any cell group or fiber tract can rarely be found in the literature. To identify a structure in practice, it is usually necessary to find the center, where its characteristic features are obvious, and then to establish as well as possible the borders, taking into account the location of neighboring structures; there is no way to avoid the fact that this is a very time-consuming exercise in three-dimensional reconstruction.

On the other hand, it is important to emphasize that while new data should be mapped with respect to the older literature (e.g., the maps in this atlas), it should be mapped accurately, which means that it may well not conform to previously established borders. Perhaps enough

information about a new peptide like the one discussed above would force new boundaries to be established on the basis of a new cell type or types.

Coordinate Systems. The approach taken thus far to describe locations in the brain involves referring to named structures like cell groups, fiber tracts, or parts of the ventricular system. This is like referring to countries and highway systems in geographical maps. However, it is also helpful in some instances to define locations in the brain with a three-dimensional Cartesian coordinate system. The most common use of such coordinate systems today is in stereotaxic surgery, although they will play an increasingly important role in computer graphics models of the brain, where local, non-linear coordinate systems may also prove useful.

Two sets of coordinates are provided for the maps in this atlas. One is a set of physical coordinates related directly to the tissue sections themselves; these are the coordinates on the maps themselves. In this system, zero in the rostrocaudal dimension is the rostral tip of the olfactory bulb; zero in the mediolateral dimension in the midline; and zero in the dorsoventral dimension is the flat surface that the brain is lying upon. The other system is a calculated set of stereotaxic coordinates derived from the atlas of Paxinos and Watson (1986). The rostrocaudal coordinate is given in parentheses after the corresponding physical coordinate, and the other two (dorsoventral and mediolateral) can be obtained with the transparent overlay provided in Appendix B. The derivation of these coordinate systems will be described in the next section. While the accuracy of stereotaxic coordinate systems provided in different atlases varies for a number of reasons, it is helpful to bear in mind that stereotaxic coordinates derived from any atlas are really only useful first approximations for experimental work; they obviously vary as a function of age, strain, surgical technique, and so on, and must in the end be determined empirically.

In addition to the rat brain atlas already mentioned (Paxinos and Watson 1986), 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). All of the problems discussed here will become apparent when comparing these atlases critically.

E. How the Atlas Was Produced

An ideal atlas would present the brain 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 central nervous system (particularly the lower brainstem and spinal cord) changes with head and body movements (see text fig. 6). 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.

The most difficult technical problem 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 purposes because they can be produced rapidly, there is 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 current 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 or fold 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. This problem may be greatly reduced as tape-transfer methods for cryostat sections are refined (Ornstein 1986). Third, small pieces of the brain may be lost in the mounting process, 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 easy to obtain, various pieces of the tissue section are held firmly in place, and (relative to frozen sections) there is little 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 g 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 g 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 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 perpendicular to a line that approximates the base of the brain provides sections that are approximately transverse to the long axis of the central nervous system (text fig. 6).

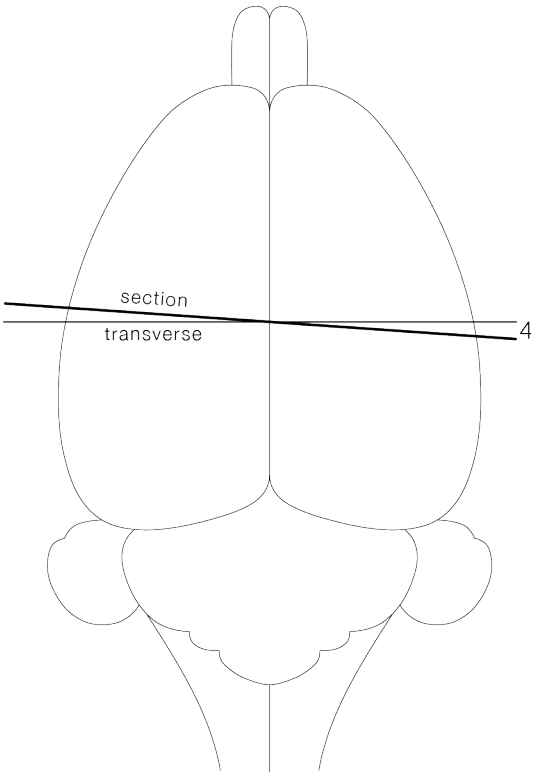
Every section through the brain was collected, stained, and mounted. The first 133 sections (through the olfactory bulbs) were 30 μm thick, whereas the last 423 sections (to the transitional region between the medulla and first cervical segment of the spinal cord) were 40 μm thick. Relatively thick sections 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 the sections were cut. 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 size of our sections was 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).

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) 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. 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° (text fig. 8).



Text fig. 8. The atlas brain was not cut perpendicular to the longitudinal axis; there was an angle of about 4° between the transverse plane and the sections themselves.

The 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" 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 maps 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 were carefully examined before they were drawn. The drawings were printed with an Agfa Compugraphic ProSet 9800 imagesetter with Emerald RIP at 1200 dpi.

It is important to point out that the drawings are not faithful renderings of the sections in one 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) was eliminated in the drawings.

In a general sense, the maps were designed to show regions of gray matter in gray, and regions of white matter in white. Obvious regions of dense Nissl-staining have been indicated with darker shades of gray, although this was done in a very qualitative way with only three shades of gray.

Level Selection. Short of illustrating every section through the brain, a reasonable number of levels must be chosen that nevertheless adequately shows all of the various parts. Since 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 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.