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II. Structural Differentiation of the Brain

This section provides an overview of *pattern formation* in the developing brain from the neural plate stage through establishment of the primary brain vesicles, complementing and extending the schematic treatment presented in section III of our adult rat brain atlas (Swanson 1992a). As before, the emphasis is on a description of structural changes, as a framework for interpreting cellular and molecular mechanisms underlying neuron differentiation, migration, and settling, and the subsequent formation of neuronal circuitry, which includes the aggregation of neurons into nuclei and cortical layers, differentiation of adult neuronal phenotypes, and regressive phenomena such as neuronal cell death and axonal pruning (see Cowan 1978). Insightful work on mechanism underlying these processes is now advancing rapidly, but is beyond the scope of the present volume.

How can a purely descriptive account of brain structure differentiation be justified in the Age of Molecular Biology? The following points are offered in no particular order of importance. First, as just mentioned, structural information provides a scaffolding for the interpretation of functional information—it is a sometimes forgotten truism that structure and

function are inseparable, as shown throughout the history of biology. The structural work of Vesalius (1543) and Fabricius (1603) was essential for Harvey's (1628) conception and experimental verification of the circulation of the blood, just as Cajal's (1894) work on neuronal morphology provided the framework for Sherrington's (1906) interpretation of reflex physiology, and today's attempts to identify molecules responsible for constructing spinal circuitry. In brief, structural constraints are essential for the interpretation of functional data, and a sure guide to the formulation of interpretable hypothesis that can be tested experimentally. Second, at least since the morphological work of Baer (1828), it has been suspected that there is a common basic structural plan of the vertebrate brain. The extent to which this may be true, and the nature of this plan, are fundamental issues that deserve consideration from time to time. And third, reviews of brain structure are useful for refining and standardizing anatomical nomenclature, which has always been confusing and inconsistent. A detailed, synthetic analysis of brain structure from the neural plate to the adult in one species that has received a great deal of experimental attention (the rat) should be useful from this point of view, especially when compared eventually with similar analyses of other mammals, and of other vertebrate classes as well.

Below, we shall review briefly the differentiation of the neural plate, the subsequent formation of the neural tube with its vesicles and neuromeres, the birth of neurons from proliferation zones in the neural tube, and the migration of these young neurons. The later differentiation of major brain regions will be considered in section III. Together, this information will be used to construct flattened maps of the brain in section IV.

A. Neural Plate Differentiation: Polarity, Symmetry, and Regionalization

The brain is the first recognizable organ to differentiate in the embryo, and the *neural plate* is its first manifestation. The neural plate is a thick part of the *ectoderm* in the *trilaminar embryonic disc* that is induced by underlying midline mesoderm (the *notochordal plate* then *notochord*) and perhaps later by differentiating cells of the *primitive node and streak* (Ruiz i Altaba 1992). In the rat, the first mesoderm appears about three-quarters of the way through embryonic day 8 (Huber 1915), and the neural plate region can be observed during the first half of embryonic day 9, before the first pair of somites appears (Adelmann 1925; Schwind 1928). In rodents, the midline notochordal plate is embedded in the roof of the archenteron (primitive gut endoderm) early on, and then pinches off dorsally to form the definitive notochord, at about the 8 somite stage in rats (Fig. 1; Schwind 1928; Jurand 1974; Sulik et al. 1994; Sausedo and Schoenwolf 1994).

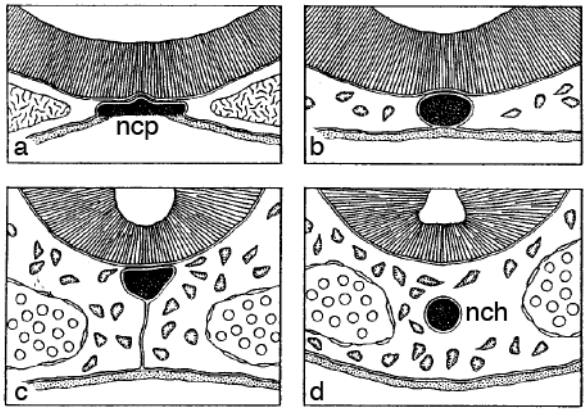

Fig. 1. Differentiation of the definitive notochord (nch in d) from the notochordal plate (ncp in a) in the mouse embryo. In these schematic drawings, which are from transverse sections just caudal to the forelimb fields, part a is based on the e9 embryo, part b on the e9.5 embryo, part c on the e10.5 embryo, and part d on the e12.5 embryo. See text for details. (Relabeled from Jurand, 1974).

As shown in Fig. 2 (upper left), the early neural plate is centered in the ectodermal layer, so that the *neuroectoderm* is surrounded by *somatic ectoderm*. In rodents, the neural plate becomes thicker than the somatic ectoderm because cells in the latter become relatively shorter, unlike the situation in chicks, for example, where neuroepithelial cells become relatively taller (see Morris-Kay 1981). Thus, at the earliest stages of neural induction, the rat neural plate is indistinguishable from surrounding ectoderm (Schwind 1928).

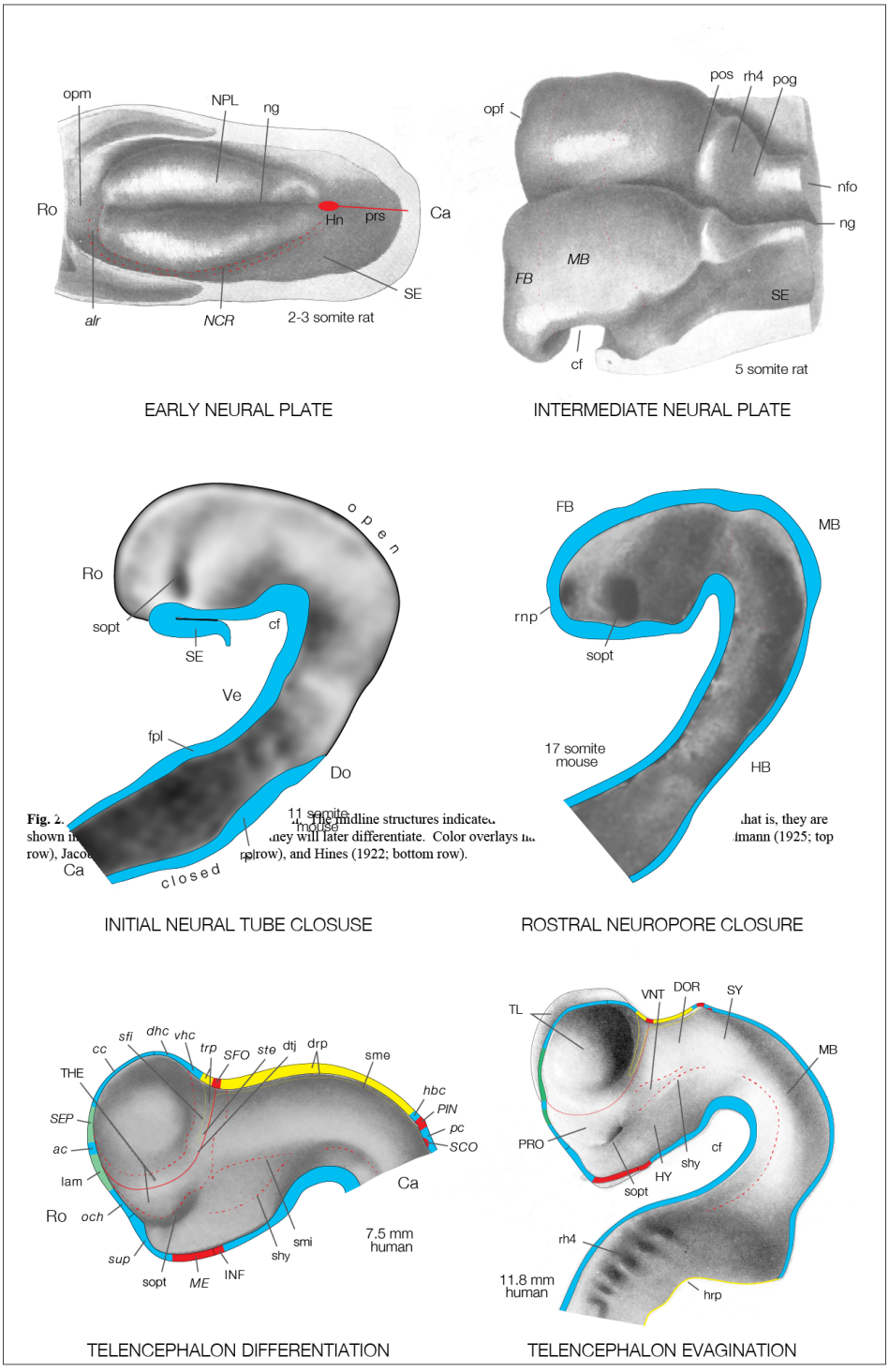

Fig. 2. ...midline structures indicated... ...hat is, they are shown in... ...ney will later differentiate. Color overlays ha... ...mann (1925; top row), Jacob... ...row), and Hines (1922; bottom row).

FIG. 2 *Neural Tube Differentiation*

Fig. 2. An overview of mammalian neurulation. The midline structures indicated in the lower two figures are presumptive; that is, they are shown in the approximate location where they will later differentiate. Color overlays have been added to figures from Adelmann (1925; top row), Jacobson and Tam (1982; middle row), and Hines (1922; bottom row).

The neural plate is rendered bilaterally symmetrical (consisting of right and left *neural folds*) by a midline depression, the *neural groove*, which also defines a longitudinal axis bounded rostrally by the *oropharyngeal membrane* and caudally by the *primitive (Hensen's) node*. According to Källén (1952), the mouse neural groove has a dual origin, appearing first (at the one somite stage; also see Adelmann 1925 for rat) rostrally; shortly thereafter a caudal groove appears, the two being separated by a length of neural plate with no groove and slight lateral constrictions; and shortly thereafter the two grooves fuse. In addition, the neural plate has dorsal and ventral surfaces, the former adjacent to the overlying *amnionic cavity* and the latter adjacent to the underlying *mesoderm*. Thus, the major anatomical axes of the brain, and body as a whole, are evident at the neural plate (trilaminar embryonic disc) stage of development.

Experimental evidence suggests that the neural plate differentiates progressively from rostral to caudal (see Nieuwkoop 1991; Alvarez and Schoenwolf 1991; Slack and Tannahill 1992). Because the neural plate from early stages is spoon-shaped, with the rostral end wider than the caudal end, it would appear that the brain region of the neural plate is formed before the spinal cord, coccygeal segments of which would thus differentiate last. Conversely, in view of the discussion below on the formation of primary brain vesicles, it would appear that the presumptive forebrain is the first region of the nervous system to differentiate.

As development progresses, the neural plate begins to show certain differentiations that are well-illustrated in the 5 somite rat embryo (Fig. 2, upper right) (Adelmann 1925; Schwind 1928). The rostral end of the neural plate bends ventrally around the primitive *cephalic flexure*, along with the *head fold* of the embryo, and three features can now be identified: the *optic pits* rostrally, the *primitive infundibulum* in the rostral midline, and the *otic rhombomere* caudally, which is indicated by a deep, rostral transverse indentation, the *preotic sulcus*, and a shallower,

caudal indentation, the *postotic groove*. In addition, two shallow indentations appear in the lateral margins of the *brain plate* (the broad, rostral part of the neural plate, as opposed to the caudal *spinal plate* area of the neural plate as a whole). These notches appear to indicate future transverse boundaries between the forebrain, midbrain, and hindbrain vesicles. Thus, the neural plate appears to be divided into presumptive forebrain, midbrain, hindbrain, and spinal domains (the basic divisions of the *central nervous system*); the optic stalk has begun to differentiate in the forebrain region; and neuromeres have begun to differentiate in the hindbrain region. There is some indication that in the rat the optic (five somite stage) and otic (three somite stage) differentiations may take place slightly earlier than formation of the presumptive boundaries between midbrain, forebrain, and hindbrain (Adelmann 1925; 1936a,b; Bartelmez 1962), although the precise sequence of events must now be reexamined thoroughly with more sensitive histochemical methods.

As the neural plate develops, the lateral margins of the neural folds gradually extend dorsally, relative to the neural groove, and eventually they begin to fuse—leading to the next major stage, formation of the *neural tube*. However, before they fuse, a narrow longitudinal strip of ectoderm at the border between neural and somatic ectoderm, caudal to the level of the optic pits, begins to differentiate at about the 4 somite stage in rats (Adelmann 1925). This strip, the *neural cres*t, eventually generates neurons of the *peripheral nervous system*, as do a series of *placodes* that differentiate from somatic ectoderm lateral to it (see Bartelmez 1962; Verwoerd and van Oostrom 1979; Altman and Bayer 1984; Tan and Morriss-Kay 1986; Hall 1988; Le Douarin and Smith 1988). The placodes, however, differentiate somewhat later, between embryonic days 11 and 12 in the rat, when the neural tube has formed (see Altman and Bayer 1982; Fig. 1 in Swanson 1992a).

It is important to point out that in mammals, unlike amphibians and birds, for example, cephalic neural crest cells begin migrating before closure of the neural tube. In the rat, this migration begins at the 4-5 somite stage, whereas the laterodorsal edges of the neural folds begin to fuse about 10 hours later at the 10-11 somite stage. In addition, this migration does not follow a simple rostral to caudal temporal gradient (for details see Tan and Morriss-Kay 1985, 1986; Morriss-Kay and Tan 1987; Fukiishi and Morriss-Kay 1992).

Histologically, the neural tube is a one-cell thick pseudostratified columnar epithelium. So far as is known, it contains a single cell type--progenitor cells that undergo symmetrical divisions by way of interkinetic migration, and that will eventually form the stem cells for the major cell types of the central nervous system, neurons and glia (see Jacobson 1991). However, it is now clear that the neuroepithelial cell population at this stage is not homogeneous. For example, homeobox POU-domain genes are differentially expressed in the neural plate, in a way suggestive of future structural divisions (Alvarez-Bolado et al. 1995).

Fundamental insights can be expected soon into molecular mechanisms underlying the induction of neuroepithelium; regional differentiation of the neural plate, presumably induced by gradients of diffusible morphogens; and changes in the shape of the neural plate as it progresses toward a neural tube. It cannot be overemphasized that to understand clearly the development of the brain, it is necessary to begin at the earliest stages.

B. Neural Tube Formation: Invagination

It is well-known that the vertebrate central nervous system becomes internalized by a process of invagination, in contrast to the delamination typical of invertebrates. This process of *neurulation* has been described in the rat, and is illustrated in Fig. 3. In the transverse plane, it is

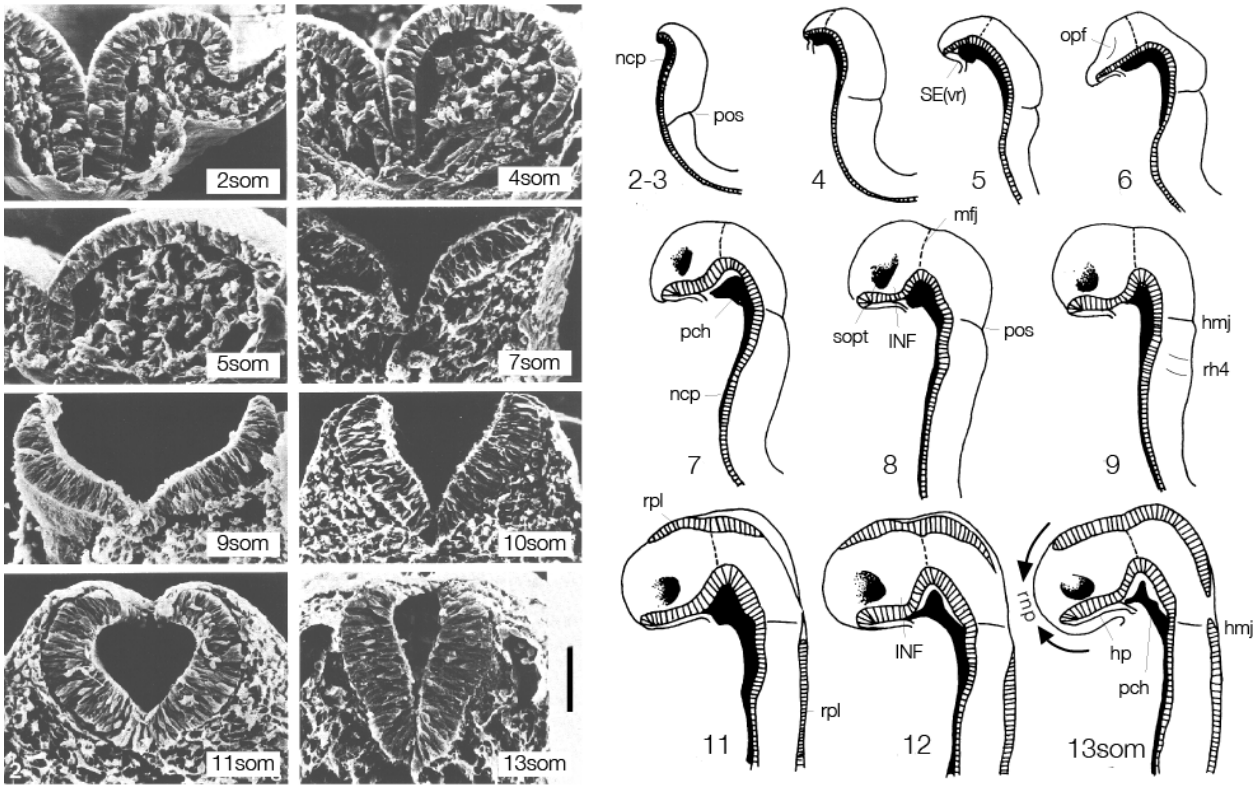

Fig. 3. Neurulation in the rat between the 2-13 somite stages on embryonic days 9 and 10. The scanning electron micrographs on the left show the fractured surface of the neural plate and tube in embryos transversely cut across the region roughly at the midbrain-hindbrain junction. The drawings on the right show the pattern of longitudinal growth in the brain part of the neural plate and tube at the same stages. The notochordal plate and notochord are shown in black. Arrows in the 13 somite stage drawing indicate two lines of fusion involved in closing the rostral neuropore. (Slightly modified from Morris-Kay and Tuckett, 1987).

obvious that the neural folds undergo a complex change in shape as the lateral margins gradually meet dorsally to fuse, leaving the region of the midline neural groove to lie ventrally in the embryo (Fig. 3, left). In other words, the topologically flat ectodermal neural plate becomes internalized, with the midline of the plate assuming a ventral position and the lateral margins of the plate fusing dorsally. Discussion of cellular mechanisms underlying this transformation may be found in Morriss-Kay (1981), Morriss-Kay and Tuckett (1985, 1987), and Jacobson (1991).

The exact progression of dorsal fusion varies in different species. In the rat (Fig. 3, right), fusion begins at two sites around the 10-11 somite stage (middle of embryonic day 10)(but see

Christie 1964). One site is associated with the transitional region between the brain and spinal cord, that is, in the cervical region where the initial somites differentiate from rostral to caudal at the rate of about a pair every two hours (Schwind 1928; Butcher 1929; Christie 1964). The second site, which may fuse slightly later (see Fig. 2, middle left; and Jacobson and Tam 1982), lies in a region around the border between the presumptive midbrain and forebrain vesicles, and there is a gap between these two regions of fusion that lasts on the order of 6 hours.

The caudal end of the caudal fusion region extends toward the end of the spinal plate, and the *caudal neuropore* is obliterated around the 23-25 somite stage (Christie 1964). Closure of the *rostral neuropore* occurs sooner, and is apparently more complex. It would appear that fusion in the forebrain region is accomplished by rostral fusion progressing simultaneously from dorsal and ventral parts of the neural plate (see Fig. 3, right, 13 somite stage). The rostral neuropore closes at about the 15-18 somite stage in the rat (Schwind 1928; Bartelmez 1962; Christie 1964), and some of the complex morphological changes associated with separation of the somatic and neural ectoderm in this region have been described by Hoving et al. (1990). The exact site of rostral neuropore closure is a matter of long debate, and may in fact vary in different animals of the same species. However, it is almost certain that it occurs in the region of the adult lamina terminalis, between the presumptive optic chiasm and anterior commissure (see Müller and O'Rahilly 1985).

Formation of the rostral end of the neural tube--the presumptive forebrain vesicle--has not been described in great detail. At the earliest stages of neural plate formation, the notochordal plate probably extends all the way to the oropharyngeal membrane, that is, to the rostral pole of the neural plate (see Fig. 13, e9; figs. 58-61 in Hamilton and Mossman 1972). However, the area of the rostral neural plate soon expands considerably, bulging rostrally on

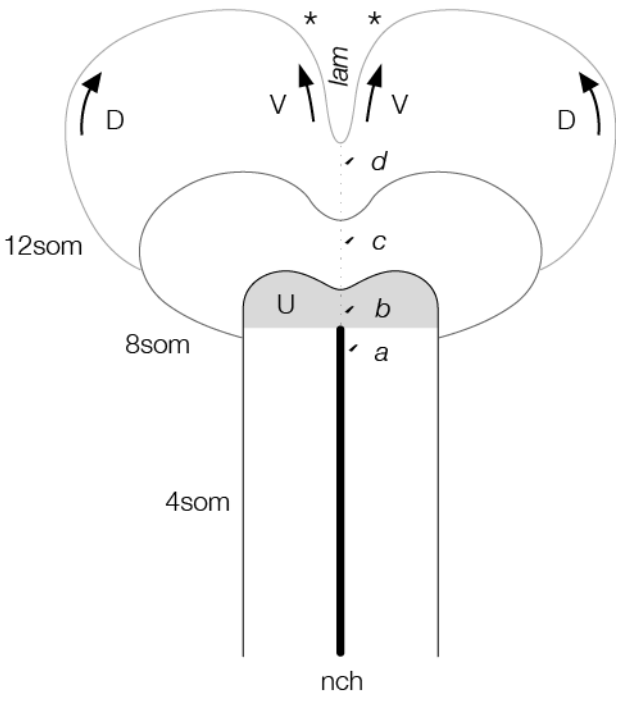

Fig. 4. Growth of the forebrain region in the rat neural plate between the 4 and 12 somite stages. The rostral end of the notochord (associated with the prechordal plate) ends at point a, just caudal to the infundibulum, and just ventral to the mammillary region of the hypothalamus. At the 4 somite stage, the rostral shaded area, U, of the neural plate may or may not be paired. By somite stage 8, this rostral area (between a and c) has begun to expand greatly, and this process continues through the 12 somite stage, when the rostral neuropore is beginning to close by a process of ventral (V) and dorsal (D) fusion (arrows; compare with Fig. 3). The ventral fusion produces the definitive terminal lamina, which begins just rostral to the future site of the optic chiasm (d). Asterisks indicate the approximate location of the presumptive anterior commissure. (Modified from Morris-Kay and Tuckett, 1987).

either side of the oropharyngeal membrane (Fig. 4), and bending ventrally with the head fold (see Fig. 13, e10, 5 somites). Beginning caudally, the medial (and ventral) and lateral (and dorsal) edges of the bulge on each side fuse and two fusion zones eventually meet rostrally (arrows *V* and *D* in Fig 4; Johnston 1909; Müller and O'Rahilly 1985). However, as a result of the rostral bulging, the prechordal plate eventually comes to lie ventral to the caudal hypothalamus, just caudal to the infundibulum and Rathke's pouch (see Fig. 13 and section IIIA2).

According to the analysis of Morriss-Kay and Tuckett (Morriss-Kay 1981; Tuckett and Morriss-Kay 1985; Morriss-Kay and Tuckett 1987), the number cells in the midbrain-rostral hindbrain neuroepithelium remains constant during rat neurulation, whereas the number of cells in the presumptive forebrain region of the neural plate increases more than can be accounted for by the rate of mitosis (an approximately 6 hour cell cycle). This would imply that disproportionate growth of the presumptive forebrain region (rostral end) of the

neural plate takes place by a combination of intrinsic mitoses and the rostral migration of cells generated in the brainstem. How far this rostral migration may extend remains to be determined (that is, to the presumptive telencephalic region? See below).

C. Brain Vesicles and Neuromeres: Transverse Regionalization

As the rostral neuropore closes, the brain region of the chick neural tube displays three rostrocaudally arranged swellings (Malphigi 1673), which have since been identified in all vertebrates. These *three primary brain vesicles* are now known as the *forebrain (prosencephalon)*, *midbrain (mesencephalon)*, and *hindbrain (rhombencephalon)*, and are recognizable during the second half of embryonic day 10 in the rat. Next, the forebrain vesicle divides into *endbrain (telencephalic) and interbrain (diencephalic) vesicles*, and the hindbrain displays a *pons (metencephalon) and medulla (myelencephalon)*—the *five secondary brain vesicle* stage first identified by Baer (1828) in the chick, and apparent on embryonic day 11 in the rat. Through early parts of the five vesicle stage, the wall of the entire neural tube remains a pseudostratified epithelium that has yet to generate neurons or glia.

Based on the discussion thus far, it seems clear that the neural plate and early neural tube demonstrate a pattern of histological regionalization before the first generation of neurons. The topography and nature of this regionalization has been one of the most contentious issues in neuroembryology. There are two fundamental questions that remain to be answered satisfactorily: what is the extent of transverse regionalization in the neural tube, and what is the relationship of transverse regionalization to segmentation of the neural tube?

1. Extent of Transverse Regionalization

Two features of the neural plate seem well-established. First, the rostral end is wider and corresponds to the brain, whereas the caudal end is narrower and corresponds to the spinal cord, although it is essential to point out that no histological feature separates the two until much later in development (with differentiation of the rhombic lip). At the simplest possible level of analysis, this implies the early existence of two transversely (rostral to caudal) arranged regions that could be explained by the well-known rostral to caudal elongation of the notochord by addition of cells from the region of the primitive node, combined with the localized rostral and/or caudal release of morphogens (see Gilbert and Saxén 1993). Thus, the early neural plate (Fig. 2, upper left) displays three cardinal features, *polarity* (rostrocaudal), *bilateral symmetry* (because of the midline neural groove), and *regionalization*. And second, the first two clear differentiations of the brain plate are the optic pits and the otic rhombomere. The interpretation of these two primordial features capsulize a host of problems in developmental neurobiology.

Leaving aside the entirely unknown mechanisms responsible for the appearance of these features, and turning to pure description, they have often been referred to as *neuromeres*. According to the classical work of Orr (1887), a neuromere may be defined as a transverse bulge in the neuroepithelium that is separated from adjacent regions by a relatively cell-poor septum and displays a higher rate of mitosis in the central region. Since then, hindbrain neuromeres have come to be known as *rhombomeres*, midbrain neuromeres as *mesomeres*, and forebrain neuromeres as *prosomeres* (Meek 1907). As considered in more detail below, the otic rhombomere corresponds well to Orr's definition of a neuromere. In contrast, the optic pit or neuromere does not: it is not a clearly transverse differentiation, and it is not separated from surrounding regions by an obvious cell-poor zone or septum.

Very shortly after the appearance of these two features, early indications of boundaries between the future forebrain, midbrain, and hindbrain vesicles appear as slight constrictions on the lateral margins of the neural plate (section IIIB), and also contribute to regionalization of the neural plate. Thus, when the rostral neuropore closes, 5 brain regions can be distinguished: the optic stalk and one or more rhombomeres, and the forebrain, midbrain, and hindbrain vesicles.

While the most synthetic review of early work on neuromerism was provided by Kupffer (1906), the most comprehensive and penetrating analysis of the problem to date has been carried out by Bergquist and Källén (see Bergquist and Källén 1954; Källén 1956). Based on work in a wide range of vertebrates, they divided the process into three distinct phases: (a) *proneuromerism*, in the neural plate; (b) *neuromerism*, in the early neural tube before neurogenesis; and (c) *postneuromerism*, in the later neural tube and involving the generation and migration of neurons. They agreed with Kupffer that these transverse bands correspond to *proliferation zones*, so that differentiation of the central nervous system would consist of three consecutive waves of proliferation intensities that proceed from rostral to caudal. Furthermore, the successive patterns of proliferation zones are formed such that the second subdivides the first, and the third subdivides the second--that is, a basic pattern (proneuromeres) is progressively subdivided. Specifically, they proposed that the vertebrate neural plate displays one or two proneuromeres in the presumptive forebrain, one in the presumptive midbrain, and three in the presumptive hindbrain. This description corresponds reasonably to what was outlined above, with the three hindbrain proneuromeres corresponding to the otic rhombomere and one on either side, except that it fails adequately to deal with the optic pit in the presumptive forebrain. At the classical neuromere stage of the early neural tube, they recognized four prosomeres (the most caudal

corresponding to the synencephalon or pretectal region), one mesomere, and six rhombomeres (each of the 3 prorhombomeres being divided in two).

The Bergquest-Källén model of CNS differentiation is attractive in its simplicity and elegance. However, because so little has been done on this problem, their observations need to be confirmed with similar methods, and extended with newer methods (such as spatiotemporal expression patterns of homeobox genes). For example, their claim that prosomeres disappear before neuromeres appear has not been confirmed, although no one has examined the problem with as closely spaced developmental stages and closely spaced series of histological sections. In addition, their analysis of forebrain development, at least in the rodent, is seriously flawed (section IIIA), and the work of Adelmann (1925) suggests that the otic rhombomere (caudal) differentiates before the optic pits (rostral).

Rhombomeres are by far the clearest indication of transverse differentiation in the early CNS (see Guthrie and Lumsden 1991), although they are a transitory feature. In rats, the first indication of this formation is the appearance of an otic rhombomere in approximately the center of the hindbrain region, perhaps as early as the 3 somite stage, early on embryonic day 9 (above). The exact stage at which the rhombomeres disappear in the rat has not been determined precisely, although based on evidence from the human (Hines 1922) and mouse (Källén and Lindskog 1953), this likely occurs on embryonic day 13. There now seems general agreement that in mammals, three primary rhombomeres (proneuromeres), called A-C by Bartelmez (1923), are replaced by or subdivided into 7 secondary or definitive rhombomeres (Figs. 5, 17) (Adelmann 1925), although the order in which they appear and disappear is not linear (for details see Adelmann 1925; Tuckett et al. 1985). In the rat, they are shaped irregularly, and the secondary otic rhombomere (number 4, associated with the facial nerve) is the only one that

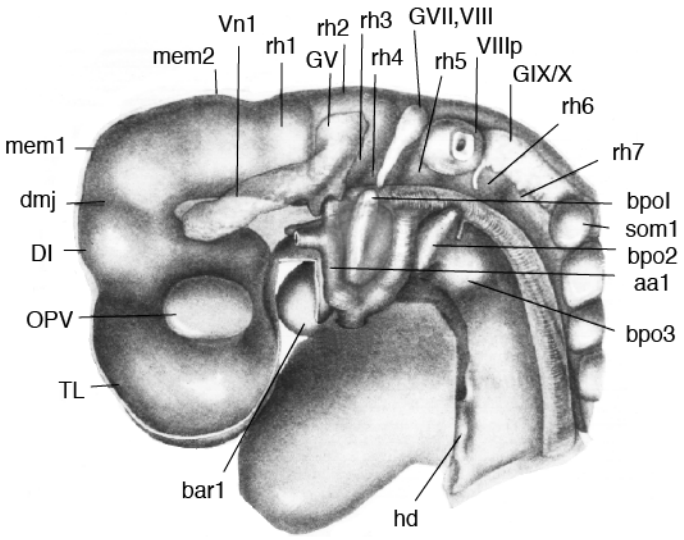

Fig. 5. A lateral view of a model of the head of an 18 somite rat embryo. The head was dissected to show the neural tube and anlagen of the cranial ganglia. (Relabeled from Adelmann, 1925).

forms a complete ring around the hindbrain vesicle, extending from roof plate to floor plate (Adelmann 1925). Incidentally, there remains some confusion about the numbering of rhombomeres. Adelmann's scheme for the rat, illustrated in Figs. 5 and 17, may be related to the scheme used by Noden (1991) in the following way: rhombomeres 1-4 the same; Adelmann's 5 includes Noden's 5 and 6; Adelmann's 6 equals Noden's 7, and Adelmann's 7 is caudal to Noden's 7, and is related to the vagus nerve.

Mechanisms responsible for triggering the development of rhombomeres—whether intrinsic or extrinsic—are unclear although an intimate relationship with the adjacent *branchial arches* is obvious, and it is generally accepted that rhombomeres differentiate along with motoneuron pools of the cranial nerves, although there is not a one-to-one correspondence between the two (see Keynes and Lumsden 1990; Noden 1991; Gilland and Baker 1993). A reasonable model (Fig. 6) of cellular events leading to the formation of rhombomeres in the rat has been proposed by Tuckett and Morriss-Kay (1985). In essence, the model proposes that bulges are formed by the occurrence of fan-shaped arrays of cells with microtubules aligned perpendicular to the luminal surface in the septa between neuromeres, and by microfilaments localized to the luminal end of cells in the neuromeres themselves. Figure 6 also emphasizes the

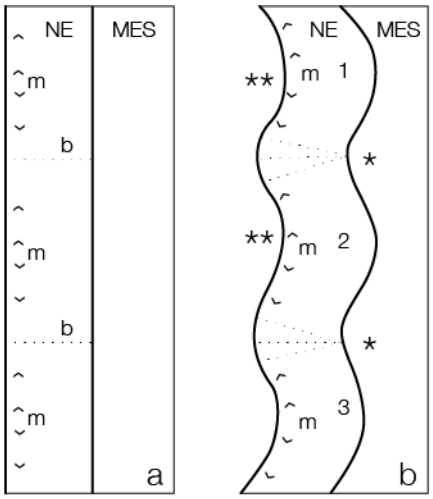

Fig. 6. A model of rhombomere formation in the rat embryo. a. Before neuromere formation, the luminal surface is rich in microfilaments (m), which contract in the direction of arrows, and while growth of the neural plate/tube is generated by cell division in the longitudinal plane, the neuroepithelium (NE) is fixed at certain points, forming "blocks" (b) to elongation. b. During neuromere formation the lengthening neuroepithelium bulges along lines of least resistance, with the microfilament-rich luminal border (opposite the mesenchyme, MES) preventing inward bulging. In addition, fan-shaped arrangements of cells with microfilaments aligned perpendicular to the luminal surface differentiate, and as a result of these changes sulci form at the interneuromeric junctions on the outside of the neural tube (*), and in the middle of neuromeres on the inside of the neural tube (**). Finally, neuromeres appear to represent proliferation, and later migration, zones in the wall of the neural tube. (Redrawn from Tuckett and Morris-Kay 1985).

fact that neuromeres are recognized as bulges externally and depressions internally. Recent transplantation experiments in chick suggest that molecular signals generated within, and transmitted longitudinally through, the neural tube are critical for the establishment of rhombomeres (Grapinbotton et al. 1995).

Based on scanning electron microscopy, Tuckett et al. (1987) concluded that at the three primary vesicle stage of rat development, one prosomere can be identified with certainty, and that two mesomeres, which differentiate from one, are also clear.

2. Neuromeres versus Segments

Neuromeres are often equated with *segmentation* of the developing central nervous system. However, a clear distinction between segmentation and *regionalization* should be made. Historically, and properly speaking, segmentation is synonymous with *metamerism*, that is, the formation of serially repeating, initially similar morphological units. Segments in annelid worms and insects are familiar, unequivocal examples, as are the somites characteristic of vertebrates. These repeating units undoubtedly share a common genetic program that reduces the total amount of DNA required for

morphogenesis, although individual units commonly show different patterns of terminal differentiation (for a good introduction to molecular mechanisms see Lawrence 1992). There is no compelling evidence to date that neuromeres are metameres, and until this is established (or refuted), it seems appropriate to refer instead to regionalization of the neural plate and tube. Thus, our use of the word 'neuromere' in this book implies a morphologically distinct region of the developing brain, whether or not surrounded by a cell-poor zone or septum (observed only in rhombomeres), not a segment of the developing brain. In summary, the evidence to date suggests that they are proliferation centers that later generate localized groups of neurons, and are the fundamental building blocks or divisions of the central nervous system. They provide a topographical description of the latter, but fail to explain the organization of functional neuronal systems (circuits). The synthesis of topographic and systems approaches is a long-range goal of systems neuroscience.

D. The Birth of Neurons: Longitudinal Regionalization

Just after the five vesicle stage has begun, the differentiation of neurons and radial glial cells starts in the neuroepithelium. The generation of neurons leads to further regionalization of the neuromeric pattern, and at least in the brainstem this regionalization tends to be oriented longitudinally rather than transversely. A brief overview of this process will be given here, with more details given in the next section.

It is perhaps best to start in the spinal cord, where it is established that neurogenesis begins ventrally and spreads dorsally, with a slight rostral to caudal temporal gradient (from embryonic day 10 to 13 in the rat; Altman and Bayer 1984). This precocious differentiation of a *mantle layer* in ventral parts of the spinal cord apparently leads to the formation of a longitudinal

groove on the ventricular surface of the spinal tube—the *limiting sulcus*, which divides it into *basal (ventral)* and *alar (dorsal) plates* (His 1888). However, the limiting sulcus only becomes clear around embryonic day 14 in the rat (Altman and Bayer 1984), and it now seems clear that the limiting sulcus does not distinguish clearly between a 'purely' motor ventral part of the spinal cord and an interneuronal/sensory dorsal part. Instead, it provides a rough guide to the dorsal border of the prospective motoneuron pools in the ventral horn. One other feature of spinal cord development deserves mention in view of the earlier discussion of neuromery. Whereas neuromeres have been described in the spinal cord, they disappear before the generation neurons, which takes place in the form of uninterrupted longitudinal columns (see Bergquist 1952; Källén and Lindskog 1953).

The gross anatomy of early hindbrain morphogenesis is treated in almost every relevant textbook. All that needs to be reiterated here is that, first, the limiting sulcus extends unequivocally to the rostral end of the hindbrain, with cranial nerve motoneuron pools generated ventromedial to it and cranial nerve sensory nuclei generated dorsolateral to it. As in the spinal cord, the limiting sulcus, defining basal and alar plates, appears after the neuromeres (rhombomeres) disappear (see Hines 1922; Hochstetter 1919, 1929, for human brain). And second, a distinguishing feature of the hindbrain vesicle is the appearance of a *rhombic lip*, which extends longitudinally in the most dorsal region of the alar plate (see below).

The overall morphological differentiation of the midbrain vesicle has received less attention than any other division of the central nervous system. It is usually stated in textbooks that the limiting sulcus divides the midbrain vesicle into *tectal* (dorsal, alar) and *tegmental* (ventral, basal) parts. However, careful examination has failed to trace the hindbrain limiting sulcus without interruption into the midbrain vesicle (see Keyser 1972). On the other hand, two

studies of the developing mouse (Palmgren 1921; Bengmark et al. 1953) agree on basic features. First, soon after the mantle layer differentiates, two longitudinal sulci divide the midbrain into three zones. The dorsal sulcus (*lateral tectal sulcus* of Palmgren, *tectal sulcus* here) indicates the approximate ventral extent of the tectum, whereas Palmgren's *lateral midbrain sulcus* (*midbrain sulcus* here) is quite ventral and divides a *subtectal region* into dorsal and ventral halves. There is some evidence to suggest that the oculomotor nucleus and substantia nigra originate from neuroepithelium ventral to the (lateral) midbrain sulcus (Palmgren 1921; Bengmark et al. 1953; Kawano et al. 1995). Recall that after rhombomeres disappear, the hindbrain is divided into three longitudinal zones by the limiting sulcus (ventrally) and the rhombic lip (dorsally).

Forebrain differentiation is much more complex than regions of the neural tube caudal to it. As noted above, the optic pits and then stalks are the first morphological features to appear, and they dominate early on. Then, on embryonic day 11 in the rat, two major events occur. First, the endbrain and interbrain vesicles are distinguished by the *torus hemisphericus*, an internal bulge, and the *hemispheric sulcus*, a corresponding external groove. This boundary courses just rostral to the *optic sulcus*. And shortly thereafter, two longitudinal grooves appear in the interbrain: the *middle diencephalic* and *hypothalamic sulci*. Then, on rat embryonic day 12, the two parts of the forebrain vesicle are further divided by additional sulci: the *corticostriatal sulcus* in the endbrain, and the *habenular sulcus* in the interbrain. At this stage, the endbrain is divided into cortical and basal nuclear regions, whereas the interbrain is divided into habenular, dorsal thalamic, ventral thalamic, and hypothalamic regions, as defined on comparative morphological grounds by Herrick (1910)(Fig. 2, lower row). Additional details about forebrain differentiation are provided in section IIIA; here we need only point out that birthdating evidence indicates that the pattern of neuronal differentiation in the forebrain vesicle is much different than in the spinal

cord and hindbrain: it does not proceed from ventral to dorsal, and is not arranged in a clear longitudinal way (reviewed in Alvarez-Bolado et al. 1995).

E. Nonradial Migration and Fate Mapping

Differentiation of the early mantle layer to the cytoarchitecture of the adult brain is obviously a very complex problem. However, the problem can be simplified by imagining that each neuron is derived from a point in the neuroepithelium and then migrates to its final adult position. That is, under normal circumstances, a neuroepithelial cell with a particular address eventually generates one or more neurons that migrate to their final addresses in the brain. The exercise of determining experimentally the neuroepithelial origin of adult neuronal cell groups is referred to as *fate mapping*. Thus, an important goal of developmental neuroscience is to correlate regionalization of the neuroepithelium, and then regionalization of the mantle layer, with adult structure. The ultimate goal is to produce a map of the neural plate, an essentially two-dimensional structure, that displays the origin of each adult neuronal cell group.

This problem is greatly simplified by evidence that a majority of neurons migrate perpendicular to the neuroepithelial surface along, or in the direction of, a scaffolding of radial glial cells (see Fig. 7a; and Rakic 1987). On the other hand, some neuronal populations migrate tangentially at some stage of development. The best evidence for this comes from the rhombic lip, where some cells migrate dorsally to form the granule cell layer of the cerebellum, and other cells migrate ventrally to form the inferior olive, raphé nuclei, basal pontine gray, and other cell groups in the brainstem (Fig. 7b; and His 1890; Harkmark 1954; Bourrat and Sotelo 1990a). Furthermore, neuronal migrations can be even more complex. For example, Levi-Montalcini (1950) demonstrated that sympathetic preganglionic neurons first migrate radially from the

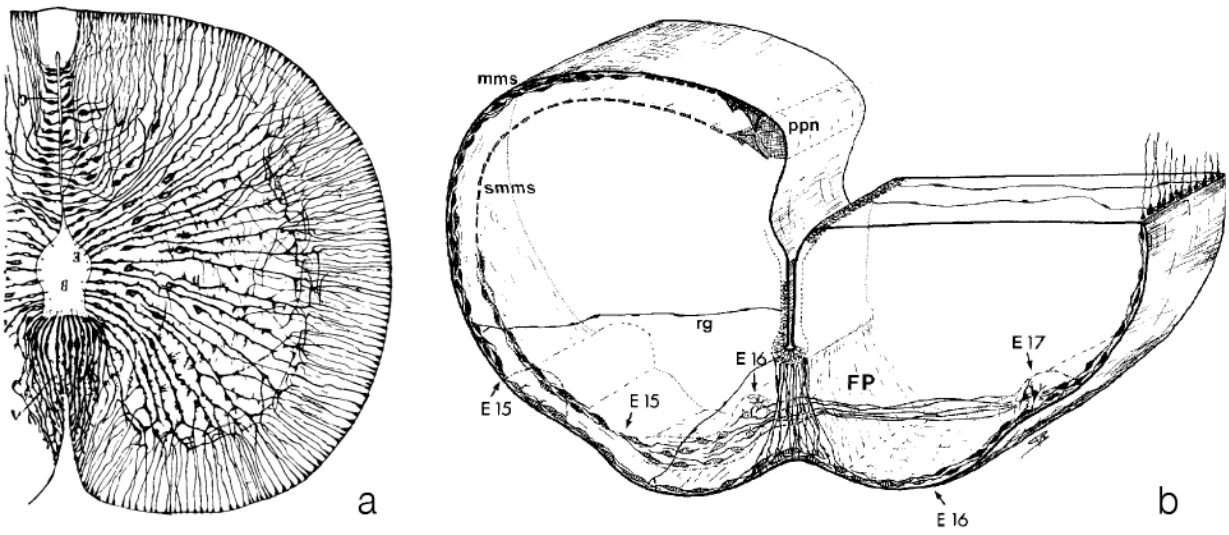

Fig. 7. a. The arrangement of radial glial fibers in the spinal cord of a 44 mm. human embryo as seen with the Golgi method. The illustration is printed upside down so that dorsal it at the top. b. Nonradial migration in the embryonic rat hindbrain. Young neurons generated in the rhombic lip (ppn, precerebellar neuroepithelium) migrate ventrally in two streams (mms, marginal migratory stream; smms, submarginal migratory stream) to form cell groups such as the inferior olive, lateral reticular nucleus, external cuneate nucleus, and basal pontine gray. The progression of cell migration at various embryonic days (E15-17) is indicated. Other abbreviations: FP, floor plate; rg, radial glial fiber. (a, from Cajal, 1909-11; b, from Bourrat and Sotelo, 1990b).

ventral spinal neuroepithelium along with young somatic motoneurons, but then after settling briefly undergo a dorsal *secondary migration* to the chick equivalent of the intermediolateral column.

At least two complications need to be superimposed on these basic principles of radial and nonradial migration. First, because mantle layer differentiation often involves considerable differential growth of adjacent regions, the underlying radial glial cell scaffolding may be severely distorted while remaining topologically radial. A good example of this involves formation of the dentate gyrus (see section IIIA). And second, it appears certain that more than one cell type, and more than one cell group, may be generated from the same patch of neuroepithelium, usually, although not necessarily always, in a temporally sequential way. The best example of this principle is the sequential formation of layers in the cerebral cortex (see

section IIIA). All of these features must be taken into account in refining a fate map of the neural plate.