

This file has Chapter III: Later development of major brain regions

- *Forebrain*
- *Midbrain and hindbrain*
- *Differentiation of the midline*

III. Later Development of Major Brain Regions

In this section we shall consider in more detail the further differentiation of major brain regions, as well as structures associated with the midline, as a prelude to the discussion of map design in the next section.

A. Forebrain

After formation of the endbrain and interbrain vesicles, differentiation of the forebrain progresses rapidly, leading to by far the most complex division of the nervous system, responsible for the elaboration of anticipatory behavior. Figure 8 illustrates the spatiotemporal pattern of mantle layer formation in the rat, and from this it is clear that there is no simple way to describe this pattern. The earliest mantle layer is formed in the retrochiasmatic area of the hypothalamus, followed closely by the anlagen of the accessory olfactory bulb in the cortical mantle (for a review of forebrain birthdating literature see Alvarez-Bolado et al. 1995). For the sake of convenience, we shall consider the endbrain first, followed by the interbrain.

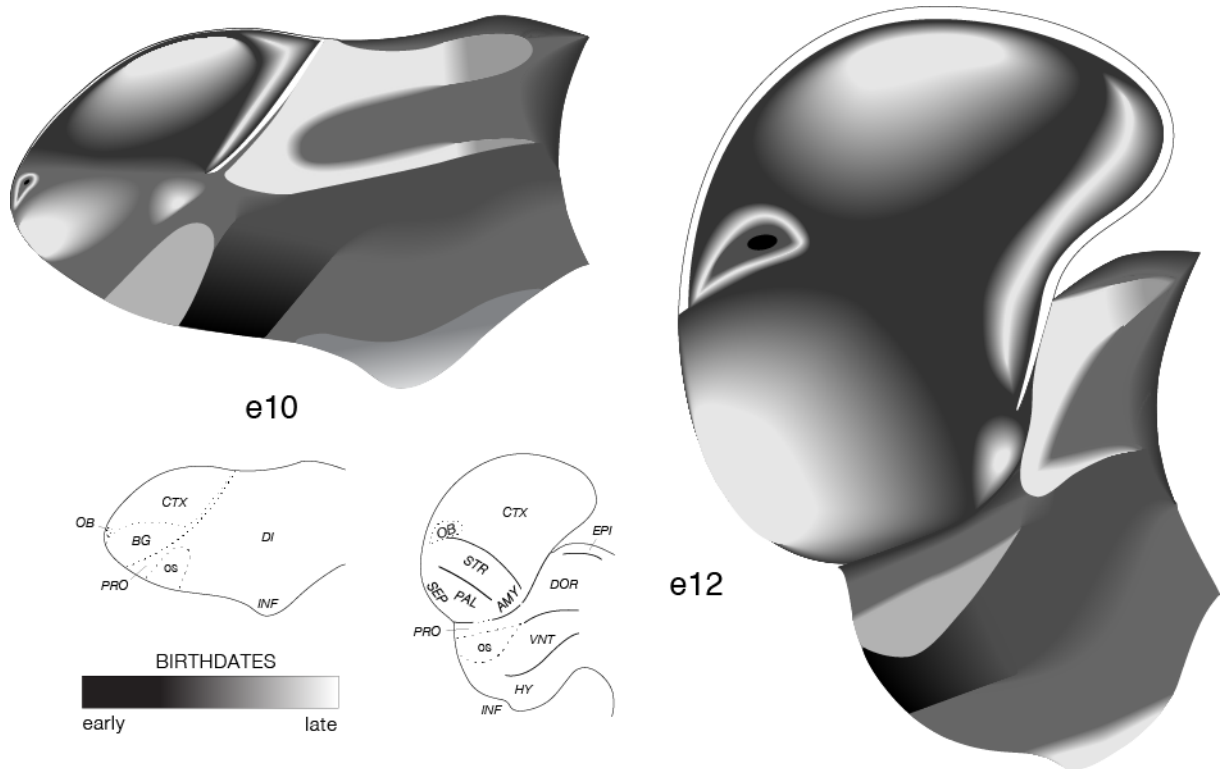


Fig. 8. Summary of earliest neuronal birthdates in the rat forebrain neuroepithelium. The complex pattern of regionalization is displayed on fate maps of the flattened forebrain vesicle for embryonic days 10 and 12. (From Alvarez-Bolado et al., 1995).

1. Endbrain: Cortex and Basal Nuclei

The earliest mantle layer formation in the endbrain takes place in the presumptive *accessory olfactory bulb* (Fig. 9, e11), and differentiation of the rodent *olfactory bulb* as a whole has been well-described (see Hinds 1968a, b). It seems most parsimonious to regard the olfactory bulb as a highly differentiated region of the cortical mantle (for example, see Brodmann 1909), unique in that it receives sensory information directly from the vomeronasal (accessory olfactory bulb) and olfactory (main olfactory bulb) nerves, rather than by way of relays through the

thalamus. The presumptive olfactory bulb region lies adjacent to the ectodermal *olfactory*

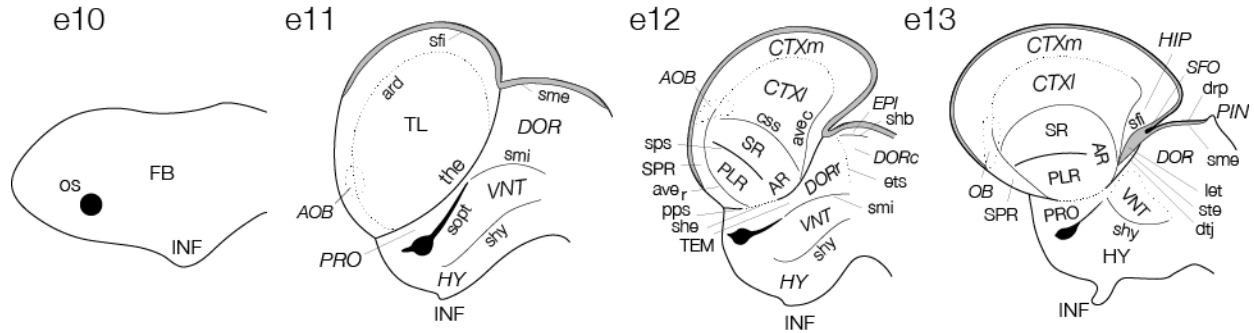


Fig. 9. Flat maps of the early rat forebrain vesicle, between embryonic days 10 and 13, showing the relative position of obvious morphological features. (From Alvarez-Bolado et al., 1995).

placode, and evagination of the olfactory bulb starts after the first axons from the placode arrive, and after the first neurons have been generated in the presumptive bulb region (see Humphrey 1966). Furthermore, ablation experiments and pathological material indicate that the olfactory bulb does not evaginate without olfactory nerve input, and transplantation experiments suggest that olfactory placode influences induce differentiation in the forebrain vesicle (see Burr 1916, 1924; Dryer and Graziadei 1994). The olfactory placodes lie in a region commonly referred to as the *rostrolateral* or *anterolateral ridge* (see Couly and Le Douarin 1985, 1990), which also contains the *hypophysial placode* in the midline (see section IIIA2). The anterolateral ridge forms a U-shaped zone around the rostral end of the neural plate, rostral to what is classically regarded as the neural crest (see Fig. 1 in Swanson 1992a). Finally, it should be noted that evagination of the olfactory bulb leads to a major distortion of the radial glial scaffolding that must be accounted for when interpreting the migratory pattern of late-forming granule cells (see Kishi 1987).

The region of the basal nuclei is the next part of the endbrain vesicle to generate a mantle layer, which leads to the formation of a *corticostriatal sulcus* (lateral striatal sulcus) between the two major parts of the endbrain or *cerebral hemispheres*, the *basal nuclei* or ganglia and *cerebral*

cortex or *pallium*. A great deal has been written about the development of the vertebrate basal nuclei, although general principles have yet to emerge and a confusing terminology has evolved. A synthetic account of early basal nuclei development in the rat has recently been presented (Alvarez-Bolado et al. 1995). On embryonic day 12, when the basal nuclei region is distinguished from the cortical area dorsally by a very shallow corticostriatal sulcus, and from the interbrain vesicle ventrally by the hemispheric sulcus and torus hemisphericus, the appearance of a clear *striatopallidal sulcus* (interstriatal sulcus) may be observed. This sulcus runs down the middle two-thirds of the basal nuclei region without extending to either end (Fig. 8, e12), thus dividing the basal region into *four ridges* (also called ventricular or ganglionic ridges or eminences)—*striatal (dorsal)*, *pallidal (ventral)*, *septal (rostral)*, and *amygdalar (caudal)*. The striatopallidal sulcus appears to form as a result of precocious mantle layer generation in the pallidal ridge. The four ridges are named for the major adult structures that appear, in a general way, to be generated from the corresponding regions of neuroepithelium. (During the short time between formation of the corticostriatal and striatopallidal sulci, it is perhaps most accurate to refer to the former as the *corticobasal sulcus*).

The sequence of events leading to formation of adult basal nuclei structures is poorly understood. However, one general principle may provide an important clue to an eventual solution. Birthdating evidence suggests that there are two major waves of neurogenesis in the basal nuclei (see ten Donkelaar and Dederen 1979; ten Donkelaar et al. 1979). The earlier wave generally contains the largest and most superficial neurons, whereas the later wave generally contains smaller, more deeply situated neurons. Structures generated by the first wave include the magnocellular basal system (including the substantia innominata and medial septal-diagonal band complex), globus pallidus and bed nuclei of the stria terminalis, and corticomедial and

central amygdalar nuclei; whereas the second wave generates the striatum and olfactory tubercle, lateral septal nucleus, and basolateral amygdalar complex. A model to explain certain anomalies in this pattern (for example, the superficial location of the adult olfactory tubercle and the deep location of the globus pallidus and bed nuclei) has been presented elsewhere (Alvarez-Bolado et al. 1995).

A valid distinction between basal nuclei and cortex is not always clear. This is particularly obvious in two regions: the corticomедial ‘nuclei’ of the amygdala and the olfactory tubercle. Although the amygdala is often regarded as a separate component of the endbrain, the corticomедial group is the only recipient of information from the accessory olfactory bulb (and thus the vomeronasal nerve, which transmits pheromonal information), and thus must be analogous to the main olfactory cortical regions innervated by the lateral olfactory tract. That is, at least parts of the corticomедial nuclei are pheromonal cortical. The other problem area is the olfactory tubercle, which is in direct receipt of main olfactory bulb information by way of the lateral olfactory tract. However, it displays architectonic and connectional features of both cortex and striatum.

The *cerebral cortex* or *pallium* is clearly laminated, and broadly speaking is conveniently divided into an isocortical region—in the lateral wall of the hemisphere—that displays 6 layers, and a limbic (allocortical) region—in the medial wall of the hemisphere—that surrounds it and displays much simpler lamination patterns (see section IV). It is often stated that cortical differentiation (for example, neurogenesis) begins rostroventrally, near the olfactory bulb, and then spreads caudoventrally. However, this applies only to the isocortex; limbic regions including the hippocampal formation and cingulate gyrus begin neurogenesis earlier than

adjacent regions of isocortex, and neurogenesis in the hippocampal formation displays an unusually complex pattern (see Smart and McSherry 1982; Smart 1984; Figs. 8 and 9).

The sequence of events leading to formation of the six-layered adult isocortex has been studied extensively, and while there is still disagreement about certain features, and inconsistencies in the terminology used by different investigators, the main features can be summarized as follows (Fig. 10). Taking the rat parietal region as an example, the simple

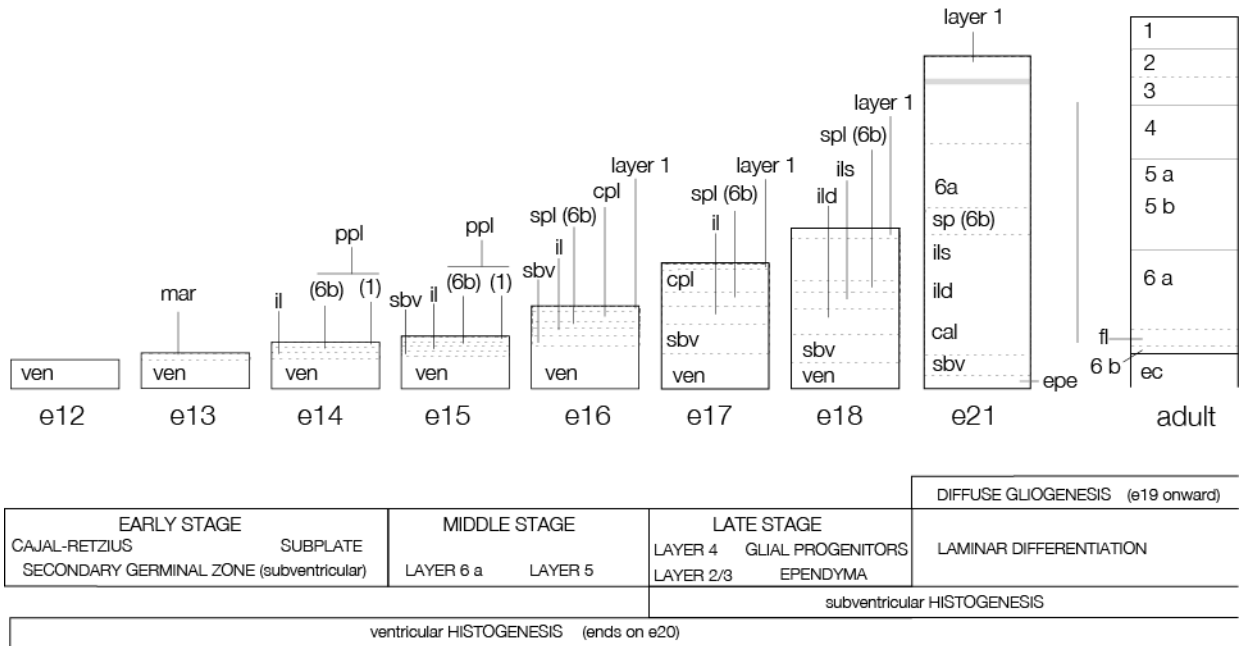


Fig. 10. A schematic overview of the major stages involved in differentiation of isocortical lamination in the rat. (From Alvarez-Bolado et al., 1995).

neuroepithelium comes on embryonic day 13 to display clear *ventricular* and *marginal* layers, with the latter containing tangentially oriented axons (Marín-Padilla 1978). A day later, the first neurons are born and establish an incipient *mantle layer* between the ventricular and marginal layers. The early mantle layer has been referred to as the *cortical preplate* (Rickmann and Wolff 1981). Two additional features of the neural tube wall at this stage of development are important to point out. First, the preplate contains tangentially oriented neurons superficially and

polymorphic neurons deep to them. Furthermore, there is evidence to suggest that the superficial neurons tend to be generated earlier than the deep neurons (Raedler and Raedler 1978). Thus, preplate neurons may be generated in an ‘outside-in’ gradient. And second, the preplate (mantle layer) is separated from the ventricular layer by an incipient *intermediate layer*, which is currently thought to contain young neurons migrating to the preplate (Sánchez et al. 1992).

The next obvious morphological event in isocortical development is the appearance on embryonic day 15 of a *subventricular layer* just superficial to the ventricular layer and deep to the intermediate layer. The subventricular layer is a secondary proliferation zone, and is much thicker in the basal nuclei region. Another feature of this stage is a differentiation of the intermediate layer into deep and superficial sublayers (for this stage see Stensaas 1967a-e; Smart and McSherry 1982; Van Eden et al. 1989; Cobas et al. 1991).

Around this time (embryonic day 15), the first neurons destined for the *cortical plate* are also born, and they begin to migrate through the intermediate layer and settle in the middle of the preplate, between the superficial tangential cells and the deep polymorphic cells. A distinguishable cortical plate is established on embryonic day 16, when the pallial wall now displays a *molecular (plexiform) layer* that contains the scattered, superficial tangential neurons (Cajal-Retzius cells) associated originally with the preplate; the cortical plate; a layer referred to as the *subplate* (Kostovic and Molliver 1974) that contains the deep polymorphic neurons of the preplate and becomes layer 6b of the adult isocortex (Valverde et al. 1989); the intermediate layer; the subventricular layer; and the ventricular layer. This fundamental organization is maintained through the prenatal period, although by embryonic day 18 superficial and deep parts of the intermediate layer can be distinguished easily, and the cortical plate becomes progressively thicker throughout its development. The mantle layer on embryonic day 13 along

with the preplate before cortical plate formation have sometimes been considered together as the primordial plexiform layer (Marín-Padilla 1978), and the subplate has been regarded as a component of the intermediate layer (for example, Kostovic and Molliver 1974; Shatz et al. 1988).

It is well-known that neurons of the cortical plate are generated in an ‘inside-out’ pattern, so that more superficial layers of the adult are generated later than deeper layers, although generation times overlap in adjacent layers (for review see Bayer and Altman 1991). Taken together, the evidence thus suggests a two stage model of cortical differentiation where the preplate arises first, and is followed by the cortical plate, which settles in the middle of the preplate. If this is correct, Cajal-Retzius cells in layer 1 are the earliest-generated neurons, neurons in layer 6b are generated next, and neurons in layer 2 are generated last. The order of layer generation would thus be: 1, 6b, 6a, 5, 4, 3, 2.

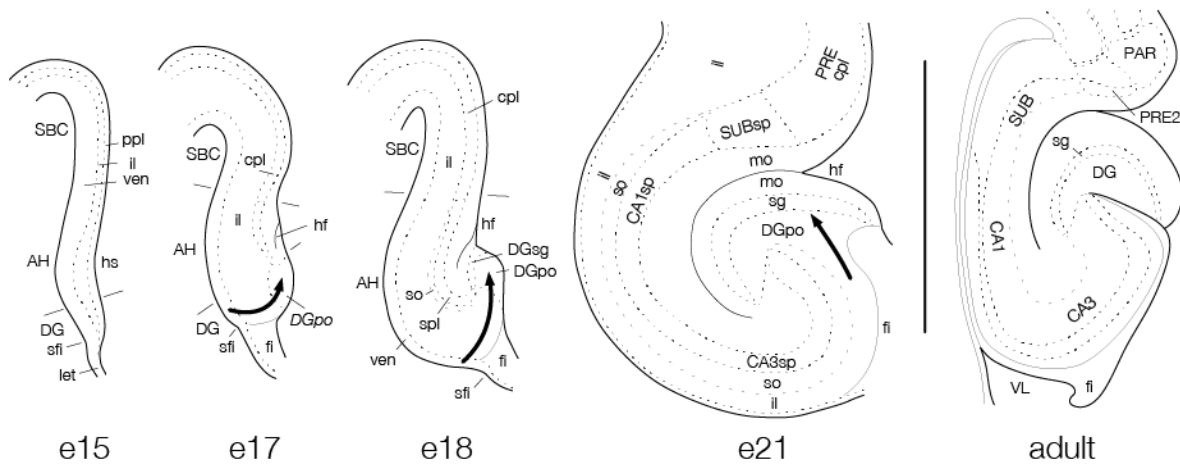


Fig. 11. A summary of hippocampal development in the rat. (Modified from Alvarez-Bolado et al. 1995).

Hippocampal formation development requires separate consideration because of its unusual pattern of neurogenesis and complex folding pattern (Figs. 8 and 11). To start, it is essential to appreciate that the presumptive hippocampus stretches along the entire medial edge of the cortical mantle, from the temporal pole caudally to the olfactory bulb rostrally (Hines 1922; see Fig. 21). During the course of development, the rostral end of this strip grows little, and remains as the induseum griseum and fasciola cinerea in the adult, whereas the caudal end in the temporal region expands greatly and is commonly referred to as the hippocampus in the adult. The hippocampal formation, which is defined by an intricate series of association connections (Swanson et al. 1987), consists of 9 adjacent, longitudinally arranged (along the axis of the medial wall of the cortical mantle) cortical areas. Beginning at the medial edge of the cortical mantle, and progressing laterally, they include the dentate gyrus and fields CA3, CA2, and CA1 of Ammon's horn (together, the hippocampus); the subiculum, presubiculum, and parasubiculum (together, the subicular complex); and the medial and lateral entorhinal areas.

Next, it is important to note that early stages of hippocampal development are similar if not identical to those of the isocortex (Stensaas 1967a-e), although the adult dentate gyrus and Ammon's horn consist of three essential layers (plexiform or molecular, pyramidal, and polymorph) rather than 6. In the dentate gyrus they include the molecular layer, granular layer (granule cells are modified pyramidal cells), and polymorph (hilar) layer; whereas in Ammon's horn they include a molecular layer (*stratum lacunosum-moleculare* and *stratum radiatum*), a pyramidal layer, and a polymorph layer (*stratum oriens*)(see Cajal 1909-11). Neurons throughout the hippocampal formation display an 'inside-out' pattern of generation (see Angevine 1965; Schlessinger et al. 1975, 1978; Bayer 1980).

When the hippocampal mantle layer first appears on embryonic days 14 to 15 in the rat, it is not folded, and an incipient *hippocampal fissure* (the *hippocampal sulcus*) indicates an approximate boundary between the presumptive dentate gyrus and Ammon's horn (Fig. 11a). Neurons in the presumptive dentate gyrus are arranged more irregularly, and their generation begins earlier than those in the presumptive Ammon's horn. The large polymorphic neurons of dentate gyrus layer 3 are generated first (see Schlessinger et al. 1978). At this stage, the wall of the neural tube in this region has been described as consisting of ventricular, intermediate, and preplate layers (Stensaas 1967b-d). By embryonic day 17, the first indication of the hippocampal fissure itself can be observed, and it appears to be formed by rapid growth of the dentate gyrus. Young neurons from the most medial part of the ventricular layer must cross a much thicker intermediate layer, although they still follow the orientation of elongated and distorted radial glial cells (Stensaas 1967c; Eckenhoff and Rakic 1984; arrow in Fig. 11b). The presumptive dentate gyrus ventricular layer lies adjacent to the *epithelial lamina* (Hines 1922), which now contains the incipient *fimbria*, and the border between the dentate ventricular layer and epithelial lamina is indicated by the *fimbrial sulcus* (*sulcus ventralis; taenia fornicis et fimbriae*; see Hines 1922). At this stage, the wall of this part of the neural tube has been described as containing ventricular, intermediate, cortical plate, and molecular layers (Stensaas 1967c).

As hippocampal development progresses, this basic geometry does not change, although the migratory route followed by neurons to the dentate gyrus elongates considerably (Stensaas 1967c; Eckenhoff and Rakic 1984; arrows in Fig. 11c, d), and it would appear that a 'proliferation zone' for dentate granule cells forms along this migration route (see Stanfield and Cowan 1988). This proliferation zone may be a specialized extension of a dentate subventricular

layer, a rudiment of which continues to generate neurons into adulthood (see Stanfield and Trice 1988), similar to that found in the developing olfactory bulb (see Kishi 1987).

2. Interbrain: Retina, Thalamus, Hypothalamus, Pituitary

After the neural groove, the *optic pits* are the first major differentiation of the forebrain neural plate, although the precise extent and location of neuroepithelium dedicated to generating the retina at the stage just preceding pit formation remains unknown (see Adelman 1936a, b). In the rat, optic pits begin to form at around the 5 somite stage on embryonic day 9, just after the head fold appears at the 2-3 somite stage (Adelman 1925). As the pits elongate into *optic stalks*, their location on the ventricular side of the neuroepithelium is indicated by the *optic sulcus* (of Grönberg 1901; *sulcus intraencephalicus anterior* of Kupffer 1906). The optic sulcus lies rostroventrally as the forebrain vesicle forms, and it lies caudal to the hemispheric sulcus when the latter differentiates endbrain from interbrain. Thus, the optic stalk at the 5 vesicle stage lies in the rostral hypothalamus, between the presumptive preoptic and anterior hypothalamic regions, just dorsal to the presumptive optic chiasm (see below). Interestingly, whereas the optic pits, stalks, and cups differentiate quite early, neurogenesis in the optic cups does not begin until embryonic day 14 in the rat (see Reese and Colello 1992), at least three days later than the earliest neurogenesis in the rest of the forebrain. The general differentiation of the rat optic stalk, and eye as a whole, appears to be similar to that in other mammals, although we are unaware of a detailed account of this morphogenesis in the rat.

Mantle layer formation in the interbrain has recently been clarified (see Alvarez-Bolado et al. 1995). It begins on embryonic day 11 (rat) in the midline between the presumptive optic chiasm and median eminence, in the *retrochiasmatic area*, and then spreads rapidly in a

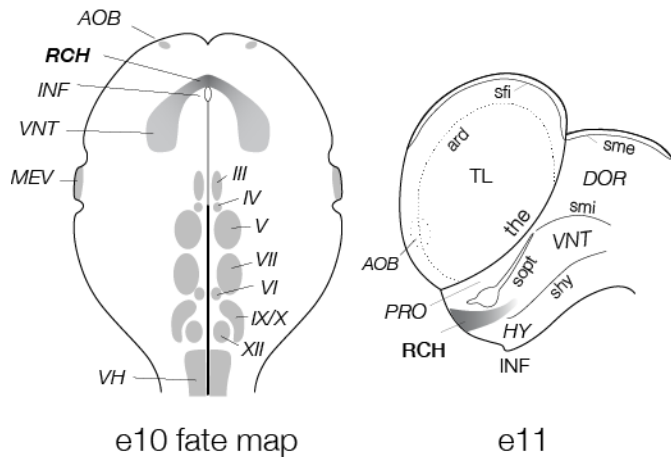


Fig. 12. Schematic views of early mantle layer formation in the rat. The sites of early mantle layer formation are projected on a bilateral fate map of the neural plate (left), and the site of initial mantle layer formation in the forebrain on embryonic day 11 (in the retrochiasmatic area, RCH) is shown on the right. (Drawing on left from Alvarez-Bolado et al., 1995).

dorsocaudal direction through the presumptive *ventral thalamus* (Fig. 12). This mantle layer formation is associated with the appearance of the *hypothalamic and middle diencephalic sulci*, ventral and dorsal, respectively, to the ventral thalamus. Curiously, the ventral thalamus is the only part of the interbrain to display a relatively clear ‘inside-out’ pattern of neurogenesis (Angevine 1970; Keyser 1972; Altman

and Bayer 1986).

With the appearance of the *habenular sulcus* (dorsal diencephalic sulcus of Herrick 1910) on rat embryonic day 12, the four classic longitudinal regions of the interbrain defined by Herrick (1910) can be recognized—*epithalamus*, *dorsal thalamus*, *ventral thalamus*, and *hypothalamus* (Fig. 9). It is important to point out that at these early stages (rat embryonic days 11 and 12) neither the hypothalamic sulcus nor the middle diencephalic sulcus is continuous unequivocally with the optic sulcus; and that the hypothalamic and middle diencephalic sulci cannot be followed uninterrupted into the midbrain vesicle. One enigmatic region of the early interbrain vesicle lies just caudal to the ditelencephalic border, near the rostral end of the middle diencephalic sulcus (see e12 in Fig. 9). Known as the *thalamic eminence*, it lies at the rostral end of the dorsal thalamic region and caudal end of the preoptic region (see below). However, no obvious morphological features define a border between the dorsal thalamus and preoptic region

at these early stages. By rat embryonic day 13 the middle diencephalic sulcus has disappeared and the hypothalamic sulcus has shifted dorsally because of rapid mantle layer formation in the hypothalamus.

The dorsal thalamus is the last major part of the interbrain to differentiate, and neurogenesis proceeds mainly between embryonic days 13 and 16 (rat) via an ‘outside-in’ gradient that is not strictly correlated to individual adult nuclei (see Angevine 1970; Altman and Bayer 1979b, c). Except for the region of the medial and dorsal lateral geniculate bodies (*metathalamus*), which can be distinguished on embryonic day 14 in the rat, differentiation of recognizable cell groups takes place very late in the development of the dorsal thalamus (between embryonic days 18 and 20 in the rat). The morphological differentiation of the dorsal thalamus has been described in a variety of mammals (see Droogleever Fortuyn 1912; Miura 1933; Gilbert 1934; Rose 1942; Kuhlenbeck 1948, 1951; Kahle 1956; Ströer 1956; Niimi et al. 1962; Coggeshall 1964; Keyser 1972), but these accounts are often difficult to follow and contradictory. It is safe to say that the topological rearrangements involved in transforming the simple wall of the dorsal thalamic part of the neural tube into the adult thalamus are unknown. This is due partly to the fact that a relatively small area of neuroepithelium generates a relatively very large adult region, partly to the very large number of distinct cell groups in the dorsal thalamus, and partly to a lack of molecular markers expressed in neurons before they form specific cell groups. Current information suggests that the various dorsal thalamic nuclei differentiate at a relatively late stage from an earlier, undifferentiated ‘dorsal thalamic primordium’.

Little of interest can be said about *differentiation of the epithalamus* (or at least about the *habenular part* of the epithalamus), except that neurogenesis here begins prior to that in the

dorsal thalamus, hence the appearance of the habenular sulcus; and that lateral habenular neurogenesis starts before medial habenular neurogenesis (see Altman and Bayer 1979a, b). However, the caudal extent of the epithalamus is controversial. In short, some authors (for example, Kupffer 1906; Rose 1942) consider the *pretectal region* (roughly equivalent to the dorsal *synencephalon* of Kupffer) as a caudal part of the epithalamus, whereas many others regard it as a rostradorsal, nontectal, part of the midbrain. Because the connections of the pretectal region are quite different than those of the habenula, and because structures ventral to the pretectal region (ventral synencephalon) include the periaqueductal gray and substantia nigra, for example, it seems likely that the synencephalon is a component of the midbrain.

Hypothalamic differentiation is an unusually complex, little understood process, although an interesting model has been presented by Altman and Bayer (1986), who point out that, overall, it displays an ‘outside-in’ pattern of neurogenesis. However, its adult cytoarchitecture is much less regular than that of the thalamus, and much remains to be learned about its embryonic construction. As a starting point, the adult hypothalamus has been divided into *three longitudinal zones* (lateral, medial, and periventricular) and *four rostrocaudal regions or levels* (preoptic, anterior, tuberal, and mammillary; see Swanson 1987). Based on the ‘outside-in’ principle, the lateral zone tends to differentiate first, followed by the medial zone, and then the periventricular zone, although there are exceptions. For example, mantle layer formation begins in the retrochiasmatic area (see above, and Fig. 12), and neuroendocrine motoneurons associated with the periventricular zone are born quite early (Ifft 1972; Markakis and Swanson 1996).

Whereas the adult hypothalamic lateral zone displays little obvious rostrocaudal cytoarchitectural differentiation, the medial zone is divided into a series of distinct nuclei that define the rostrocaudal levels or regions. Early in hypothalamic differentiation the approximate

location of the four levels are indicated by a pair of transitory grooves, the *optic* and *infundibular sulci*.

The *preoptic region* has been particularly difficult to understand. Our own interpretation, based on the incomplete evidence now available (see Alvarez-Bolado et al. 1995), is as follows (Fig. 9). On rat embryonic day 12, when the forebrain vesicle divides into endbrain and interbrain vesicles, the preoptic region lies caudal to the hemispheric sulcus (external) and rostral to the optic sulcus (internal); thus it lies in the caudal half of the torus hemisphericus (the internal ridge formed by the hemispheric sulcus). This topology has led to endless controversy about whether the preoptic region is telencephalic or diencephalic because at this stage it is clearly part of what has been called the medial ventricular ridge or ganglionic eminence (mostly in the ventral half of what here is referred to as the pallidal ridge). However, several lines of evidence argue for a diencephalic origin. First, the hemispheric sulcus appears to be an obvious demarcation between endbrain and interbrain; second, homeobox POU-III gene expression during embryogenesis clearly defines a cryptic boundary in the middle of the torus hemisphericus between the preoptic region and basal telencephalic nuclei; third, the preoptic region appears to be generated from the third ventricular neuroepithelium; and fourth, preoptic connections are very similar in principle to other parts of the hypothalamus.

Nevertheless, one problem remains—the dorsal border of the preoptic region, which in the adult involves the bed nuclei of the stria terminalis and the rostroventral tip of the thalamus, and is rather indistinct. It now seems clear that the bed nuclei are derived from lateral ventricular neuroepithelium (and are thus derived from the endbrain), although exactly where the ventral border of the bed nuclei should be drawn in the adult is not obvious.

Caudal to the preoptic region, the *anterior* and *tuberal* regions lie between the optic and infundibular sulci, whereas the *mammillary region* lies caudal to the latter. During embryogenesis, the anterior region forms at the level where the optic chiasm and supraoptic

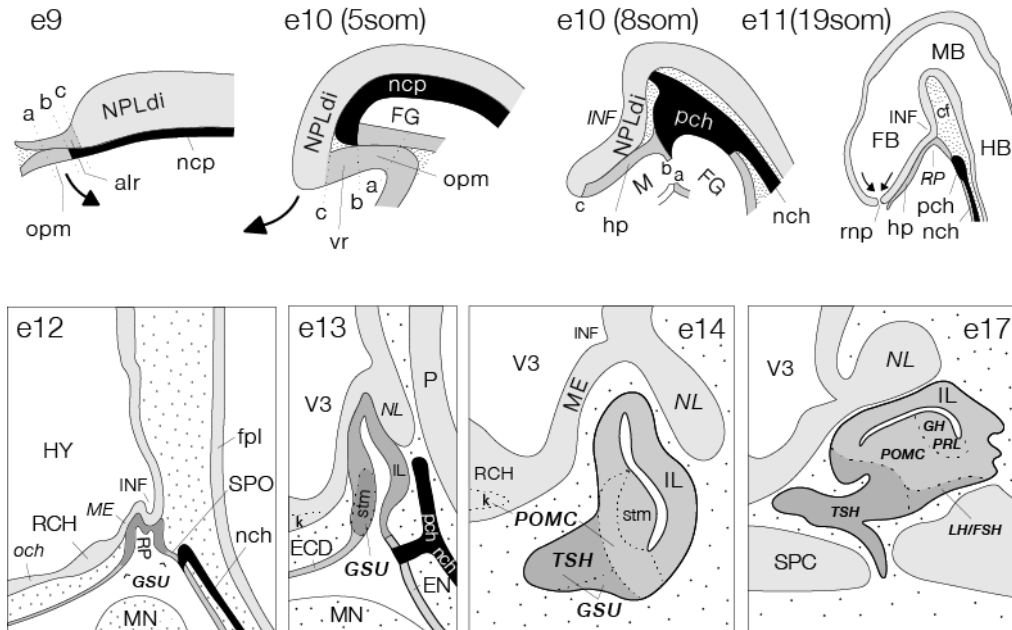


Fig. 13. A summary of rat pituitary development as viewed in midsagittal sections. Differentiation of Rathke's pouch (RP) is accompanied by the expression of various pituitary-specific genes (FSH, follicle-stimulating hormone; GH, growth hormone; GSU, α -glycoprotein subunit; LH, luteinizing hormone; POMC, pro-opiomelanocortin; PRL, prolactin; TSH, thyrotropin-stimulating hormone). (Redrawn from Schwind, 1928; Simmons et al., 1990; Swanson, 1992b).

commissures differentiate, whereas the tuberal region forms at the level of the median eminence and infundibulum (see Fig. 2, lower left).

The *infundibulum* is an integral part of the *pituitary gland*, which will be considered in some detail because of its intrinsic interest and because its formation helps clarify certain difficult topological transformations in neural plate and early neural tube morphogenesis (Schwind 1928; Hamilton and Mossman 1972; Simmons et al. 1990; Swanson 1992b). As mentioned in section IIB, the notochordal plate probably extends all the way to the oropharyngeal membrane at the earliest stages of trilaminar embryonic disc development (Fig.

13, e9). By the 5-somite stage early on rat embryonic day 10 (Fig. 2, upper right; Fig. 13), the rostral end of the neural plate region has folded ventrally, perpendicular to more caudal parts of the neural plate, and the oropharyngeal membrane has folded even more, so that it comes to lie perpendicular to the rostral neural plate, parallel and ventral to the caudal neural plate. In essence, the rostral end of the trilaminar embryonic disc has undergone a U-shaped folding, with the center of the U occupied by the rostrally extending foregut.

It is very important to realize that the anterolateral ridge (section IIIA1) extends around the front of the neural plate, just caudal to the oropharyngeal membrane in the midline and just lateral to presumptive parts of the forebrain region more laterally (Fig. 13; Fig. 1 in Swanson 1992a; Schwind 1928). The hypophysial placode later differentiates within midline parts of this ridge, which, topologically at least, is a rostral extension of the neural crest.

On rat embryonic day 10, at about the 8 somite stage (Fig. 13), the rostral end of the neural plate continues to grow rapidly, now in a rostral direction, with the angle between 'vertical' and 'horizontal' rostral growth indicating the approximate site of the future infundibulum. This rostral growth of the neural plate is accompanied by an elongation of the underlying anterolateral ridge region. In the midline, the rostral neural plate and the underlying (somatic) ectoderm of the anterolateral ridge lie adjacent to one another, whereas more laterally they are separated by a thin layer of paraxial head mesoderm. Two other important events occur at this stage. First, the oropharyngeal membrane ruptures, forming an opening between the oral cavity and foregut. And second, the definitive, internalized notochord has replaced the notochordal plate, except in the region just caudal to the ruptured oropharyngeal membrane. This region, which is called the *prechordal plate*, expands considerably and appears to contain a

mixture of cells associated with the rostral notochordal plate and the oropharyngeal membrane (Fig. 13, stage e10, 8 somites; Schwind 1928).

By rat embryonic day 11 (Fig. 13) the edges of the forebrain neural plate fuse, the rostral neuropore closes, and the forebrain vesicle thus emerges (section IIB). At this stage, the somatic ectoderm is separated completely from the neuroectoderm—when the rostral neuropore closes, the forebrain part of the neural tube is completely internalized along with the rest of the neural tube. Furthermore, somatic ectoderm in the roof of the oral cavity (derived from the anterolateral ridge) displays an inverted V-shape, stretching from the approximate site of rostral neuropore closure rostrally to the rostral end of the prechordal plate caudally. A shallow evagination in the midline floor of the forebrain vesicle indicates the site of the infundibulum, and gene expression studies indicate that the V-shaped region of midline somatic ectoderm lying adjacent to the floor of the forebrain vesicle contains the hypophysial placode (the presumptive Rathke's pouch) giving rise eventually to the anterior and intermediate lobes of the pituitary (Simmons et al. 1990).

By embryonic day 12 the growing infundibulum produces an indentation in the apex of the hypophysial placode, and a definitive *Rathke's pouch* is now visible (the limits of which are shown by arrows in Fig. 13, based on gene expression studies; Simmons et al. 1990), with presumptive anterior (rostral) and intermediate (caudal) lobes. The tiny ventrally concave region of ectoderm between the caudal end of Rathke's pouch and the rostral end of the notochord is known as *Seessle's pouch*. Thus, at this stage the hypophysial placode has differentiated into Rathke's pouch, and is surrounded by the somatic ectoderm that forms the epidermis of the oral cavity roof.

On embryonic day 13 the infundibulum has extended ventrally along the caudal wall of the greatly elongated Rathke's pouch. The embryonic infundibulum ultimately contains the neural (posterior) lobe of the pituitary gland and the hypothalamohypophysial portal system. At this stage of development, a region of stem cells for the anterior lobe differentiates in ventral parts of the rostral wall of Rathke's pouch, whereas the caudal wall (presumptive intermediate lobe) remains undifferentiated (Simmons et al. 1990). A day later, Rathke's pouch pinches off from the somatic ectoderm to form a vesicle, and the anterior lobe has begun to differentiate, with separate presumptive thyrotrope, corticotrope, and gonadotrope regions (Fig. 13, e14; and Simmons et al. 1990). By embryonic day 17, another anterior lobe region for somatotropes and lactotropes has differentiated, and the disposition of the pituitary gland as a whole is approaching that seen in the adult.

B. Midbrain and Hindbrain

The adult midbrain and hindbrain contain many fewer known subdivisions than the forebrain, and their development is thus probably simpler. However, less work has been devoted to mammalian hindbrain development as compared to forebrain development, so that little will be added here to the overview provided in section II.

Perhaps the simplest way to characterize the brainstem is with reference to the spinal cord. There are two obvious differences. First, the alar and basal plates of the brainstem give rise to cell groups associated with cranial rather than spinal nerves; and second, the hindbrain is distinguished by the presence of the rhombic lip. Thus, brainstem sensory and motor nuclei are more differentiated because of the special sensory modalities associated with the caudal cranial nerves, and with the motor innervation of the branchial arches, extraocular muscles, and tongue.

Associated with this sensorimotor differentiation, the brainstem reticular formation is greatly expanded as compared to the spinal cord.

The rhombic lip stretches throughout the length of the most dorsal part of the hindbrain. A complete fate map of the rhombic lip would be very useful to have, but little work is being done on this as yet obscure problem. What can be said is that the cerebellum is generated from approximately the rostral half, through a well-known sequence of events (see Jacobson 1991; and Acirón 1951, for development of foliation in the rat). Progressively more caudal parts of the rhombic lip may generate the vestibular and cochlear nuclei, the raphé nuclei, the precerebellar nuclei (basal pontine gray, inferior olive, lateral reticular nucleus, external cuneate nucleus), and dorsal column nuclei (see Harkmark 1954; Taber Pierce 1966, 1967; Bourrat and Sotelo 1990a, b). Topologically, the rhombic lip appears to lie dorsal to the part of the alar plate that generates the sensory nuclei associated with the trigeminal, glossopharyngeal, and vagus nerves (which might be thought of as the rostral extension of corresponding columns of the spinal cord). For a review of rat brainstem nuclear differentiation see Altman and Bayer (1982).

C. Differentiation of the Midline

No other single concept is as useful in understanding the three-dimensional growth of the neural tube—and in constructing flattened maps of the central nervous system (next section)—as differentiation of the midline, primarily because the midline can be regarded at the first level of analysis as a two-dimensional structure throughout development (Figs. 2 and 14). At the point in time when the rostral neuropore closes (Fig. 2, middle right), the midline has been divided into a ventral *floor plate* and a dorsal *roof plate* (Burckhardt 1894). However, there is uncertainty about the limits of these segments.



Swanson 1996

FIG. 14 *CNS Development (midsagittal)*

Fig. 14. Schematic midsagittal views of rat central nervous system development, with color coding of the major brain vesicles. In the bottom row, rotation of the longitudinal axis (red line) is indicated. Embryos are arranged so that the longitudinal axis of the forebrain is approximately horizontal.

Early on, the neural plate becomes divided into right and left halves by the floor plate, a narrow, histologically distinct region of the neuroepithelium that never generates neurons, and consists of what have been referred to as median hinge-point cells (see Alvarez and Schoenwolf 1991). The rostral limit of the floor plate has been controversial for many years and is not yet settled. Thus, the following discussion is designed to point out major issues and offer a reasonable synthesis based on current evidence. As will be seen, this is an important topic because it bears on the fundamental divisions of the central nervous system.

In the most careful, widely-quoted, histological analysis of this problem, Kingsbury (1934) concluded that in all vertebrates a uniform, definitive floor plate extends the length of the spinal cord and hindbrain to end at the caudal boundary of the midbrain. In related work, Kupffer (1906) had previously suggested that the brain can be divided into two fundamentally different parts, *archencephalon* (rostral to the notochord; that is *prechordal*) and *deuterencephalon* (associated with the notochord; that is, *epichordal*), and more broadly that the central nervous system as a whole may be divided into *archencephalic* and *deuterencephalospinal parts*. Kingsbury concluded that the notochord and floor plate end rostrally at approximately the same level, and thus together define even more clearly the archencephalon and deuterencephalon. However, it is clear (Fig. 13) that notochordal tissue (associated with the prechordal plate; section IIIA2) extends rostrally, to the level of the infundibulum, and thus underlies the midbrain vesicle and mammillary region of the forebrain (also see Schoenwolf 1985; Puelles et al. 1987, for the chick).

This raises the question of how far rostrally the floor plate actually extends, and there is controversy about this feature, although it seems clear that the uniform histological feature described by Kingsbury ends at the rostral border of the hindbrain (see Keyser 1972). Whether

the floor plate continues more rostrally (for example, to the infundibulum) in a modified form remains to be determined but seems likely because the definitive notochord is more closely opposed to the neural tube in the hindbrain region, whereas the prechordal plate underlies the midbrain region, and the floor plate and notochord are essentially coextensive (G.C. Schoenwolf, personal communication). If the definitive floor plate and notochord end at the rostral border of the hindbrain vesicle, and the prechordal plate ends just caudal to the infundibulum, then to a first order of approximation the spinal cord and hindbrain are associated with definitive floor plate and notochord, the midbrain is associated with prechordal plate and a poorly defined floor plate, and most of the forebrain is associated with neither.

The *roof plate* is formed by fusion of the lateral edges of the neural plate during neural tube formation, and like the floor plate, it contains a strip of neuroepithelial cells that does not generate neurons. In essence then, the roof plate extends along the apex of the neural tube from the caudal end of the spinal cord to the rostral end of the floor plate, which as we have just seen probably lies at the level of the infundibulum. Except for the diencephalon, there is little controversy about the fundamental divisions of the roof plate. Caudally, it lies at the apex of the spinal cord; next, it forms the thin, broad dorsal wall of the fourth ventricle (*hindbrain roof plate*); next, it courses through the apex of the midbrain (*midbrain roof plate*); and then it forms the narrow dorsal wall of the third ventricle (*diencephalic roof plate*).

Extending rostral to the ditelencephalic junction, the interpretation of roof plate extent (and associated terminology) becomes progressively more obscure. Hines (1922) has presented the best analysis of the region between the ditelencephalic junction and presumptive optic chiasm, which she defined as the *telencephalon medium*, and divided into a dorsal *choroidal area* and a ventral *terminal plate (lamina terminalis)* separated by the terminal angle. As pointed

out below, the initially thicker, ventral part of the terminal plate forms the rostral wall of the third ventricle, and thus is diencephalic rather than telencephalic.

We now come to a particularly difficult issue: formation of the midline between the infundibulum and terminal plate (as defined by His 1895; and by Hines 1922), which in the adult includes the median eminence, retrochiasmatic area (and supraoptic commissures), and optic chiasm. On the one hand, Kingsbury (1934), Morriss-Kay and Tuckett (1987), Alvarez and Schoenwolf (1991) and others have suggested that the rostral end of the very early neural plate is unpaired rostral to an ill-defined point associated with the rostral end of the floor plate and/or notochordal tissue (presumably the infundibulum, see area *U* in Fig. 4, and 8 somite stage in Fig. 13). On the other hand, it is clear that the rostral neuropore closes in the terminal plate by rostrally directed fusion proceeding from both the ventromedial and dorsolateral edges of the neural plate (arrows *V* and *D* in fig. 4). According to Müller and O’Rahilly (1985; see their Fig. 7), the ventromedial fusion process that forms the terminal plate begins just rostral to the presumptive optic chiasm and proceeds rostradorsally. If this is true, the region between the infundibulum and terminal plate (area *U* in Fig. 4), which lies roughly between the presumptive optic and infundibular sulci, has neither floor plate nor roof plate. However, it remains to be demonstrated whether or not this midline region (between *a* and *d* in Fig. 4) forms by fusion of the most medial edge of the neural plate (starting at point *a* in Fig. 4) or whether it is truly unpaired.

Having outlined what is known about the midline of the early neural tube, we shall now move on to an overview of structures that differentiate within the midline, and thus provide topographic landmarks or anchor points through the course of development. For this, it is

convenient to begin caudally in the roof plate and progress rostrally, ending at the caudal tip of the floor plate. Special attention will be paid to the forebrain because of its unusual complexity.

In the spinal cord the roof plate is a narrow, unremarkable strip. However, near the rather indeterminate boundary with the medulla, the roof plate expands rapidly into the rhomboid sheet (hindbrain roof plate) that forms the dorsal wall of the fourth ventricle, which differentiates into the *caudal* and the *rostral medullary vellum*. The transitional region between spinal and hindbrain roof plate is associated with the *area postrema*, a circumventricular ‘organ’ (nucleus lacking a blood-brain barrier). Rostrally, the hindbrain roof plate converges toward the hindbrain-midbrain junction, known as the *isthmus* in the older literature (see Gillilan 1943) and the site of the *trochlear nerve decussation*, and then continues as the narrow *midbrain roof plate*, between the right and the left tectum.

The region near the midbrain-interbrain junction is quite differentiated (Fig. 2, lower row; see Warren 1917). The *posterior commissure* and associated *subcommissural organ*, a differentiation of ependymal cells that secretes Reissner’s fiber (Wislocki and Leduc 1954), differentiate at the junction of the pretectal region (dorsal synencephalon) and interbrain. The *pineal gland* evaginates from the roof plate just rostral to the posterior commissure and just caudal to the *habenular commissure*. The *diencephalic roof plate*, which is bounded laterally by the *sulcus medullaris*, forms the dorsal aspect of the thalamus, and extends to the interbrain-endbrain junction, which is occupied by the *paraphyseal arch* (immediately adjacent to the adult *subfornical ‘organ’*, another circumventricular nucleus lacking a blood-brain barrier; see Kappers 1955).

In early stages, there is a region in the telencephalon (just rostral to the paraphyseal arch) that is similar to the diencephalic roof plate called the *tela choroidea telencephalon medii* (the

‘midline telencephalic roof plate’), although it later becomes relatively very short as the cerebral hemispheres expand and it never forms choroid plexus, unlike the diencephalic roof plate (Hines 1922) and caudal medullary vellum. Rostral to this, the *terminal plate* stretches to the optic chiasm. The terminal plate is an important feature. The hippocampal commissures and corpus callosum cross through the caudal (dorsal) end of the terminal plate very late in development. Until cortical fibers begin to cross, the presumptive site of these commissures is very small and has been referred to as a general *commissural plate* region of the lamina terminalis (for details see Abbie 1939; Rakic and Yakovlev 1968; Wahlsten 1981; Valentino and Jones 1982). Rostral to this, there is a segment of lamina terminalis associated with the *septal region* (Hines 1922), and then, at the point where the torus hemisphericus and hemispheric sulcus converge on the midline is the site, called the *torus transversus* (see Keyser 1972), where the *anterior commissure* crosses (see Keyser 1972). As mentioned above, this is the midline boundary between endbrain and interbrain, and the fact that the rostradorsal aspect of the anterior commissure is surrounded by the very thin median preoptic nucleus suggests that the commissure actually passes through the rostradorsal tip of the hypothalamus.

Ventral to the anterior commissure, the roof plate continues as the classic terminal plate (lamina terminalis). This is almost certainly the region where the rostral neuropore usually seals (see above), and it forms the rostral wall of the third ventricle adjacent to the preoptic region of the hypothalamus. Another circumventricular organ, the *vascular organ of the lamina terminalis*, differentiates immediately rostral to the optic chiasm. The *presumptive optic chiasm* is initially a very thin region in the midline that has often been confused with the caudally adjacent *retrochiasmatic area*, which is much thicker until optic nerve fibers reach the presumptive chiasm on embryonic day 15 in the rat (Lund and Bunt 1976). As pointed out above, the

retrochiasmatic area contains the earliest-generated neurons in the forebrain, and the resulting mantle layer extends across the midline (see Alvarez-Bolado et al. 1995; Fig. 12). The precocious *supraoptic* (or more properly, the *postoptic*) *commissures* also cross in this region (see Tsang 1940). The presumptive *median eminence* extends between the retrochiasmatic area and infundibulum, and it is yet another circumventricular region lacking a blood-brain barrier; it contains the proximal end of the *hypothalamohypophysial portal system*. As discussed above, current evidence suggests that the *floor plate* extends from the infundibulum to the caudal tip of the spinal cord, and that the floor plate is relatively indistinct until the midbrain-hindbrain junction, where it assumes a characteristic, uniform appearance.

Before closing this journey along the midline, it is useful to clarify one related feature: differentiation of the *telencephalic roof plate* (Hines 1922). This process begins on embryonic day 13 in the rat (Fig. 9) with ventrolateral extension of the midline *epithelial area*, beginning at the interbrain-endbrain junction (see Fig. 14 in Hines 1922, for a particularly clear illustration in the 14 mm. human embryo). In essence, the initially straight epithelial layer forming the midline diencephalic roof plate and rostrally continuous, short telencephalic choroidal area (see above) becomes T-shaped (for the presumptive site of this transformation see Fig. 2, lower row). The stem of the T is known in the adult as the telencephalic roof plate or epithelial area and it extends about half way down the rostral wall of the torus hemisphericus. Its caudal border with the mantle layer is indicated by the *sulcus terminalis*, which meets the rostral end of the sulcus medullaris dorsally; whereas its rostral border is indicated by the *fimbrial sulcus* (*sulcus ventralis*, *sulcus limitans hippocampi*, *taenia fornicis et fimbriae*), which curves rostrally when it reaches the midline, and then continues as far as the olfactory bulb, medially adjacent to the primordial hippocampus. The sulcus terminalis does not form the boundary between interbrain

and endbrain. Instead, a narrow, dorsally tapering extension of the amygdala (containing neurons and the stria terminalis) lies between the sulcus terminalis and interbrain-endbrain junction, which lies at or slightly caudal to the apex of the torus hemisphericus (in the caudal wall of the torus; see Alvarez-Bolado et al. 1995). The *lateral ventricular choroid plexus* develops from the endbrain roof plate and is continuous with the *third ventricular choroid plexus* at the *subfornical organ*, to which it is attached in the adult *interventricular foramen*. In the early embryo, the short region of midline roof plate just caudal to the paraphyseal arch, essentially at the junction of the endbrain and interbrain, is known as the *velum transversum* (see Hines 1922); it lies at the apex of the telencephalic epithelial area extending along the torus hemisphericus.