CELL MIGRATION DOMAINS IN THE CHICK TELENCEPHALON

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For my mother,
Evelyn Lerner Montgomery

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> Punishment... is born at the same instant with the sin... Wickedness forges torments against itself.
>
> Montaigne

> In the wound they make they leave their lives.
>
> Virgil

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ABSTRACT

Little is known about the process by which the vertebrate forebrain (the diencephalon and telencephalon) becomes regionalized during development. In studies reported here, DiI injections were used to label the embryonic day 3 (stage-16) chick telencephalon *in ovo* , and the migration patterns of labelled cells were analyzed in relation to various molecular markers, including the regulatory genes Cash-1 and Sonic hedgehog (Shh). Cells generated in the ventral telencephalon (basal ventricular ridge, or BVR) were found to migrate widely, but were restricted from crossing into the more dorsal telencephalon (dorsal ventricular ridge, or DVR), and the more caudal diencephalon. The cell migration boundary between the BVR and DVR correlates with a Cash-1 expression boundary, and the cell migration boundary between BVR and diencephalon correlates with a Shh expression boundary. In addition, cell migration patterns were dramatically different in the BVR and DVR territories. These results suggest that the BVR represents a "cell migration domain," or a true unit of telencephalic compartmental organization, which is distinct from cell migration domains in both the DVR and diencephalon. In addition, two lines of evidence in the early embryo are shown to support the proposal that avian BVR is homologous to mammalian basal ganglia, and that avian DVR is homologous to mammalian cerebral cortex: regulatory gene expression patterns in the chick and mouse telencephalon are very similar; and the cell migration patterns in the chick telencephalon demonstrated here are found to correspond closely to those previously reported in the mouse telencephalon.

Using the same DiI labelling technique, a regional fate map of the stage-16 chick telencephalon was derived. This fate map can now be used to guide transplantation or misexpression experiments, and to interpret gene expression patterns in the stage-16 telencephalon. For example, though Cash-1 is expressed in the entire BVR at E5-E7 (stage 24-30), superimposition of its expression pattern onto the stage-16 fate map shows that it is only expressed in a subregion of the presumptive BVR at stage-16.

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Ring the bells that still can ring
forget your perfect offering
there is a crack
a crack in everything
that's how the light gets in

Leonard Cohen

I come from the place where I desire to return.
Love moved me and makes me speak.

Dante, The Inferno

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INTRODUCTION

The aim of developmental genetics is to understand how genetic information is recast into function and form. This general problem can be conceptually divided into two major developmental phenomena: the acquisition of specific cellular phenotypes, and the acquisition of pattern in organs and appendages. This thesis addresses the process by which the forebrain (the telencephalon and diencephalon) acquires its basic pattern during the earliest stages of its development.

Pattern formation in *Drosophila*

Many of the paradigms which have motivated studies of pattern formation have grown out of experiments originally performed in the fruit fly, *Drosophila melanogaster* . Two major aspects of *Drosophila* pattern formation have been intensively studied: the patterning of the early embryo, and the patterning of the imaginal discs. The cells which will later form the imaginal discs are set aside during embryonic development, and then expand during larval life, eventually forming the appendages of the adult fly. The *Drosophila* wing imaginal disc system provided some of the earliest insights into how an initially uniform field of cells may become subdivided into distinct territories that may then go on to acquire unique identities (Garcia-Bellido, 1975). Cell marking studies found that, from early blastoderm stages, the pool of cells which gives rise to the anterior wing remains separate from those giving rise to the posterior wing; even when challenged by differential cell growth rates in either the anterior or posterior region, the two neighboring pools of cells do not mix with one another (Garcia-Bellido, 1975).

In flies carrying a mutation for the engrailed gene, the posterior region of the wing resembles a mirror image of the anterior wing, developing vein patterns and bristles normally only found in the anterior region (Morata and Lawrence, 1975). In engrailed

mutants, where the posterior wing blade is transformed into an anterior identity, marked cells in the posterior wing no longer remain confined to the posterior cell field, and instead mix with cells originating in the anterior field of the imaginal disk. Based on these studies, Garcia-Bellido (1975) hypothesized that the anterior and posterior regions of the wing each represent "developmental compartments" which are separated by a compartmental restriction maintained by the action of engrailed, the "selector gene." The expression of a selector gene was postulated to be limited to a polyclone of compartmental progenitors and to be stably inherited; its expression would then act as a switch which both restricts cells to a compartment during growth (presumably by decreasing their affinity for non-compartmental cells) and specifies the identity of distinct compartments. The selector-affinity model therefore predicted that the loss of a selector gene should disrupt both lineage restriction and compartment-specific identity (Garcia-Bellido, 1975; Morata and Lawrence, 1975). This model, in a sense, made the problem of pattern formation appear to be conceptually much simpler: an animal could be seen as being a collection of distinct units, which to some extent interact and to some extent are autonomous, and which could be acted upon by evolution independently (Garcia-Bellido, 1975).

The dorso/ventral boundary in the *Drosophila* wing

In the Drosophila wing imaginal disc, in addition to an antero/posterior (A/P) cell lineage restriction, there is also a dorso/ventral (D/V) restriction (Blair, 1993). After it was found that a zone of cells with low replication rates was present near the D/V boundary, but not the A/P boundary, in the wing, it was suggested that these cells may act as a barrier to the spread of clones across the D/V wing margin (Blair, 1995). It has

since been shown, however, that the D/V restriction, like the A/P restriction, is mediated by a selector gene, apterous (Diaz-Benjumea and Cohen, 1993), and in cell marking experiments, the limit of apterous expression often coincides exactly with marked cells (Blair, 1993). Furthermore, both the apterous boundary and the cell-mixing boundary were shown to run along the middle of the zone of "non-proliferating" cells previously proposed to act as a barrier. These experiments essentially disproved the barrier hypothesis (Blair, 1993).

When ectopic ventral cells are created in the dorsal wing margin, characteristic boundaries are created around their perimeter, effectively isolating the "ventral " cells from the surrounding dorsal cells (Blair,1993). This phenomenon is similar to findings in the hindbrain, where boundaries are created when specific rhombomeres are juxtaposed in transplants (Guthrie, 1991; see later). Blair et al. (94) also found that different integrin genes are normally expressed in the dorsal and ventral compartments. Integrins have been shown to mediate cell migration and adhesion in a number of systems (Galileo et al., 1992; Baver et al., 1992; Bronner-Fraser, 1993). In apterous$^-$ flies, cell mixing between the dorsal and ventral compartments correlates with the expression of the same integrin gene in the dorsal and ventral cells (Blair et al., 1994), supporting the idea that cell-adhesion differences are responsible for the observed cell-mixing restrictions in the wing disc.

Boundaries do not appear to form as a consequence of the presence of specialized cells; rather, it has been proposed that at least one purpose of boundaries may be to create regions of specialized cells near the location of the boundaries (Blair, 1995). In the parasegments and imaginal discs of *Drosophila*, such regions appear to formed by inductive interactions between the cells in each compartment; boundary regions may play specific roles in the patterning of cells within each compartment, in some sense defining the axes of appendage development (Heemskerk and DiNardo, 1994; Basler and Struhl,

1994). In *Drosophila* , it has been shown that transcompartmental inductive events are necessary to induce expression of a number of regulatory genes present near compartmental boundaries (Blair, 1995; Basler and Struhl, 1994; Kim et al., 1995; Ingham, 1993; Zecca et al., 1995; Diaz-Benjumea and Cohen, 1993).

Patterning in the vertebrate CNS

In amphibians and other vertebrates, neural development is thought to be induced in the ectoderm by signals arising from the dorsal mesoderm during gastrulation (reviewed in Doniach, 1993; Kelly and Melton, 1995). While classical embryological experiments indicated that these signals follow a "vertical" path, from the involuted dorsal mesoderm to the overlying ectoderm, recent work has revealed the existence of "planar" neural-inducing signals, which pass within the continuous sheet formed by the dorsal mesoderm and presumptive neuroectoderm (Papalopulu and Kintner, 1993).

In CNS regions caudal to the forebrain, neurons in the ventral neural tube are thought to be induced by signals arising from the notochord and/or the floorplate (reviewed in Placzek, 1995). These inducing activities are mimicked by the amino-terminal domain of the Shh protein (Roelink et al., 1994, 1995; Echelard et al., 1993). Based on both cell morphology, and on the absence of diagnostic markers, the forebrain is thought to lack both a notochord and a floorplate (Ericson et al., 1995; Doniach, 1993). At early developmental stages, Shh expression in the forebrain is confined to the ventral midline of the diencephalon and caudal telencephalon (Ericson et al., 1995; Marti et al., 1995). Sonic hedgehog (Shh) has been found to induce the differentiation of ventral neuronal cell types in explants derived from forebrain regions, including the telencephalon, in a contact-independent manner (Ericson et al., 1995). Shh therefore appears to mediated induction of distinct ventral neuronal cell types along the entire rostro/caudal extent of the embryonic neural tube.

The hindbrain

Antero/posterior (A/P) patterning in the vertebrate CNS has been most extensively studied in the hindbrain. Early in its development, the hindbrain is divided into "rhombomeres," which are first visible as periodic undulations that develop on either side of the unsegmented floor plate. Later, boundaries which separate each rhombomere become morphologically detectable (Lumsden and Keynes, 1989). It has been suggested (Martinez-Arias and Lawrence, 1985; Lumsden, 1990) that to be true developmental compartments, rhombomeres, or any other "neuromeres" in the CNS, should optimally have four characteristics: 1) a neuromeric pattern corresponding to an underlying pattern of cell or molecular differentiation; 2) differentiation patterns which are matched by equivalent patterns of cell proliferation; 3) neuromeres boundaries which act as barriers to cell movement; 4) expression of genes with possible regulatory roles in patterns that relate in some way to the neuromeric pattern. Each of these characteristics appear to be met, at least to some extent, in the developing hindbrain: differentiated neurons first emerge in r4, r2, and r6, and only later appear in odd-numbered segments (Lumsden and Keynes, 1989); rhombomeres are centers of cell proliferation, while boundaries contain populations of relatively static cells (Guthrie et al., 1991b); cell movements appear to be restricted at rhombomere boundaries (Fraser et al., 1990), though these restrictions are not absolute (Birgbauer and Fraser, 1994); various Hox genes (Lumsden, 1990), a zinc-finger gene (Nieto, 1991), a tyrosine kinase receptor gene (Nieto, 1992), and other cell-surface markers (Kuratani, 1991; Wijnholds et al., 1995) are expressed in specific rhombomeres. Null mutations in some of these genes produce distinct defects in specific rhombomeres (Schneider-Maunory et al., 1993; Mark et al., 1993). In addition, the early partitioning of rhombomeres appears to be mediated by adhesive differences between

adjacent rhombomeres (Guthrie and Lumsden, 1991), with the observed cell surface properties supporting, at least in part, the idea of a 2-segment periodicity. In support of the idea that differential expression of regulatory genes can influence adhesive behavior, a number of transcription factors have been shown to have downstream effects on adhesion molecules (Valarché et al., 1993; Gould and White, 1992; Jones et al., 1992) and to control the direction of cell movements (Garriga et al., 1993; Salser and Kenyon, 1992).

In a series of donor-to-host transplant experiments which confronted tissue from different axial levels within the hindbrain, Guthrie et al. (1991, 1993) showed that new rhombomere boundaries are generally only created when rhombomeres from adjacent positions or positions 3 rhombomeres distant from each other are juxtaposed. Combinations of 2 odd- or 2 even-numbered rhombomeres usually failed to generate a new boundary (Guthrie, 1991). When new boundaries were formed in these transplants, cells generally were restricted from mixing between the apposed rhombomeres; however, when a new boundary was not created, e.g., by the apposition of r3 and r5, cells mixed relatively freely between the two rhombomeres (Guthrie et al., 1993). These results suggest that a rhombomere boundary is not simply a physical barrier which impedes the mixing of cells from adjacent rhombomeres. Instead, cells from adjacent rhombomeres appear to possess distinct cell surface differences which lead to the creation of a boundary (Guthrie and Lumsden, 1991; Guthrie et al., 1993). Though lineage restrictions exist in the spinal cord, the restrictions are not intrinsic to the neuroepithelium, as they have been shown to result from interactions with neighboring mesodermal somites (Lim et al., 1991; Stern et al., 1991).

Later in hindbrain development, after the lineage restrictions have become apparent, specialized cells form at rhombomere boundaries, as indicated by, for instance, increased levels of chondroitin sulfate proteoglycan and vimentin immunoreactivity, as well as upregulation of certain regulatory genes such as Pax-6, Fgf-3, and the zinc-finger

gene PLZF (Heyman et al., 1995). Boundaries are also characterized by specific cell cytoarchitectures and low cell densities (Guthrie and Lumsden, 1991; Guthrie et al., 1991b), and are pathways for commissural axons (Lumsden and Keynes, 1989). While specialized cells do develop at the rhombomere boundaries, the available evidence indicates that the specialized boundaries are a consequence, rather than a cause, of lineage restrictions (Guthrie, 1995).

The diencephalon

The vertebrate diencephalon has been traditionally divided into 4 regions: dorsal thalamus, ventral thalamus, epithalamus, and hypothalamus (Herrick, 1910). These 4 areas can be clearly homologized in all existing vertebrates (Alvarez-Bolado et al., 1995). Figdor and Stern (1993) have proposed that the diencephalon is composed of four compartments, D1-D4, and provide evidence that lineage restrictions distinguish the borders between these regions. In addition, cell surface markers such as peanut lectin are upregulated in alternate compartments, and alternate compartments appear to have different schedules of neurogenesis, much like the hindbrain (Figdor and Stern, 1993; Layer and Alber, 1990; Layer et al., 1988; Puelles et al., 1987). Many potential regulatory genes, including genes coding for both transcription factors and cell-surface and secreted proteins, have been shown to be expressed in various subregions of the diencephalon (reviewed in Puelles and Rubenstein, 1993), and many of these genes appear to have expression boundaries which correlate with the D1-D4 boundaries proposed by by Figdor and Stern (1993). Based on gene expression and morphological data, Rubenstein et al. (1994) have proposed a model of diencephalic compartmentalization which is significantly different from the model proposed by Figdor and Stern (see Chapters 3 and 4). In the "neuromeric" model of Rubenstein et al., there are three proposed neuromeres which contain only diencephalic derivatives, and three

additional neuromeres which contain both diencephalic and telencephalic derivatives (Bulfone et al., 1993 ; see Chapters 3 and 4). Putative "segmental" distributions of molecular markers have also been reported in the cerebellum (Oberdick et al., 1993; Hatten and Heinz, 1995) and the midbrain (Wurst et al., 1994).

Histogenesis in the telencephalon

Telencephalic neurons are generated in the ventricular zone (VZ) and, when postmitotic, leave the VZ, migrate into the intermediate zone (IZ), and then into the cortical plate (Rakic, 1988), apparently by forming "migration junctions" with radial glial fibers (Hatten, 1990). The developing cortex contains long glial guides with highly complex branching patterns (Rakic, 1988). Antibody perturbation experiments suggest that the astrotactin protein may provide a neural receptor for migration on radial glial fibers (Fishman and Hatten, 1993).

Cell migration plays a fundamental role in many normal biological processes, such as embryonic development, wound healing, and the immune response, as well as in many pathological conditions such as metastasic tumor spread (reviewed in: Huttenloche et al., 1995; Hynes and Lander, 1992; Stossel, 1993). A network of subcortical actin is thought to provide mechanical support for the surface of a cell, enabling the cell to undergo the shape changes that are associated with locomotion (Rivas and Hatten, 1995). Cell surface adhesion molecules are central to the migratory process, as they couple interactions with the substratum to the intracellular cyoskeletal apparatus. Pathways involving receptor tyrosine kinase signalling appear to be involved in cell migrations in both invertebrates (Murphy et al., 1995; DeVore et al., 1995; Reichman-Fried et al., 1994) and vertebrates (Klemke et al., 1994). Ion channel activities have also been shown to modulate cell migration dynamics in the vertebrate brain (Komuro and Rakic, 1992, 1993).

Prior to the onset of neurogenesis, the telencephalic progenitor pool appears to be expanded through a series of symmetrical divisions in which both daughter cells reenter the cell cycle after mitosis (Caviness, 1995). Early progenitor cells appear to divide asymmetrically in a stem cell fashion, with each division producing a neuron and another progenitor cell. Studies in which cortical progenitor cells were labelled with DiI and imaged in living slices through the VZ have supported this idea (Chenn, 1995). The early developing VZ is a pseudostratified single-cell layer composed of mitotic neuroblasts which span the ventricular and pial surfaces of the neuroepithelium (Caviness, 1995). During each mitotic division, neuronal cell bodies are translocated, so that neuroblasts break and then reform their pial connection each time the cell body divides. After the final cell division occurs, the cell migrates toward the marginal zone by breaking its connection with the ventricular surface, and finally by losing its endfoot attachment to the pia (Takahashi et al., 1993). At early developmental times, the vast majority of cells in the cortex reenter the cell cycle after mitosis without generating any postmitotic cells, resulting in a steady expansion of the proliferative population. At about E14 in the mouse, half of all cortical cells exit the cell cycle after mitosis, and neurons are produced in large numbers as the ventricular population begins to be depleted (Caviness, 1995; Takahashi et al., 1995, 1995b). In the mouse neocortex, cell-cycle duration increases from about 8 hours at E11 to nearly 20 hours at the end of neurogenesis (about E17) (Takahashi et al., 1994).

Cell identity in the telencephalon

There are three primary aspects to cell fate in the early developing mammalian cerebral cortex: cell identity, laminar fate, and cytoarchitectonic (regional) fate. While in most regions of the CNS, single progenitors may produce clones that contain both neurons and glial cells (Turner, 1987; Wetts and Fraser, 1988; Leber, 1990; Gray et al.,

1990; Gray and Sanes, 1992), lineage studies in the cerebral cortex have suggested that neurons, astrocytes, and oligodendrocytes may largely arise from distinct sets of progenitors (Walsh and Cepko, 1992; Grove et al., 1993). In addition, white matter and grey matter astrocytes appear to be generated from distinct precursor-cell populations (Grove et al., 1993), as are pyramidal and non-pyramidal neurons (Parnavelas et al., 1991; Götz et al., 1995). In vivo studies have shown that specified precursor cell types make up 80-90% of precursor cells and that the remaining precursors are bipotential, generating either neurons and astrocytes, or neurons and oligodendrocytes (Grove et al., 1993). In vitro studies have largely confirmed these results (Williams et al., 1991, 1995). However, the analysis of clones in these studies has usually relied on replication-incompetent retroviruses, which can often result in "splitting errors" in which distant but clonally related cells are not recognized as having originated from the same clone (Walsh and Cepko, 1992). The use of large libraries of retroviral vectors that allow individual cells to be assigned to a clone regardless of their position within the cortex, has indicated that 90% of widespread clones contain cells of multiple phenotypes, e.g., both neurons and glia, or both pyramidal and nonpyramidal neurons. But when cell phenotypes are compared in local subpopulations of clones, clustered cells that are clonally related usually possess a common phenotype (Reid et al., 1995).

When cortical VZ cells are grown in single cell culture, only 7% of the cells behave like stem cells (Davis and Temple, 1994), suggesting that in the cortical VZ, a small population of stem cells exist in the presence of larger numbers of more restricted precursor cells. The existence of stem cells suggests that as development proceeds in the cortex, precursor cells are generated that are more restricted in their developmental potential, similar to cell diversification mechanisms which may operate in the neural crest (Anderson, 1989; Stemple and Anderson, 1992).

Recent studies have shown that different growth factors have opposing actions on rat cortical cells in culture. While bFGF stimulates proliferation of progenitor cells, NT-3 stimulates differentiation of progenitor cells into neurons (Ghosh and Greeenberg, 1995; Vicario-Abéjon et al., 1995). A homozygous null mutation in BF-1, a winged helix transcription expressed specifically in the telencephalon, results in dramatic decreases in the number of proliferating progenitors in the telencephalon, suggesting that BF-1 may normally affect the timing and number of progenitor cell divisions in the telencephalic VZ (Xuan et al., 1995).

Laminar determination in the cerebral cortex

While little is known about the mechanisms that may restrict progenitors to producing, for instance, neurons or glia, some progress has been made in studying the process by which cortical neurons acquire specific laminar fates. The neurons in the preplate, just below the pial surface, are the earliest generated neurons of the cerebral cortex. In the cat, the early-generated preplate is split in two by migrating cortical plate neurons, creating the superficial marginal zone, which will become layer 1, and a deep subplate (Allendoerfer and Shatz, 1994; Carlos and O'Leary, 1992). These cortical neurons then form layers 2-6 in an inside-first, outside-last fashion (Caviness, 1976; Bayer and Altman, 1991). Molecular markers have been identified which distinguish between different cortical layers (Frantz et al., 1994). In the mouse mutant reeler, the preplate is not split in two; instead, the cortical plate neurons accumulate in an outside-in fashion, such that the cerebral cortex forms beneath the early-generated cells with layer 2 proximal and layer 6 distal to the VZ (Bayer and Altman, 1991; Caviness, 1978). Despite the dramatic changes in laminar pattern found in the reeler mutant, or similarly when the cortex is irradiated, the basic topographic connections between the thalamus and cortex develop essentially normally (Caviness, 1978; Jensen, 1984), and neurons in all classes,

though in abnormal positions, appear to survive (Rakic and Caviness, 1995). Recently, the gene mutated in the reeler mutant, reelin, has been found to code for a protein related to extracellular matrix proteins (D'Arcangelo et al., 1995; Ogawa et al., 1995). In the developing cerebral cortex, reelin is expressed specifically in the earliest generated neurons in the preplate. Thus a single mutation affecting cells in the preplate is apparently sufficient to convert inside-out histogenesis into outside-in histogenesis (see Chapter 4).

The normal inside-out pattern of cortical neurogenesis results in a strong correlation between a neuron's birthday and its normal laminar position. Transplantation experiments have indicated that the laminar identity of cortical neurons is determined just prior to the cell's final mitotic division within the VZ (McConnell and Kaznowski, 1991). When embryonic progenitor cells were transplanted into older host brains at various times after labelling with ^{3}H-thymidine, the laminar fate of transplanted neurons was in some cases appropriate to the normal fate of the host (deep layer), and in other cases appropriate to the normal fate of donor cells at the time of their transplantation (upper layer fate). This laminar fate decision was found to correlate with the position of the progenitor in the cell cycle at the time of transplantation, suggesting that cell-cell interactions within the cortical VZ do determine the laminar fates of young neurons, but that these interactions must occur during a critical period during the S phase of the cell cycle which precedes the birth of a neuron (McConnell and Kaznowski, 1991). While transplanted early progenitors can acquire either the deep or upper-layer laminar fate, more recent results suggest that late progenitors, which normally produce only upper-layer neurons, may have a more restricted potential. When late progenitors are transplanted into younger brains, they will produce only upper-layer neurons (Frantz et al., 1995; McConnell, 1995).

Cell migration in the cortex

In addition to migrating through the IZ and the cortical plate radially, cortical neurons in both proliferative and non-proliferative regions appear to migrate tangentially. While tangential migration in non-proliferative regions has been consistently observed in a number of studies (Walsh and Cepko, 1993; O'Rourke et al., 1992; Roberts et al., 1993), tangential migration in the proliferative cortical layers has been a subject of some debate (Austin and Cepko, 1990; Walsh and Cepko, 1993; McConnell, 1995b, Fishell et al., 1993; Reid et al., 1995). In the chick spinal cord, it has been shown that cells labelled at early neural tube stages spread widely in the VZ, along both the dorso/ventral and rostro/caudal axes, but that clones labelled later are confined to narrow domains, suggesting that the displacement of cells within the VZ becomes progressively restricted during development (Leber et al., 1990, 1995). Tangential movements in the cortical VZ may similarly depend on developmental stage.

In the non-proliferative cortical regions, by contrast, studies indicate that cells move primarily radially at early developmental times, and then undergo progressively more tangential movements as development proceeds (Austin and Cepko, 1993; Walsh and Cepko, 1993; see Chapter 2). Recent experiments using LacZ markers in transgenic mice indicate that cortical cell migration contains both radial and tangential components (Tan et al., 1993, 1995). While some experiments have stressed cases in which at least some cells within cortical clones are widespread, other studies stress the predominance of clones in which cells are distributed primarily radially (Tan et al., 1993, 1995; Bayer et al., 1991; Kornack and Rakic, 1993).

Regionalization of the telencephalon

Little is known about the process by which the telencephalon becomes regionalized during development. A large collection of both transcription factors

(reviewed in Puelles and Rubenstein, 1993) and cell-surface or secreted molecules (Tole et al., 1995b; Parr et al., 1993; Stainier et al., 1991) have been found to be expressed in distinct subregions of the murine forebrain. Gene expression boundaries within the forebrain are often found near ventricular sulci, and may also coincide with other histotypic boundaries (Puelles and Rubenstein, 1993; Kuhlenbeck, 1910; Källén, 1953; see Chapters 2 and 4). Within the telencephalon, a large number of potential regulatory genes, such as Mash-1 (Lo et al., 1991), Dlx-1 and Dlx-2 (Price, 1993; Porteus et al., 1991), are expressed in the presumptive basal ganglia, but not in the presumptive cerebral cortex; other genes, such as Pax-6 (Stoykova and Gruss, 1994), Emx-2, and Otx-1 (Simeone et al., 1992, 1992b) are expressed in the cortex but not in the basal ganglia. Emx-1 appears to be expressed in all of the cerebral cortex except the olfactory cortex (Simeone et al., 1992, 1992b). Nkx2-1 (Price, 1993), Gbx-2 (Puelles and Rubenstein, 1993), and Sonic hedgehog (see Chapter 2) are expressed in the medial, but not the lateral, ganglionic eminence within the basal ganglia. LAMP (Limbic System-Associated Membrane Protein), an Ig superfamily member (Pimenta et al., 1995), is expressed specifically in cortical cells of the limbic system, but not in nonlimbic cortical neurons (Barbe and Levitt, 1991). Other molecular markers, such as Shh (see Chapter 2), FORSE-1 (Tole et al., 1995b; Allendoerfer et al., 1995), and Mash-1 (Lo et al., 1991) label the lateral, but not the medial, walls of the telencephalon. BF-1, a transcription factor (Tao and Lai, 1992), and Telencephalin, an adhesion molecule (Yoshihara et al., 1994), are both expressed specifically in the entire telencephalon.

Null mutations in mice have recently been generated for Mash-1, Dlx-2, BF-1, and Otx-2. While Dlx-2$^-$ mice show some neuronal deficits in the olfactory bulb (Qiu et al., 1995), Mash-1$^-$ mice have no detectable phenotype in the telencephalon (Guillemot et al., 1993). Otx-2$^-$ mice show a complete absence of the rostral head (Matsuo et al.,

1995), and BF-1$^-$ mice show a dramatic reduction in overall telencephalic size, particularly in the ventral telencephalon (Xuan et al., 1995).

Matsuo et al. (1995) argue that the Otx-2$^-$ phenotype indicates that it plays a gap-gene-like role in the rostral head. The *Drosophila* homologues of the vertebrate Emx and Otx genes are also expressed in the head, and seem to be required both to establish contiguous blocks of segments, and to specify segmental identity in the head - that is, these genes appear to have properties of both the gap and homeotic genes as they function in the trunk of the Drosophila embryo (Cohen and Jürgens, 1990; Martinez-Arias and Lawrence, 1985). Mutations in Drosophila Otd and Emx similarly eliminate, rather than homeotically transform, entire regions in the head, as do gap-gene mutations in the *Drosophila* trunk (Hirth et al., 1995).

The BF-1 gene appears to specifically affect cell proliferation in the telencephalon, as, in its absence, telencephalic precursor cells fail to proliferate actively after E9.5. Though regional markers, like Emx-2 and Pax-6, are expressed normally in the BF-1$^-$ mice, the ventral telencephalon (the presumptive basal ganglia) is much more severely affected than the dorsal telencephalon (presumptive cerebral cortex) (Xuan et al., 1995; see Chapter 2, discussion).

Cytoarchitectonic fate in the mammalian cortex

Three general kinds of studies have addressed the issue of cytoarchitectonic cell fate in the mammalian cortex: transplantation studies which ask whether specific regions can change their fate when transplanted into ectopic regions; studies asking whether cells generated in one cytoarchitectonic region are capable of crossing into different cytoarchitectonic regions during normal development; and studies analyzing cell behavior in different cortical regions.

One transplantation study involved the protein LAMP, which is specifically expressed in the limbic cortex (Barbe and Levitt, 1991). When non-limbic or limbic cortical areas are transplanted to ectopic locations prior to E12, their LAMP-expression phenotype reflects their new location rather than their embryonic origin. For instance, when sensorimotor cortical regions are transplanted at E12 into the presumptive limbic region, they express LAMP, indicating the acquisition of a limbic phenotype. If sensorimotor cortex is transplanted after E14 into the presumptive limbic region, it will no longer acquire LAMP expression (Barbe and Levitt, 1991). Transplantation experiments using a transgenic marker for mouse somatosensory cortex similarly indicate that by E14-E16, the cytoarchitectonic fate of the somatosensory cortex has been specified (Cohen-Tannoudji et al., 1994). When striatal VZ cells are transplanted into cortex, they can acquire morphologies and axonal projections specific to cortex rather than to the striatum (Fishell, 1995). Other transplantation experiments show that when cortical regions are transplanted to ectopic locations within the cortex at early developmental times, they also acquire thalamic input connections typical of their new location, rather than their site of origin (O'Leary, 1989; Roe et al., 1992; O'Leary et al., 1994).

A number of studies performed using replication-incompetent retrovirus expressing LacZ have indicated that cells move between distinct cytoarchitectonic areas during cortical development. Grove et al. (1992) showed that marked clones crossed borders within the hippocampus of the rat. Distinct regions such as CA1-CA4 contained clones which traversed the boundaries between the regions, seemingly without constraint. Walsh and Cepko (1992), using a library of genetically distinct retroviruses, found that the majority of clonally-related neurons in cerebral cortex contained cells which were widely dispersed across distinct functional areas, such as motor cortex and somatosensory cortex. Neuronal cells have also been shown to undergo long-distance migrations in the adult rodent telencephalon (Lois and Alvarez-Buylla, 1994).

Other studies have analyzed broad regions within the telencephalon to see if differences exist in different aspects of cell behavior, such as adhesivity or cell cycle kinetics. Acklin and van der Kooy (1993) reported that the cortical germinal zone appears to be segregated into three spatially distinct populations of precursor cell lineages, which differ in both cell cycle kinetics and amount of cell death. Whitesides et al. (1995) performed dissociation/reaggregation assays with lateral, ventral, and medial aspects of the embryonic mouse telencephalon, and found calcium-independent differential adhesion between these regions; for instance, cells from the lateral telencephalon were shown to specifically sort out from cells isolated from the medial telencephalon. In primates, the presumptive primary visual cortex appears to have distinctly different cell cycle kinetics than other visual areas (Dehay et al., 1993).

In the last century, various segmentation models have been proposed for the developing forebrain. These models, as well as other issues concerning forebrain evolution, will be discussed in Chapter 4.

Chapter 2

Cell Migration Domains in the Chick Forebrain

ABSTRACT

The compartmental plan of the vertebrate forebrain (the telencephalon and diencephalon) has been inferred primarily from morphological features and gene expression boundaries. By labelling cells in the chick telencephalon *in ovo*, we show here that the ventral telencephalon (basal ventricular ridge, or BVR) represents a distinct cell migration domain. Migrating cells respect two gene expression boundaries which define the borders between BVR and DVR (dorsal ventricular ridge), and between BVR and diencephalon. Similarities in both gene expression and cell migration patterns may support a broad homology between avian BVR and mammalian basal ganglia, and avian DVR and mammalian cerebral cortex. These results appear to be inconsistent with a recently proposed model of forebrain segmentation.

INTRODUCTION

A major problem in developmental neurobiology is understanding the process by which the central nervous system becomes regionalized during development. In the hindbrain, the boundaries between "rhombomeres" correlate with both gene expression boundaries and cell mixing restrictions (Fraser et al., 1990; Lumsden, 1990), though these restrictions are not absolute (Birgbauer and Fraser, 1994). These cell movement restrictions may allow adjacent regions of the neuroepithelium to develop semi-autonomously and consequently to acquire unique identities (Guthrie, 1995). Adjacent rhombomeres also appear to have different cell adhesion properties (Guthrie et al., 1991), and distinct schedules of neurogenesis (Lumsden and Keynes, 1989), and may constitute distinct developmental units, or "compartments." In addition, null mutations in regulatory genes specifically affect distinct rhombomeres in which these genes are

expressed (Schneider-Maunoury, 1993; Mark et al., 1993). Regionalization of the vertebrate forebrain (the telencephalon and diencephalon) has been more difficult to examine due to its relative inaccessibility. Cell marking experiments in the diencephalon indicate that, like the hindbrain, the diencephalon is organized into compartments (Figdor and Stern, 1993), though putative compartmental boundaries have been delineated according to different schemes (Bulfone et al., 1993; Figdor and Stern, 1993). The prospective compartmental organization of the telencephalon has been an issue of particularly intense interest (Bulfone et al., 1993; Swanson et al., 1995; Karten, 1991). Though various subregion-specific regulatory genes have been described in the telencephalon (Puelles and Rubenstein, 1993; Lo et al., 1991; Stoykova and Gruss, 1994; Simeone et al., 1992), a major issue has been whether their expression boundaries correlate with cell mixing boundaries.

Here we perform *in vivo* cell marking experiments in the chick telencephalon and directly compare cell migration patterns to the domains of two regulatory genes, Cash-1 and chick Sonic hedgehog (Shh), which are expressed in subregions of the telencephalon. We find that the majority of tangential cell movements appear to occur in regions of the telencephalon which contain differentiated neurons. Two gene expression boundaries which define the dorsal and caudal limits of the basal ventricular ridge (BVR) in the ventral telencephalon correlate with cell migration boundaries, while another gene expression boundary within the BVR does not appear to represent a cell migration boundary. Cells generated in the BVR do not generally migrate, therefore, into either the more dorsal DVR (dorsal ventricular ridge) or the more caudal diencephalon. The patterns of cell migration in the BVR and the neighboring DVR are strikingly distinct. In addition, an axon bundle invades at the site of a cell migration boundary between the BVR and DVR two days after the migration boundary is first evident. These data appear to argue against a model of forebrain compartmentalization proposed by Bulfone et al.

(1993), and, at least in part, favor other proposed models (Karten, 1991; Alvarez-Bolado et al., 1995). Finally, comparison of homologous regulatory gene expression patterns in the mouse and chick, and comparison of telencephalic cell migration patterns in the chick described here, and those previously reported in the mouse (Tan et al., 1995; Walsh and Cepko, 1993; Halliday and Cepko, 1992), provide developmental genetic support for a proposed homology between avian BVR and mammalian basal ganglia, and avian DVR and mammalian cerebral cortex.

MATERIALS AND METHODS

Embryos and DiI injection

Fertile White Leghorn chicken eggs were incubated and opened essentially as previously described (Birgbauer and Fraser, 1994). Eggs were obtained from local suppliers and maintained at 38° C in a humidified incubator until the embryos reached Hamburger-Hamilton stage-16 (Hamburger and Hamilton, 1992). A circular hole was cut on the top of the egg shell with curved scissors, and India ink was injected beneath the surface of the embryo to improve contrast. The vitelline membrane was deflected using a tungsten needle, and a micromanipulator was used to position electrodes for DiI-labelling. Electrodes of 5-10MΩ resistance were pulled from Al-Si glass capillaries (with filament) using a Sutter P-80/PC Micropipette Puller. The electrodes were back-filled with 0.5% DiI in ethanol, pre-warmed at 37°C, and placed in a holder with a silver wire immersed in the DiI solution. The pipette was driven past the telencephalic mesenchymal layer (Fig. 1C), and the DiI was injected into the neuroepithelium by iontophoretic injection with a maximum of 90 nA current through a 100MΩ resistor using a box powered by a 9 volt battery.

Histology and immunohistochemistry

After incubation, embryos were washed in PBS, the eyes and body were removed, and the heads were fixed for 10-20 hours at 4°C with 4% paraformaldehyde, pH 7.4. After fixation and washing in PBS, the embryos were infiltrated with 7.5% gelatin/15% sucrose for 2-4 hours and then embedded. Frozen serial sections of 20um were cut using a cryostat and placed on TESTA-coated slides. Some of these sections were then imaged without cover slips, generally within 2-4 hours after sectioning, in order to record the presence of DiI label.

DAB immunohistochemistry was performed essentially as described previously (Groves et al., 1995). Sections were washed several times in PBS after fixation, incubated in PBS containing 5% goat serum, 0.4% Triton X-100 and 0.25% BSA for 1-3 hrs. at room temperature. Primary antibodies were diluted in the same buffer and incubated with the slides 4° overnight. The slides were washed several times in PBS and endogenous peroxidase activity was quenched by incubating the slides in 0.3% H_2O_2 in methanol for 30 minutes at room temperature. The slides were washed again in PBS, and incubated with either IgG or IgM goat anti-mouse secondary antibodies conjugated to horseradish peroxidase (HRP) overnight at 4° C. The slides were then washed and processed for the HRP reaction by using diaminobenzidine as a substrate. In this study, antibodies were used against: GFAP (GA5) from Boehringer Mannheim; vimentin (3A7) from Dr. Vance Lemmon; laminin (L-9393 from Sigma); chondroitin sulfate (CS-56) from Sigma; peanut lectin (L-7759) from Sigma; and Neurofilament-M (NF-160, RMO 270.3) from Virginia Lee. For anti-laminin and anti-chondroitin sulfate antibodies, triton was not used in any of the buffers.

In situ hybridization

Serially-sectioned embryos were processed for non-radioactive in situ hybridization using digoxigenin-labelled complementary RNA (cRNA) probes by a modification of the protocol of Harland (1991) as previously described (Groves et al., 1995). Whole-mount in situ hybridizations were performed as described previously (Groves et al., 1995). cRNA probes were made from: chick Shh (C. Tabin) mouse Shh (Vhh-1; T. Jessell), chick SCG-10 (P. Jeffrey), Cash-1(T. Reh). NF-160, 270.3 antibody from Virginia Lee.

Imaging

Immediately after injection, the DiI injection position was recorded by taking both fluorescence and bright-field images with a SIT camera (Hamamatsu) in ovo.
The fixed specimens (both whole-mount and sectioned) were imaged for DiI-labelled cells by using either a SIT camera or, in some cases, a Biorad MRC600 laser confocal microscope mounted on a Zeiss axiovert microscope. In both cases, the epifluorescence and bright-field images were superimposed using false color. Confocal fluorescent images were collected at 1-5um intervals and then compressed computationally into a single plane and overlaid onto a single bright-field image. In situ hybridizations and DAB immunohistochemistry were imaged using a Roche CCD camera. Adobe photoshop was used to enhance contrast.

RESULTS

In situ hybridization with telencephalic markers

In the E11.5 mouse telencephalon, in situ hybridization for Mash-1 mRNA shows expression in the ventral telencephalon, in the medial and lateral ganglionic eminence, but

not in the more dorsal cerebral cortex (Fig. 1A) (Lo et al., 1991). Within the ventral telencephalon, Mash-1, a transcription factor (Johnson et al., 1992), is expressed in the ventricular zone (VZ) and not the intermediate zone (IZ). Mouse Sonic hedgehog (Shh), a secreted molecule that influences pattern formation in various systems (Ericson et al., 1995; Ingham, 1995), is expressed in the medial, but not the lateral, ganglionic eminence (Fig. 1B). Because cell marking experiments are difficult to perform in the embryonic mouse telencephalon *in vivo*, we utilized the embryonic chick (Fraser et al., 1990; Birgbauer and Fraser, 1994). We first analyzed the expression patterns of the chick homologues of Mash-1 and Shh. In the E5.5 (stage-26; Hamburger and Hamilton, 1992) chick telencephalon, the chick homologue of Mash-1, Cash-1, is expressed in the ventral telencephalon (the basal ventricular ridge or BVR), but not in the more dorsal telencephalon (the dorsal ventricular ridge, or DVR; Fig. 1C). Like Mash-1, Cash-1 is expressed in the VZ (Jasoni et al., 1994). Chick Shh is expressed in a ventral territory in the telencephalon, in a similar relative position to that of mouse Shh, with a dorsal boundary of expression which lies within the BVR (Fig. 1D, black arrowhead). Between E5 and E7 in the chick, Cash-1 and Shh appear to have stable domains of expression within the telencephalon (not shown).

DiI injections

To analyze telencephalic cell migration patterns in relation to these regulatory gene expression domains, we performed focal injections of DiI *in ovo* into various regions of the E3 (stage-16) chick telencephalon and analyzed the specimens after 2-4 days of incubation (E5-E7, stages 24-30). Fig. 2A shows an image taken *in ovo* immediately following DiI injection. The position of this injection site is shown relative to the domain of Cash-1 expression (Fig. 2B, black arrow; white arrows in 2A and 2B indicate the developing eye) (Jasoni et al., 1994). A section through an embryo injected with DiI and

then incubated for 10 hrs. demonstrates that the injections are focal and that the labelled cells remain tightly clustered at this developmental stage (Fig. 2C). Because the neuroepithelium is only a single cell thick at the stage of injection, the injections appear to consistently label its entire depth at the injection site (Fig. 2C).

Telencephalic cell migration related to Cash-1 expression pattern

In whole-mount images of DiI-injected specimens analyzed three days after marking (E6), cells labelled in the dorsal telencephalon (DVR) remain tightly clustered (Fig. 2D), while those labelled in the ventral region (BVR) demonstrate substantial dispersion (Fig 2E). Sections of labelled specimens demonstrate more clearly that cell migration in the DVR is predominantly radial, while cells labelled in the BVR show widespread tangential dispersion (Fig. 3). This tangential dispersion occurs primarily within the ventral domain marked by Cash-1 expression, as seen in adjacent serial sections (Fig. 3B and C). Tangentially migrating cells were detected primarily in the IZ and MZ (marginal zone), as marked by expression of SCG10 mRNA (for neuronal cell bodies) (Anderson and Axel, 1985; Groves et al., 1995) and neurofilament-M (NF-M, for neuronal processes; Fig. 3D and E). Double-injections with DiI (red, Fig. 3A, high magnification, and 3B, lower magnification) and DiI(C5) (green, Fig. 3A and B), analyzed at E5, are shown in Fig. 3. DiI(C5) is a red-excited, infrared-emitting dye. Cells are radially aligned in the Cash-1$^-$ DVR (Fig. 3A, top, white arrowhead) in contrast to the tangential migration in the Cash-1$^+$ BVR (red in Fig. 3A, bottom, and Fig. 3B). Between E3 and E5, there is no significant tangential dispersion in the BVR (not shown). The initial injection site in DiI-labelled specimens is indicated by cells in the VZ which remain labelled after the incubation period (e.g., Fig. 3B, white arrow). Though the injection site in Fig. 3B (white arrow) is just ventral to the Cash-1 expression boundary (Fig. 3C, black arrowhead), and cells disperse a significant distance in a ventral direction,

no labelled cells are observed to migrate dorsally across the Cash-1 boundary in this specimen (Fig. 3B and C).

Specimens injected at E3 in various dorso/ventral (d/v) locations and analyzed at E6 demonstrate the basic cell migration patterns found in the BVR and DVR at this developmental stage (Fig. 4). The BVR has been divided into three regions relative to the total d/v extent of the Cash-1 expression domain: 85-100% ventral, 50-85% ventral, and 0-50% ventral (see Fig. 5). A specimen labelled with DiI just ventral to the Cash-1 expression boundary shows extensive migration ventrally, but no cells move dorsally to cross the Cash-1 expression boundary (Fig. 4C, black arrowhead). Ventral migration appears to follow the wedge-shaped pattern of SCG-10 expression in the Cash-1$^+$ domain (Fig. 4D). Injections into the 50-85%V region (Fig. 4F-J) show labelled cells migrating in both dorsal and ventral directions (Fig. 4G), but not crossing the Cash-1 boundary (Fig. 4H). Injections into the 0-50%V region (Fig. 4K-O) result in cells migrating predominantly in a dorsal direction (Fig. 4L). Injections into the Cash-1$^-$ DVR (Fig. 4P-T) show radial alignment of cells in the DVR, with cells migrating tangentially only in the lateral, NF$^+$ subpial region (Fig. 4Q, white arrowhead, and 4T). The migration patterns in these different regions are schematized in Fig. 5.

A total of 150 specimens were injected with DiI into the presumptive BVR at E3 and analyzed at E5-E7. In 96% of the specimens, no cells crossed the Cash-1 boundary (Table 1). In the 6 cases where crossing was seen, only 1 or 2 cells out of approximately 50-150 cells sampled per specimen were observed to cross the boundary. Taken together, these data indicate that the progeny of cells labelled in the BVR generally remain confined within its territory, though some cells do cross into the DVR at an extremely low frequency. While tangential migration within the VZ was occasionally observed, the majority of tangential migration appears to occur within the regions of the telencephalon

which contain differentiated neurons (e.g., Fig. 3A , B, D) (Fishell et al., 1993; O'Rourke et al., 1992; McConnell, 1995).

Nuclear staining with DAPI confirms that the subpial label seen in injections into the DVR represents cell bodies. In Fig. 6B, a high power image of a subpial cell shown at lower power in Fig. 6A (indicated by the more dorsal of the two white arrowheads) indicates a cell morphology consistent with a migratory neuron, with leading and trailing processes (Fig. 6B). White arrowheads in Fig. 6C and D point to the same 2 DiI-labelled cells indicated in Fig. 6A. In Fig. 6D, in which both DAPI and DiI were imaged, arrowheads point to DiI-labelled cells which appear to be $DAPI^+$ in panel C (imaged only for DAPI label), consistent with the DiI label being associated with cell nuclei.

The difference in the patterns of cell migration in the DVR and BVR is more dramatically illustrated by specimens analyzed at E7 (Fig. 7A-J). Cells labelled in the DVR (Fig. 7A and B) migrate radially through the $SCG10^+$ IZ (Fig. 7D) and generally begin to migrate tangentially only in the most lateral, NF^+ subpial region (Fig. 7B and F, white arrowheads). Cells labelled in the BVR (Fig. 7K and L) show widespread dispersion within the IZ, with the most migration apparently occurring within strongly $SCG10^+$ regions (Fig. 7I). Though labelled cells fill the entire dorso-ventral extent of the BVR, no cells are observed to cross the Cash-1 expression boundary dorsally into the DVR (Fig. 7H, black arrowhead; Table 1).

A nerve fiber bundle, the lamina medullaris dorsalis (LMD), highlighted by NF staining (Fig. 3E, black arrow), invades at the site of the Cash-1 expression/cell migration boundary at E7 (Fig. 3C), thus clearly dividing the telencephalon into the ventral BVR and the more dorsal DVR (Fig. 3E) (Tsai et al., 1981). While the LMD is not detectable until E7, the cell migration boundary is clearly observed two days earlier, at E5 (Fig. 3A-E). Prior to E7, markers for N-CAM, peanut lectin, laminin, chondroitin sulfate, vimentin, and GFAP (Oakley and Tosney, 1991; Oakley et al., 1994; Lumsden

and Keynes, 1989; Figdor and Stern, 1993; Kálmán et al., 1993) fail to highlight the Cash-1/cell migration boundary (data not shown), suggesting that the boundary does not represent an obvious physical barrier to cell movement.

Telencephalic cell migration related to Sonic hedgehog expression pattern

Cell migration patterns were also analyzed in relation to two Shh expression boundaries: the dorsal boundary of expression within the BVR (e.g., Fig. 1D, black arrowhead), and an expression boundary between the caudal telencephalon (Shh^+), and the rostral diencephalon (Shh^-; Fig. 8H, black arrow). Fig. 8A-E shows a series of adjacent sections in which the DiI injection site (white arrows in Fig. 8A and B) is clearly dorsal to the Shh^+ domain (Fig. 8C), but ventral to the Cash-1 expression boundary (marked by a white arrowhead in Fig. 4B). Dispersing cells appear to migrate ventrally from the Shh^- region into the Shh^+ region (Fig. 8B and C). All other specimens injected at a similar location showed cells apparently migrating into the Shh^+ domain (Table 2). In the majority of these cases, many cells (about 10-20) were observed to cross the Shh expression boundary. Almost 50% of specimens injected within the Shh^+ domain also showed cells migrating dorsally into the Shh^- region (Table 2).

In contrast to the Shh expression boundary within the BVR, the Shh expression boundary at the telencephalon/diencephalon (t/d) boundary appears to represent a cell migration boundary. Fig. 8F-J shows serial adjacent sections in a specimen injected with DiI in the telencephalon just rostral to the t/d boundary (Fig. 8F, G, and H, arrow). The t/d boundary is highlighted both by a flexure (Fig. 8G, white arrowhead), and by a Shh expression boundary (Fig. 8H, black arrow). Though many cells migrate away from the injection site, no cells are observed to move caudally across the Shh boundary (Fig. 8G and H; note horizontal orientation of sections). Out of 21 specimens injected in the telencephalon near the t/d boundary at various dorso/ventral levels, only one specimen

contained cells crossing the Shh boundary (Table 2), and in this one case only a single cell had crossed (not shown).

DISCUSSION

Tangential cell migration in the early embryonic telencephalon

We have shown here that in the dorsal and ventral regions (DVR and BVR) of the early embryonic chick telencephalon, cell migration occurs in two distinct patterns: in the DVR, cells migration migrate largely radially through the intermediate zone (IZ), and show tangential movements only in the most lateral, subpial region; in the BVR, migrating cells disperse widely within the IZ. Experiments performed in the mouse both in vitro (Fishell et al., 1993) and in vivo (Walsh and Cepko, 1993) indicate that cells can move tangentially within the telencephalic ventricular zone (VZ) (but see also O'Rourke et al., 1992, and McConnell, 1995b). In the chick telencephalon, we show here that tangential cell migration, while occasionally observed in the VZ, appears to occur predominantly within regions containing differentiated neurons.

Early regionalization of telencephalon into dorsal and ventral territories

We have analyzed cell migration patterns in the embryonic chick telencephalon and related these patterns to three gene expression boundaries. The telencephalic Cash-1 expression boundary, which marks the presumptive BVR/DVR boundary, appears to correlate with a cell migration boundary from E5-E7. While one Shh expression boundary at the telencephalon/diencephalon border appears to represent a cell migration boundary, a Shh expression boundary within the ventral telencephalon (the BVR) does not appear to represent a cell migration boundary.

Four lines of evidence presented above suggest that the chick BVR represents a cell migration domain, or a true unit of telencephalic development: 1) cells generated in

the BVR do not cross dorsally into the DVR, or caudally into the diencephalon; 2) these cell migration boundaries correlate with regulatory gene expression boundaries; 3) a nerve fiber bundle, which divides the BVR and DVR, invades at the site of one of these cell migration boundaries several days after the boundary is first detected; 4) the pattern of cell migration in the BVR is strikingly different from that in the neighboring DVR territory.

In the mouse, many potential regulatory genes, including Mash-1 (Lo et al. 1991), Dlx-1 and Dlx-2 (Price, 1993; Porteus et al., 1991), are expressed in the basal ganglia but not in the cerebral cortex, while many others, such as Pax-6 (Stoykova and Gruss, 1994), Emx-2, and Otx-1 (Simeone et al., 1992, 1992b), are expressed in the cortex, but not in the basal ganglia. A compartmental division between the cortex and basal ganglia is also supported by the phenotype of a null mutation in the BF-1 gene, a winged-helix transcription factor. In the BF-1 mutant, the entire presumptive basal ganglia is essentially absent, while the presumptive cerebral cortex, though also reduced in size, is much less severely affected (Xuan et al., 1995).

The establishment of cell migration boundaries

Developmental "compartments" were classically defined in the developing imaginal disc of the Drosophila wing (Bellido, 1975). While the telencephalic "compartment" described here, the BVR, has many similarities to compartments in the Drosophila wing, there are some interesting differences. Unlike either the D/V or A/P boundaries in the Drosophila wing, cells do occasionally cross compartmental boundaries (Bellido, 1975; Blair, 1993) (Tables 1 and 2). In the hindbrain, though cell migration is generally restricted between rhombomeres (Fraser et al., 1990), extensive cell crossing across rhombomere boundaries has been observed, even after morphological and cellular boundaries are detectable (Birgbauer and Fraser, 1994). However, in the Drosophila wing imaginal disc, cells do not migrate significantly (Blair, 1995), so direct comparisons

with vertebrate CNS boundaries, which divide regions undergoing widespread cell migrations, may be inappropriate.

While the wing A/P boundary forms within an apparently featureless terrain (Bellido, 1975), the boundary, for instance, between BVR and DVR, is often marked by a discontinuity in SCG-10 expression (e.g., Fig. 4S) which may highlight a relatively "cell-free" zone (Tsai et al., 1981). In both the D/V Drosophila wing boundary, and boundaries between rhombomeres, specialized cells form at the boundaries, but their presence appears to be a consequence, rather than a cause of, cell-mixing restrictions and adhesive differences (Blair, 1995; Guthrie and Lumsden, 1991; Guthrie, 1995; Heyman et al., 1995). It has been proposed that one purpose of compartmental boundaries may be to create regions of specialized cells near the boundaries which may then play special roles in growth and patterning through inductive interactions (Blair, 1995). Boundaries dividing regions in both the hindbrain and diencephalon become populated with transversely oriented axon bundles (Lumsden and Keynes, 1989; Figdor and Stern, 1993). Similarly, a nerve fiber bundle, the lamina medullaris dorsalis, navigates through the cell-migration boundary between the DVR and BVR several days after the boundary is established. In the zebrafish, pioneer neurons often extend their axons along regulatory gene expression boundaries, and the absence of specific expression boundaries correlate with improper axonal extension (Macdonald et al., 1994; Wilson et al., 1993). At E5, when the cell migration boundary between the DVR and BVR is first apparent, no morphological correlate is detectable at the boundary, and markers for peanut lectin and chondroitin sulfate, which can act as barriers to cell migration and axon advance (Oakley and Tosney, 1991; Oakley et al., 1994), fail to reveal the boundary.

Telencephalic compartmentalization

Our study suggests that it may be insufficient to use gene expression data alone to establish the compartmental plan of the vertebrate forebrain (see also Chapter 3 and Chapter 4). Recently, a "neuromeric" model of the forebrain, based primarily on gene expression data, has been proposed (Bulfone et al., 1993). In this formulation, the telencephalic region is divided into 3 "segments": P4, P5 and P6. P5 contains the basal ganglia, portions of the cerebral cortex, and portions of the diencephalon; P4 and P6 also contain both telencephalic and diencephalic derivatives (Bulfone et al., 1993). Our results, and those of Figdor and Stern (1993), who showed that cells generated in the diencephalon do not generally migrate into the telencephalon, appear to be inconsistent with this model. Instead we suggest that the diencephalon, the ventral telencephalon (BVR or basal ganglia), and the more dorsal telencephalon (DVR or cerebral cortex) may each represent distinct cell migration domains. Our results support, at least in part, regionalization models in which the telencephalon itself is primarily divided dorso/ventrally (Karten, 1991; Alvarez-Boladoet al., 1995), rather than rostro/caudally, and in which the telencephalic and diencephalic vesicles are divided into distinct regions (Figdor and Stern, 1993).

While the BVR appears to represent a cell migration domain, the exact extent and nature of the DVR cell migration domain is more difficult to ascertain. There is a clear and consistent pathway of subpial migration in which cells migrate along NF^+ axon fibers into both dorso-lateral and ventro-lateral regions (e.g., Fig. 4Q and 7B). Double-labelling experiments generally support the idea that cells generated in the BVR remain spatially separate from those migrating ventrally from the DVR, though cell-mixing could not be scored definitively without appropriate lateral markers (J. M. M. et al., unpublished data). The anatomy of the late embryonic and early adult telencephalon is, however, consistent with the proposal that cells migrating ventro-laterally from the DVR

may contribute to the olfactory cortex (see Chapter 3 and Chapter 4). More long-term cell labelling studies, either by genetic means, or with non-diluting cell markers, will be needed to resolve this issue. Since cell migration through the main cell mass of the DVR (and more dorsal regions - see Chapter 3) is predominantly radial at these early stages, it is also difficult to assess whether the DVR cell migration has a dorsal boundary, though its ventral boundary is clearly at the Cash-1/cell migration boundary at the division between the DVR and BVR. Longer-term cell labelling experiments, analyzed in combination with dorsal and lateral regulatory gene markers, may resolve this issue.

Of the three gene expression boundaries analyzed, one Shh expression boundary within the BVR does not represent a cell migration boundary. We cannot exclude the possibility that the BVR is further subdivided at the Shh boundary into two separate cell migration domains after the developmental stages analyzed here. The Drosophila wing imaginal disc, for example, is divided into A/P compartments early in development and is later subdivided into additional D/V compartments. It is intriguing that in Drosophila, the engrailed gene, which like Drosophila hedgehog is a member of the segment-polarity class of genes, has two boundaries of expression within the embryonic parasegments, and that, like chick Shh in the BVR, one expression boundary represents a lineage restriction and the other does not (Vincent and O'Farrell, 1992).

Telencephalic homologies between avians and mammals

The evolutionary lines leading to avians and mammals diverged from a common ancestor about 300 million years ago (Ulinski, 1983). The proposed homologies between avian BVR and mammalian basal ganglia, and avian DVR and mammalian cerebral cortex, have been based primarily on immunohistochemical and connectional similarities between the adult telencephalon of existing avians, reptiles, and mammals (Karten 1991; Källén, 1953; Ulinski, 1983; Northcutt and Kaas, 1995). Here we provide

developmental genetic data supporting these homologies. Mash-1, mouse Shh, and their chick homologues are expressed in very similar patterns in the embryonic telencephalon of the mouse and the chick (Fig. 2). In the chick, the Cash-1$^-$ DVR exhibits strikingly radial cell migration at early stages through the IZ, while the Cash-1$^+$ BVR shows widespread tangential cell movements within the IZ. Similarly, cell migration in the embryonic mouse cerebral cortex has been observed to be largely radial (Tan et al., 1995; Walsh and Cepko, 1993; Austin and Cepko, 1990), while cell migration in the basal ganglia appears to involve much more significant tangential spread (Halliday and Cepko, 1992). In the mouse, cells migrate from the more dorsal cerebral cortex into ventro-lateral regions (the "lateral migratory stream," Bayer and Altman, 1991) in a non-radial pattern (Tan et al., 1995; Walsh and Cepko, 1993; Austin and Cepko, 1990). In the chick, cells also migrate from the DVR non-radially into a defined ventro-lateral region which appears to be distinct from the more medial BVR (Fig. 3G). These commonalities in both embryonic gene expression and cell migration patterns in the chick and the mouse support a homology between the dorsal and ventral telencephalon of avians and mammals, and suggests that these homologies may be particularly apparent in the early embryo (Källén, 1959).

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Table 1.

Table showing number of specimens in which DiI-labelled cells in BVR respected the Cash-1 expression boundary. All injections were at E3, and embryos were analyzed at E5-E7 (after 2-4 days of incubation). Specimens analyzed at E6 are presented both as a total value (row 2), and divided into three categories indicating the injection site relative to d/v extent of the Cash-1$^+$ domain (see Fig. 7): 85-100%V, 50-85%V, and 0-50%V (rows 3-5). Injections were also performed at various rostro/caudal levels within the BVR (not shown).

time of fixation / site of injection	# specimens respecting Cash-1 boundary
E5 / BVR	11/11 (100%)
E6 / BVR total	120/125 (96.0%)
E6 / BVR: 85-100% ventral	39/40 (97.5%)
E6 / BVR: 50-85% ventral	33/34 (97.1%)
E6 / BVR: 0-50% ventral	48/51 (94.2%)
E7 / BVR	13/14 (92.9%)

Table 2.

Table showing number of specimens in which DiI-labelled cells in BVR violated (column 2) or respected (column 3) the Shh expression boundaries. Column 2 refers to Shh boundary within BVR, and column 3 refers to Shh boundary at telencephalon/diencephalon (t/d) boundary. NA, not applicable.

time of fixation / site of injection	# specimens violating Shh boundary within BVR	# specimens respecting Shh boundary at t/d boundary
E5-5.5 / dorsal to Shh boundary in BVR	11/11 (100%)	NA
E5-5.5 / ventral to Shh boundary in BVR	9/19 (47.0%)	NA
E5.5-6 / caudal tel near t/d boundary	NA	20/21 (95.3%)

FIGURE LEGENDS

Figure 1. Mash-1, Cash-1, and Shh expression in embryonic mouse and chick telencephalon. In E11.5 mouse, Mash-1 is expressed in the ventricular zone (VZ) of the medial ganglionic eminence (MGE) and the lateral ganglionic eminence (LGE) (A), while Shh is expressed in the intermediate zone (IZ) of the MGE (B). Similarly, Cash-1 is expressed in the VZ of the basal ventricular ridge (BVR) in E5.5 chick (C), and chick Shh is expressed in the IZ in a restricted ventral domain in the BVR (D). bars, 100u.

Figure 2. In vivo focal injections of DiI. (A) E3 (Hamburger-Hamilton stage 16) chick telencephalon immediately following DiI (shown in red) injection in ovo. (B) Whole-mount in situ with Cash-1 shows expression in a defined ventral domain of the telencephalon at this same stage. White arrows in A and B point to developing eye. (C) A section through a DiI-labelled specimen following ten hours of incubation shows focal labelling and largely radial alignment of cells. Labelled cells are in neuroepithelium, not in more lateral (L) mesenchyme (mesench). (D, E) Whole-mount images of DiI-labelled telencephalon following three days of incubation (at E6, with eyes and facial mesenchyme removed to reveal neuroepithelium) show little spread of labelled cells in DVR (D), and more significant tangential spread in BVR (E). M, medial; bars, 100u.

Figure 3. Cash-1 expression boundary correlates with a cell migration boundary and divides two regions which display different cell migration patterns. (A-E) Double injection with DiI (red) and DiIC5 (green) analyzed at E5 (A and B) with adjacent sections showing in situ hybridizations with Cash-1 (C), and SCG-10 (D) and antibody staining for NF (E; see text). Cells in BVR migrate tangentially from injection site (white arrows

in A and B), but are restricted from crossing the Cash-1 boundary (black arrowhead in C). Cells labelled in DVR (white arrowheads, A and B) maintain radial alignment.

Figure 4. Specimens labelled at E3 at various dorso/ventral levels, and analyzed at E6. The d/v extent of BVR has been divided into four regions relative to total d/v extent of Cash-1 expression: dorsal to Cash-1 boundary, 85-100% ventral, 50-85% ventral, 0-50% ventral (see Fig. 7). In A-E, DiI label is just ventral (85-100%V) to Cash-1 expression boundary (B, white arrow, and C, black arrowhead). Cells migrate ventrally, but do not cross dorsally into Cash-1$^-$ domain (B, C). Migrating cells appear to follow contours of SCG10$^+$ region (D). In F-J, DiI injection is in 50-85%V region. Cells migrate in both dorsal and ventral directions, but do not cross Cash-1 boundary (G, white arrow, and H, black arrowhead). In K-O, DiI injection is in 0-50%V region. Cells migrate primarily in dorsal direction, but do not cross Cash-1 boundary (L, white arrow and M). In P-T, DiI injeciton is in dorsal region. Labelled cells in Cash-1$^-$ region (R, black arrowhead) are radially aligned, but migrate ventrally in lateral, NF$^+$ subpial region (Q and T) (see Fig. 5 and Fig. 6).

Figure 5. Cartoon summarizing migration patterns in telencephalon at E6-E7. Ventral region had been divided into three dorso/ventral regions relative to d/v extent of Cash-1 expression: 85-100%V, 50-85%V, and 0-50%V (left panel). Injections in 85-100%V region show migration primarily ventrally, in 50-85%V region, both dorsally and ventrally, and in 0-50%V region, primarily dorsally (center panel). Cells labelled in all three regions disperse widely, but generally remain confined to BVR (see Table 1). Cells labelled in dorsal, Cash-1$^-$ region show radial alignment through IZ, and migrate tangentially in lateral, subpial region (right panel).

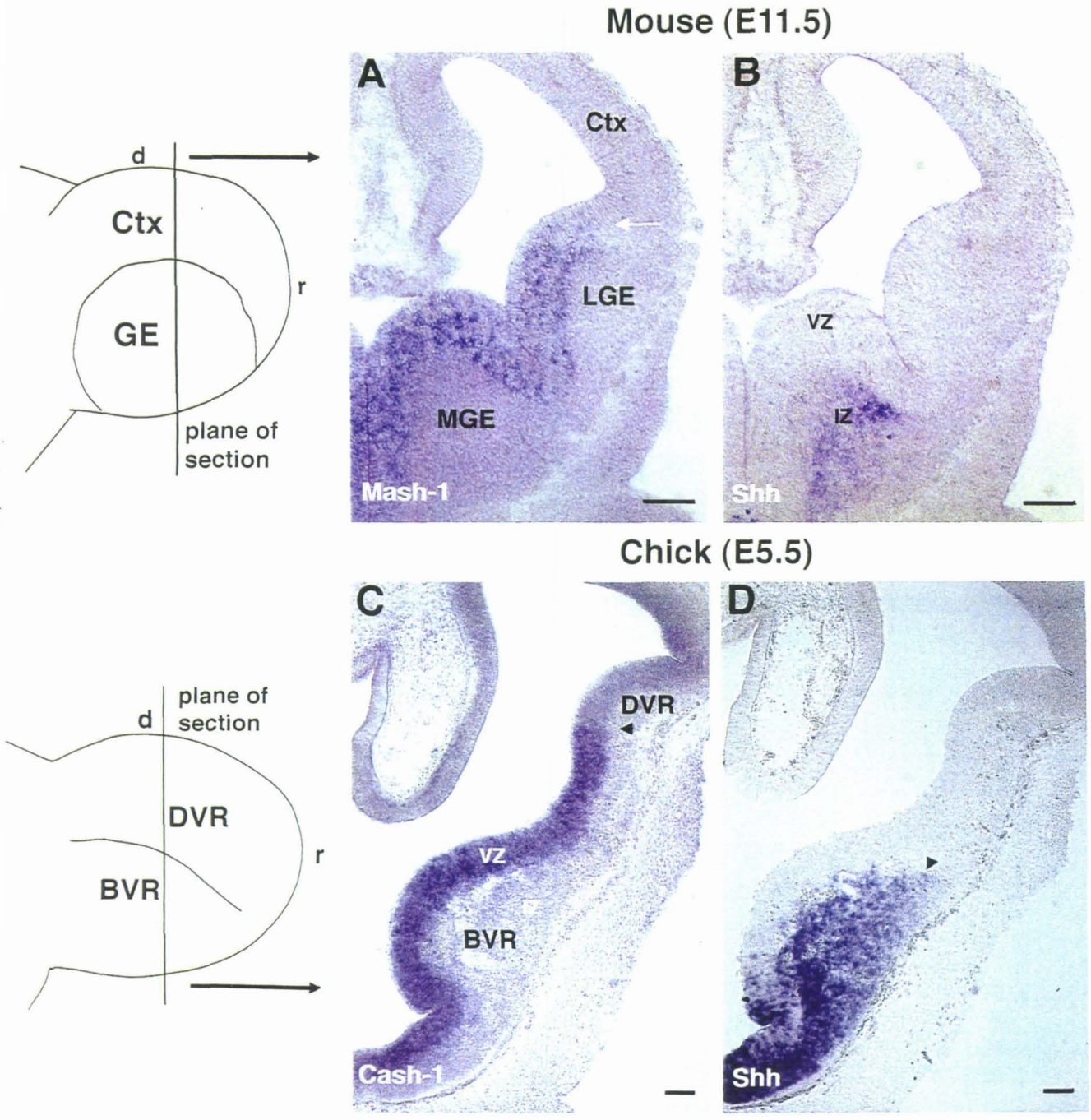
Figure 6. Double-labelling with nuclear dye DAPI suggests subpial DiI label represents discrete cells. Panel A shows same specimen as in Fig. 4, P-T, labelled with DiI in dorsal, Cash-1$^-$ region. Labelled cells are migrating ventrally in subpial region (A, white arrowheads). A labelled cell in panel A (more dorsal white arrowhead) is shown at higher power in B. In C and D, white arrowheads show the same two cells as those indicated in A. In C, arrowheads point to apparent DAPI-labelled cell nuclei which have discrete DiI label in D.

Figure 7. (A-J) Specimens injected with DiI at E3 and analyzed at E7. In DVR, cells migrate radially through SCG-10-positive IZ (A-D) and migrate tangentially only in most lateral, NF-positive region (B and E, white arrowheads). (E) A fiber bundle, the lamina medullaris dorsalis (LMD), invades between E6 and E7 at the site of this Cash-1 expression/cell migration boundary. (F-J) Injection into BVR shows widespread tangential migration within BVR, but no cells crossing Cash-1 expression boundary (H, arrowhead) into DVR. bars, 100u.

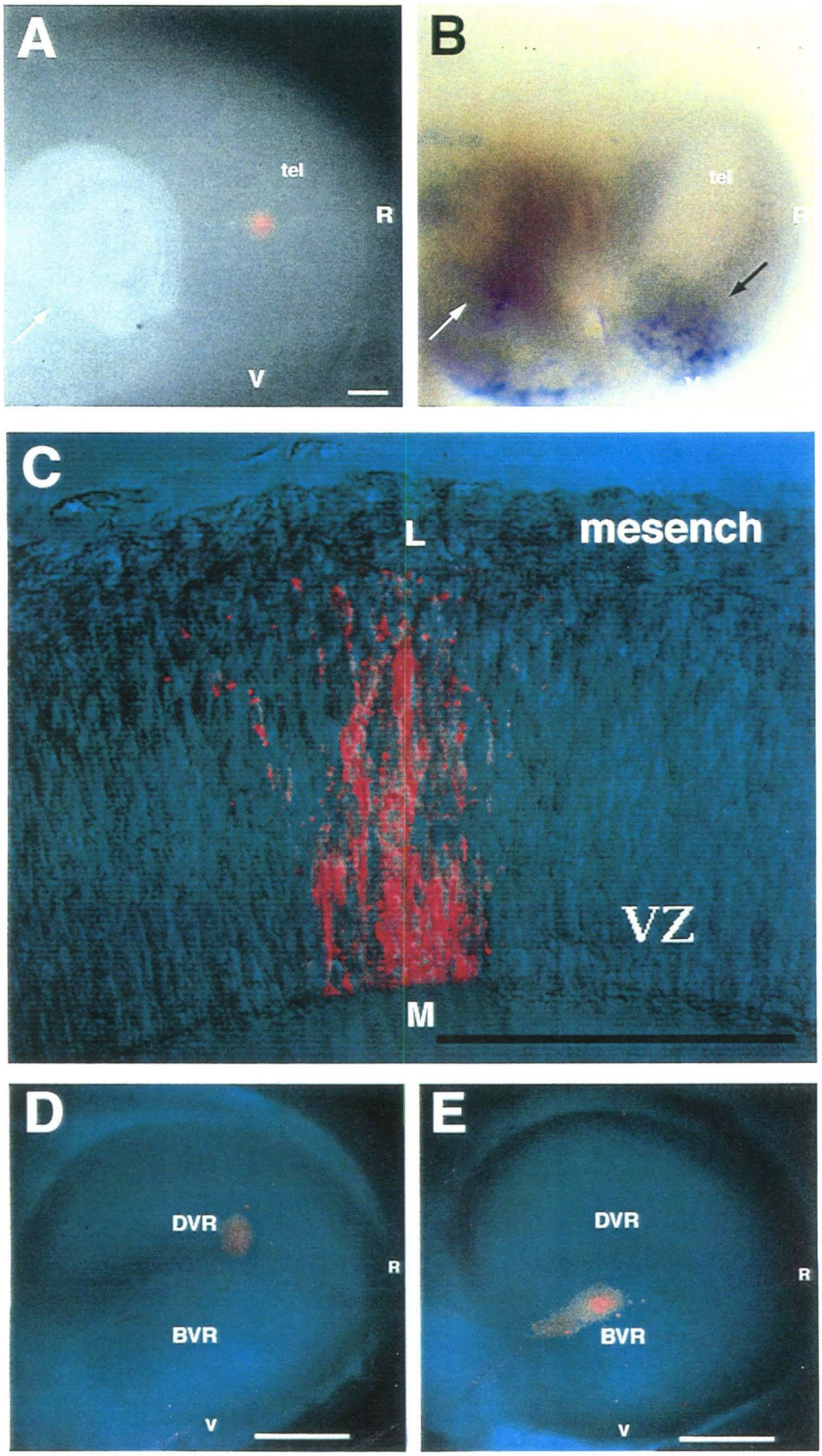
Figure 8. (A-E) The dorso-ventral Shh expression boundary within the BVR does not represent a cell migration boundary. In the specimen analyzed at E5, injection site (white arrow, A and B) is clearly dorsal to Shh domain (C), but cells migrate into Shh-expressing region (B and C). (F-J) Shh expression boundary at rostro-caudal telencephalon/diencephalon (t/d) boundary correlates with a cell migration restriction. Adjacent sections in specimen fixed at E6 and injected with DiI near t/d boundary, indicated by a flexure (G, white arrowhead). Cells migrate tangentially but do not cross Shh expression boundary at t/d boundary (G and H, black arrow). (For purposes of direct comparison, panels D, E, and B (with and without green label) are the same in

Figs. 3 and 4). Diagram indicates transverse planes of section for A-E, horizontal sections for F-J. bars, 100u.

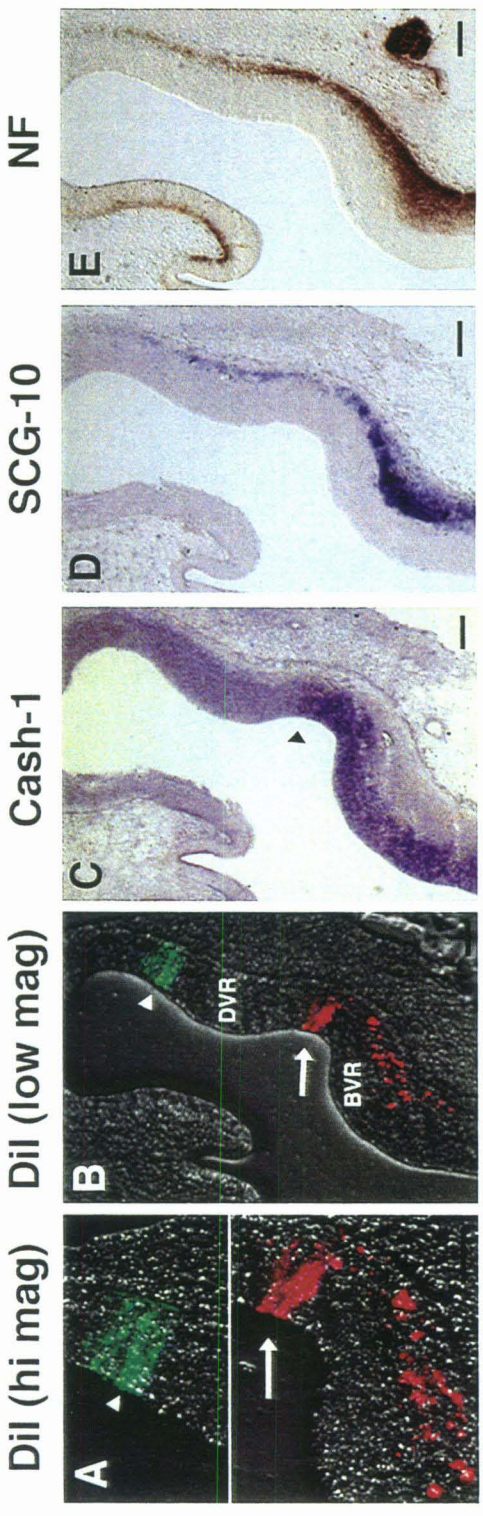
Chapter 2
Figure 1



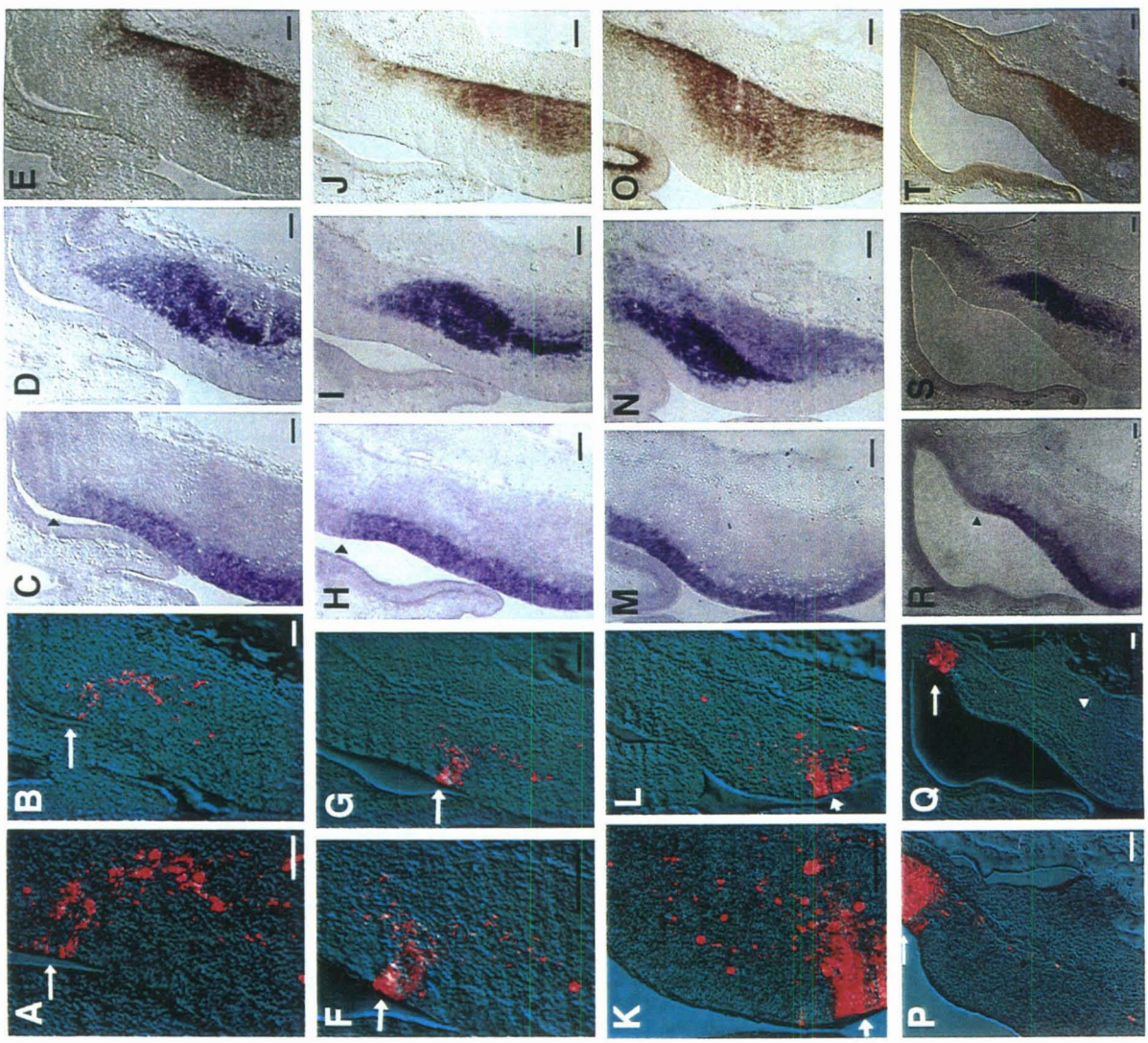
Chapter 2
Figure 2



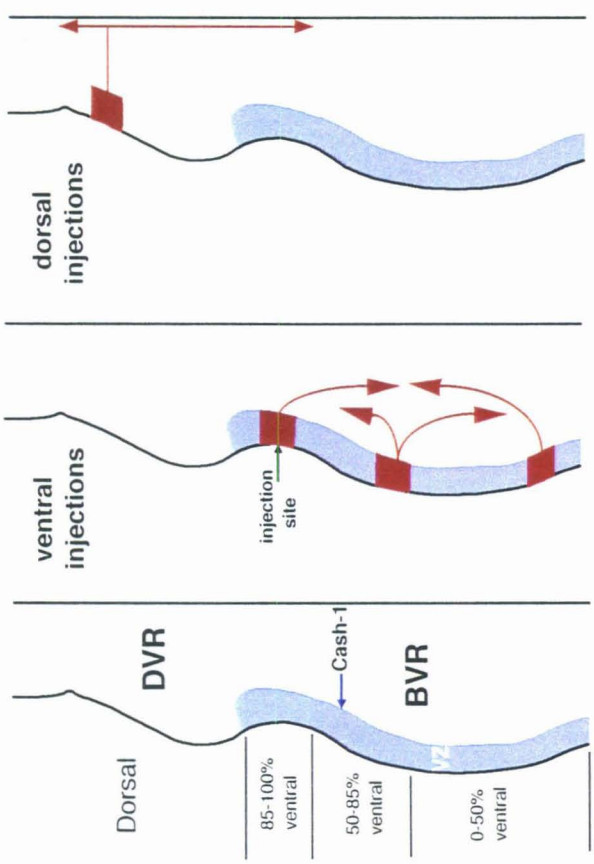
Chapter 2
Figure 3



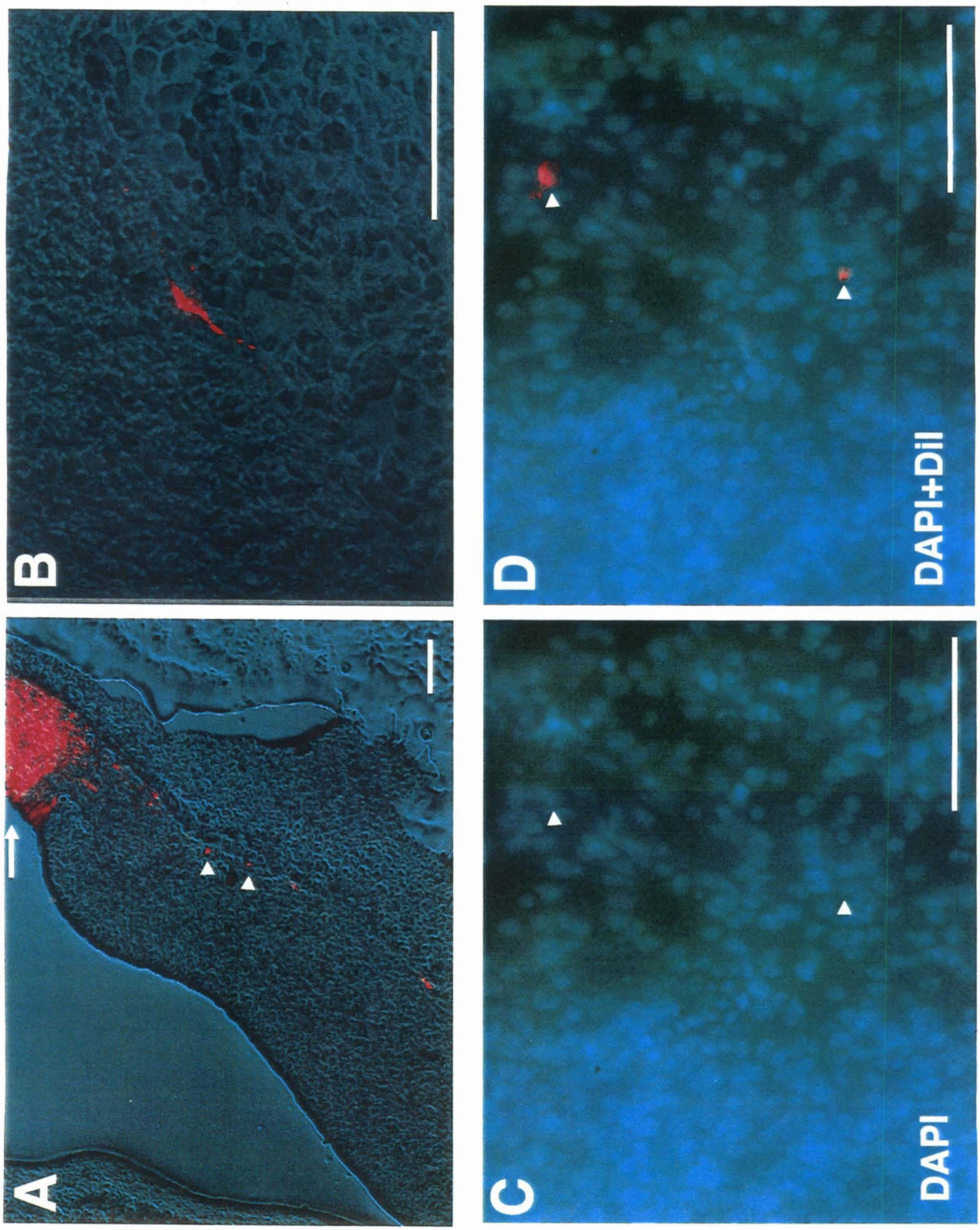
Chapter 2
Figure 4



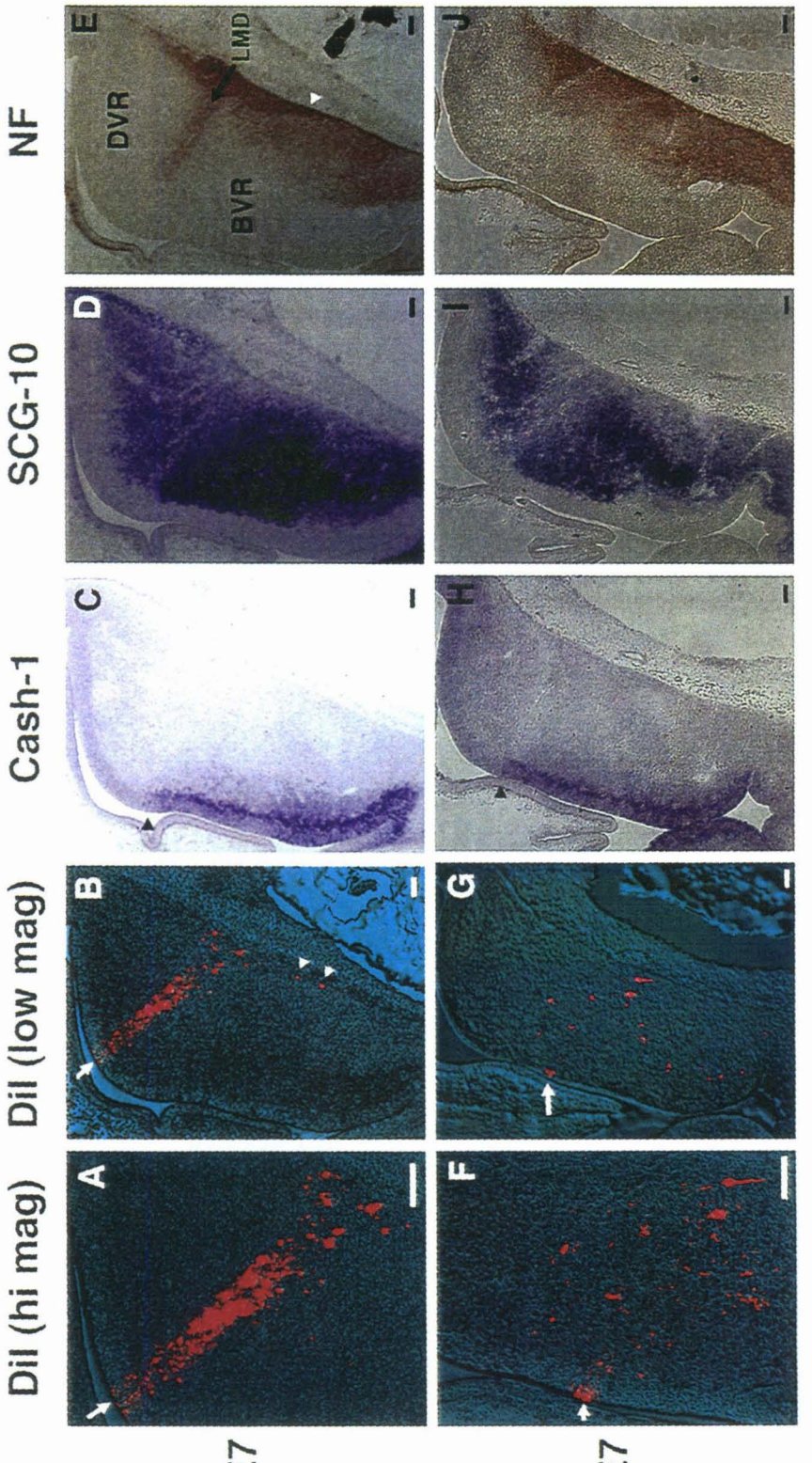
Chapter 2
Figure 5



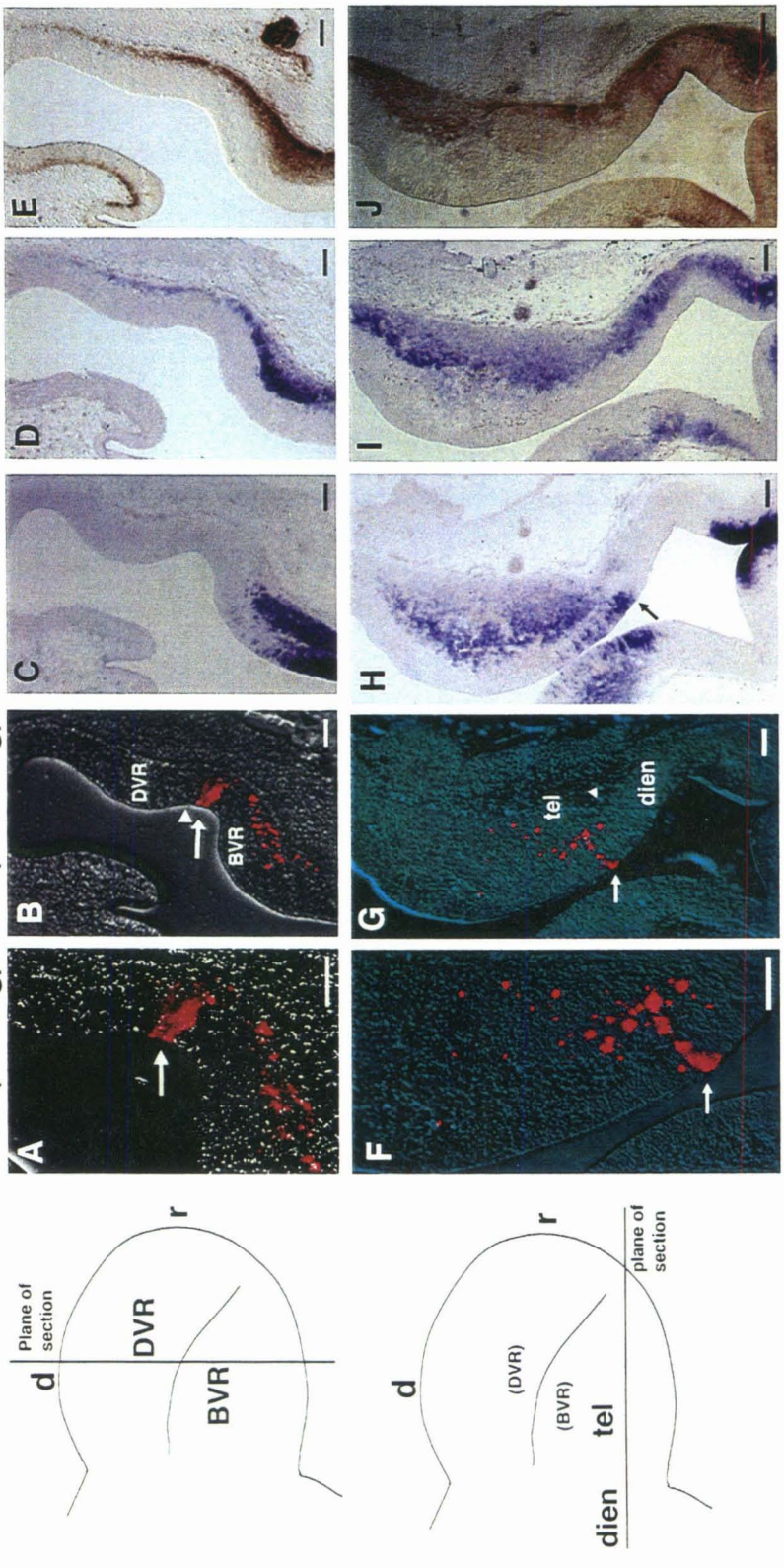
Chapter 2
Figure 6



Chapter 2
Figure 7



Chapter 2
Figure 8



Chapter 3
A Fate Map for the Stage-16 Chick Telencephalon

ABSTRACT

Fate maps are useful for a number of experimental purposes, such as transplant and misexpression experiments, and are essential for precise interpretations of gene expression patterns. Here we present a regional fate map for the stage-16 (E3) chick telencephalon. Distinct telencephalic regions were recognized based on both morphology and specific patterns of regulatory gene expression. When the regulatory gene Cash-1 is superimposed onto this fate map, it is expressed in only a subregion of the presumptive BVR territory at stage-16, although it is expressed in the entire BVR at stage 24-30 (E5-E7). We also show that these telencephalic regions display distinct cell migration patterns.

INTRODUCTION

A complete understanding of the development of any patterned structure requires the construction of a fate map. Fate maps are essential or desirable for a number of studies, including transplant and explant experiments, and for interpretations of molecular markers which are expressed before the overt patterning within a structure is detectable. Fate maps have been derived using many different techniques, including laser ablation (Lohs-Schardin et al., 1979), genetic mosaics (Struhl, 1981), chick-quail chimeras (Couly and LeDouarin, 1987, 1988; Hallonet and LeDouarin, 1993; Balaban et al., 1988), and by using vital dyes (Keller, 1975; Selleck and Stern, 1991; Woo and Fraser, 1995). Here, we use the carbocyanine dye DiI to make a regional fate map of the E3 (stage-16; Hamburger and Hamilton, 1992) chick telencephalon. Embryos were injected at E3 and then analyzed after 2- 4 days of incubation (E5-E7). The map was derived by assigning a regional fate to injection sites in the E3 telencephalon aaccording to the eventual location of labelled cells in the E5-E7 chick telencephalon. Areas within the E5-E7 telencephalon were scored by morphology, and also in relationship to the expression

domains of two genes, Cash-1 and Shh, which are stably expressed in subregions of the telencephalon at E5-E7 (see Chapter 2).

We have used our data to derive two different fate maps: one which maps only the initial position of an injection in the neuroepithelium at E3 according to its eventual location at E5-E7, and another map which accounts for both initial injection position and subsequent cell movements at E5-E7. When we analyzed the expression pattern of Cash-1 at E3 and at E5-E7, we found that while Cash-1 is expressed in the entire basal ventricular ridge, or BVR, at E5-E7, it is only expressed in a subregion of the presumptive BVR at E3. That is, though Cash-1 is expressed in the entire BVR at E5-E7, it does not simply "mark" this territory at E3, but instead is expressed initially in only a subregion of the presumptive BVR. By E5, however, Cash-1 is expressed in the entire BVR region.

MATERIALS AND METHODS

Fate map construction

A coordinate system (Fig. 1C) was used to map the position of DiI injection sites in the E3 telencephalon. After calculations of relative dorso/ventral and rostro/caudal percentages, coordinates were calculated and plotted onto a canonical E3 telencephalon by using Adobe Photoshop. Positions to which each injection site mapped was determined relative to expression patterns of both Cash-1 and Shh, and on the basis of morphology at E5-E7. Canonical E3 telencephalon dimensions were calculated by taking average of various dorso/ventral and rostro/caudal measurements of the telencephalon from 30 representative embryos. Error in measuring boundaries of regions (e.g., BVR/DVR boundary) was determined by measuring spread of points mapping to BVR/DVR boundary. This total spread was taken as the error for each boundary region. This very

likely resulted in an overestimation of error, as the calculation includes all of the most deviant points.

Scanning Electron Microscopy

Chick embryos at E3 and E6 were dissected, rinsed in PBS, and fixed in 2% paraformaldehyde and 2.5% gluteraldehyde, pH 7.4, at 4° C overnight. After rinsing in PBS, embryos were incubated for 1 hour in 2% OsO_4, rinsed again in PBS, dehydrated, transferred to acetone and critical-point dried overnight. Specimens were mounted on stubs, sputter coated, and viewed with an ETEC CT001 SEM.

Hematoxylin/eosin staining

Hematoxylin and eosin staining was performed using Mayer's hematoxylin (1g hemotoxylin, 1 liter dH_2O, 0.2g sodium iodate, 50g, aluminum potassium sulfate, 1g citric acid, and 50g chloral hydrate). After hydration with water, slides were incubated in Mayer's hematoxylin for 15 minutes, washed in dH_2O for 20 minutes, and counterstained with eosin solution (1g eosin in 100 ml dH_2O) for 1 minute. Sections were then dehydrated in 95% and 100% EtOH, with 2 changes of 2 minutes each, cleared in xylene, with 2 changes of 2 minutes each, and mounted in Permount.

Embryos and DiI injections

Fertile White Leghorn chicken eggs were incubated and opened essentially as previously described (Birgbauer and Fraser, 1994). Eggs were obtained from local suppliers and maintained at 38° C in a humidified incubator until the embryos reached Hamburger- Hamilton stage 16 (Hamburger and Hamilton, 1992). A circular hole was cut on the top of the egg shell with curved scissors, and India ink was injected beneath the surface of the embryo to improve contrast. The vitelline membrane was deflected

using a tungsten needle, and a micromanipulator was used to position electrodes for DiI-labelling. Electrodes of 5-10MΩ resistance were pulled from Al-Si glass capillaries (with filament) using a Sutter P-80/PC Micropipette Puller. The electrodes were back-filled with 0.5% DiI in ethanol, pre-warmed at 37°C, and placed in a holder with a silver wire immersed in the DiI solution. The pipette was driven past the telencephalic mesenchymal layer (Fig. 1C), and the DiI was injected into the neuroepithelium by iontophoretic injection with a maximum of 90 nA current through a 100MΩ resistor using a box powered by a 9 volt battery.

Histology

After incubation, embryos were washed in PBS, the eyes and body were removed, and the heads were fixed for 10-20 hours at 4°C with 4% paraformaldehyde, pH 7.4. After fixation and washing in PBS, the embryos were infiltrated with 7.5% gelatin/15% sucrose for 2-4 hours and then embedded. Frozen serial sections of 20um were cut using a cryostat and placed on TESTA-coated slides. Some of these sections were then imaged without cover slips, generally within 2-4 hours after sectioning, in order to record the presence of DiI label.

Imaging

Immediately after injection, the DiI injection position was recorded by taking both fluorescence and bright-field images with a SIT camera (Hamamatsu) in ovo.
The fixed specimens (both whole-mount and sectioned) were imaged for DiI-labelled cells by using either a SIT camera or, in some cases, a Biorad MRC600 laser confocal microscope mounted on a Zeiss axiovert microscope. In both cases, the epifluorescence and bright-field images were superimposed using false color. Confocal fluorescent images were collected at 1-5um intervals and then compressed computationally into a

single plane and overlaid onto a single bright-field image. In situ hybridizations and DAB immunohistochemistry were imaged using a Roche CCD camera. Adobe photoshop was used to enhance contrast.

In situ hybridization

Serially-sectioned embryos were processed for non-radioactive in situ hybridization using digoxigenin-labelled complementary RNA (cRNA) probes by a modification of the protocol of Harland (1991) as previously described (Groves et al., 1995). Whole-mount in situ hybridizations were performed as described previously (Groves et al., 1995). cRNA probes were made from: chick Shh (C. Tabin) mouse Shh (Vhh-1; T. Jessell), chick SCG-10 (P. Jeffrey), Cash-1(T. Reh). NF-160, 270.3 antibody from Virginia Lee.

RESULTS

Construction of telencephalic fate map

We labelled a total of 236 embryos at E3 (stage-16) and analyzed the embryos after 2-4 days of incubation (at E5-E7, or stages 24-30; see Chapter 2). Fig. 1A shows an *in ovo* image of an E3 embryo taken immediately after DiI injection. Fig. 1B shows the Cash-1 expression domain in the ventral telencephalon at stage-16 (see Chapter 2). The other gene expression marker used in this study, Sonic hedgehog (Shh), is expressed in a restricted region at the ventral midline in the caudal telencephalon at E3 (Ericson et al., 1995; J.M.M. et al., unpublished). All embryos were serially sectioned after incubation, and in situ hybridization with Cash-1 and Shh was performed on adjacent sections from most specimens. In Fig. 1A and B, the white arrow indicates the developing eye, which was used as the primary landmark to target injections into different telencephalic regions. Fig. 1C shows how the eye was used to divide the telencephalon

into three regions: dorsal to the horizontal plane of the eye (DE), ventral to the horizontal plane of the eye (VE), and within the horizontal plane of the eye. All injections, such as the one shown in Fig. 1A, were measured for the following values: the location of the injection site along the dorso/ventral (D/V) axis expressed as a percentage of the entire dorso/ventral distance in that particular specimen; and the distance along the rostro/caudal (R/C) axis expressed as a percentage of the total R/C distance in that specimen. These two relative distances where then plotted onto the canonical E3 (stage-16) telencephalon shown in Fig. 1C. The dimensions of this canonical E3 telencephalon were calculated by measuring the telencephalon in 30 specimens for a number of D/V and R/C values, and then averaging these values (not shown). The dimensions should therefore reflect the dimensions of the average chick telencephalon at stage-16.

The grid which has been overlayed on the specimen in Fig. 1C is a simple two-dimensional grid and does not account for the curvature of the specimen. Distortion within the map is therefore expected to be substantial where curvature is greatest in the most dorsal, ventral, and rostral telencephalic regions. The map should therefore be most accurate within the center portion of the telencephalon, within the horizontal plane of the eye, and along most of the R/C dimension. This is the region to which the DVR/BVR boundary, previously shown to represent a cell migration boundary (see Chapter 2), maps. Because misalignment of specimens in relationship to one another at the time of documentation could cause significant degrees of error, some specimens which were misaligned were not included in the fate map derivation (not shown).

Hematoxylin/eosin and SEM analysis

Fig. 2A and B show hematoxylin/eosin stains of a parasagittal section through the stage-16 chick telencephalon at lower (A) and higher (B) magnifications. Nuclei are

much more dense in the ventricular zone (VZ) of the neuroepithelium than in the surrounding mesenchyme. Fig. 1C shows a scanning electron micrograph (SEM) at E3 (stage 16), and Fig. 1D shows an SEM at E6. At both E3 and E6, the specimens were bisected mid- or para-sagittaly, and turned on their sides to reveal the ventricular zone (VZ) of the neuroepithelium. While at E3, the inner surface of the neuroepithelium is completely featureless, at E6 two prominent eminences separated by a sulcus (white arrow) are clearly visible. This furrow or sulcus is just ventral to the presumptive BVR/DVR boundary, which correlates with the boundary of Cash-1 expression and also with a cell-migration restriction at E5-E7 (see Chapter 2). Therefore, between the time at which the telencephalon was labelled with DiI (E3), and analyzed (E5-E7), the regionalization of the telencephalon has become morphologically detectable.

"Injection Site" fate map

Two types of fate map were derived from the DiI-labelling data. Previously, we showed that Cash-1 is expressed in the entire BVR at E5-E7, but not in the DVR, and that Shh is expressed in a ventral subregion of the telencephalon at E5-E7 (see Chapter 2). In the "Injection Site" fate map shown in the left two panels in Fig. 3, each injection site has been color-coded with respect to where the initial injection site at E3 maps onto the telencephalon at E5-E7. The initial injection site in DiI-labelled specimens is indicated by cells in the VZ which remain labelled after the incubation period (e.g., Fig. 5B). Six regions, as well as the boundaries betwen these regions, were scored in all: 1) the Shh^+ ventral region of the BVR (coded in light blue); 2) the more dorsal region of the BVR which is Cash-1^+, but Shh^- (dark blue); 3) the DVR, scored based on morphology, and designated as such if the injection site was dorsal to a region expressing Cash-1 and Shh (peach); 4) the Wulst, scored based on morphology (dark gray); 5) the Septum, scored based on both morphology, and designated as such when the injection site was in a Shh^-

region at a location ventral to the Shh$^+$ region (yellow); 6) the diencephalon, scored based on morphology, and designated as such when the injection site was caudal to regions which are Shh$^+$ (purple).

The clearest designations in this map are the Cash-1$^+$ and Shh$^+$ region of the BVR, because these regions can be unambiguously identified based on gene expression patterns alone, and therefore do not require morphological interpretations. The more ventral regions of the DVR in the more caudal telencephalon can also be clearly identified as lying dorsal to Cash-1$^+$ and Shh$^+$ regions, but ventral to the Wulst/DVR border. The Wulst/DVR border cannot be unambigously identified at this stage on morphological grounds, and we do not have an appropriate molecular marker for this region. However, a dorsal sulcus, proximal to the presumptive lamina frontalis superior, which will later divide the DVR and the Wulst (Tsai et al., 1981), is clearly visible, and we have designated this sulcus as the division between the DVR and the Wulst. It may well be that, as in the case of the DVR/BVR boundary, the actual DVR/Wulst boundary is slightly offset from this sulcus. The presumptive Wulst as designated here includes regions that will give rise to the hyperstriatum intercalatum, hyperstriatum augmentatum, and hyperstriatum dorsale (Ebbesson, 1980; Deng and Wang, 1992; Tsai et al., 1981b; see Chapter 4).

We have designated as "Septum" Shh$^-$ regions which are ventral, or medial, to Shh$^+$ regions. At E5-E7, the Septum, when scored as such, occupies a small, defined region within the rostral third of the telencephalon. The injections mapping to Shh$^-$ areas in the most rostral region of the E5-E7 telencephalon, which do not lie ventral, medial, or dorsal to Shh$^+$ or Cash-1$^+$ regions, have been designated as "rostral." Again, the precise extent of the septal nucleus is difficult to ascertain with the available markers at these early developmental stages. We have, however, classified the injection sites unambiguousy on the basis of gene expression markers. Injection sites were classified as lying on a

boundary (e.g., violet for the DVR/BVR boundary), when the injection site straddled the boundary. In this case, the DVR/BVR boundary is defined as the boundary of Cash-1 expression.

"Cell Dispersion" fate map

The "Cell Dispersion" fate map (Fig. 3, right panels) color-codes the injection sites with four different designations: when injection site maps to the BVR, and all labelled, dispersing cells remain confined to the BVR, the initial injection site is color-coded as light blue; when injection site maps to the BVR, but one or more dispersing cells migrate into the DVR, it is color-coded violet; when injection sites map to the BVR, but one or more dispersing cells migrate into the diencephalon, it is coded as green; when injection sites map to the DVR, and cells stay either within the DVR, or within the DVR and the most lateral portion of the neuroepithelium (see Chapter 2, Figs 4Q and 7B), the initial injection site is coded as peach. Cell dispersions were not scored in other regions.

Final fate map and Cash-1 superimposition

The data from the "Injection Site" fate map is summarized in Fig. 4A. This map details the approximate limits of the presumptive BVR, DVR, Wulst, and Septum. The regions between each area represent the boundary regions. Because the actual boundaries between regions are likely to be quite sharp, these boundary regions as designated in the fate map can be seen as approximations of experimental error. In the case of the DVR/BVR boundary, the error was approximated by measuring the spread of points which mapped to the DVR/BVR boundary, and then using this spread as representing the entire extent of the boundary, as measured experimentally. Because the mapped boundary regions include all of the most outlying points, it should result in an

overestimation of the actual error. We estimate the error as about 10% of the dorso/ventral extent of the eye.

In Fig. 4B, the Cash-1 expression domain at E3 (stage-16) has been superimposed onto the fate map. It is clear from the map that the Cash-1 domain includes only a restricted ventral subregion of the presumptive BVR, although the entire BVR expresses Cash-1 at E5-E7. Therefore, Cash-1 does not simply "mark" the presumptive BVR when it is first expressed; rather, its domain of expression spreads further dorsal until, at E5, it is expressed in the entire presumptive BVR. Cash-1 begins to be expressed in the telencephalon at about stage-15 (data not shown; Jasoni et al., 1994).

Cell migration patterns in distinct telencephalic regions

We also analyzed the cell migration patterns found in the different regions indicated in the fate map. Previously, we showed that labelled cells in the BVR disperse widely, primarily within the intermediate zone (IZ), and that labelled cells in the DVR migrate predominantly radially through the IZ, and move tangentially only in the most lateral, subpial region (see Chapter 2). Fig. 5 shows an injection which is just ventral to where we have positioned the DVR/Wulst boundary (indicated by a black arrowhead in Fig. 5E), in which the embryo is analyzed at E7. Cells migrate primarily radially through the SCG-10$^+$ IZ, and disperse widely in the subpial region (Fig. 5B, gray arrow). However, a number of cells disperse tangentially in the lateral surface of the ventricular zone (Fig. 5A, white arrowheads). This migration pattern was observed in a number of specimens labelled in the more dorsal portion of the DVR (not shown).

Fig. 6 shows some of the cell migration patterns found in other telencephalic regions. Fig. 6A and B show an example of an injection into the Septum analyzed at E6. The injection site (white arrow in Fig. 6A) is in a Shh$^-$ region which is medial to the Shh$^+$ region in the ventral BVR (Fig. 6B). At this stage (E6) there is very little tangential

movement of labelled cells in the septum. Fig. 6C and D show lower (C) and higher (D) magnification images of an injection into the more medial telencephalon in a very rostral, Shh$^-$ and Cash$^-$ region, and analyzed at E6. Cells disperse widely along the lateral telencephalon (white arrowheads in Fig. 6C and D) away from the initial injection site (white arrow in Fig. 6C and D). This dispersion pattern, which predominates throughout the region we have designated as "rostral," is similar to that found in the DVR, which is also Shh$^-$ and Cash-1$^-$. Fig. 6E shows the typical migration patterns of cells labelled in the presumptive Wulst. Cells migrate in the lateral telencephalon (white arrowhead, Fig. 6E) far ventral to the initial injection site (white arrow, Fig. 6E). Fig. 6F shows an injection site (white arrow) into the ventral BVR. This represents a more caudal section at E6 which does not include the Shh$^-$ septum, which is only present in the more rostral regions at this stage. Cells migrate predominantly dorsally within the IZ. Though some specimens with injection sites similar to the site in Fig. 6F showed labelled cells crossing the telencephalic midline (not shown), labelled cells more commonly migrate away from the midline, as they do in this specimen.

DISCUSSION

Potential uses for fate map

We have derived a regional fate map of the stage-16 (E3) chick telencephalon and superimposed the expression pattern of Cash-1 at stage-16 onto the fate map. In addition, we have provided examples of typical cell migration patterns in the different regions described in the fate map. This fate map can now serve a number of useful functions in future studies. The expression pattern of any gene which is expressed in the chick telencephalon at stage-16 can now be superimposed onto this map, as we have done for Cash-1. Though Cash-1 is expressed in the entire BVR at E5-E7, its expression

domain at stage-16 clearly encompasses only a subregion of the presumptive BVR. Without a fate map, it may have been assumed that Cash-1 expression simply "marked" the entire presumptive BVR region at stage-16. When further genes are analyzed in relationship to this map, some of their expression domains may in fact encompass an entire presumptive region and, if the gene is expressed in this same region at later stages, the gene may then be considered as a reliable marker for this particular area.

The fate map also provides the basis for future transplantation or misexpression experiments. The existence of such a map would make it much easier, for example, to transplant presumptive BVR tissue into the presumptive DVR region, or vice versa. The map will also facilitate experiments in which, for example, retroviral constructs are used to express genes which are normally expressed only in the BVR, in the DVR, or vice versa (Galileo et al., 1992).

DiI labelling for fate map construction

DiI has been used to label cells in many systems (Serbedzija et al., 1989; Honig and Hume, 1986, 1989; Fraser, 1992) and appears to become incorporated into the inner layer of cell membranes (Axelrod, 1979; Schlessinger et al., 1977). The general method of focally injecting DiI to derive a fate map can, in principle, be applied to any region of the chick neuroepithelium; it is technically easier than performing single-cell injections, and it also labels small groups of cells, which can facilitate the derivation of a fate map (Birgbauer and Fraser, 1994; Woo and Fraser, 1995). The main advantages of DiI-labelling when compared to using chick-quail chimeras are: 1) greatly improved resolution - as few as 5-10 cells may be labelled with DiI injections, whereas small regions are very difficult to graft in chimeras; 2) experiments are performed in situ, and do not require surgical removal and replacement of tissue; 3) DiI-labelling experiments can be performed at later stages in development, whereas grafting experiments are

difficult to perform after the region of interest has become heavily vascularized, as bleeding during and after surgery is usually lethal to the developing embryo. The main disadvantages of DiI labelling are: 1) it is a short-term label; and 2) it is difficult to label single cells with DiI.

Experimental error in fate map construction

Experimental error in deriving the fate map probably arose from a number of sources: 1) alignment at the time of documentation is not precisely the same for all specimens; 2) a two-dimensional grid was used to map a three-dimensional structure; 3) chick specimens contain significant variability in the relative size of their telencephalon, and it is very likely that the exact location of presumptive regions in relation to morphological landmarks, such as the eye, is also somewhat variable from specimen to specimen. Experimental error was estimated as roughly 10% of the total dorso/ventral extent of the eye. This error is reflected in the extent of the boundaries between discrete regions on the fate map.

The region of the map which was most saturated with individual injections was near the boundary of the presumptive DVR and BVR, which we have shown previously to represent a cell migration boundary (see Chapter 2). While the limits of the presumptive BVR could be firmly determined by correlations with Cash-1$^+$ and Shh$^+$ regions, other regions, such as the Septum and the Wulst, relied on the absence of Shh and Cash-1 expression and on morphological features, and therefore are more likely to be inexact. The availability of other specific markers for these regions will greatly facilitate the construction of a more precise and comprehensive fate map.

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FIGURE LEGENDS

Figure 1. Mapping DiI-injection sites at E3. Panel A shows *in vivo* image of the E3 (Hamburger-Hamilton stage-16) telencephalon taken immediately after DiI (shown in red) injection. Panel B shows restricted expression of Cash-1 in ventral telencephalon at this same stage. White arrows in A and B point to developing eye. Panel C shows the coordinate system used to map the position of DiI injection sites in the telencephalon. Injections were divided into three categories: those ventral to the dorso/ventral (D/V) plane of the eye (VE), those within the D/V plane of the eye, and those dorsal to the D/V plane of the eye (DE). Injections were also measured along the rostro/caudal (R/C) plane (see text for more details).

Figure 2. Panels A and B show a hematoxylin/eosin stain of a sagittal section through the E3 chick telencephalon at lower (A) and higher (B) power. Cells in the ventricular zone (VZ) represent a pseudostratified neuroepithelium at this stage. Panels C and D show scanning electron micrographs (SEMs) of the E3 (C) and E6 (D) telencephalon. In both cases, the specimen has been bisected mid-sagitally and then placed on its side to reveal the inner ventricular surface of the telencephalon. While the ventricular zone (VZ) is completely featureless in the E3 telencephalon (white arrow in C points to optic recess in diencephalon, just caudal to telencephalon), the SEM at E6 (D) shows a prominent sulci (white arrow) separating dorsal and ventral eminences. d, dorsal. r, rostral. v, ventrtal. bars, 100u.

Figure 3. Two representations of regional fate in telencephalon. (Left panels) "Injection site" fate map color-codes each injection site at E3 with respect to the region where the initial injection site at E3 maps at E5-E7. The five primary domains within the E5-E7 telencephalon are shown in the lower left panel. Injection sites coded in yellow (top left) map to the Septum at E6. Injections sites in light blue map to Shh^+ domain in BVR at E6. Dark blue injection sites map to Cash-1^+, Shh^- domain within BVR (dorsal BVR). Injection sites in peach map to DVR at E5-E7. Wulst is color-coded in dark gray. Legend at upper left also shows color-codes for injection sites which straddled the boundary regions found between distinct telencephalic areas at E5-E7. For instance, injection sites coded in violet mapped to the DVR/BVR boundary at E5-E7, and injections sites coded in brown mapped to the DVR/Wulst boundary at E5-E7 (Youngren and Phillips, 1978). Injection sites which were dorsal and rostral to the Shh^+ domain are color-coded in green. Injection sites scored as mapping to DVR mapped to regions dorsal to Cash-1^+ and Shh^+ regions, but ventral to flexure scored as separating DVR and Wulst (lower left; also see Fig. 5, black arrowhead). Injection sites mapping dorsal to this flexure were scored as mapping to the Wulst.

(Right panels) "Cell Dispersion" fate map color-codes injection sites according to both where the injection site maps at E6, and also whether the progeny of labelled cells remain within the same region. If injection site is color-coded in light blue, the injection site maps to the BVR, and migrating cells remained within the BVR. Injection sites color-coded in violet indicate cases where injection site was in BVR, but one or two cells migrated out of the BVR into the DVR. Injection site color-coded in green indicates case where injection site mapped to BVR at E5-E7, and one cell migrated into the diencephalon. In all injections mapping to DVR, cells remained confined to DVR and lateral telencephalon (dorsal and ventral). This map does not include injections which mapped to the BVR/DVR or BVR/diencephalon boundaries.

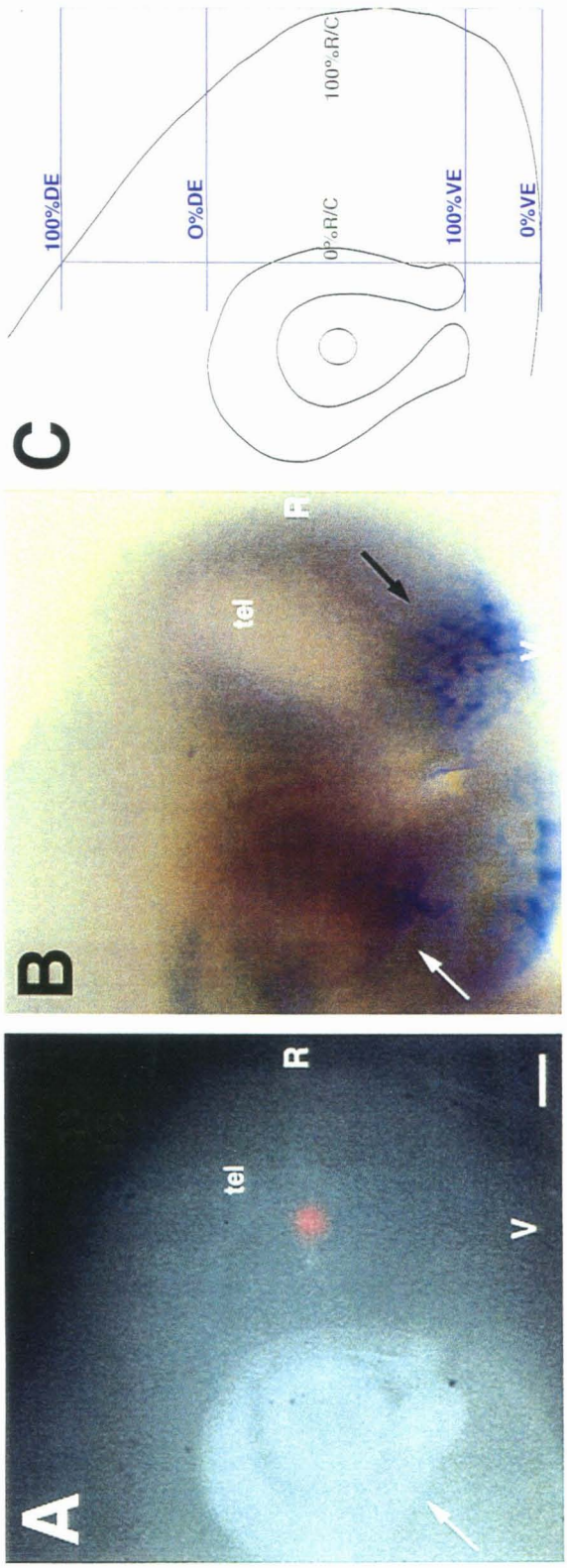
Figure 4. (A) Summary diagram of the "Injection Site" fate map data. Injections into regions color-coded in light blue will map to BVR at E5-E7; regions in peach will map to DVR; dark gray maps to Wulst; and regions in yellow will map to Septum. Intermediate regions represent error (roughly 10% of dorso-ventral extent of eye) in mapping the boundaries between these regions. (B) Cash-1 expression domain superimposed onto fate map. Note that while Cash-1 is expressed in the entire BVR at E5-E7, it is only expressed in a ventral subdomain of the presumptive BVR at E3 (see text).

Figure 5. DiI injection in dorsal DVR at E3 analyzed at E7. Serial adjacent sections show Cash-1, SCG-10, and neurofilament expression (see Chapter 2). Injection site is dorsal to Cash-1 expression boudnary (C, black arrowhead), and to LMD (E, arrow), but just ventral to flexure separating DVR and Wulst (E, black arrowhead). Specimens labelled in the more ventral region of the DVR (see Chapter 2, Fig. 7B) may show migration patterns indicative of the presumptive neostriatum (see Chapter 4, Figs. 1 and 3). The injection shown above in panel E may label the presumptive hyperstriatum ventrale (HV), though no specific marker for either the neostriatum or for HV is available at this developmental stage. Cells migrate predominantly radially through SCG-10$^+$ intermediate zone (IZ), and then show subpial migration in lateral telencephalon (B, gray arrow). In this specimen, a few cells are seen to migrate dorsally on the lateral surface of the ventricular zone (VZ) (white arrowheads, A and B).

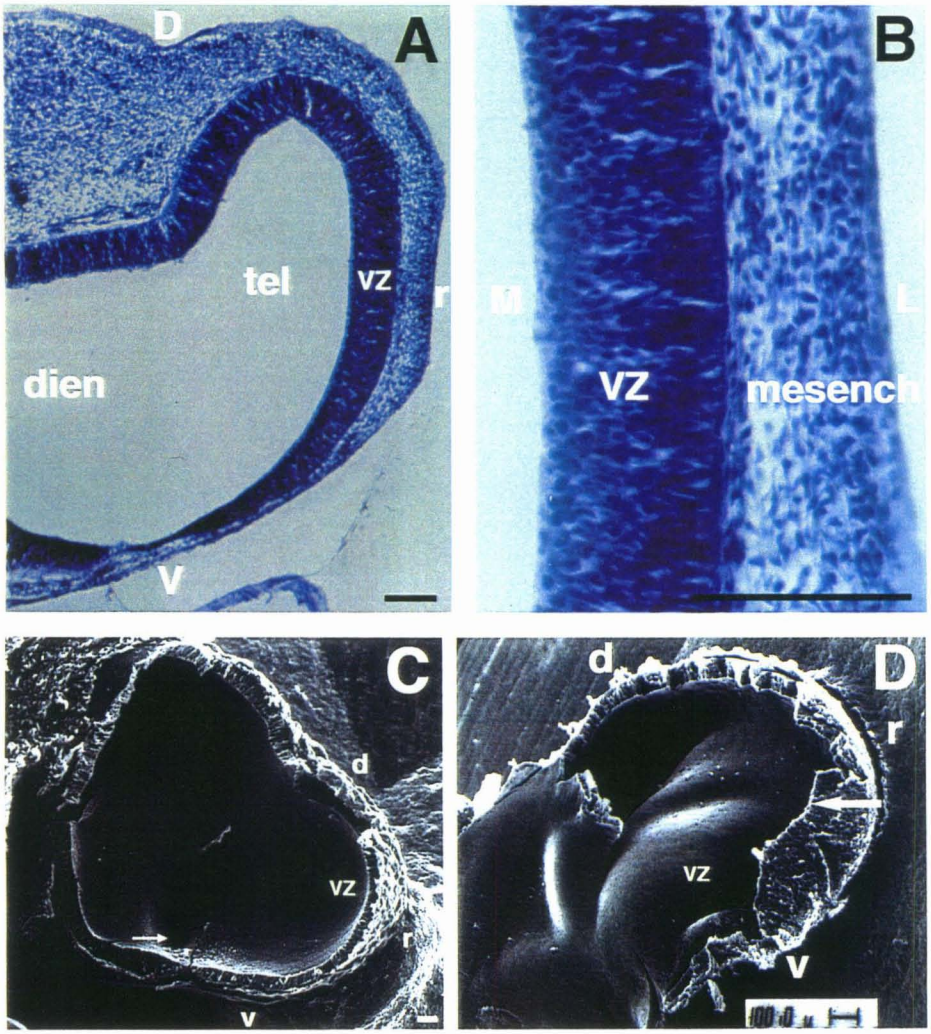
Figure 6. Migration patterns in other regions of telencephalon. Panels A and B show a DiI injection into the Septum (A, white arrow), which is in the more rostral telencephalon in the Shh$^-$, medial region (B). Labelled cells are aligned radially (A). Panels C and D show low power (C) and high power (D) images of specimen injected in Shh$^-$ region in

rostral telencephalon (rostral to presumptive BVR). Injection site is in medial wall of telencephalon (white arrows, C and D), and cells migrate tangentially in lateral neuroepithelium (white arrowheads, C and D). Panel E shows injection into presumptive Wulst (white arrow). Cells migrate substantial distances away from injection site in lateral region of neuroepithelium, with labelled cells moving into ventro-lateral regions (E, white arrowhead). Panel F shows DiI injection into ventro-caudal telencephalon (white arrow). Cells migrate predominantly dorsally in this specimen, though some specimens with similar injections sites showed some labelled cells crossing midline of telencephalon (not shown).

Chapter 3
Figure 1

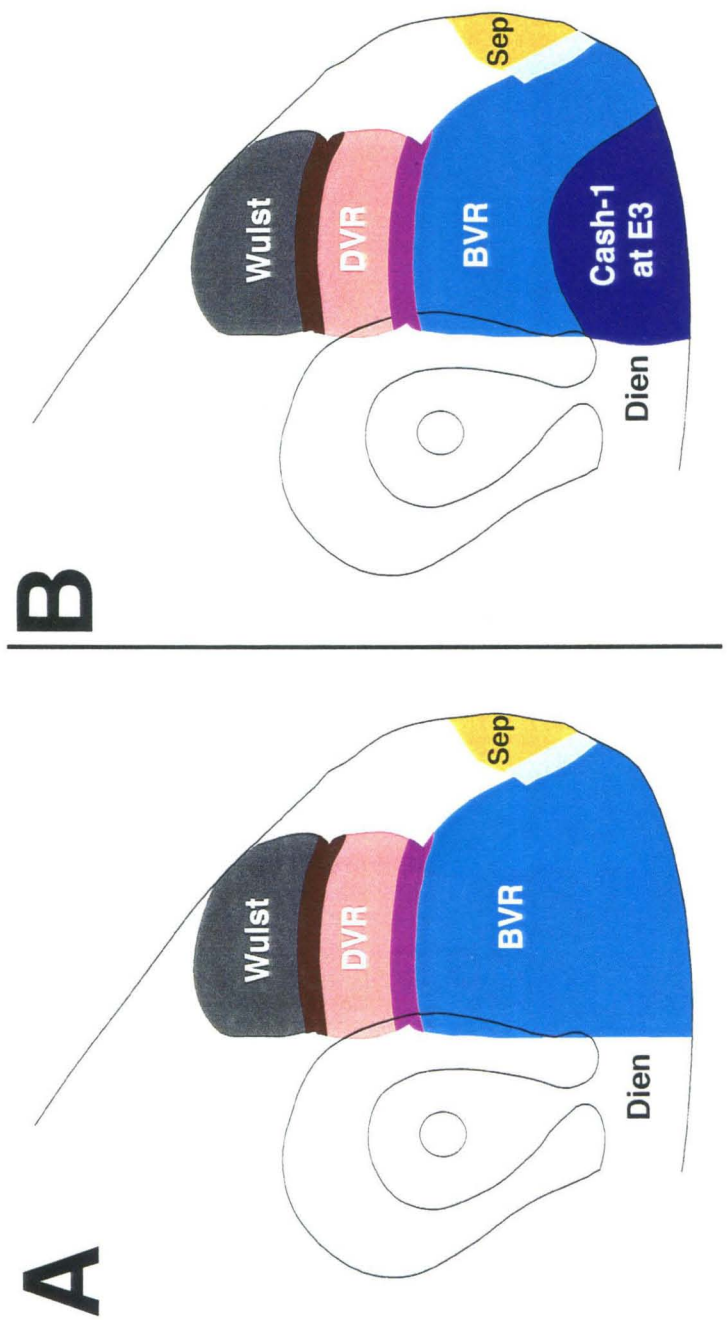


Chapter 3
Figure 2

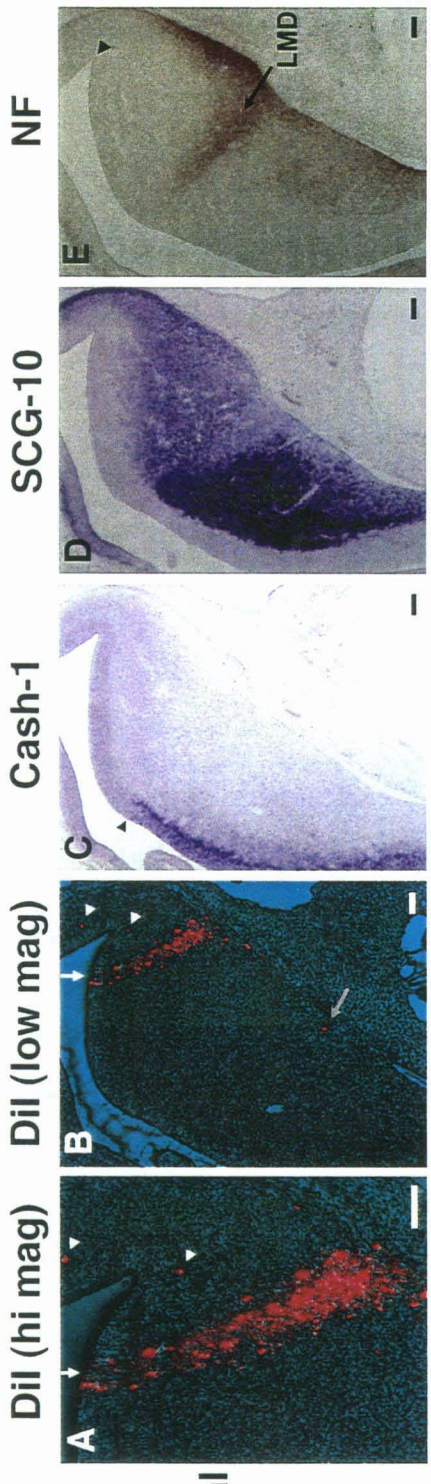


Chapter 3
Figure 3

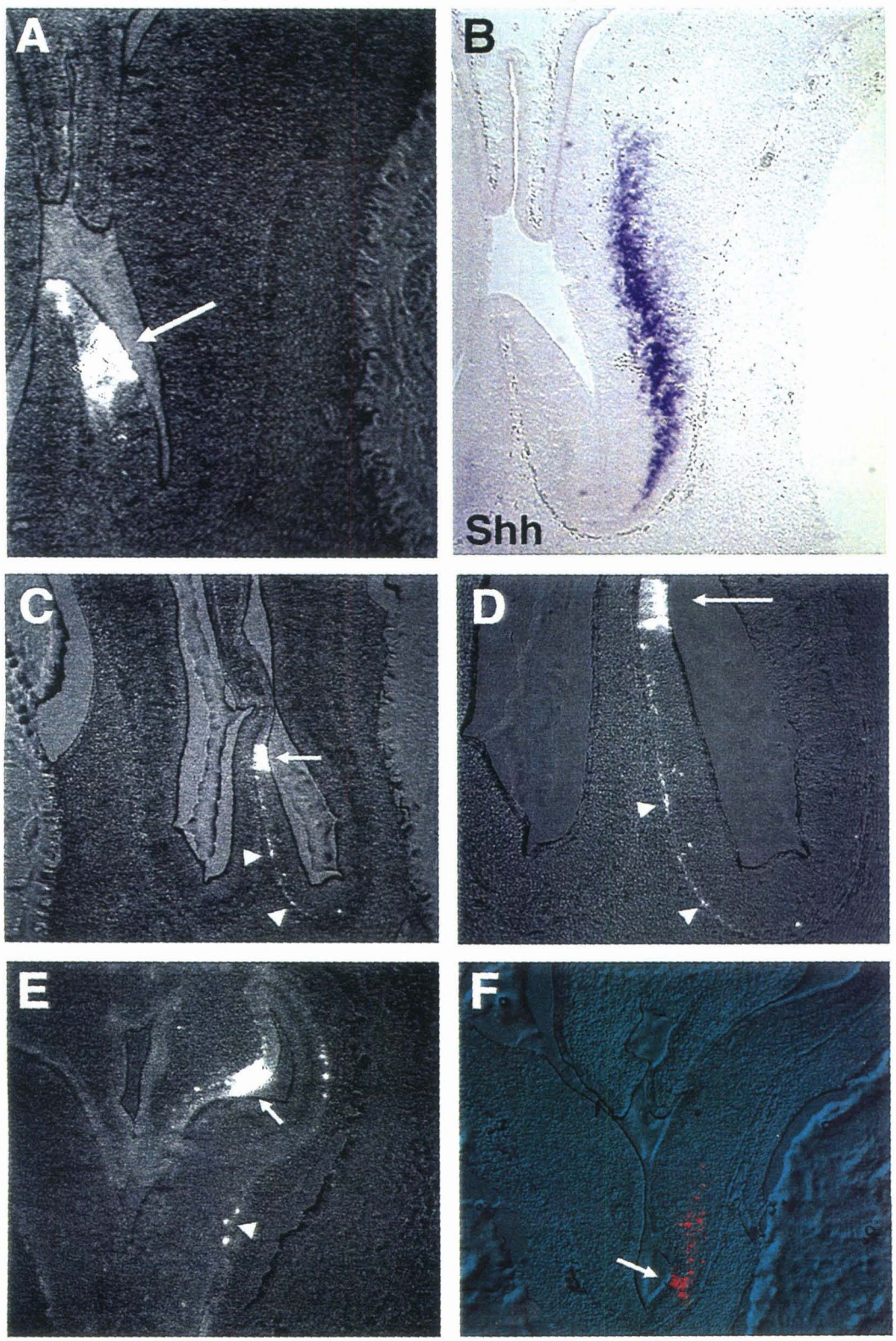
Chapter 3
Figure 4



Chapter 3
Figure 5



Chapter 3
Figure 6



Chapter 4

The Evolution and Compartmental Plan of the Amniote Telencephalon

Evolutionary homology in the central nervous system

Two major goals of evolutionary biologists are to describe phylogenetic changes in specific organismal attributes or characters, and to elucidate evolutionary mechanisms in general. Both pursuits require that homologous characters (a character and its subsequent phylogenetic transformation) be differentiated from homoplastic characters (characters that appear similar but have different evolutionary histories). Of the various phylogenetic definitions of homology, the most widely quoted may be that of Simpson (1961), which states that "homology is resemblance due to inheritance from common ancestry;" homoplasy is defined as resemblance not due to inheritance from a common ancestry. Campbell and Hodos (1970) propose that "structures and other entities are homologous when they could, in principle, be traced back through a geneological series to a stipulated common ancestral precursor irrespective of morphological similarity."

Since the time that Darwin proposed his theory of evolution, homology has been defined one way, but nearly always tested in a very different way. Though definitions of homology are based on common ancestry, in practice homologies have traditionally been based on the existence of detailed similarities between characters, and the pattern of variation of these characters, in living organisms (Northcutt, 1984; Striedter and Northcutt, 1991). Since brains, and most other organismal attributes, do not generally fossilize, determination of homologies in the nervous system must depend on the study of living organisms.

Four primary criteria have been most widely used to determine neuroanatomical correspondence or homology between specific structures: 1) similarities in topology, or relative position, between the two structures; 2) similarities in cytoarchitecture, i.e. cellular composition and organization; 3) similarities in histochemical or

immunohistochemical staining; and 4) similarities in afferent and efferent connections. No single "essential" criterion is widely recognized as being of cardinal importance in the determination of morphological homology. While the developmental criterion (similarities in the development of a structure) was classically used for identifying homologies (Kuhhlenbeck, 1937; Herrick, 1910), many evolutionists have viewed developmental data with suspicion when applied to the establishment of homology (Striedter and Northcutt, 1991; Wagner, 1989, 1994). Others, however, have challenged this view (Källén, 1959; Karten, 1991; Collazo and Fraser, 1996; Goodwin, 1994) (see later).

Primitive and derived characters

Neural characters, or features, are often thought of as being either "primitive" or "derived": primitive characters are retained with little or no modification over the course of evolution, while derived characters are highly modified in specific evolutionary lines. In reality, the brains of all living vertebrates are a mosaic of both primitive and derived characters. Despite its shortcomings, the principle of parsimony, stating that the hypothesis involving the smallest number of changes be accepted, usually must be implemented in order to draw evolutionary conclusions (Stewart, 1993).

To address whether neural characters are primitive or derived, it is essential to perform an out-group analysis for that character: a step-wise examination of a given character, beginning with the closest geneological relative and progressing to more distantly related taxa. For instance, the middle temporal visual area (MT) , which was first described in owl monkeys (Allman and Kaas, 1971), has been clearly identified in all primates which have been investigated. But a similar visual area has not been identified in any non-primate species (Kaas, 1991). In this case, therefore, the out-group analysis suggests that MT emerged early in primate evolution and was not present in the common

ancestor of primates and other placental mammals, or that it is a primate character which is "derived" (Northcutt and Kaas, 1995). It is also possible that the anlage of MT is in fact present in all mammals, but that it has been transformed so greatly in primates that it has not been possible to recognize its homologue in other mammals.

A few basic areas of cerebral cortex appear to be present in most or all mammals; these include the primary and secondary visual areas (VI and VII) and the primary and secondary somatosensory areas (SI and SII) (Kaas and Krubitzer, 1991). The most parsimonious explanation is that these areas were present in the most recent common ancestor of all mammals, i.e., that these are "primitive" characters.

It is also important to determine whether similar regions in different species are homoplastic, rather than truly homologous. For instance, both cats and monkeys have at least 10 distinct cortical visual areas, and perhaps as many as 15-20. Detailed comparative studies have suggested, however, that most of these visual areas are not homologous, but rather that the lines leading to cats and monkeys independently increased the number of visual areas (Kaas, 1989).

The amniote telencephalon

Forebrain organization has been widely studied in both amniotes (mammals, reptiles, and birds) and anamniotes (fish and amphibia). After inspecting the telencephalon in a number of vertebrate species, Kappers (1922) concluded that the vertebrate telencephalon has evolved in steps, and proposed his linear model of evolution: in anamniotes, the telencephalon is dominated by the olfactory or "paleo" cortex; the "neopallium," which contains thalamic input, then evolved in reptiles; finally the neopallium was elaborated into the neocortex in mammals, and added to the existing complex. Kappers also proposed a related model for striatal evolution. He concluded that reptiles and birds are dominated by striatal structures, and that their largely instinctive

and sterotyped behavior correlates with an absence of "cortical" derivatives. Kappers introduced a nomenclature to fit his theory of the evolution of the telencephalon. Though his theory has now been thoroughly discredited, Kappers' nomenclature is still widely used, despite many efforts to install a more evolutionarily neutral terminology (Jones and Levi-Montalcini, 1958). Most of the subdivisions of the avian telencephalon are still referred to with the suffix "-striatum," although most of these regions are clearly not striatal in origin. Similarly, the terms "paleo-," "archi-," and "neo-," reflect the incorrect premise that the telencephalon evolved in a linear sequence, but the terminology is still frequently used (Northcutt and Kaas, 1995).

The evolutionary history of living reptiles and birds is as extensive as that of the radiation leading to mammals, and therefore modern reptiles and avians cannot be viewed as simply representing a more primitive form of vertebrate organization than mammals. All recent comparative studies have demonstrated that the pallium (the roof of the telencephalon) and the subpallium (the basal telencephalon) are characterized by cytologically distinct cell groups that are identifiable in every vertebrate radiation. The pallium can be divided into lateral, dorsal, and medial formations, and the subpallium can be divided into striatum and septum in essentially all vertebrates (Northcutt, 1981; Wicht, 1992; Ulinski, 1983), although in some highly derived fish the basic pallial subdivisions are difficult to homologize (Northcutt, 1981). Contrary to the "scala naturae" proposed by Kappers, therefore, essentially all major telencephalic anlagen appear to have been present in the common ancestor to all vertebrates. The lateral pallium (pyriform cortex) has been identified in different vertebrate species by topology and by secondary olfactory inputs; the medial pallium (hippocampal formation) has been identified primarily by topology and afferent and efferent connections; and the dorsal pallium, which lies between the lateral and medial pallium, has often been defined by exclusion (see Fig. 1). In mammals, the dorsal pallium is called neocortex. The neocortex is a layered structure

which receives afferents primarily from the dorsal thalamus. In reptiles and birds, the dorsal pallium includes the DVR, or dorsal ventricular ridge, which is not layered.

The amniote basal ganglia

The line leading to mammals probably diverged from the line leading to reptiles and birds not long after the origin of amniotes about 300 million years ago (Fig. 2) (Ulinski, 1983; Allman, 1990; Novacek, 1992). It is now widely recognized that the basal ganglia contains similar or homologous neuronal subpopulations in all amniotes (Reiner et al., 1984b; Karten and Dubbeldam, 1973; Rehkämper and Zilles, 1991). These homologies have been determined primarily by the transmitters that specific neuronal subpopulations use, and by their afferent and efferent connections. Prior to modern histochemical studies, the DVR was considered to be a "striatal" structure, as was most of the hyperstriatum (see Figs. 1 and 3). Karten and Dubbeldam (1973) showed that the paleostriatum of the avian telencephalon, but not the neostriatum or hyperstriatum, is rich in catecholaminergic and acetylcholinesterase-(AChE-) containing fibers. The mammalian basal ganglia is usually considered to consist of the nucleus accumbens and the caudate-putamen ("the striatum") and the globus pallidus ("the pallidum") (Reiner et al., 1984b). Like the avian paleostriatum, the mammalian striatum is characterized by distinctly higher levels of AChE and catecholamines than mammalian neocortex. This finding suggested that the border between avian paleostriatum and neostriatum separates "striatum" from "non-striatum" (Reiner et al., 1984b) (see Figs. 1 and 3). The augmentatum and mammalian striatum contain high AChE and catecholamine levels, while the primitivum and pallidum contain low levels of both. Findings in reptiles have been essentially similar to those in birds (Parent, 1979; Ulinski, 1983). This histochemical data further suggests that the two paleostriatal subfields in birds, the

augmentatum and primitivum, are comparable to the mammalian striatum and pallidum, respectively.

DVR and cerebral cortex

The DVR is a ridge in the lateral hemispheric wall of birds and reptiles that bulges into the lateral ventricle. Classical comparative neuroembryologists often interpreted DVR as occupying a position comparable to that occupied by the lateral ganglionic eminence (LGE) in mammals, which is of striatal origin (Ulinski, 1983; Kappers, 1922). Eliot Smith (1919) first proposed that DVR was pallial in origin. After looking at embryological data in different amniotes, Källén (1953b, 1962) also concluded that the DVR is of pallial origin, and was the first to propose a direct homology between the avian DVR/Wulst complex and mammalian neocortex. The histochemical data of Karten and Dubbeldam (1973), showing restriction of AChE and catecholamine staining to the paleostriatum (see Figs. 1 and 3), provided the first strong evidence that the DVR is of pallial origin, and that the BVR (basal ventricular ridge) in avians alone contains all the components of the basal ganglia in mammals. Karten et al. (1973) also showed that the avian DVR, like the mammalian cerebral cortex, receives specific auditory and visual projections from the LGN, further suggesting a homology between the DVR and mammalian cerebral cortex. Karten (1969) proposed that both the DVR and the Wulst in avians are together homologous to neocortex in mammals.

All living mammals have a 1-3mm thick, multi-layered cortical sheet, which is interposed between the more lateral olfactory cortex and the more medial hippocampal cortex. Because the avian and reptilian DVR are not layered structures, and as the layering of mammalian neocortex is so exquisitely refined and apparently integral to neocortical function, many comparative anatomists do not accept that the DVR of avians and reptiles and the cerebral cortex of mammals can be called homologues. So, while

striatal homologies among all amniotes are widely accepted by comparative neuroanatomists, there are two divergent hypotheses which address the issue of pallial homologues: the "out-group hypothesis" and the "recapitulation hypothesis" (Northcutt and Kaas, 1995).

The out-group hypothesis holds that the cerebral hemispheres in a putative ancestor of terrestrial vertebrates was comparable to the cerebral hemispheres found in living amphibians, and that portions of the dorsal hemisphere enlarged independently in sauropsids (living reptiles and birds) and in synapsids (mammal-like reptiles and mammals). The DVR is proposed to have arisen in sauropsids by the differentiation and enlargement of the ancestral lateral pallium. The neocortex is proposed to have arisen in synapsids by an independent enlargement of the ancestral dorsal pallium and, possibly, a portion of the lateral pallium. In either case, the DVR in sauropsids and the neocortex in synapsids would be non-homologous, or homoplastic, structures (Northcutt and Kaas, 1995; Northcutt, 1981).

The recapitulation hypothesis does not accept that the cerebral hemispheres of living amphibians represent the ancestral amniote condition, and instead holds that the cerebral hemispheres of living amphibians are highly derived structures. The histochemical and connectional similarities between DVR and neocortex mentioned above are proposed as supporting the following scenario: that DVR existed in the earliest terrestrial vertebrates, was retained in living reptiles and birds, and was transformed, along with a portion of the dorsal cortex, into the multi-layered neocortex in mammals. The cerebral hemispheres in living reptiles and birds would therefore represent the primitive condition, and the mammalian pallium would represent a derived condition. Since the scenario requires only one "transformation" from the same anlage, the sauropsid DVR and the mammalian cerebral cortex would then be homologous.

The developmental genetics of DVR and cortex

In general, there are three different developmental criteria that can be used to determine morphological homology: 1) the developmental origin of a structure; 2) the gene expression patterns within a structure; and 3) the general pattern of cell behavior in a structure. Although the developmental criterion has not been widely utilized in the recent past due to its perceived unreliability (Striedter and Northcutt, 1991; Wagner, 1994), it has been argued that this perception has arisen from simplistic assumptions about both development and evolution (Collazo and Fraser, 1996). The developmental criterion supports a proposed homology between two structures if their development is similar (Collazo and Fraser, 1996; Källén, 1959; Goodwin, 1994; Wilson, 1894). Wagner (1989) has said that one must identify the correct developmental unit in order to make appropriate comparison between different species.

Many authors have been hesitant to implement the developmental criterion of homology because patently homologous structures, it has been claimed, can often develop differently. However, the determinations of differing developmental mechanisms have usually been based on a superficial knowledge of the developmental mechanisms operating in the tissue of interest. One classic example of homologous structures which have been proposed to have non-homologous development, is the neural tube of different vertebrates (Striedter and Northcutt, 1991; Collazo and Fraser, 1996). In many vertebrate groups, the initial formation of the neural tube occurs by the rolling up of the neural plate until the lateral edges meet, a process called primary neurulation. Teleosts, in a process called secondary neurulation, form a neural tube directly from a superficially amorphous mass of cells by cavitation, in which no groove or fold is visible (Jacobson, 1991). Though these processes seem to be distinct on a gross level, recent single-cell labelling suggests that neurulation in the zebrafish (a teleost) occurs by a mechanism very similar to that of primary neurulation (Papan and Campos-

Ortega, 1994). In this case, therefore, development in different species which seemed superficially different using classical embryological techniques, was revealed as being much more similar using modern biological techniques (Collazo and Fraser, 1996). As our knowledge of development becomes increasingly detailed, it seems likely that a broadly comparative developmental criterion will become critical for the determination of homologies. A truly persuasive argument about the homology of any complex structure (like the forebrain) is likely to require a reconciliation of both the embryological data and its final phenotypic manifestation (Karten, 1991). B. Källén (1962) has said that "from whatever point of view a comparison of different forms is attempted - whether it is a comparative-anatomical, comparative-embryological, or phylogenetical - the findings must be analyzed in the light of embryology." And Roth (1984) concluded: "ultimately, genetically controlled development as a process is the real criterion of homology."

Developmental genetic data presented in Chapter 2 of this thesis appears to bear on the issue of the proposed homologies between avian DVR and mammalian cerebral cortex, and avian BVR and mammalian basal ganglia. Two general lines of evidence are compared between the chick and the mouse: regulatory gene expression patterns and cell migration patterns. In both cases, the similarities between the chick and mouse are striking. At the early stages analyzed here (see Chapter 2, Fig. 1), the mouse and chick telencephalon also appear morphologically very similar (Ulinski, 1983). Cash-1 is expressed in the ventricular zone (VZ) of the presumptive BVR, but not the DVR, in chick. Similarly, Mash-1 is expressed in the VZ of the medial and lateral ganglionic eminence (MGE and LGE, which constitute the presumptive basal ganglia), but not in the presumptive cerebral cortex. Shh is expressed in the intermediate zone (IZ) of the mouse MGE, but not the LGE. Similarly, chick Shh is expressed in the IZ of a defined ventral region of the presumptive BVR.

It seems likely that morphological diversity through phylogeny has been based primarily on changes in mechanisms controlling the expression patterns of genes, rather than the specific sequences of proteins (Carroll, 1994, 1995; Patel, 1994). Gene expression patterns are likely to be highly dynamic through phylogeny, and gene-knockout experiments in the mouse have indicated that genes are often expressed in tissues in which they serve no apparent function (Guillemot et al., 1993; Qiu et al., 1995). Evolutionary experiments leading to morphological changes may therefore often involve different genes expanding their expression domains into new regions; some of these new expression domains will ultimately lead to morphological change and some will serve no useful function. It is therefore likely that homologous structures in different species will sometimes show divergent, but functionally insignificant, gene expression patterns in homologous structures (see Chapter 3, Tole, 1994). Nevertheless, as the amount of data concerning regulatory gene expression patterns in different organisms is accumulating rapidly, this vast amount of information should be implemented for determinations of homology, particularly if the genes being studied are found to be functionally significant in the tissues of interest.

The second line of evidence presented in Chapter 2 supporting avian and mammalian telencephalic homologies concerns cell migration patterns, and, as this reveals a basic cellular phenomenon, it is likely to be of greater general significance than the expression pattern of any particular gene. The data presented in Chapter 2, when compared to previously published studies in the mouse, shows striking similarities in cell migration patterns in the early development of the telencephalon in the chick and mouse. The data presented in Chapter 2 shows that cells migrate radially in the DVR through the IZ, and then tangentially in the lateral neuroepithelium. Cells migrate significant distances, often into the ventral telencephalon, but are always confined to the lateral region. Various studies in the mouse, using replication-incompetent retroviruses (Walsh

and Cepko, 1988; Austin and Cepko, 1990), transgenic markers (Tan et al., 1995), and pulse-labelling with ^{3}H-thymidine (Bayer and Altman, 1991b), have also shown that cells migrate radially in the cerebral cortex, and non-radially into ventro-lateral regions in the telencephalon. In the chick BVR, cells disperse widely within the IZ (see Chapter 2). In the mouse basal ganglia, experiments using replication-incompetent retroviruses show significantly more dispersion than in the cerebral cortex. In many cases, dispersion is sufficiently extensive in the basal ganglia, that after only a few days of development, the full extent of labelled clones may not be reliably identified (Halliday and Cepko, 1992). Both the gene expression and cell migration data presented in Chapter 2 therefore support the argument that avian DVR and mammalian cerebral cortex, and avian BVR and mammalian basal ganglia, are homologous, and furthermore suggest that the general pattern of radial cell migration in the pallium and more dispersive cell migration in the basal ganglia may be an organizational property which is common to all amniotes, and perhaps to all vertebrates. The cell migration data presented here can only suggest a general homology between broad regions in the dorsal and ventral telencephalon, as more defined regions within the telencephalon, such as ectostriatum or paleostriatum primitivum (in the chick), are not recognizable at these early stages without the availability of established markers.

The DVR and cerebral cortex have a number of similarities, including: 1) early cell migration and gene expression patterns; 2) similar thalamic inputs from the LGN; and 3) similar topologies, as both are located just dorsal to the pallium/subpallium boundary (highlighted in the adult chick and mouse by AChase and catecholamine expression, and in the embryo by the expression patterns of Cash-1 and Mash-1). Despite the similarities between DVR and cerebral cortex, there are a number of distinct differences. The cerebral cortex is a layered structure, while the DVR is composed of discrete nuclei which are not overtly layered. In addition, the cerebral cortex shows an "inside-out" pattern of

histogenesis (Bayer et al., 1991; Smart and Smart, 1982; Smart, 1983), whereas Tsai et al. (1981, 1981b) have shown, and we have confirmed (data not shown), that histogenesis in the DVR is "outside-in."

I would argue that if thorough studies of early development demonstrate that two structures arise from the same anlage, or primordium, in a similar fashion, that these two structures should then be thought of as being homologous, even if they appear distinctly different in the adult animal. In this view, studies of early development are paramount in establishing morphological homologies (Goodwin, 1994; Roth, 1984). Though the formalisms of comparative evolution tend to view structures as either homologous or homoplastic, another view is that structures which clearly arise from similar or identical anlagen are similar to one another to a greater or lesser extent. For instance, Northcutt and Kaas (1995) frame the distinction between whether DVR and cerebral cortex are homologous or homoplastic as hinging on whether the ancestral amniote pallium underwent either only one "transformation" in the line leading to mammals (warranting the label of "homologous"), or whether there were two "transformations" from the ancestral pallium, one in the line leading to mammals, and one in the line leading to birds and reptiles (warranting the label "homoplastic"). But what exactly is a "transformation"? Structures arising from homologous anlagen diverge from one another by degree, not by the tripping of a binary switch. The progressive transformation during development of the neocortex from a transient reptilian-like structure into a distinctly mammalian organization has been noted previously, and Haeckel's law states that the more general characters within a group of related animals appear earlier in development than the more specialized characters (Ulinski, 1983). Although the available data may currently support the view that avian DVR and mammalian cerebral cortex arise from homologous anlagen, and then diverge morphologically in later development, a deeper molecular-genetic and

cell-biological understanding of early development in the forebrain will be necessary to fully resolve this issue.

In the reeler mutation, the cerebral cortex is essentially converted from a structure with inside-out histogenesis to one with outside-in histogenesis. The gene at the reeler locus, called reelin, which is related to molecules with adhesive functions, is expressed specifically in the preplate (D'Arcangelo et al., 1995; Ogawa et al., 1995). In mammals, the preplate is split in two by cells which will form the cortical plate, resulting in a superficial layer 1, and a deep subplate. In the chick DVR, there is a strip of cells similar to the mammalian preplate which differentiate earliest along the pial surface (shown by SCG-10 expression in, e.g., Chapter 2, Fig. 3D), that may be homologous to cells in the mammalian preplate. In the chick, this early-forming strip of differentiated cells does not appear to be split in two, but cells instead accumulate medially in an outside-in fashion (Tsai et al., 1981, 1981b; J.M.M. et al., unpublished). In mammals, layer 1 is generated first, and, in a clear example of outside-in histogenesis, becomes the most lateral layer in the neocortex. Mammalian cortical histogenesis, therefore, is initially outside-in (in producing layer 1), but then proceeds in an inside-out fashion thereafter (in producing the rest of the cortical plate) (Alvarez-Bolado et al., 1995). Furthermore, a mutation in a single gene (reelin), apparently affecting only cells in the preplate, is capable of transforming subsequent histogenesis from an inside-out pattern to an outside-in pattern. Interestingly, in the reeler mutation, thalamo-cortical connections still form normally (Caviness, 1976), suggesting that differences in cortical histogenesis can, at least to some extent, be compensated for by thalamic innervation patterns. One plausible (and at least partially testable) evolutionary scenario is that the reelin gene, or a gene with a comparable function, became operative or functional in the line leading to mammals, after the split with reptiles and birds (see Fig. 2). Consequent changes in the adhesive properties of the preplate resulted in inside-out cortical migration patterns, and perhaps

this specific and novel mode of cell migration eventually became associated with the laminar cortical structure found in living mammals.

The homology between avian BVR and mammalian basal ganglia is widely accepted, and so the similarities in gene expression and cell migration patterns shown here, though they provide perhaps the first solid evidence in the early embryo for this homology, only reinforce an already strong case. Yet there are some notable differences between avian BVR and mammalian basal ganglia. The most dramatic is that sauropsids and mammals appear to have undergone a divergence in the major outflow target of the "pallidum." In mammals, the pallidum influences the function of motor cortex via a thalamic relay, while in sauropsids, the pallidum apparently exerts its major effects on motor function by influencing the tectum via a pretectal relay (Reiner et al., 1984b). Thus, though these two structures have clearly diverged significantly during evolution, it is still widely recognized that they are homologous. The similarities are again quite noticeable in early development, and the differences are far more apparent in later development. It seems quite possible that, as developmental data accumulates, information about early gene expression patterns and various aspects of cell movement and differentiation will become the touchstones for the establishment of morphological homology.

The compartmental plan of the amniote forebrain

Various models of forebrain segmentation have been proposed in the last century. Classically, Herrick (1910) divided the forebrain into various regions with external bulges, or "neuromeres," on the basis of various internal or external sulci that were suggested to be associated with divisions between proliferative zones. Neuromeres were thought to be separated to some extent by cell-poor zones at their constrictions, and to demonstrate higher rates of mitosis in the central regions of the neuroepithelial bulge.

Based on his comparative work, Herrick divided the diencephalon into four longitudinal zones: the epithalamus, dorsal thalamus, ventral thalamus, and hypothalamus. The telencephalon has been similarly divided into the dorsal laminated cortex, and the ventral nonlaminated basal nuclei (Herrick, 1910; Kuhlenbeck, 1937). Kuhlenbeck (1937) also proposed a model of telencephalic segmentation and similarly based his proposals for different "cell columns" on the positions of ventricular sulci. This notion was disputed by Källén (1953) who claimed that ventricular grooves are not located at the boundaries between areas of high proliferative and migration activity, but instead lie in the center of them. For that reason, argued Källén, the use of these grooves in segmentation schemes is of little value. Källén further proposed that the DVR (or Cortex) and BVR (or basal ganglia) represent distinct "cell migration areas" as well as cell proliferation areas (1953b, 1962). Karten (1991) elaborated on Kallen's ideas and proposed that the avian telencephalon is divided into at least three neuromeres or "prosomeres": BVR, DVR, and DLVR (or dorso-lateral ventricular ridge).

Recently, a more novel segmentation or "neuromeric" scheme has been proposed by Puelles and Rubenstein (1993) (Fig. 4). Rather than considering telencephalic and diencephalic derivatives as separate, as essentially all workers had previously, these authors proposed that the forebrain is composed of six neuromeres, p1-6, with p1-3 containing only diencephalic derivatives, and p4-6 containing both telencephalic and diencephalic derivates. Similarly, the cerebral cortex and the basal ganglia, as well as diencephalic derivatives, are combined into p5, although the cortex and basal ganglia have been considered by nearly all previous workers to be separate neuromeres (Ulinski, 1983). This "neuromeric" model was based primarily on gene expression patterns, but also on morphological considerations, and the model was apparently motivated by a desire to symmetrically extend the rostro-caudal orientation of the rhombomeres in the hindbrain into the telencephalon and diencephalon.

The data presented in this thesis represents perhaps the most rigorous test to date of neuromeric models of the forebrain. Here, cell migration patterns and gene expression patterns were, for the first time, directly correlated in single specimens. In general, the data supports, at least in part, the models of Figdor and Stern (1993), Alvarez-Bolado et al. (1995), Källén (1962), and Karten (1991), and argues strongly against the model proposed by Puelles and Rubenstein (1993). Figdor and Stern (1993) performed a series of cell labelling experiments and concluded that the diencephalon is divided into four neuromeres, which are in part manifested as cell-mixing compartments. While Figdor and Stern found that cells in the diencephalon do not mix with cells in the telencephalon, we have shown that cells originating in the telencephalon do not generally cross into the diencephalon, and that cells generated in the BVR (basal ganglia) do not generally cross into the DVR (cerebral cortex) (see Chapter 2, Fig. 8G and H).

The p5 segment of Rubenstein and Puelles contains most of the dorsal telencephalon (cerebral cortex), ventral or basal telencephalon (basal ganglia), as well as derivatives of the diencephalon (see Fig. 4). The work presented in this thesis, taken together with the work of Figdor and Stern (1993), suggests that these three regions in fact appear to occupy three distinct cell migration domains, or "neuromeres." Although the Puelles and Rubenstein model was based primarily on gene expression patterns, analysis of gene expression patterns have not supported the conclusions of these authors (Alvarez-Bolado et al., 1995; see Chapter 2). The regional fate map of the telencephalon shown in Chapter 3 also supports, contrary to the Puelles and Rubenstein model, a general dorso-ventral organization of at least the more caudal aspect of the telencephalon. It is generally acknowledged that of the numerous criteria needed to support the existence of a neuromere (see Introduction), the two most cardinal features are that neuromeres should correspond to cell mixing domains and should have unique patterns of regulatory gene expression (Guthrie, 1995; Lumsden, 1990; Fraser, 1993). For the BVR, the

DVR, and the diencephalon, these conditions now appear to have been satisfied (see Chapter 2).

My own model of telencephalic compartmentalization is presented in Fig. 5. It seems clear from the data presented that cells generated in DVR migrate into ventral regions lateral to the BVR (e.g., Fig. 7B). Telencephalic morphology in the adult chick suggests that this region may give rise to olfactory cortex (see Fig. 3). Although the eventual fate of these ventro-lateral cells is not known, it seems clear from the data that cell mixing does occur between the DVR and the ventro-lateral telencephalon. I have therefore combined the ventro-lateral telencephalon with the DVR into one cell migration domain. Regulatory gene expression patterns which may correspond to this migration path have not yet been reported in the chick, but candidate genes in the mouse have been identified which may be expressed in a comparable fashion (T. Saito, personal communication). The more medial aspect of the telencephalon, the BVR, is represented as a single cell migration domain. This also appears to be well-supported by the data, as cell mixing does not occur between BVR and DVR, or BVR and diencephalon, and the regulatory gene Cash-1 is expressed in the entire region of the presumptive BVR before it becomes morphologically distinct from the neighboring DVR territory. The diencephalon is considered to be a distinct cell migration domain from the telencephalon based both on the results presented in Chapters 2 and 3, and on the work of Figdor and Stern, who have divided the diencephalon further into four compartments. Though a Shh expression boundary within the BVR does not correlate with a cell migration boundary at the developmental times analyzed in this study, the possibility cannot be excluded that the BVR is further subdivided into two distinct domains later in development at a site which corresponds to the Shh boundary.

There are at least three regions of the telencephalon which, because the available data do not address their compartmental nature, are not included in the model which I

have proposed: the Septum, the dorso-medial telencephalon (the avian Wulst), and the hippocampal region. Cell labelling was performed in all three regions, but in all cases there was insufficient cell movement, except in the most lateral portion of the neuroepithelium, to assess whether cells from adjacent regions were capable of mixing. In the presumptive Wulst, cells migrate for substantial distances laterally, often reaching the ventro-lateral telencephalon (Chapter 3, Fig. 6E). Cells may therefore mix in the lateral telencephalon throughout the dorso-ventral extent of the telencephalon. Similarly, in the Septum, at the early developmental stages analyzed in this study, tangential cell movements are not significant enough to assess whether cells generated in the Septum mix with those generated in the BVR, and vice versa. The BVR is Shh^+, and the septum is Shh^- until at least E8 (not shown), and thus Shh is a marker which could be used to address this issue in more long-term cell labelling studies.

Previous workers have suggested that the basal ganglia is of dual origin: that the "striatum" originates from the telencephalon, and the "pallidum" originates from the diencephalon (Stoykova and Gruss, 1994). Shh is expressed in the MGE in mouse, suggesting that it may be a marker for the more ventral pallidum. Similarly, Shh is expressed in the ventral BVR, in a location which suggests that it may be a marker for the presumptive paleostriatum primitivum (PP) (see Fig. 3). It has been suggested, based primarily on immunohistochemical staining, that the mammalian pallidum may be homologous to the avian PP (Karten, 1969; Reiner et al., 1984b). The study of Figdor and Stern (1993) shows that cells do not migrate from the diencephalon into the telencephalon, at stages in which the anlage of the PP is becoming apparent. In the study presented here, numerous DiI injections into the presumptive BVR were performed, and these injections as a whole appear to mark essentially all locations within the BVR. These two studies taken together suggest that the basal ganglia (or BVR) is in fact entirely of telencephalic origin.

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FIGURE LEGENDS

Figure 1. Schematic representation of adult mammalian and avian telencephalon. Both diagrams represent frontal sections of a single telencephalic hemisphere, with medial (M) to the left, and lateral (L) to the right. Similar hatching patterns in mammalian and avian telencephalon indicate established morphological homologies, including: the mammalian striatum and the avian paleostriatum (Paleostr.); the mammalian and avian septum (Sep.); the hippocampus (Hip.); and the piriform cortex (Pirif.). In avians, the sagittal Wulst, ventral hyperstriatum (Vent. Hyperstr.), and neostriatum (Neostr) occupy topological positions comparable to that of the mammalian neocortex (Neo). The avian DVR includes the ventral hyperstriatum, the neostriatum, and (usually) the archistriatum (Archi). The sagittal Wulst includes the hyperstriatum intercalatum, hyperstriatum accessorium, and dorsal hyperstriatum. Am, amygdala; D, dorsal; V, ventral. (Diagram based on lecture notes from G. Striedter.)

Figure 2. Simplified diagram of the evolution of amniotes (birds, reptiles, and mammals) and anamniotes (fish and amphibia). Branch points indicate approximate time (in millions of years ago, mya) at which lines leading to living amniotes and anamniotes diverged.

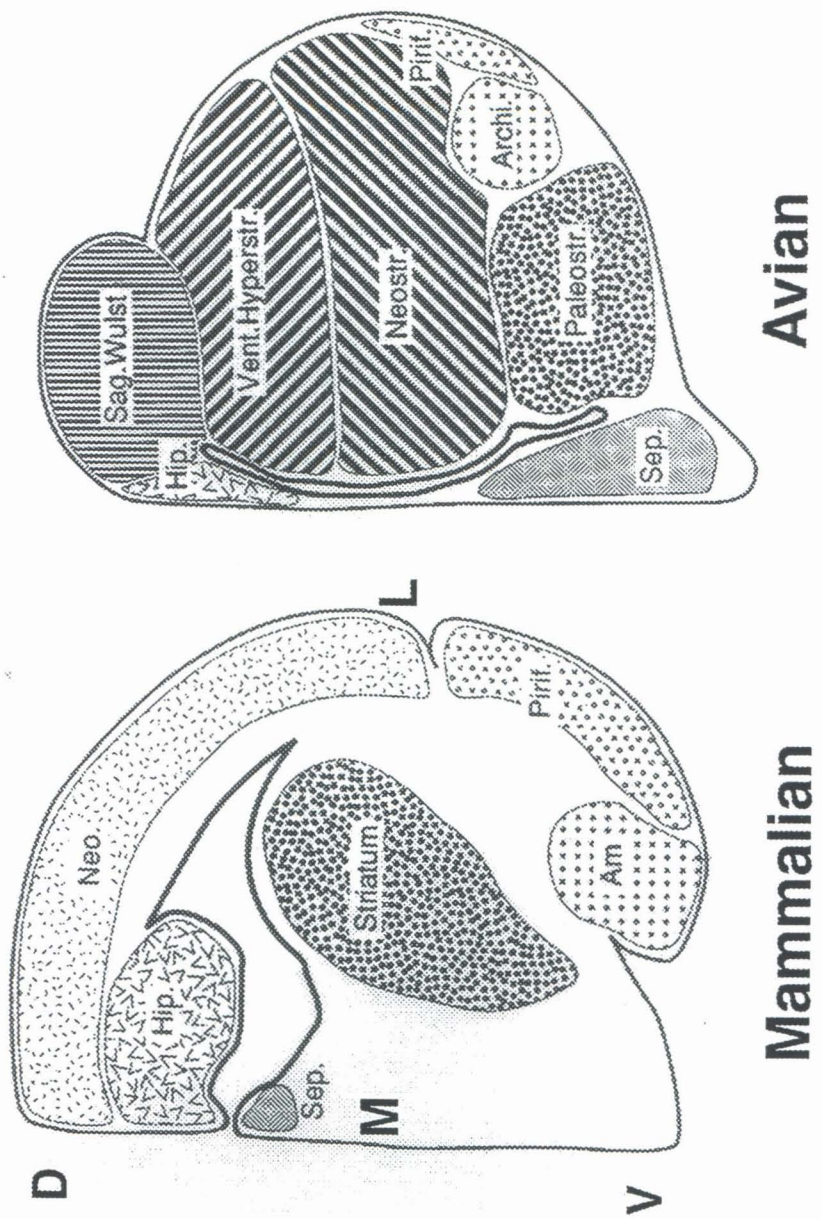
Figure 3. Two representations of the adult chick telencephalon. The left diagram is more schematic, while the right diagram is more anatomically accurate. Both represent frontal sections of a single telencephalic hemisphere, with dorsal up, ventral down, medial to the left, and lateral to the right. The BVR (left) includes the paleostriatum augmentatum and paleostriatum primitivum (right, PA and PP). The DVR (left) includes the neostriatum (N, right), the ventral hyperstriatum (HV, right) and the archistriatum.

TPO (right), olfactory cortex (Olf. Ctx, left), and DLC (dorso-lateral cortex, left), all refer to olfactory regions. HP, hippocampus; HA, hyperstriatum accessorium; "L," field L; SL, septal nucleus. (Diagrams from Tsai et al., 1981, and Karten, 1991.)

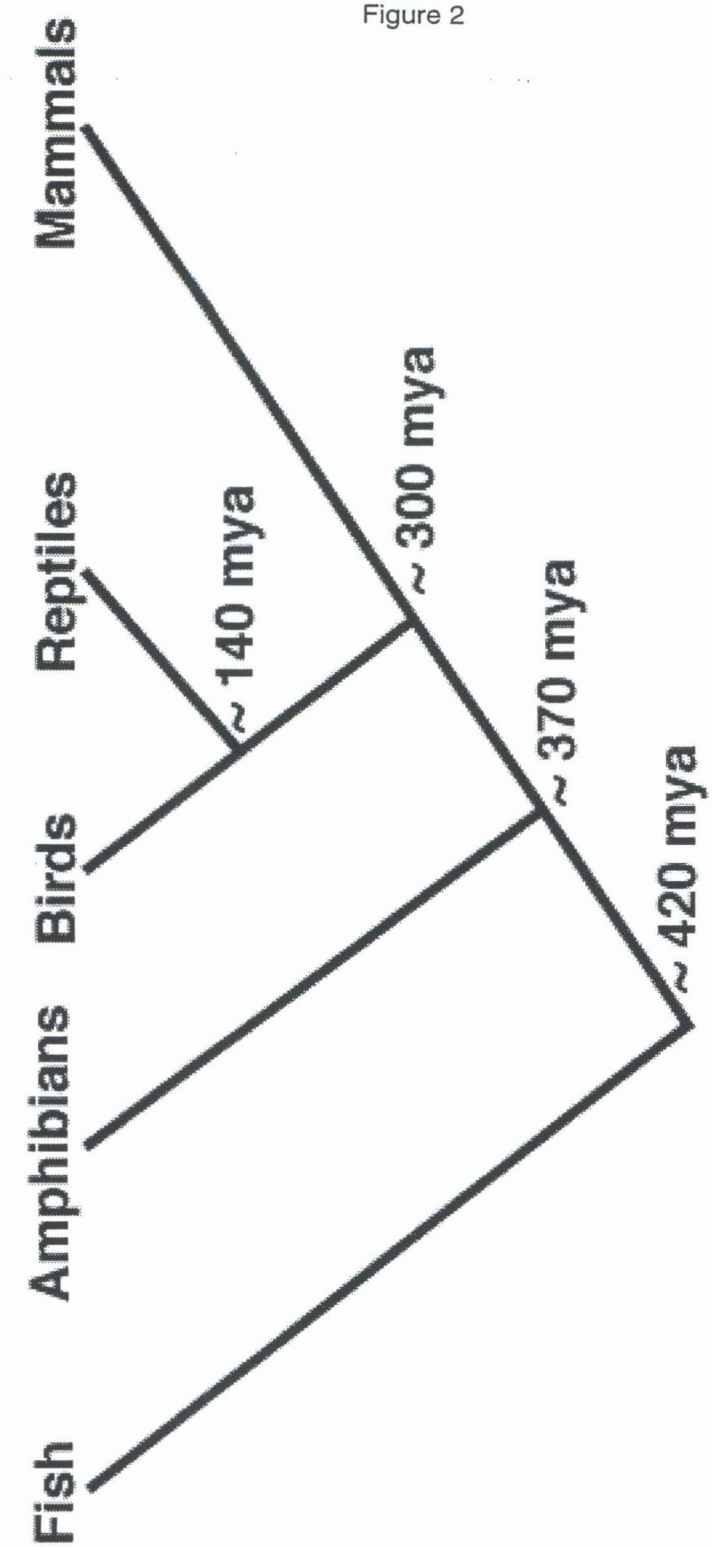
Figure 4. Puelles and Rubenstein's "neuromeric" model of forebrain segmentation. The forebrain is divided into six rostro-caudally aligned "prosomeres," p1-6. Diagram shows anatomical approximation (top) and more schematic diagram of prosomeres (bottom). p1 contains the pretectum (PT); p2 contains the epithalamus (ET), and the dorsal thalamus (DT); p3 includes the ventral thalamus (VT); p4 includes the archicortex (ACX), a telencephalic derivative, as well as the eminentia thalami (EMT), a diencephalic derivative; p5 includes the neocortex (NCX), the lateral and medial ganglionic eminence (LGE and MGE), the anterior hypothalamus (AH), and the hypothalamic cell cord (HCC); p6 includes the olfactory bulb (OB) and the septum (SE), both telencephalic derivatives, and the suprachiasmatic area (SCH) and retrochiasmatic area (RCH), both diencephalic derivatives. (See Bulfone et al., 1993, for other abbreviations).

Figure 5. Schematic representation of proposed model for cell migration domains in the chick forebrain. Three separate domains are noted: the diencephalon, the BVR, and the DVR/lateral telencephalon (lateral tel). At E5-E7, the septum is located rostral to the plane of section indicated.

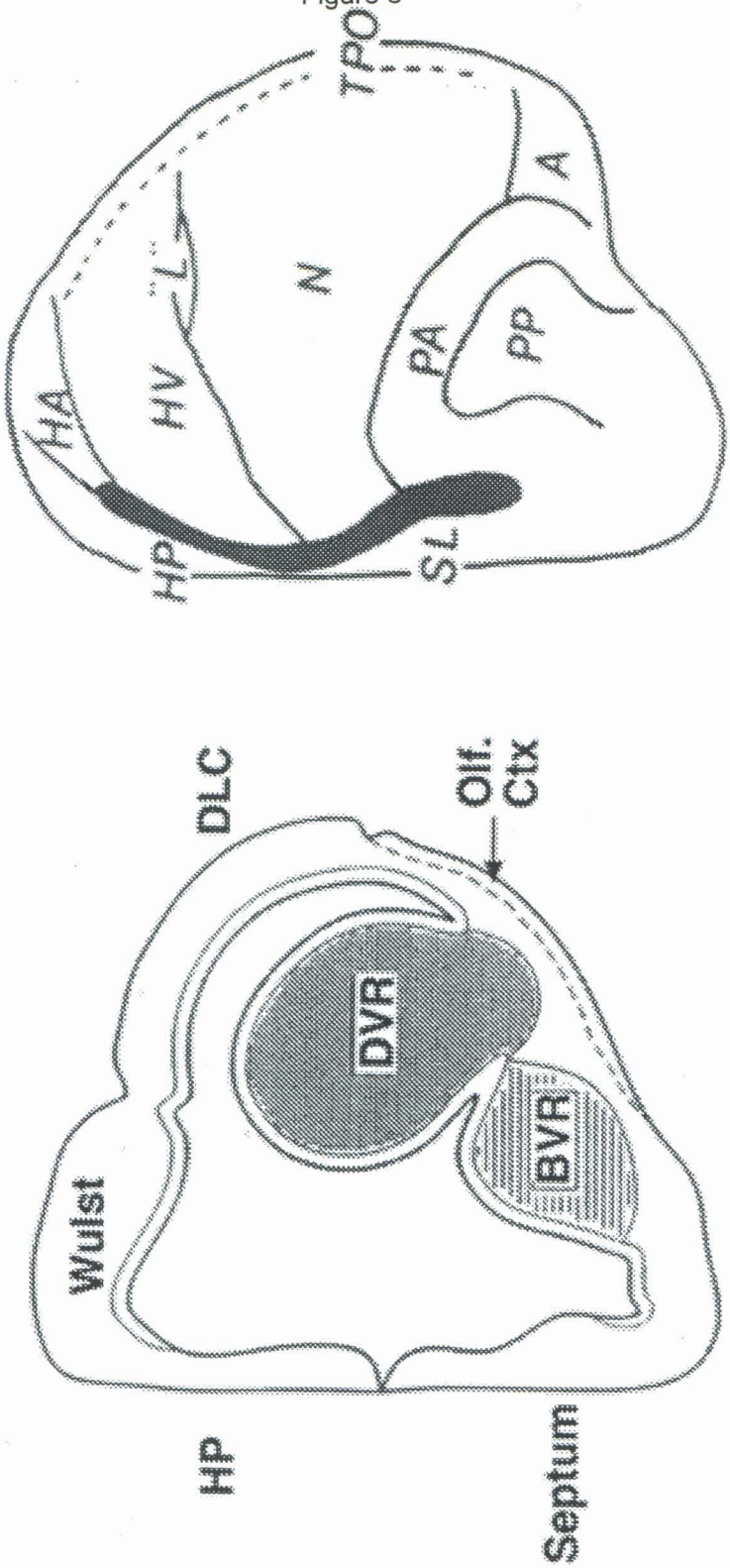
Chapter 4
Figure 1



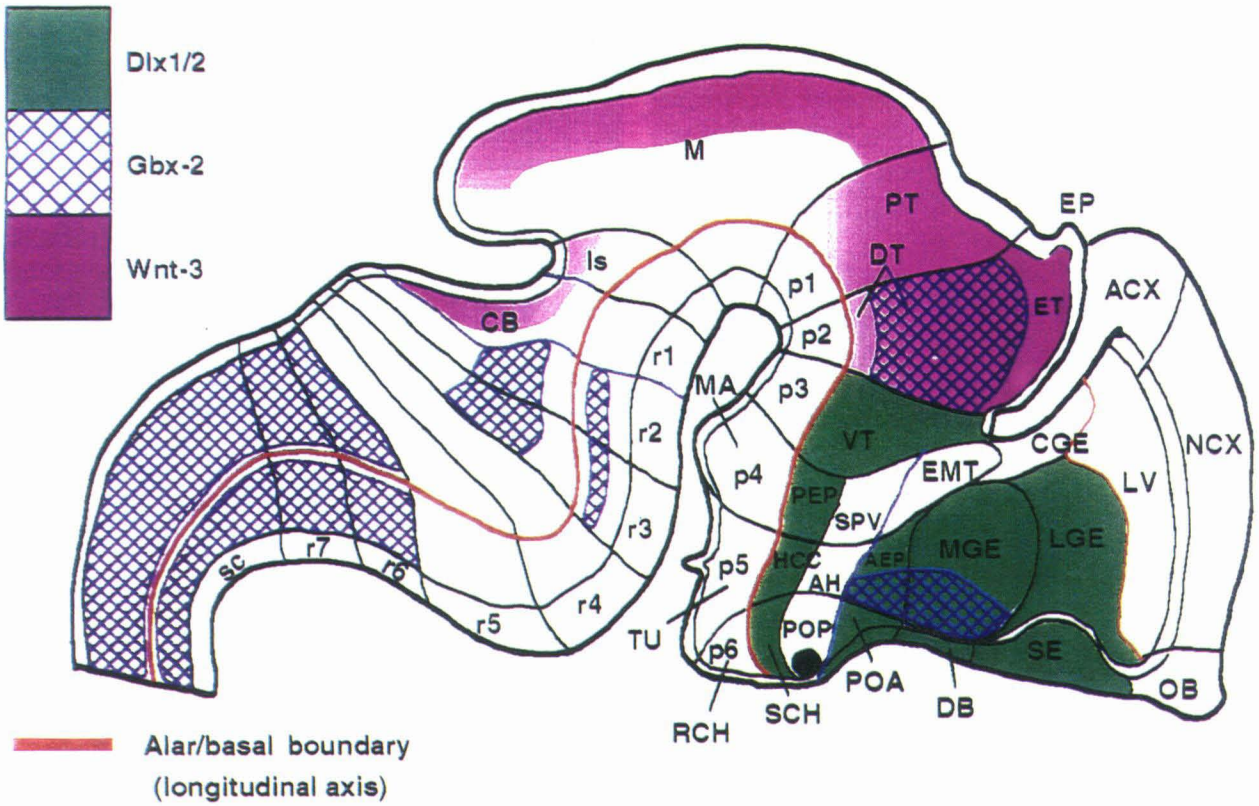
Chapter 4
Figure 2

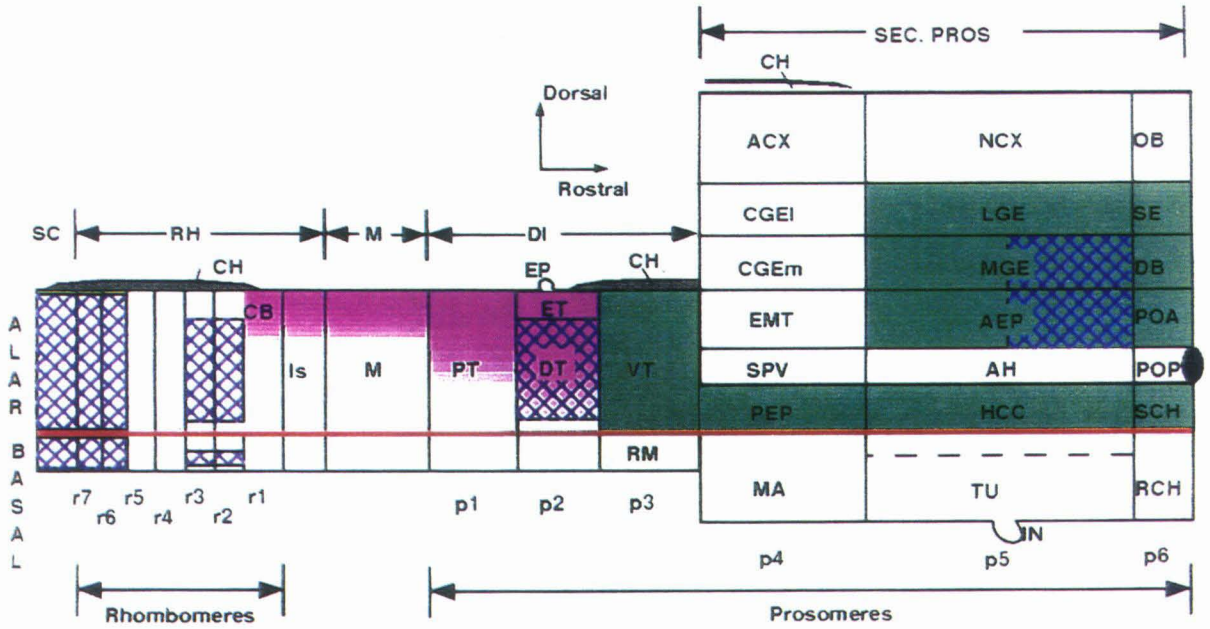


Chapter 4
Figure 3

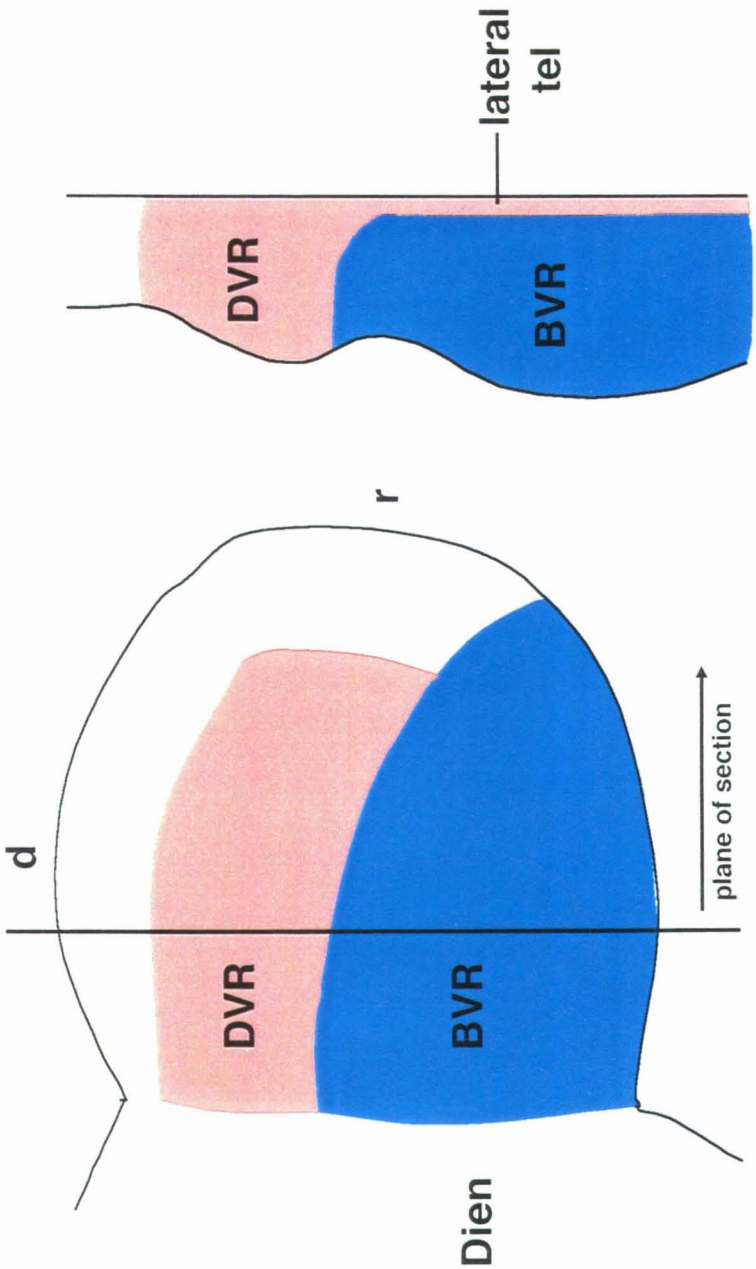


Chapter 4
Figure 4





Chapter 4
Figure 5



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