

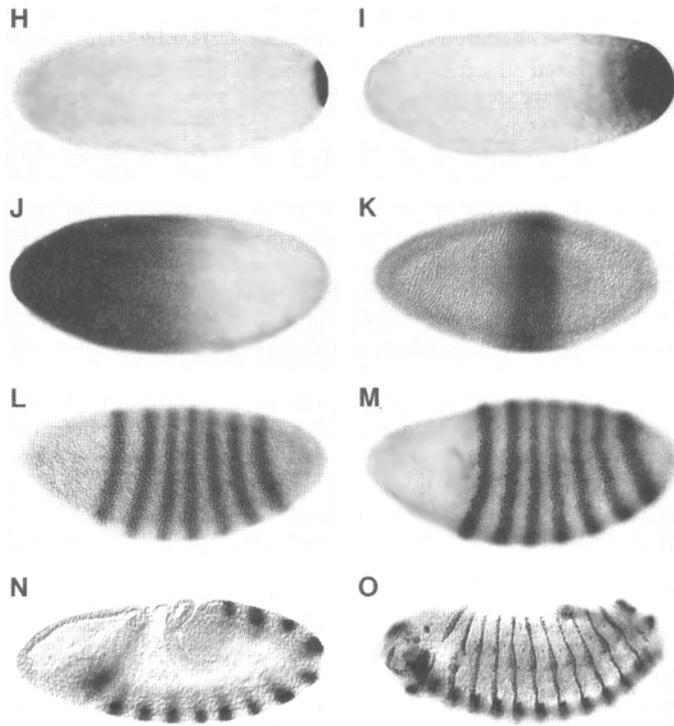
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#### **IV. Mapping Developmental Patterns**

Because the developing nervous system undergoes profound changes in shape and complexity, it has proven unusually difficult to interpret and illustrate experimental results. Ultimately, this problem may be solved largely by the use of three-dimensional computer graphics methods, where an animated model of the growing neural plate and tube would be extremely useful. The recent development of very high resolution magnetic resonance imaging could provide the digital starting point for such an approach.

However, conceptualization of pattern formation is also facilitated by the use of simple two-dimensional flat maps of the developing nervous system, and such maps are useful for summarizing morphological information in the adult as well. Spectacular examples of pattern formation, as reflected in restricted spatiotemporal patterns of gene expression, have been provided by work on the development of segmentation in the fly, where the results are typically presented as photographs or drawings of whole, histochemically-stained embryos that may be interpreted as flat maps (Fig. 15).

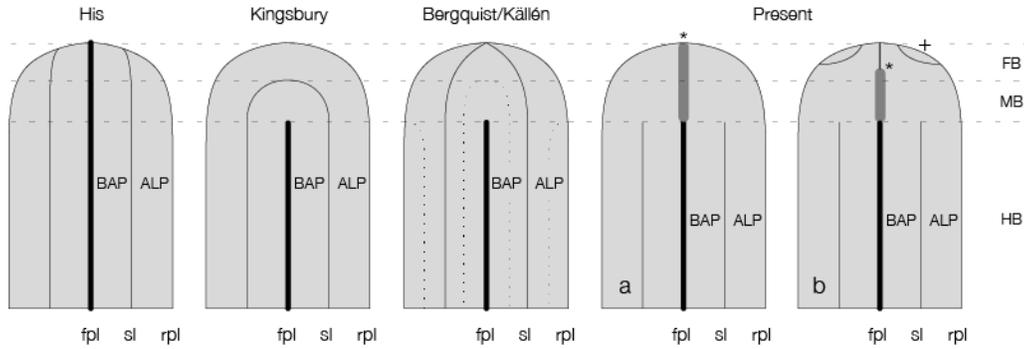


**Fig. 15.** Rostrocaudal pattern formation in the *Drosophila* embryo, as indicated by the expression of various proteins and mRNAs. Spatiotemporal patterns are easy to appreciate in these whole mounts subjected to immunohistochemistry for nanos protein (I), maternal hunchback protein (J), zygotic gap gene Krüppel protein (K), pair rule gene even-skipped protein (L), pair rule gene fushi tarazu protein (M), and segment polarity gene engrailed protein (O); and in situ hybridization for transcripts of the nanos gene (H), and of the segment polarity gene gooseberry (N). (From Patel, 1994).

Attempts to develop flat maps of the nervous system have a checkered history. One major stumbling block is the tremendous growth and fissuration of the human cerebral hemispheres. An early, especially ingenious, approach to this problem was supplied by Gall (see Spurzheim 1826, section *Of the Structure of the Convulsions, and of the possibility of unfolding them*), who realized that the smooth cortical surface in cases of adult human hydrocephalus reflect the basic structural plan, and even attempted to unfold normal hemispheres by inflation. Eerily, the latter has now

been achieved conceptually, using computer graphics methods (Sereno et al. 1995); and other mathematical methods are being used to unfold systematically various more restricted cortical regions (Drury et al. 1996). However, these approaches have not yet dealt with how to attach the unfolded hemispheres to the rest of the brain.

Herrick, in his classic book *The Brain of the Tiger Salamander* (1948), provided a schematic flat map of the adult amphibian central nervous system, which was possible because



**Fig. 16.** Various models of the fundamental plan or fate of the vertebrate neural plate. Very early (a) and slightly later (b) stages of development for the model proposed here (present) are shown on the right, where the asterisks (\*) indicate the site of the infundibulum; the plus-sign (+) indicates the presumptive endbrain; and a thicker gray line in the midline of midbrain (and caudal forebrain) indicates that the floor plate in this region is less distinct, and perhaps different, than that in the brainstem and spinal cord. See text for details. (Three drawings on left modified from Keyser, 1972).

of its relative simplicity: the wall of the brain is thin and the fundamental divisions are not massively enlarged. Thus, the adult brain is similar, to a first order of approximation, to the embryonic neural tube, and is rather easily flattened. This map was used to display schematically the major neural pathways on a common template. A similar approach to the mammalian brain was later taken by Nauta and Karten (1970), although none of this work attempted to provide bilateral maps like those worked out by Nieuwenhuys for the cranial nerve nuclei (see Nieuwenhuys 1974).

An important conceptual starting point for developing bilateral flat maps of the embryonic and adult central nervous system was provided by Keyser (1972) in an attempt to summarize various hypotheses about its fundamental plan (Fig. 16). In essence, he projected these hypotheses onto the early neural plate to yield simple fate maps. This approach was extended to the neural tube stage, and then to the adult, in our atlas of the adult rat brain (Swanson 1992a). In the following discussion we shall examine in more detail the construction

of flat maps for each major stage of neural tube differentiation, and their use in mapping experimental results.

### **A. Major Axes (longitudinal and transverse) and Plane of Section**

The adult brain is almost universally described in terms of three cardinal planes of section—frontal (or transverse), sagittal, and horizontal, although this approach is more successful in animals such as quadrupeds (for example, the rat) and fish with an approximately longitudinal central nervous system, than it is in bipedal animals (for example, the human) where the brain undergoes an approximately 90° bend in the midbrain region (see Swanson 1992a). However, because the longitudinal axis of the neural plate and tube is so convoluted, and undergoes such radical changes during embryogenesis, the horizontal plane is essentially meaningless in histological sections, and the longitudinal and transverse planes need careful definition.

As background for the following discussion, midsagittal views of the developing rat brain tube (the right side) are presented in Figure 14, where the approximate extent of the vesicles is color coded for ease of comparison. In addition, this figure shows in a general way how the longitudinal axis of the brain tube changes during embryogenesis. From this figure it should be obvious immediately that the most straightforward way to describe location in the developing neural tube is in terms of position along the longitudinal axis, and of position in a section transverse to this axis. In common parlance, this translates into sagittal sections for the longitudinal dimension, and relative transverse sections (that is, sections transverse to the longitudinal axis at a particular point). Through at least embryonic day 15 in the rat, ‘horizontal’

sections are essentially meaningless for the brain tube as a whole, although they are more useful within a particular vesicle, for example.

This problem is solved conceptually by straightening out the neural tube, a common textbook approach. In essence, one must imagine unbending the *cephalic*, *pontine*, and *cervical* flexures. When this is done, the standard transverse, sagittal, and horizontal planes of section are again relatively straightforward. Undoubtedly, it will be possible in the future to accomplish this systematically using computer graphics methods. For now we must be content to suggest qualitative approaches to developing idealized and simplified brain maps.

## **B. Flat Maps of the Neural Tube**

As mentioned above, fate maps of the neural plate offer an elegant solution to the problem of constructing central nervous system flat maps. However, even the neural plate is not entirely straightforward because while it is a sheet rather than a tube, it is not flat *in vivo* (see Figs. 2 and 3, and embryonic day 9), and in fact the embryo may be curved such that the midline is not straight. Thus, even the neural plate must be flattened, and the midline straightened, to produce an idealized flat map (Fig. 17, e9).

Cartographers have long dealt with the problem of flattening curved surfaces (see Snyder 1987, 1993). In essence, a curved surface cannot be flattened without *distortion*. There are three fundamental properties associated with the curved surface—*area* of a feature, *distance* between two points, and *shape* of a feature—and only one of the three can be preserved without distortion. This leads to the production of *equivalent* (equal area), *equidistant*, and *conformal* maps, respectively; or to the production of maps that employ compromises between the various distortions. *Gore maps*, which involve cutting the curved surface, reduce overall distortion,

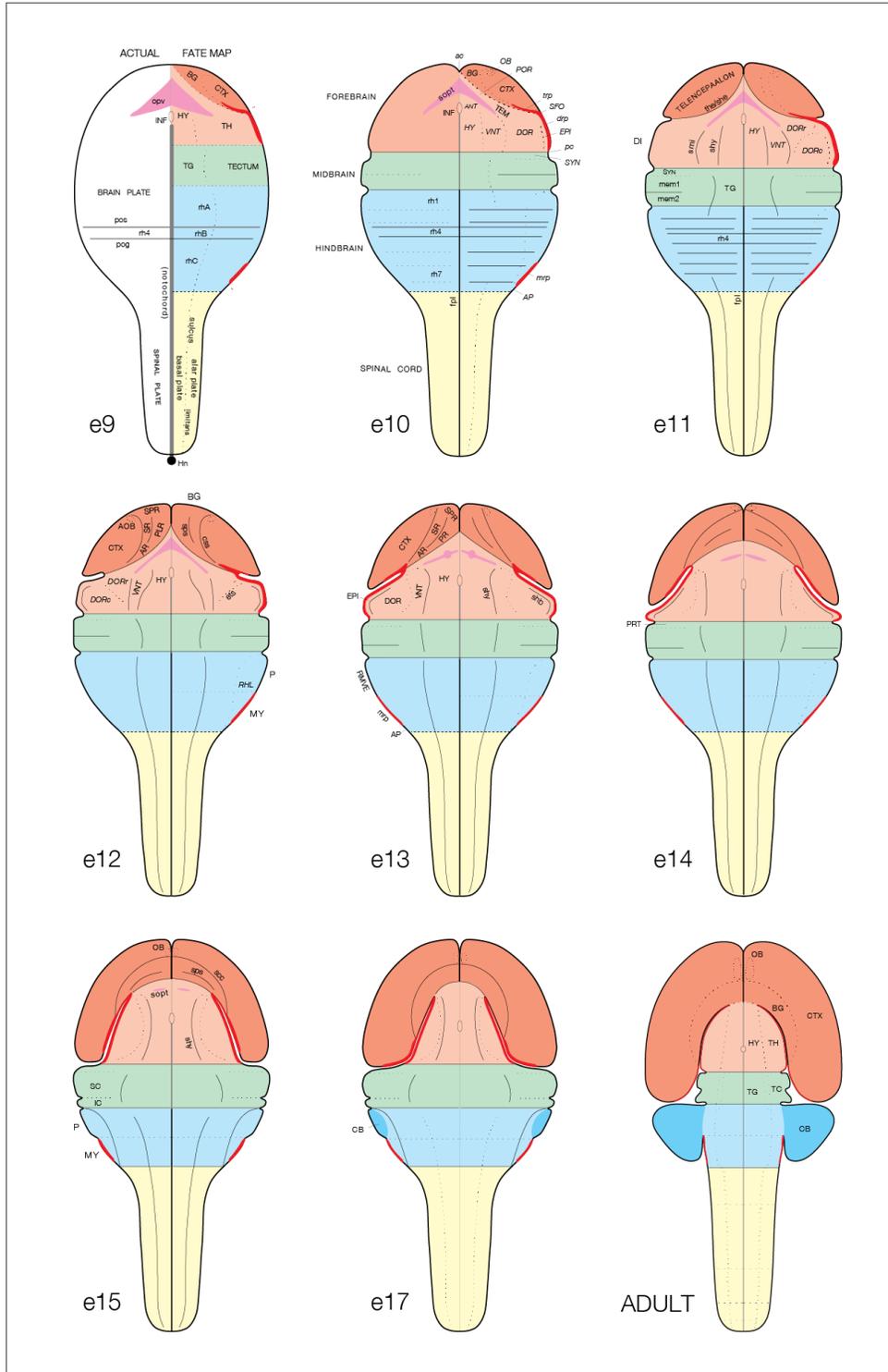


FIG. 17 *CNS Development (bilateral flattened)*

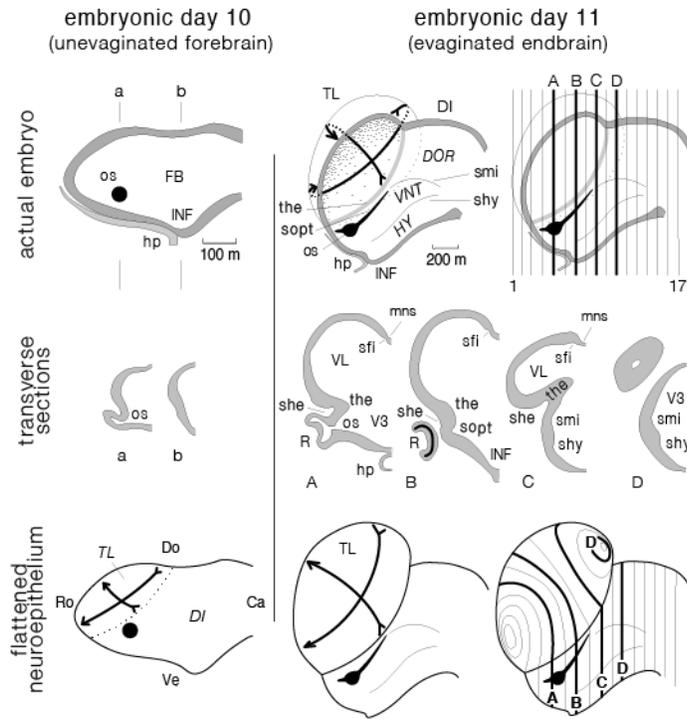
Fig. 17. Schematic overview of rat central nervous system development illustrated on bilateral flat maps. Area of major subdivisions in the flat maps is proportional to corresponding volume in the embryos (see Fig. 14). Adult flat map is based on Swanson, 1992a.

although adjacent points on either side of the cut become artificially separated and overall

patterns are difficult to appreciate. In contrast, *continuous maps* (no cuts) have more distortion but are much easier to interpret (see Appendix Fig. 8 in Alvarez-Bolado et al. 1995). Taking all of this into account, it is easy to see that *map design is inextricably linked to intended use*. Incredibly, over 200 projections of the earth's surface, an approximate sphere, have been described although none are ideal.

*The formal construction of a map* involves placing a *graticule*, mesh, or system of lines on the curved surface and then transforming that graticule *topologically* onto a flat surface, with the caveat that only one line can be represented accurately, although the others can be displayed in an orderly way with respect to it. Longitude and latitude form the common graticule used in the construction of world maps. A possible graticule for the neural plate consists of the following two sets of lines: (a) the location of a series of transverse histological sections (a regular series of parallel lines), and (b) obvious histological features (for example, its lateral border, the floor plate, the optic pit, and the otic rhombomere).

Designing a continuous (uncut), equivalent (equal area) flat map of the early neural tube, at the three vesicle stage, involves another step, but conceptually is still rather straightforward. Because the initial tube—which after straightening is essentially a cylinder with rounded ends—has just formed by fusion of its lateral margins, it can be reflattened by cutting the dorsal midline (the roof plate), which would then constitute the lateral margins of a flattened map, with the floor plate constituting the midline, longitudinal axis. As with the neural tube, the ventral midline is not straight in the dorsoventral plane (it forms the ventral, outwardly convex part of each vesicle), and it must be straightened to form the axis of bilateral symmetry for a flat map (Fig. 17, e10; also see Figs. 3-5 in Swanson 1992a). Again, the area of the flat map is made equal to the surface area of the neural tube, with the reservation that the surface area of the rapidly



**Fig. 18.** Early differentiation of the endbrain (telencephalic vesicle) from the forebrain vesicle. On embryonic day 10 in the rat, the entire surface of the right forebrain vesicle can be viewed from a midsagittal perspective (upper left), and from the length of each histological section in a transverse series (middle left), it is easy to reconstruct a map of the surface (leaving out, as here in lower left, the surface of the optic stalk). However, with the bulging or evagination of the endbrain vesicle, parts of the neuroepithelium become hidden by medial parts of the vesicle and the interbrain (diencephalon). One way to display the entire surface in a flat map is to perform deevagination—stretching the midline rim of the endbrain vesicle to approximate the appearance of the endbrain before evagination (lower left). A method for constructing the flat maps at the lower right from transverse sections at the middle right is illustrated in Figs. 19 and 22. The maps in the lower part of each column are designed to maintain equal areas and topological relationships (indicated by arrows). (From Alvarez-Bolado et al., 1995).

expanding optic stalk and cups is typically not included in the area of the interbrain. In principle, the length of the neuroepithelium as viewed in a series of histological sections can be measured and plotted on the flat map (Fig. 18, embryonic day 10, middle row), with the distance between sections proportional to the surface area of the neural tube between them.

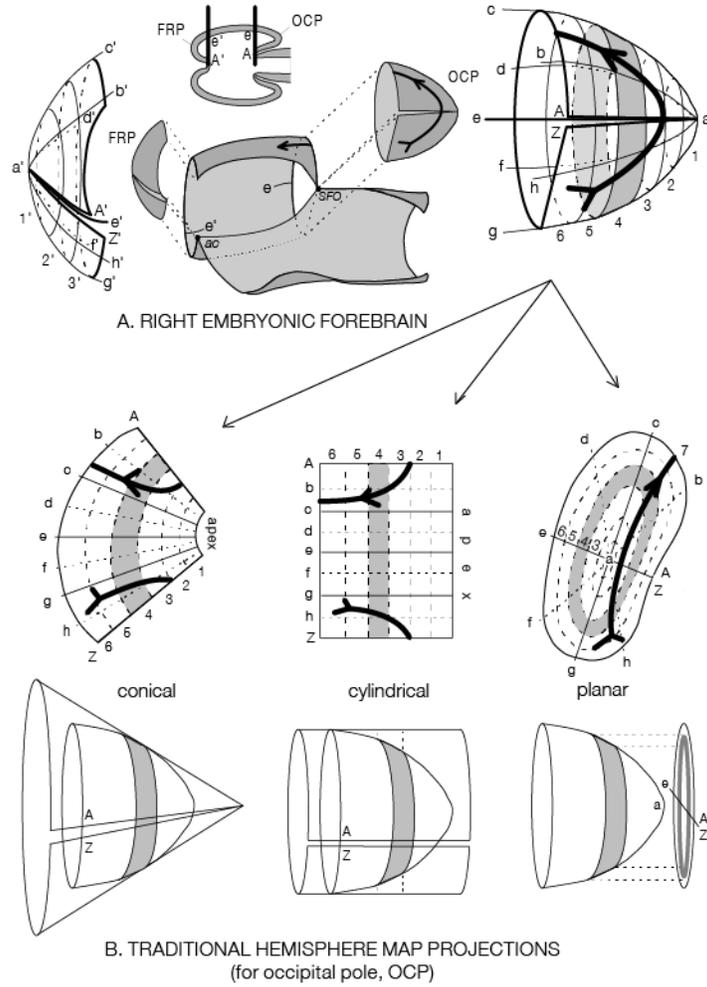
Evagination of the endbrain vesicle presents the next major problem in flattening the neural tube. As shown in Figure 18, as the cerebral hemisphere rapidly evaginates, some transverse sections contain two separate parts, one for the interbrain and another for the endbrain vesicle (Fig. 18, embryonic day 11, section D), although of course

the surface of the forebrain vesicle as a whole is continuous. The simplest way conceptually to flatten the endbrain vesicle at this stage is to perform a *deevagination*—that is, to simulate the previous unevaginuated stage by stretching the midline as illustrated in Figure 18 (embryonic day

11, bottom row), which also indicates the type of distortion involved in this operation. When this is done, the two parts of a section through an evagination fit onto the flattened map (Fig. 18, embryonic day 11, middle and lower rows). As always, the area of the flattened map is equal to the surface area of the neural tube, and now the areas of the endbrain and interbrain vesicles, for example, are equal to the surface area of these two parts of the neural tube. Finally, the midline is straightened, and a bilateral representation of the flattened neural tube may be constructed (Fig. 17, e11).

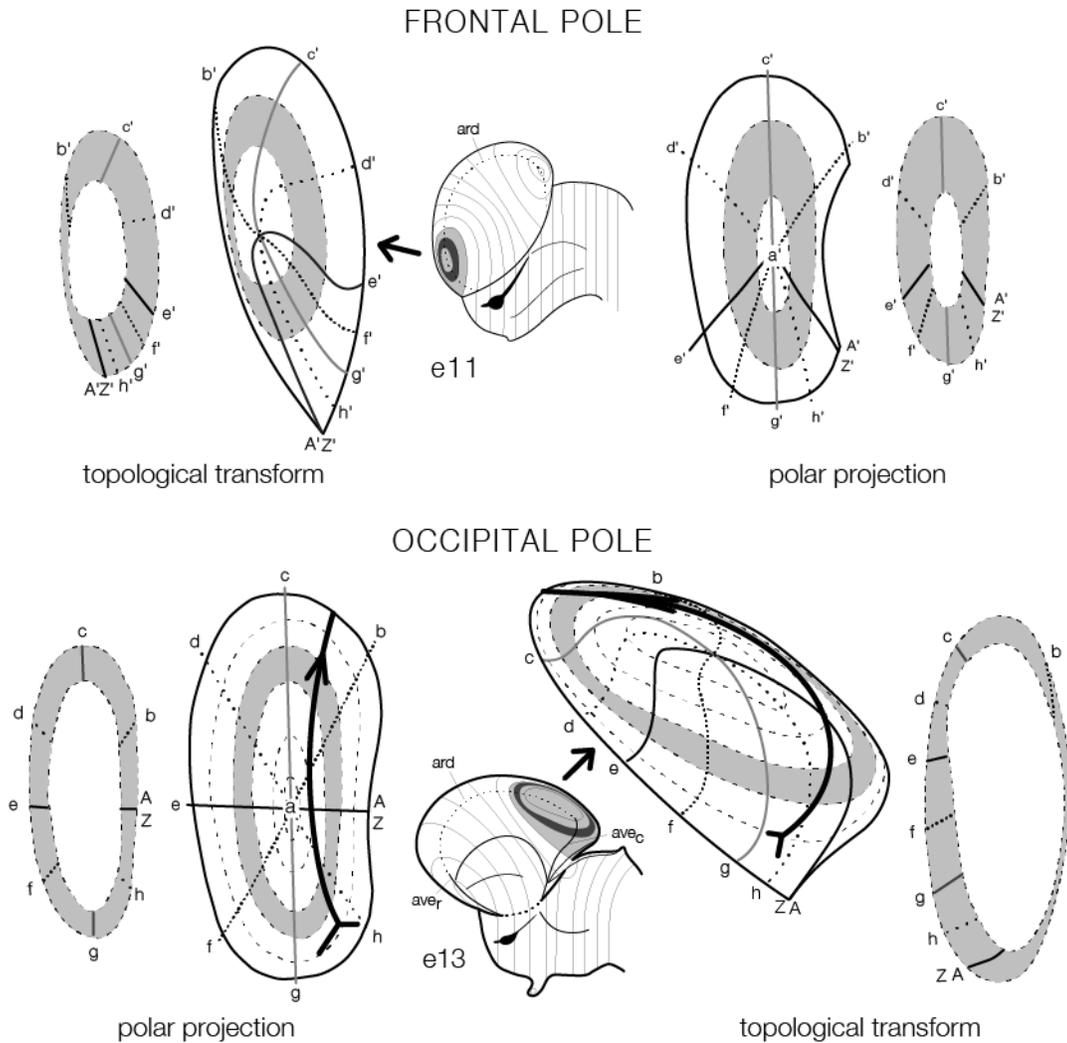
It is important to consider how information from tissue sections can be transferred to flat maps, or more generally, how flat maps can be generated from a series of tissue sections. As the cerebral hemispheres evaginate, the frontal and occipital poles may be viewed initially as approximate hemispheres, with parallels or latitude circles represented by evenly spaced sections (Fig. 19, top). Continuous maps of hemispheres are most easily generated by a planar (azimuthal) projection, like star charts visible from a point on the earth, whereas gore maps can be generated by conical or cylindrical projections (see Fig. 19, middle and bottom, for distortions associated with each projection method). For the equivalent map developed here, the surface area of the planar projection is made equal to the surface area of the original hemisphere (frontal or occipital pole), and the latitude circles (tissue sections) are adjusted such that the surface area between sections is represented accurately.

How are the two hemispheres (frontal and occipital poles) fitted into the complete map, that is, onto the continuous part of the endbrain and interbrain (Fig. 19, top)? The first step involves generating a set of meridians to complete the graticule, although the meridians are imaginary because so few morphological landmarks are available at this stage of development. This graticule allows transformation of the planar hemisphere map to the overall flat map in a



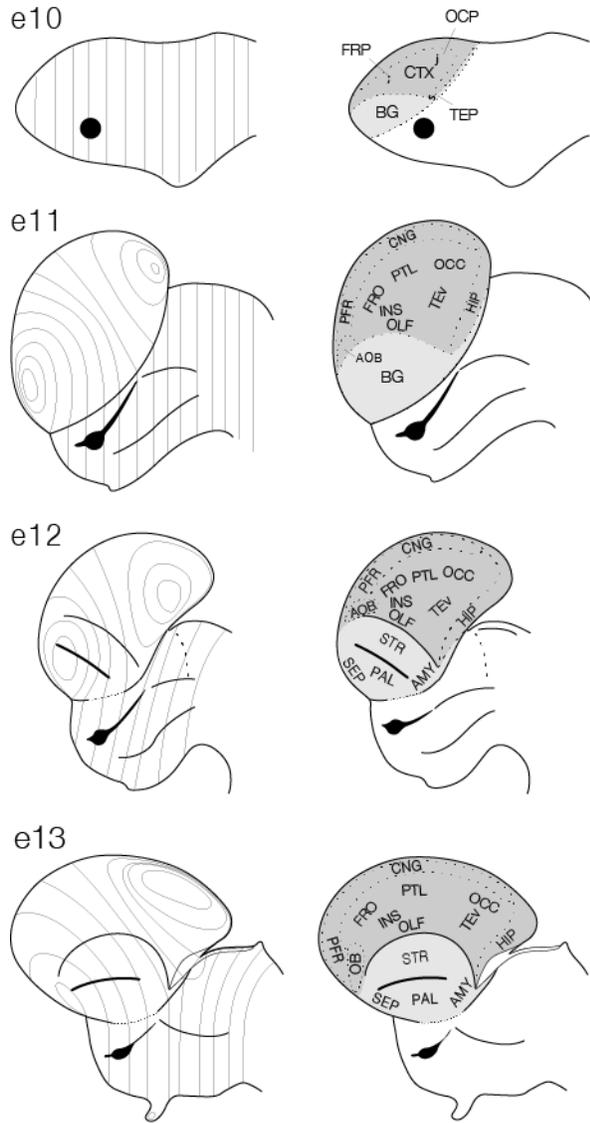
**Fig. 19.** Projection methods for mapping simple evaginations such as the rostral (frontal) and caudal (occipital) poles of the early endbrain vesicle. When cut transversely, the poles are not continuous with the interbrain and may be treated as approximate hemispheres. Thus, it is relatively easy qualitatively to map their surfaces with traditional cartographic methods. Conical and cylindrical projections are useful, although because they must be cut (e.g., at AZ) the resulting maps contain a major discontinuity. In contrast, planar projections are continuous (no cut) because they are projected directly onto a flat surface. In these approximate hemispheres, transverse histological sections provide a series of parallels (between sections 1 and 6), and a series of meridians (AZ-e, b-f, c-g, and d-h) may be constructed to complete a useful graticule. The thick arrows indicate topological transformations. (From Alvarez-Bolado et al., 1995).

smooth, continuous way (see, for example, line e in Figs. 19 and 20). Of course, this process further distorts shape and distance, but maintains equal areas and correct boundary relationships. It is worth noting that the polar maps are attached to the whole flat map at points where the rostral and caudal evaginations become detached from the midline. The resulting flat map can



**Fig. 20.** A method for warping planar projections and tissue sections to fit into a continuous map of the forebrain. These projections and sections need to be warped to produce a smooth, continuous map as indicated by the thick arrows and graticules. If a point is kept fixed (e.g., point AZ, which indicates the presumptive anterior commissure for the frontal evagination, and the presumptive subforminal organ for the occipital evagination), and if the lines b, c, d, e, f, g, and h must run through the rest of the endbrain to link the rostral and caudal evaginations, the distortion rule illustrated here is unique. Note that whereas the shape of each “square” or polygon in the graticule is distorted, its area remains constant. (From Alvarez-Bolado et al., 1995).

then be used to plot data from transverse sections, taking into account distortions of marker position in a section as compared to marker position in the representation of that section within the flat map (Fig. 20). This approach can be generalized to any plane of section through the neural tube (Appendix Fig. 5 in Alvarez-Bolado et al. 1995).



**Fig. 21.** General principles for constructing uncut flat maps of the forebrain from transverse histological sections are independent of age, at least early in development before extensive differentiation of the mantle layer (left). Using such maps, one can indicate the approximate presumptive location of the major endbrain regions (right). (From Alvarez-Bolado et al., 1995).

By extending this general approach, a series of flat maps from the neural plate to the adult can be developed (Fig. 17). Before leaving this section, however, it is important to discuss further parcellation of the flat maps, which is relatively straightforward for cortical structures but problematic for most of the brainstem where nuclei are stacked in the wall of the neural tube. Perhaps the most interesting exercise involves designing a presumptive fate map of the cerebral cortex, based on known adult boundary conditions between areas (for the adult rat and human brain, see Swanson 1992a, 1995). The general approach is illustrated in Figure 21, where the relative position of the standard adult cortical lobes is illustrated. The advantage of using flat maps to illustrate such patterns is shown in Figure 22, where the endbrain vesicle

can be seen from a midsagittal perspective as compared to a flat map.



tangentially to eliminate stacking, both can be represented unambiguously on a flat map. However, if they remain stacked, both occupy the same area in a flat map (the same applies, of course, to the layers of a cortical structure). There are three solutions to this problem. First, separate maps can be used to indicate the position of stacked features; this unsatisfying approach is the only reasonable way to show the various layers of a cortical structure on a flat map. Second, stacked features can be nested, which of course eliminates depiction of actual boundary conditions. However, this is a useful way to illustrate a small structure adjacent to a much larger one (for example the subthalamic nucleus and lateral hypothalamic area, respectively). And third, stacked features may be shifted, which is a common approach that again eliminates natural boundary conditions. Thus, the design of central nervous system flat maps involves a series of compromises, guided by the intended use of the map. For example, *shifting and nesting in flat maps designed to illustrate circuitry are guided by arrangements that allow pathways to be as short, straight, and nonoverlapping as possible.*

### **C. Mapping Experimental Results**

In the adult, it is relatively easy to map experimental neuroanatomical results onto a standard series of drawings. This is not true for embryonic material however. All available atlases, including ours, illustrate very few stages in a continuously growing organ, and the embryos are so small and difficult to mount on the stage of a microtome that planes of section are highly variable. Therefore, quantitative comparisons between embryos are exceptionally difficult, except when very small parts of the neural tube are focused on.

In contrast, displays of information transferred qualitatively from a series of sections to a flat map, using the approach outlined above, can be useful for recognizing broad patterns (Fig.

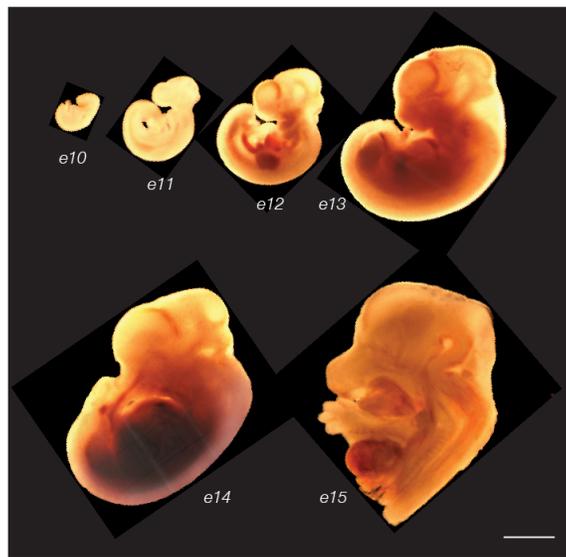
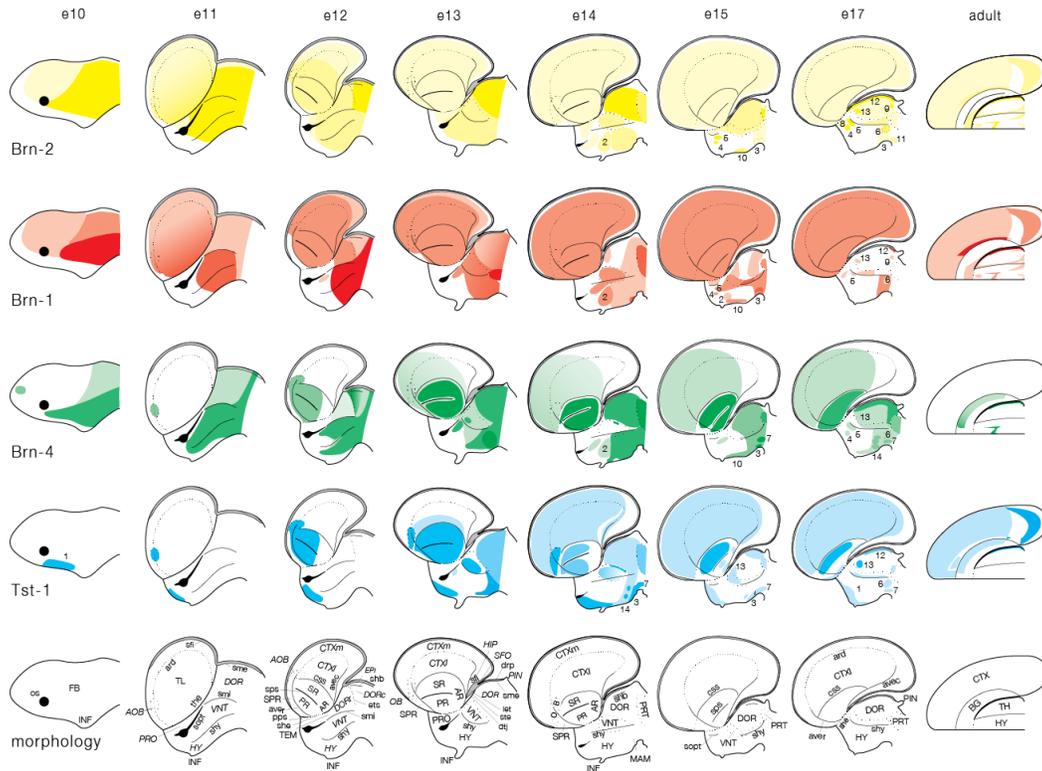


FIG. 23 *Pattern Formation in the Rat Forebrain*

Fig. 23. Patterns of POU-III homeobox gene expression in the embryonic rat forebrain, as illustrated on unilateral flat maps (top). For comparison, photomicrographs of whole rat embryos are shown to scale in the bottom panel. (Top panel from Alvarez Bolado et al., 1995).

23). Experience has shown that recognizing such patterns strictly from a large series of histological sections is an unusually difficult exercise. Of course, when strict, three-dimensional relationships are important, there is no substitute for the illustration of histological sections. Very high resolution MRI technology, now under development, offers a solution to this problem. Volumetric representations of an embryo can be used to reconstruct quantitatively the surface of the neural tube (or neural plate), and this surface could then be flattened systematically when appropriate algorithms are available. Histological sections obtained subsequently from the same embryo could then be aligned with the digital embryo and transformed systematically ('warped') to fit the digital embryo (actual shape). With this correspondence, data from the sections could be transferred systematically to the flat map.