DIRECTIONS IN NEUROGENETIC GRADIENTS
AND PATTERNS OF ANATOMICAL CONNECTIONS
IN THE TELENCEPHALON

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1. Introduction: The Neurogenetic Hypothesis

In 1974, Bayer and Altman (1974) first applied the progressively delayed cumulative labeling procedure of $^3$Hthyidine autoradiography to developmental studies of the mammalian brain, previously used by Fujita (1964) in the chick. This method allows an accurate delineation of both the onset and cessation of neurogenesis as well as the determination of the proportion of neurons produced during single days of embryonic life. The observations are made in adult brains exposed at least twice to $^3$Hthyidine at specific times during embryonic and postnatal life (methods are extensively explained in Section 2). Since 1974, cumulative thymidine labeling has been used to determine timetables of neurogenesis throughout the telencephalon (Bayer and Altman, 1974; Bayer, 1979a, 1980a, b, 1981, 1983, 1984, 1985a, b, 1986a, b). This paper reviews telencephalic neurogenesis, including some preliminary observations on the neocortex, with selected examples of the correlations between chronologies of neurogenesis and patterns of anatomical connections. Taken as a whole, these data indicate that the chronological sequence of neurogenesis throughout the nervous system is a precisely regulated event that can be related to the patterns of anatomical interconnections in the adult brain. The information obtained from these studies formed the basis for the temporal hypothesis that the sequential production of neurons is a factor in facilitating the establishment of orderly connections within and between nerve structures.

As this work in the telencephalon of adult brains is nearing completion with long survival cumulative thymidine autoradiography, we have embarked on an extensive analysis of brain development, using short and sequential survival times after single injections of $^3$Hthyidine. Observations have been completed in the spinal cord (Altman and Bayer, 1984) and are nearing completion in the diencephalon (Altman and Bayer, 1978b, 1979c, 1986 and in preparation). These data indicate that different populations of neurons, distinguishable in the adult by their different times of origin within specific structures of the nervous system, arise from discrete germinal matrices during embryonic development. The original temporal hypothesis has therefore been expanded to the neurogenetic hypothesis which postulates that during embryonic development discrete cell lines form distinct neuroepithelial zones and the progeny of these cell lines are specified as to: (a) when they will originate from their respective precursor cells; (b) what migratory routes they will follow; (c) what final location the cell body will take; and (d) where their axons will terminate. The implications of the hypothesis are that the anatomical heterogeneity of the adult nervous system is to some extent preformed in its heterogeneous germinal matrix, the neuroepithelium, and that its circuity is at least partly specified by a genetic blueprint.

The empirical derivation of the specifics of the neurogenetic hypothesis will be presented in the context of a detailed description of histological observations and experimental findings. The discussion of Section 3 provides evidence for specifications (a) and (c). Section 4 provides evidence relative to specifications (a), (b) and (c). Preliminary findings in double labeling studies combining $^3$Hthyidine radiography and horseradish peroxidase (HRP) retrograde axonal transport (Section 4.2.2) will be discussed in relationship to specification (d).
2. Methodology: The Usefulness of Various \[^{3}H\]Thymidine Autoradiographic Methods to Investigate Different Aspects of Neural Development

2.1. LONG SURVIVAL AUTORADIOGRAPHY

2.1.1. Critique of the pulse labeling method

The possibility of experimentally dating neuronal "birthdays" began with the application of \[^{3}H\]thymidine autoradiography to neuroembryological research (Miale and Sidman, 1961; Angevine and Sidman, 1961). For about 15 years, the technique most commonly used with mammals was the "pulse labeling" procedure, and some laboratories continue to use this method today. A single injection of \[^{3}H\]thymidine is administered (usually intraperitoneally) to a pregnant dam at a known gestational age. The offspring of these dams are allowed to survive into the juvenile and adult periods when the central nervous system is partly or fully mature. The question asked is: Which group of cells and what proportion of these cells become heavily labeled? The heavily labeled cells are assumed to originate on the same day that the injection is given, although the validity of this assumption has never been experimentally tested. The pulse labeling technique concentrates on intensity of label as the source of data since the birthdays of unlabeled cells are ambivalent: some neurons may be unlabeled because they are already differentiating when the injection is given, while others are unlabeled because repeated cell divisions of their precursors dilute the incorporated label beyond the detection level. But such an analysis of only the heavily labeled cells does not allow for a quantitative determination of the exact temporal order of neuron production in a specific neuronal population. First, there are problems in both defining and determining what constitutes a heavily labeled cell. This is due to the circumstance that label concentration over nuclei containing radioactive DNA depends on a host of factors other than imminent cessation of cell division. (1) Mitotic cells at the end of S-phase will incorporate less label than those in S-phase during the entire time of \[^{3}H\]thymidine exposure. Therefore, in spite of its light labeling, a cell might originate soon after the injection. (2) Cells with large nuclei often never become heavily labeled while small cells may be judged to be heavily labeled even after repeated divisions. (3) The extent of label concentration is also affected by the dose and specific activity of the radiochemical, by the sensitivity of the photographic emulsion, the duration of photographic exposure, and several other factors. The second problem with pulse labeling is that an undetermined proportion of the cells in a specific neuronal population tend to be heavily labeled (by any definition) after a single \[^{3}H\]thymidine injection, and therefore most of the cells cannot be included in data collection. Although some of these ambiguities can be resolved by serial injections of different groups of animals at different embryonic ages, the pulse labeling method has never generated reliable quantitative data in long survival studies. A modified long survival \[^{3}H\]thymidine autoradiographic procedure is now available that allows the exact quantification of the proportion of neurons generated on specific days throughout the rat nervous system.

2.1.2. Cumulative labeling method

2.1.2.1. Experimental design

The cumulative method uses multiple injections of \[^{3}H\]thymidine instead of single injections and makes a distinction only between labeled and unlabeled cells. This circumvents the label intensity and label dilution problems in the pulse labeling technique and removes the ambivalence in determining the time of origin of the unlabeled cells. With this technique, therefore, the entire cell population is used as a source of data. The procedure consists of three main steps. (1) Groups of pregnant rats or postnatal animals are injected subcutaneously with two to four successive daily doses of \[^{3}H\]thymidine (Schwarz Mann, specific activity 6 Ci/mm, 5 μCi/gram body weight). Intraperitoneal or intravenous injections result in rapid removal of labeled precursor; one hour after the injection, only 5–10% of the labeled thymidine is still present in the blood (Cleaver, 1967).
Feinendegen (1967) states that $[^3]H$thymidine is available longer after subcutaneous injections, but no specific length of time is given. The subcutaneous method of injection does not give a pulse label of $[^3]H$thymidine, and is better suited for cumulative labeling.

2. The onset of the injections is progressively delayed in each group. For animals in the prenatal groups, injections on specific embryonic (E) days are set up in an overlapping series with 24 hour delays between groups (E12 + E13, E13 + E14, . . . E21 + E22). For animals in the postnatal (P) groups, the injection groups are separated by 48 hour delays, each group receiving either four (P0–P3, P2–P5, . . . P6–P9) or two (P8–P9, P10–P11, . . . P20–P21) consecutive daily injections. These injection schedules give a complete picture of neurogenesis throughout most structures in the rat central nervous system (See Section 2.1.2.3).

3. The animals are killed as adults (P60) so that the patterns of labeled and unlabeled cells can be examined in a fully mature brain. As long as the injections in a single animal are separated by 24 hr, there is no radiation-induced cell death; brains of the injected animals appear normal in both size and cell number.

2.1.2.2. Histological and autoradiographic procedures

All animals are deeply anesthetized with pentobarbital and are killed by perfusion with 10% neutral formalin through the heart. The brains are stored in Bouin's fixative for 24 hr and are then transferred to 10% neutral formalin until further processing. To compile the data for this review, the brains of at least six males from each injection group were blocked in the coronal plane, embedded in paraffin, and were sectioned serially at 6 μm (every 15th section is saved). Four to six brains from each injection group were blocked with a midline sagittal cut before paraffin embedding. One half was serially sectioned horizontally, the other half coronally (both planes: every 15th 6 μm section is saved). The sections were mounted on slides with albumin adhesive. Before autoradiography, the sections were defatted in xylene and were rehydrated in graded alcohols to water. Under the proper safelight conditions, the surface of each slide which contains the sections was coated with a thin layer of liquid photographic emulsion (Kodak NTB-3) warmed to 40 degrees centigrade. The slides were dried upright for approximately one hour and were loaded into metal staining trays. The trays were stored in the refrigerator for a 12 week exposure period in light-tight metal boxes with a dessicant. The autoradiograms were developed in Kodak D19 and were poststained with hematoxylin and eosin.

2.1.2.3. Quantitative evaluation

The cumulative labeling procedure does not require a distinction between heavily and lightly labeled cells. All neurons within a designated area are counted and are assigned to one of two groups, labeled or nonlabeled. Cells with reduced silver grains overlying the nucleus in densities above background levels are considered labeled; obvious endothelial and glial cells are excluded. The proportion (%) of labeled cells are then calculated from these data as follows:

\[ \frac{\# \text{ labeled cells}}{\# \text{ total cells}} \times 100 = \% \text{ labeled cells} \]  

(1)

Our previous work has established that a chronological schedule of two successive $[^3]H$thymidine injections can be found that will label between 95–100% of the neurons in most rat brain structures. Table 1 gives a typical example of the data for neurogenesis of a specific neuronal population (output neurons in the accessory olfactory bulb). At the time of the maximal labeling level (E12–E13), all the precursor cells of these neurons are still proliferating (99.14% of the neurons are labeled), and none of the neurons have originated. By progressively delaying the onset of the injections in 24 hr intervals, the percentage of labeled neurons declines. This reflects the production of postmitotic neurons by their precursor cells. The proportion of neurons originating each day is equal to the daily decline in the percentage of labeled neurons. For example: (29.6% labeled cells on E14 and E15) − (8.83% labeled cells on E15 and E16) = 20.77% cells formed during E14 (or between the onset of the injections on E14 and E15). Therefore, with this procedure
Table I. Neurogenesis of the Output Neurons of the Accessory Olfactory Bulb

<table>
<thead>
<tr>
<th>Injection group</th>
<th>N</th>
<th>% Labeled cells*</th>
<th>Day of origin</th>
<th>% Cells originating†</th>
</tr>
</thead>
<tbody>
<tr>
<td>E12-E13</td>
<td>7</td>
<td>99.14 ± 0.38</td>
<td>E12</td>
<td>8.39 (A–B)</td>
</tr>
<tr>
<td>E13-E14</td>
<td>8</td>
<td>90.75 ± 5.65</td>
<td>E13</td>
<td>61.15 (B–C)</td>
</tr>
<tr>
<td>E14-E15</td>
<td>5</td>
<td>29.60 ± 4.77</td>
<td>E14</td>
<td>20.77 (C–D)</td>
</tr>
<tr>
<td>E15-E16</td>
<td>6</td>
<td>8.83 ± 2.32</td>
<td>E15</td>
<td>5.83 (D–E)</td>
</tr>
<tr>
<td>E16-E17</td>
<td>12</td>
<td>3.00 ± 1.65</td>
<td>E16</td>
<td>3.00 (E–F)</td>
</tr>
<tr>
<td>E17-E18</td>
<td>9</td>
<td>0</td>
<td>E17</td>
<td>0</td>
</tr>
</tbody>
</table>

*X ± S.D.
†Graphed in Fig. 5 (top).

The data for the output neurons of the accessory olfactory bulb are given as an example of how the data are derived for presentation in the bar graphs of the papers reviewed here. N refers to the number of animals analyzed in each injection group. The % labeled cells column (counts of the % of labeled cells to total cells as given in Eqn. 1) gives the group mean and standard deviation for the raw data for each injection group. The standard deviations are typical of the variability seen throughout the data collection. The % cells originating column lists the data that are presented in the bar graph (Fig. 5, top). To get the height of the second bar, for example, the proportion of labeled cells in injection group E13–E14 (entry B, column 3) is subtracted from the % labeled cells in injection group E12–E13 (entry A, column 3) to get the proportion of cells originating during the day on E13 (61.15%).

The onset of formation can be exactly determined and the proportion of cells formed each day can be calculated. These data allow for statistically evaluated comparisons between formation times of various neuronal populations and also for the detection of subtle neurogenetic gradients within structural subdivisions.

Throughout these quantitative analyses, it was noted that trends in cell labeling within animals were very consistent. For example, in the entorhinal cortex, the percentage of labeled cells in layer II tended to be lower than in layer III; however variability between animals in an injection group were large enough to mask this trend. Consequently, a statistical procedure is used, the sign test (Conover, 1971) to determine the consistency of sequential neuron production between paired locations ("X" and "Y") within individual animals. The comparisons are grouped into three categories: (1) X > Y, "-" comparison; (2) X < Y, "+" comparison; (3) X = Y, "0" comparison. The zero comparisons are discarded and, depending on the total number of remaining "+" and "-" comparisons, either a binomial distribution or a normal approximation is used to calculate probabilities (p).

2.2. Short and Sequential Survival Autoradiography

The pulse labeling method, fraught with technical problems in long survival studies, is ideally suited for short survival autoradiography where the period of analysis is usually restricted to the embryonic and early postnatal periods. Within two hours after a single injection of [³H]thymidine for example, the neuroepithelium and the secondary germinal matrices derived from it reveal themselves as heterogeneous patches containing regions of high or low label uptake. The pattern changes depending on the time of the injection. When the survival time is increased to daily intervals after the injection, heavily labeled migrating neurons can be followed from their germinal sources to their final locations.

The morphogenetic studies to be discussed in this paper were based on observations of an extensive "library" of histological material from over 1,200 embryos and pups. Several pregnant female rats for each embryonic day from E13 to E22 were injected with a single dose of [³H]thymidine (Schwarz-Mann; specific activity 6.0 Ci/mM; 5 μCi/g body weight). Survival times for each injection group varied from two hours (short survival) to daily...
Intervals for several days (sequential survival). For example, one dam in the E15 injection group was killed two hours after the injection, another was killed one day later on E16, another two days later on E17, and so on, until the last dam was killed on E22. All groups were treated as the E15 group. In order to get a more complete developmental series for the late-prenatal originating neurons, injection groups from E19 to E22 included some survival times extending to P5. At the time of sacrifice, the dams were deeply anesthetized with sodium pentobarbital, and their embryos were removed and killed by immersion in Bouin's fixative. After further storage in 10% neutral formalin, both the head and body of each embryo was embedded either in paraffin or in methacrylate. The blocks were serially sectioned (at 6 μm in paraffin embedded material; 3 μm for methacrylate) in either the sagittal or coronal planes. Postnatal animals were killed by transcardiac perfusion with 10% neutral formalin. The brains were stored in Bouin's fixative for 24 hours, then were transferred to 10% neutral formalin until the time of embedding. The histological and autoradiographic procedures follow those outlined above (Section 2.1.2.2) except that the exposure time for the autoradiograms was cut to six weeks.

3. Chronologies of Neurogenesis and Correlations with Anatomical Connections in Selected Telencephalic Systems

3.1. Hippocampal-Septal System

3.1.1. Neurogenetic gradients in the septum

The septal region forms the subcallosal anteromedial wall of the telencephalon. Cajal (1911), one of the first to examine this area, described three nuclei: the medial, the triangular and the “external or principal nucleus” (the lateral septal nucleus). The boundaries of the septal region were expanded by Johnston (1913, 1923) to include the nucleus accumbens, interstitial nucleus of the diagonal band of Broca and the bed nucleus of the stria terminalis. The neurogenetic gradients of Johnston's expanded septal region are summarized in Fig. J (Bayer, 1979a). Both the midline nuclear group and the bed nucleus of the stria terminalis can be described as cylinders of cells extending rostro-caudally through the basal forebrain. One lies along the midline and the other along the inferior horn of the lateral ventricle. Although the neurons in the bed nucleus form slightly later, the developmental pattern is similar to that found in the midline septal nuclei. The first originating neurons are located in the region of the crossing of the anterior commissure. The next generated, or younger, neurons are deposited both rostrally and caudally around the early forming center. The last, or youngest, neurons are located in the most rostral areas. The early forming centers in each group can be seen fusing in the embryonic rat brain before the anterior commissure crosses the midline; this may provide a bridge for the commissure (Johnston, 1923; Bayer, 1979b).

The lateral septal nucleus forms a dorsolateral cap over the medial and triangular septal nuclei and develops sequential to them. Neurogenesis in the medial and triangular nuclei is nearing completion by the morning of E16; at this time, only a few cells in the medial ridge of the lateral nucleus have been produced (E16, Fig. 1). During E16 and E17, most of the neurons in the lateral nucleus arise along a strong mediolateral gradient (E17–E18, Fig. 1). This was also observed by Creps (1974) in the mouse and by Lawson et al. (1977) in the rat. The gradient of formation between the midline and lateral septal neurons shows the classic “outside-in” pattern described by Angevine (1965). The neuroepithelial source of these nuclei lies along the medial wall of the lateral ventricle. During embryonic life, younger neurons are sequentially deposited more laterally as their perikarya shift away from the receding neuroepithelium (Bayer, 1979b).

Neurogenesis of the nucleus accumbens occurs much later than in the other septal nuclei. Although neurons in the nucleus accumbens and rostral part of the bed nucleus of the stria terminalis are similar in appearance, the break in the neurogenetic gradients indicate that the two nuclei are not developmentally related. The neurogenetic gradients in the nucleus accumbens relate it, rather, to the striatum (see Section 3.3.1.3 below).
3.1.2. Neurogenetic gradients in the hippocampal region

The "hippocampal region" includes all of the structures beyond the rhinal sulcus in the posterior telencephalic wall. These are the entorhinal cortex (area 28), the parasubiculum (area 49), the presubiculum (area 27), the subiculum, Ammon's horn, and the dentate gyrus. The term "hippocampus" applies to the combined Ammon's horn and dentate gyrus. There are three gradients of neurogenesis commonly seen within the hippocampal region: (1) deep to superficial, (2) sandwich, and (3) rhinal to dentate (Fig. 2; Bayer, 1980a).

3.1.2.1. Deep to superficial gradients

In all hippocampal structures there is a tendency for deep cells (those located nearer the white matter) to be generated before superficial cells (those located farther form the white matter). This gradient is found in the following examples: (1) Cells in layers V–VI of the entorhinal cortex are generated before those of layers II–IV (E16, Fig. 2). (2) The deep cells of the parasubiculum and presubiculum precede those in the superficial laminae (E17–E18, Fig. 2); within the outer layer of the presubiculum deep cells are generated earlier than superficial cells. (3) The subiculum has a strong deep to superficial intralaminar gradient in the pyramidal layer; this single layer is divided into deep and superficial parts (containing the letters a, b, c) in Fig. 2. (4) The cells in the stratum oriens are generated well before the pyramidal cells in Ammon's horn (E16–E18, Fig. 2). The pyramidal cells are also generated in a deep to superficial intralaminar pattern. (5) The hilar cells in the dentate gyrus form long before the granule cells (E16–E18, Fig. 2). Every developmental
GRADIENTS OF CYTOGENESIS IN THE HIPPOCAMPAL REGION

LEGEND

study in rodents has shown the deep to superficial or "inside-out" gradient in the retrohippocampal cortex, and in Ammon’s horn (Angevine, 1965; Hine and Das, 1974; Vaughn et al., 1977; Schlessinger et al., 1978).

3.1.2.2. Sandwich gradients

In many structures, later-forming cells are flanked both superficially and deeply by those originating earlier. There are several examples of this sandwich gradient throughout the hippocampal region. (1) In the entorhinal cortex, layers II and IV precede the origin of cells in layer III (E16–E18, Fig. 2). (2) The pyramidal cells in Ammon’s horn are flanked by older large and medium-sized cells in the strata oriens, radiatum and lacunosum moleculare (E16–E20, Fig. 2). (3) Similarly, the granule cells in the dentate gyrus are surrounded by early-forming large cells in the hilus and medium-sized cells in the molecular layer (E16–E21, Fig. 2). The sandwich gradient has not been described in the entorhinal cortex (Angevine, 1965; Schlessinger et al., 1978), possibly because such subtle differences are difficult to notice with the pulse labeling technique. Hine and Das (1974) noted the sandwich pattern in Ammon’s horn and the dentate gyrus.
3.1.2.3. Rhinal to dentate gradients

Within each structure of the hippocampal region, cells lying nearer to the rhinal fissure begin to arise earlier than those lying nearer to the dentate gyrus. For example in each layer of the entorhinal cortex, the cells located in the lateral part are generated first, followed by those in the intermediate part, and finally by those in the medial part (E16–E17, Fig. 2). Neurogenesis in the superficial lamina of the parasubiculum precedes that of the presubiculum (E18–E19, Fig. 2). Zone a of the subiculum begins to form first, followed by zone b, then by the prosubiculum (E17–E18, Fig. 2). Cytogenesis of the pyramidal cells in CA1a begins before that in CA1b,c (E18–E19, Fig. 2). Similarly, CA3a,b originates earlier than CA3c (E18–E20, Fig. 2).

The “rhinal to dentate” neurogenetic gradient is also prominent interstructurally in the hippocampal region. The entorhinal cortex is the oldest structural complex; here neurogenesis is completed by E18. By E19, the subiculum finishes before the CA3a,b pyramidal cells. Finally, in the dentate granular layer neurogenesis finishes postnatally. There are two notable exceptions in the rhinal to dentate gradient between structures. First, the superficial laminae of the parasubiculum and presubiculum are significantly later originating than the subiculum. Second, the pyramidal cells of CA1 are significantly later forming than those in CA3a,b. The rhinal to dentate gradient between structures and the late formation of the parasubiculum, presubiculum and CA1 pyramidal cells have been observed by Angevine (1965) in the mouse, and by Schlessinger et al. (1978) in the rat.

3.1.3. Correlations between neurogenetic gradients and afferents to restricted laminar zones on hippocampal dendrites

The rhinal to dentate neurogenetic gradient within and between hippocampal region structures is associated with discrete bands of afferent input on the apical dendrites of the pyramidal cells and on the dendrites of the dentate granule cells (Fig. 3). These zones are one of the most characteristic features of hippocampal circuitry.

3.1.3.1. Ipsilateral entorhinal cortex afferents

The entorhinal cortex sends a large ipsilateral projection, the perforant path, to the hippocampus which perforates the subiculum and runs in the stratum lacunosum-moleculare of Ammon’s horn and in the superficial dentate molecular layer (Cajal, 1911; Blackstad, 1958). Entorhinal fibers terminate in CA3 and in the dentate molecular layer (Naftstad, 1967) in a strict topographic pattern (Hjorth-Simonsen, 1972; Hjorth-Simonsen and Jeune, 1972; Steward, 1976): The lateral entorhinal cortical fibers terminate in a superficial (distal) band; below them is a band of intermediate fibers; below them, a band of medial fibers (Fig. 3). The fibers originate predominantly in layer II (Steward and Scoville, 1976). The rhinal to dentate neurogenetic gradient in the entorhinal cortex (Fig. 2) strictly correlates with its order of termination on the distal dendrites of CA3 and the dentate granule cells. It is concluded that afferents from neurogenetically older cells terminate distal to afferents from younger cells.

3.1.3.2. Commissural and associational afferents

Lesions of the contralateral hippocampus or cutting the ventral hippocampal commissure results in pronounced degeneration in the stratum oriens and stratum radiatum of Ammon’s horn and in the deep third of the dentate molecular layer (Blackstad, 1956; Raisman et al., 1965; Laatsch and Cowan, 1967). The entire field CA3 projects to the commissural zones on both CA1 and CA3 dendrites (Gottlieb and Cowan, 1973; Swanson et al., 1978); field CA3c and the large hilar cells project to the commissural zone in the dentate molecular layer (Gottlieb and Cowan, 1973; Hjorth-Simonsen and Laurberg, 1977; Fricke and Cowan, 1978; Swanson et al., 1978). These same fields also project ipsilaterally: CA3a,b to CA1 and CA3 via the Schaffer collateral system (Hjorth-Simonsen, 1973; Swanson et al., 1978); the large hilar cells to the dentate molecular layer (Zimmer, 1971;
NEUROGENESIS IN STRUCTURES SUPPLYING AFFERENTS TO THE
HIPPOCAMPUS IN RELATION TO DENDRITIC TERMINATION

Fig. 3. Order of neurogenesis in cells of origin of afferent fibers related to order of termination on dendrites in CA1, CA3 and the dentate gyrus. Cells supplying septal afferents ( # 1) begin to originate first and terminate diffusely in all dendritic areas; all other afferents represented terminate in specific laminar zones. Early originating cells from lateral entorhinal cortex ( # 2) project most distally to the cell body, later-originating cells from intermediate ( # 3) and medial ( # 4) entorhinal cortex project progressively more proximally to the cell body in CA3 stratum lacunosum-moleculare layer (SLM) and superficial part of the dentate molecular layer (ML). Cells projecting from the nucleus reuniens ( # 5) arise simultaneously (*) with medial entorhinal cortex and terminate throughout stratum lacunosum-moleculare of CA1. CA3 contains later-originating cells ( # 6) supplying the majority of commissural and associational afferents terminating in stratum radiatum (SR) and stratum oriens (SO) of CA2, CA3, and the deep part of the dentate molecular layer. Finally, the dentate granule layer has the latest-originating cells ( # 7) supplying associational afferents (mossy fibers) to the CA3 dendrite. Note that the apical dendrites of CA1 and CA3 pyramidal cells and the dentate granule cell dendrite have laminar afferents stacked according to age of the cells of origin (earliest forming cells to the most distal terminal zone; latest forming cells to the most proximal terminal zone) (from Bayer, 1980a).

Swanson et al., 1978). Thus, commissural/associational pathways have similar cells of origin and overlapping zones of termination on the dendrites of both the pyramidal and granule cells. However, the commissural associational zones are strictly isolated from the zones of entorhinal termination in CA3 and the dentate gyrus, and the zone of nucleus reuniens termination in CA1 (Fig. 3). It is important to note that the commissural-association fibers tend to have neurogenetically younger cells and terminate more proximal to the cell body, continuing the sequence begun in the entorhinal projection. For example, E17 is the last day of neurogenesis for cells projecting to restricted afferent zones in CA1 from the nucleus reuniens and to CA3 and the dentate gyrus from the entorhinal cortex, while E17 is the first day of neurogenesis for CA3 pyramidal cells (Fig. 3). Even though many hilar cells form before E17, some of the large cells are labeled up to E21, so a late neurogenesis cannot be ruled out. Finally, there is the prominent mossy fiber associational pathway running from the dentate granule cells to the CA3 pyramidal cells (Blackstad et al., 1970; Gaarskjaer, 1978; Swanson et al., 1978). The granule cells are latest to form in the hippocampal region and their fibers occupy a strictly isolated terminal zone in CA3, the stratum lucidum, most proximal to the cell body (Fig. 3). There is some evidence that the sequence of neurogenesis also corresponds to the sequence of fiber growth into the dentate molecular layer; entorhinal fibers arrive before commissural-associational fibers (Loy et al., 1977; Fricke and Cowan, 1978).
3.1.3.3. Thalamic afferents

Thalamic afferents run in the cingulum bundle to the hippocampal region (White, 1959). The anteromedial, anterodorsal and anteroventral nuclei project sparsely to the entorhinal cortex and heavily to the superficial laminae of the parasubiculum and presubiculum (Domesick, 1969, 1972, 1973). The nucleus reuniens projects lightly to the entorhinal cortex and massively to the stratum lacunosum-moleculare of CAI (Herkenham, 1978). It is interesting that all targets of heavy thalamic input form late (not finished until the morning of E20), and these structures (parasubiculum, presubiculum, and CAI) are the only exceptions to the “rhinal to dentate” interstructural gradient in the hippocampal region. Neurogenesis in the anterior nuclear complex and nucleus reuniens (Altman and Bayer, 1979a, b) is finished at about the same time as the medial entorhinal cortex. Since the thalamic nuclei are farther from the hippocampus than the entorhinal cortex, it follows that if thalamic and entorhinal fibers started to grow at about the same time the entorhinal fibers would arrive at their targets first. It is suggested that neurogenesis in thalamic targets is delayed to coincide with a possible later arrival of thalamic fibers.

3.1.3.4. Contralateral entorhinal afferents

There is a topographic entorhinal projection, predominantly from contralateral layer III, to the stratum lacunosum-moleculare of field CAI (Steward, 1976; Steward and Scoville, 1976). It is interesting that even in this sparse projection, there is a correlation between the rhinal to dentate gradient and the pattern of fiber termination. Afferent fibers from older cells of origin (lateral entorhinal cortex) project to older CAI pyramidal cells (CAIa); afferent fibers from younger cells of origin (medial entorhinal cortex) project to younger CAI pyramidal cells (CAIb,c). There is also a correlation with the sandwich neurogenetic gradient. Layer III cells are younger (on the average) than cells in layers II and IV (Fig. 2). Since their axons project contraterally, they have farther to grow to reach the targets in CAI. Possibly the late neurogenesis in CAI can be related to this late ingrowth of contralateral entorhinal afferents, similar to its relationship to the late arrival of thalamic fibers.

3.1.4. Patterns of fiber termination related to patterns of neurogenesis in target areas of hippocampal projections

The hippocampus has topographic projections to two main target structures which can be related to neurogenetic gradients. (1) The lateral septal nucleus gets prominent input from the hippocampus via the precommissural fornix (Nauta, 1956; Raisman et al., 1966; Raisman, 1966); the pyramidal cells of Ammon’s horn and the subiculum, project topographically (Fig. 4) so that dorsal levels terminate predominantly in medial zones, intermediate levels terminate in dorsolateral zones, ventral levels terminate in ventrolateral zones (Siegel et al., 1974; Swanson and Cowan, 1977; Meibach and Siegel, 1977b). The medial part of the lateral septal nucleus forms earlier than its lateral part (Bayer, 1979a); the dorsolateral part has a tendency (significant in two-thirds of the cases) to originate slightly before the ventrolateral part.

(2) The mammillary body also gets prominent hippocampal input via the postcommissural fornix (Nauta, 1956; Guillery, 1956; Raisman et al., 1966); the subiculum (Swanson and Cowan, 1975a, 1977) projects topographically so that its dorsal part terminates in a dorsal transverse zone in the pars posterior of the medial mammillary nucleus; intermediate parts terminate in a central transverse zone, ventral parts terminate in a ventral transverse zone (Meibach and Siegel, 1975, 1977b). The dorsal part of the medial mammillary nucleus pars posterior forms before its ventral part (Altman and Bayer, 1978a). These projections are diagrammed in Fig. 4.

A consistent relationship is maintained in both of these projections: The dorsal hippocampus (all fields in the lateral septal projection, the subiculum alone in the mammillary projection) always projects to zones occupied by earlier generated cells, while progressively
Fig. 4. Gradients of neurogenesis in target areas correlated with topographical hippocampal projections along the dorsoventral axis. The "dorsal" and "ventral" parts of the hippocampus are diagrammatic and may not exactly correspond the anatomical boundaries of the topographic projections [see Swanson and Cowan (1977) and Meibach and Siegel (1977b) for further details]. In the lateral septal nucleus, the dorsal hippocampus (CA1, CA3 and subiculum) projects to a zone occupied by earlier forming medially placed cells; the ventral part projects to zones occupied by later forming laterally placed cells (further dorsoventral organization within this projection is not shown). In the mammillary body, pars posterior, the dorsal subiculum projects to the dorsal transverse zones occupied by earlier forming cells: ventral subiculum, to ventral transverse zones occupied by later forming cells. Note that the dorsal projection is always to locations occupied by older cells, ventral projection to locations occupied by younger cells. This may be related to the earlier time of arrival of dorsal vs. ventral axons from the hippocampus and subiculum (from Bayer, 1980a).

more ventral parts project to zones occupied by later generated cells (Fig. 4). These relationships may be coincidental, but a hypothesis is offered. Since neurogenesis occurs simultaneously along the dorsoventral axis of both the hippocampus and subiculum, their axons may start to grow simultaneously from both dorsal and ventral levels toward the targets. The dorsal hippocampus and subiculum are closer to the targets and their fibers would arrive earlier and settle in zones where earlier forming cells are located. The same pattern would follow for axons from more ventral sources. Fiber tracing methods have to be used in developing brains to test if dorsal and ventral hippocampal fibers differ in arrival time at target structures.

3.2. Olfactory System

3.2.1. Neurogenetic gradients in the output neurons of the olfactory bulb

The olfactory bulb rests on the cribiform plate of the ethmoid bone. Axons of primary sensory neurons in either the olfactory epithelium or the vomeronasal epithelium penetrate the foramina in the cribiform plate and enter the olfactory bulb, terminating on dendrites of secondary sensory neurons in specialized structures, the glomeruli. Many of the secondary sensory neurons have axons extending beyond the bulb and are collectively called output neurons. The olfactory output neurons are unique in that they project directly to telencephalic structures instead of to thalamic relay nuclei. The olfactory projections are quite widespread, occupying a superficial terminal zone (layer Ia) along the
ventral and ventrolateral surfaces of the forebrain. The anterior olfactory nucleus, primary olfactory (piriform) cortex are structures getting the heaviest olfactory input. In addition, axons from the olfactory bulb extend to limbic structures such as the amygdala and entorhinal cortex and to a part of the ventral striatum, the olfactory tubercle. When one considers the rich supply of afferent axons that come into the bulb from the basal telencephalon, much of the ventral forebrain in macrosmatic animals is correctly termed the rhinencephalon, "nose brain".

There are four populations of olfactory bulb output neurons. The accessory bulb has a clump of medium sized to large cells lying between the superficial glomeruli and the deeper granule cells. In the main bulb, the output neurons are arranged in three laminae. The mitral cells are grouped in a layer that lies just superficial to the granule cell layer. Another group of main bulb output neurons are called tufted cells. According to Cajal's (1955) terminology, the deep half of the plexiform layer has widely scattered internal tufted cells, the superficial half of the plexiform layer has a more dense population of external tufted cells, and interstitial tufted cells are scattered between the glomeruli. Cajal's terminology is followed in Fig. 5; currently however, the external cells are often called middle tufted cells, and the interstitial cells are often called external tufted cells.

Within their respective locations throughout the main and accessory parts of the olfactory bulb, output neurons are arranged randomly with respect to age. However, there is a highly sequential pattern of neurogenesis between different populations (Fig. 5; Bayer, 1983). In the accessory olfactory bulb, some output neurons are generated on E12, and 60% of the population originates on E13. Over 80% of the main bulb mitral cells originate on E15 and E16, and neurogenesis is complete by E18. There is a strong deep to superficial gradient between the three tufted cell population which continues the sequential neurogenic pattern. Most of the internal tufted cells have originated by E18, before the peak time for neurogenesis (E18 and E19) for the external tufted cells. Still later, between E20–E22, approximately 60% of the interstitial cells originate.

3.2.2. Neurogenetic gradients in the anterior olfactory nucleus

The anterior olfactory nucleus is one of the major central olfactory processing stations, receiving heavy input from both mitral and tufted cells in the ipsilateral olfactory bulb (Broadwell, 1975a; Davis et al., 1978; Girgis and Goldby, 1967; Haberly and Price, 1977; Heimer, 1968; Meyer, 1981; Ojima et al., 1984; Powell et al., 1965; Price, 1973; Scalia and Winans, 1975; Shipley and Adamek, 1984; Sween and Hall, 1977; Turner et al., 1978). In return it projects back to the ipsilateral bulb and is the only source of contralateral projections to the opposite olfactory bulb (Alheid et al., 1984; Broadwell, 1975b; Davis and Macrides, 1981; Dennis and Kerr, 1976; de Olmos et al., 1978; Ferrer, 1969; Haberly and Price, 1978b; Luskin and Price, 1983; Macrides et al., 1981; Mori et al., 1979; Schoenfeld and Macrides, 1984; Shafa and Meisami, 1977; Shipley and Adamek, 1984). Toward the posterior parts of the anterior olfactory nucleus, the relatively undifferentiated gray matter takes on a more complex cytoarchitectonic pattern, forming transition areas (de Olmos et al., 1978; Herrick, 1924) to the primary olfactory cortex. The transition areas also get olfactory bulb input as part of the projection extending to the primary olfactory cortex. The major neurogenetic gradients seen in the anterior olfactory nucleus and the transition areas are shown in Fig. 6 (Bayer, 1986a).

3.2.2.1. Superficial–deep gradients

The segments representing the anterior olfactory nucleus in Fig. 6 have darker exterior shading and lighter interior shading indicating a superficial (older) to deep (younger) neurogenetic gradient, the “outside-in” pattern of Angevine (1965). This type of neurogenetic gradient is also found in the corticomedial amygdaloid nuclei (Fig. 9). In contrast, the ventral-lateral and dorsal transition areas show darker shading in the interior and lighter shading on the periphery indicating a deep (older) to superficial (younger) gradient.
This is the typical neurogenetic gradient seen throughout the telencephalic cortical areas, which Angevine (1965) termed the "inside-out" pattern. It will be shown below that the primary olfactory cortex located posterior to the transition areas also has the cortical neurogenetic gradient. These data suggest that the ventral-lateral and dorsal transition areas are anterior extensions of the primary olfactory cortex. On the basis of its neurogenetic gradients, the anterior olfactory nucleus is a "ganglionic" (nuclear) structure rather than a cortex, in agreement with Herrick's (1924) classification.

The lateral transition area is a true "transition" area. It is cytoarchitectonically similar to the adjacent transition areas and primary olfactory cortex, yet it does not have the typical cortical neurogenetic gradient. The deep cells originate in an unusual biphasic
3.2.2.2. Rostrocaudal gradients

While gradients along the superficial–deep plane differentiate the anterior olfactory nucleus from the transition areas, a caudal to rostral gradient is shared by all these structures. For example, the ventral lateral transition area shows darker shading at L6 than at L5 (Fig. 6), just as do the segments of the anterior olfactory nucleus between these two levels. Thus, while some parts of the olfactory peduncle differentiate into cortical structures, other parts into ganglionic structures, developmental events are linked in the rostrocaudal direction.
3.2.3. Neurogenetic gradients in the primary olfactory cortex

The primary olfactory cortex is defined as that part of the telencephalic cortical mantle which gets a direct projection from the main olfactory bulb. The projection to the piriform cortex was first described by LeGros Clark and Meyer (1947) and was confirmed by several experimental anatomical studies in the 1960's and early 1970's (Cragg, 1961; Girgis and Goldby, 1967; Heimer, 1968; Powell et al., 1965; Price, 1973; White, 1965). Many studies on various aspects of the projection are in the recent neuroanatomical literature (Broadwell, 1975a; Carlsen et al., 1982; Davis et al., 1978; Devor, 1976; Haberly and Behan, 1983; Haberly and Price, 1977; Macrides et al., 1981; Meyer, 1981; Ojima et al., 1984; Scalia and Winans, 1975; Schneider and Scott, 1983; Scott, 1981; Scott et al., 1980; Shammah-Lagnado and Negrao, 1981; Shipley and Adamek, 1984; Sineen and Hall, 1977; Turner et al., 1978). The entorhinal cortex, especially the ventral lateral part, also receives direct olfactory input (Broadwell, 1975a; Carlsen et al., 1982; Davis et al., 1978; Heimer, 1968; Kosel et al., 1981; Meyer, 1981; Price, 1973; Scalia and Winans, 1975; Shammah-Lagnado and Negrao, 1981; Shipley and Adamek, 1984; Sineen and Hall, 1977; Turner et al., 1978; White, 1965). Finally, a few of the most recent reports indicate that the posterior 2/3 of the ventral agranular insular cortex in the rhinal sulcus receives direct olfactory input (Broadwell, 1975a; de Olmos et al., 1978; Meyer, 1981; Shammah-Lagnado and Negrao, 1981; Shipley and Adamek, 1984; Sineen and Geinisman, 1984). In macromastic mammals, the primary olfactory cortex makes up a substantial part of the cerebral cortex, and in the rat it forms the entire ventrolateral wall of the telencephalon.

There is a striking similarity in the way the primary olfactory cortical areas are generated along the rostrocaudal axis (Fig. 7, Bayer, 1986b). Caudal to level A8.4 (heavy dashed line, Fig. 7), the insular piriform and lateral entorhinal cortices originate simultaneously. As a group, the three caudal parts of primary olfactory cortex originate earlier than rostral parts (indicated by the arrows running across the interface at level A8.4 in Fig. 7). All parts of the rostral primary olfactory cortex show a stepwise and more pronounced caudal to rostral neurogenetic gradient (indicated by arrows running between the segments in Fig. 7). This neurogenetic gradient is continued by the pronounced caudal to rostral gradient.

![Diagram summarizing the neurogenetic gradients seen throughout subdivisions (see legend) of the primary olfactory cortex along the rostrocaudal plane. All arrows indicate gradients of neurogenesis. The heavy dashed line represents the approximate location of level A8.4. Caudal to this level, the primary olfactory cortex originates simultaneously and earlier than the cortex located anteriorly. The anterior parts of the primary olfactory cortex, including transition areas and anterior olfactory nucleus, originate in a more prominent stepwise caudal to rostral gradient. Throughout this continuum, younger neurons are positioned nearer to the olfactory bulb; an arrangement which can be related to the primary olfactory projection (Fig. 8). The lateral (L) entorhinal area originates simultaneously with the posterior piriform cortex but earlier than intermediate (I) or medial (M) areas; this gradient is more related to the anatomical connections between the entorhinal cortex and the hippocampus (Fig. 3) than to the anatomical connections with the olfactory bulb (from Bayer, 1986b).](image-url)
throughout the olfactory peduncle (Fig. 6; Bayer, 1986a). When the anterior olfactory nucleus and primary olfactory cortex are taken as a continuum, the youngest neurons are located closest to the olfactory bulb.

Neurogenetic gradients along the rostrocaudal axis in the piriform cortex closely correlate with the observations made by Leonard (1975) in a developmental study in the hamster. Mature primary olfactory contacts (as judged by long-lasting degeneration agyrophilia) were present as early as postnatal day (P) 2 in the rostral part of the posterior piriform cortex. Olfactory axons grew rapidly throughout the posterior piriform cortex and adult levels of degeneration density were reached by P20. In line with its later neurogenesis, the anterior piriform cortex did not show any mature olfactory contacts until P5, and adult levels were not reached until P33. Also correlating with the caudal to rostral neurogenetic gradient in the piriform cortex, Schwob and Price (1984) found that associational fibers originating in the posterior piriform cortex developed in advance of those coming from the anterior piriform cortex.

3.2.4. Neurogenetic gradients in the anterior olfactory nucleus and primary olfactory cortex correlated with anatomical projections from the olfactory bulb

A hypothetical scheme of the relationship between the neurogenetic gradients in all structures receiving primary olfactory input (target structures) and their connections with the olfactory bulb is shown in Fig. 8 (Bayer, 1986b). There are caudal to rostral neurogenetic gradients (arrows, Fig. 8) in the primary olfactory cortex and anterior olfactory nucleus (Figs 6, 7). Taken together, these structures constitute the largest target area and are the most well-documented recipients of primary olfactory axons. (The anatomical literature is reviewed in Sections 3.2.2 and 3.2.3 above.)

Neuroanatomical evidence is accumulating which correlates the age of olfactory bulb output neurons with the position of their target zones along the rostrocaudal axis. For example, superficial (youngest) tufted cells project only as far as the pars externa and rostral (youngest) part of the anterior olfactory nucleus (Schoenfeld and Macrides, 1984; Schoenfeld et al., 1985). Deeper (older) tufted cells project farther back in the (older) anterior olfactory nucleus and primary olfactory cortex (Haberly and Price, 1977; Schneider and Scott, 1983). On the other hand, mitral cells project throughout the olfactory projection field, and their axons constitute the entire projection to the posterior primary olfactory cortex (Haberly and Price, 1977; Schneider and Scott, 1983; Scott, 1981). In a horseradish peroxidase (HRP) and [3H]thymidine double labeling study, Grafe (1983) found only the oldest mitral cells project to the posterior piriform cortex in the hamster. We have confirmed these observations in rats: more posterior HRP injection sites retrogradely label only the oldest mitral cells (those originating on or before E15), while more anterior HRP injection sites retrogradely label some younger mitral cells (originating on or after E16) along with older cells (Bayer, Palmore and Gerard, in preparation). We are continuing these experiments, and plan to inject more rostral parts of the primary olfactory cortex and anterior olfactory nucleus to see if even younger mitral cells become retrogradely labeled. Thus far, these data suggest that progressively younger mitral cells contribute to progressively more anterior projections, and a tentative hypothesis is offered. Within the continuum formed by the anterior olfactory nucleus and the primary olfactory cortex, the youngest output neurons project to the closest targets which contain the youngest recipient neurons. (The methods and procedures used in the double labeling studies is discussed in Section 4.2.1. below).

3.2.5. Neurogenetic gradients in the amygdala

The amygdala forms the floor of the telencephalon from the olfactory tubercle to the ventral part of the hippocampal region. Johnston (1923) was one of the first to describe the anatomy of this region, which he found to be relatively constant in a variety of mammals. Additional descriptive anatomical studies in the rat (Gurdjian, 1928; Brodal,
Fig. 8. A diagram indicating the correlation between timetables of neurogenesis in the main olfactory bulb output neurons (upper right legend) and neurogenetic gradients (arrows, lower legend) in target structures reached by primary olfactory axons. (Abbreviations: AM, amygdala, AON, anterior olfactory nucleus, MOB, main olfactory bulb; OT, olfactory tubercle; POCa and POCp, anterior and posterior parts of the primary olfactory cortex.) The projections in drawings along the diagonal show the most caudal extent of axons of youngest output neurons (top drawing), and oldest output neurons (bottom drawing). The projections thin out caudally (Haberly and Price, 1977; Shipley and Aasenek, 1984; Bayer et al., unpublished) presumably due to the decreased number of axons contributed by younger output neurons. Only the oldest mitral cells contact the amygdala and posterior primary olfactory cortex (bottom right drawing) (from Bayer, 1986b).

1947; Krettek and Price, 1978b), rabbit (Young, 1936), bat (Humphrey, 1936), cat (Fox, 1940), monkey (Lauer, 1945), and man (Crosby and Humphrey, 1941) confirmed and expanded Johnston's observations. Seven areas and/or nuclei have been traditionally included in the amygdala: (1) the anterior amygdaloid area, (2) the bed nucleus of the lateral olfactory tract, and (3) the central, (4) medial, (5) cortical, (6) basal and (7) lateral nuclei. Neurogenetic gradients in the entire amygdala are presented in detail in Bayer (1980b).

Since the cortical and medial nuclei receive direct input from the olfactory bulb, their
neurogenetic gradients are discussed here. Gradients within the corticomedial nuclei are summarized in Fig. 9 (Bayer, 1980b). The compartmentalized L-shaped areas represent anterior and intermediate parts of the complex; the box at the posterior level is that part of the posteromedial cortical nucleus which extends beneath the ventral subiculum. Shadings represent the proportion of neurons that have accumulated in specific locations by the morning of the embryonic day indicated. There are three types of intranuclear gradients. First, neurogenesis proceeds from ventral to dorsal in the medial nucleus (E14-E16, Fig. 9); second, from rostral to caudal in the dorsal part of the medial nucleus (E14-E15, Fig. 9) and in the posterolateral and posteromedial cortical nuclei (E15-E17, Fig. 9). Third, superficial neurons originate before deep ones at the intermediate posterolateral cortical nucleus, throughout the posteromedial cortical nucleus (E15-E17,

![Diagram of neurogenetic gradients in the corticomedial complex](image)

**Fig. 9.** A summary of the neurogenetic gradients in the corticomedial complex of the amygdala. Shadings indicate the percentage of cells which are unlabeled (have already originated) by the morning of the embryonic day indicated.
Fig. 9, and in the amygdalo-hippocampal area (E17–E19, Fig. 9). There are two main inter nuclear gradients. First, there is a generalized lateral to medial gradient at the anterior and intermediate levels. At the anterior level, the older ventral medial nucleus is flanked by younger neurons in the more lateral anterior cortical nucleus; neurogenesis in the anterior cortical nucleus precedes that in the anterior part of the lateral cortical nucleus (E14–E16, Fig. 9). Second there is an overall rostral to caudal gradient. Neurogenesis in each nucleus at the anterior part of the complex precedes that in nuclei at intermediate and posterior parts (E15–E16, Fig. 9).

The amygdalo-hippocampal area is unusual in that it forms significantly later than cells in the posterior part of the posteromedial cortical nucleus, placing it “out of line” in the strong anterior to posterior neurogenetic gradient (E16–E18, Fig. 9). Another exceptional feature is that it is the only part of the corticomedial complex not receiving olfactory bulb input. Both of these characteristics relate the amygdalo-hippocampal area more to the hippocampus than to other parts of the corticomedial amygdala.

The sequential order of neurogenesis between the output neurons of the accessory olfactory bulb and main bulb (arrow, Fig. 10) sets the stage for a sequential growth of their axons into target areas in the telencephalon. The corticomedial amygdala is one of the best documented targets of the olfactory projection system (LeGros Clark and Meyer, 1947; Powell et al., 1965; White, 1965; Girgis and Goldby, 1967; Heimer, 1968; Price, 1973). The main and accessory parts of the bulb project to nonoverlapping parts of the amygdala. The main bulb terminates in the nucleus of the lateral olfactory tract, anterior cortical, and posterolateral cortical nuclei (Broadwell, 1975a; Scalia and Winans, 1975; Skeen and Hall, 1977; Turner et al., 1978). The accessory bulb terminates in the nucleus of the accessory olfactory tract, medial, and posteromedial cortical nuclei (Scalia and Winans, 1975; Broadwell, 1975a; Devor, 1976; Sken and Hall, 1977). These anatomical relationships, along with the sequential growth of the olfactory fibers, are illustrated in Fig. 10. Within the amygdaloid target zones, neurons are generated in a combined anterior to posterior and medial to lateral neurogenetic gradient (Fig. 9; Bayer, 1980b). These

Fig. 10. Gradients of neurogenesis (arrows) in the olfactory bulb correlated with gradients of neurogenesis in amygdaloid target structures. Embryonic days indicated are peak days of neurogenesis. Sequential neurogenesis between accessory (AOB) and main (MOB) bulb output neurons sets up a sequential growth of fibers (solid to dashed lines) in the accessory olfactory tract (AOT) and lateral olfactory tract (LOT) toward target areas in the telencephalon (Schwob and Price, 1978); the AOT and LOT have been separated for diagrammatic purposes. Within the amygdala, neurons in the parts receiving accessory bulb input (N1OT, nucleus of the accessory olfactory tract; MED, medial nucleus; PCOm, posteromedial cortical nucleus) are generated slightly earlier than neurons in the parts receiving main bulb input (NLOT, nucleus of the lateral olfactory tract; ACO, anterior cortical nucleus; PCO1, posterolateral cortical nucleus). Throughout both target zones, anterior parts, which should receive fibers first, are generated before posterior parts (from Bayer, 1980c).
gradients may be related to the time of arrival of olfactory axons. For example, the anterior medial nucleus neurons may be ready to receive fibers from the accessory bulb earlier than neurons in the anterior cortical nucleus which get input from the main bulb. Schwob and Price (1978) found that accessory bulb fibers reach the anterior amygdala before main bulb fibers. The anterior cortical nucleus of the amygdala contains some of the oldest neurons (generated on E13) to receive input from olfactory bulb mitral cells. According to the hypothetical scheme presented in Fig. 8, the caudal part of the olfactory projection is established when axons of (presumably) older mitral cells make contacts with the early generated neurons in the anterior cortical nucleus. Both Schwob and Price (1978) and Leonard (1975) found this part of the main olfactory projection field to develop early.

3.3. STRIATOPALLIDAL SYSTEM

3.3.1. Neurogenetic gradients in the striatum

3.3.1.1. Caudoputamen complex

The striatum forms the lateral wall of the lateral ventricle and is a large expanse of gray matter that lies beneath the fiber tracts at the base of the cerebral cortex. It is penetrated by small bundles of myelinated fibers going to and from the cortex in the internal capsule, which give it a "striated" appearance. Classical anatomical descriptions of the caudate and putamen remark that these two nuclei are histologically identical (Carpenter, 1976; Kemp and Powell, 1971). In the rat, the caudate and putamen are not separated as in carnivores and primates, and the two nuclei are together referred to as the caudoputamen complex. However, there is neurogenetic evidence that in the rat too the striatum is made up of two components.

Figure 11 diagrams the two most prominent neurogenetic gradients in the striatum (Bayer, 1984). Medium sized neurons make up approximately 95% of the neuronal

![Diagram of neurogenetic gradients in the caudoputamen complex](image)

**Fig. 11.** The entire caudoputamen complex shows a prominent ventrolateral to dorsomedial gradient (solid arrows); stippled arrows indicate gradients along the superficial-deep plane. Rostral levels show a superficial to deep gradient while caudal levels show a gradient in the opposite direction. This neurogenetic shift is evidence that a "caudate" can be delineated from a "putamen" in the rat (from Bayer, 1984).
population. These cells originate in a strong ventrolateral (E14 to E18) to dorsomedial (E18 to birth) gradient throughout the entire rostrocaudal extent of the complex, confirming earlier studies (Angevine and McConnell, 1974; Fentress et al., 1981; Smart and Sturrock, 1979; ten Donkelaar and Dederen, 1979). Fewer than 10% of the medium sized cells originate after birth. There are two additional neurogenetic gradients which differentiate two areas in the caudoputamen complex. (1) The superficial (older) to deep (younger) gradient at and anterior to the plane of the crossing of the temporal limb of the anterior commissure (A7.8) switches to a deep (older) to superficial (younger) gradient posteriorly (Fig. 11). This shift in neurogenetic gradients in precommissural vs postcommissural levels had not been noted in previous [3H]thymidine autoradiographic studies of the striatum. (2) Different parts of the complex can be distinguished by neurogenetic gradients in the rostrocaudal plane diverging on either side of the decussation of the anterior commissure. The anterior part has a caudal to rostral gradient, while the posterior part has a gradient in the opposite direction. Earlier [3H]thymidine autoradiographic studies of the caudoputamen complex observed a caudal to rostral gradient (Fentress et al., 1981; Smart and Sturrock, 1979; ten Donkelaar and Dederen, 1979) but failed to notice the rostral to caudal gradient at posterior levels. Gurdjian (1928) speculated that anterior vs posterior parts of the neostriatum in rats had the characteristics of the separated caudate and putamen in other species. The neurogenetic gradients indicate that the precommissural part of the complex could be considered the caudate nucleus, and the postcommissural part of the complex, the putamen.

Shifts in neurogenetic gradients often indicate differential germinal sources during embryonic development. The basal telencephalon contains two ridges during early morphogenesis which later fuse (Lammers et al., 1980). These ridges are believed to be the sources of the striatum, globus pallidus, and possibly the amygdala. It is tempting to speculate that the putamen is derived from the more posterior of the two ridges while the caudate comes from the more anterior primordium. An extensive embryonic study of the germinal sources of various parts of the striatum is currently in progress (Bayer and Altman, in preparation).

3.3.2.2. Relationships between neurogenetic gradients and anatomical connections in the neostriatum

One of the best documented anatomical connections of the neostriatum is the projection from the substantia nigra. Rosegay (1944) found chromatolytic changes in the substantia nigra after lesions of the caudate nucleus. Later lesion studies with the Nauta technique were able to trace fine fiber degeneration into the caudate-putamen after nigral damage (Faull and Carman, 1968). Ungerstedt (1971) traced dopamine fibers from the substantia nigra into the striatum, and both lesion studies with the Fink-heimer stain (Carpenter and Peter, 1972; Hedreen and Chalmers, 1972; Maler et al., 1973; Szabo, 1971) and retrograde transport studies (Faull and Mehler, 1978; Nauta et al., 1974; Royce, 1978; Schwab et al., 1977; Sotelo and Riche, 1974) have confirmed this projection. Some of the recent studies have shown the presence of a topographic relationship such that the ventromedial substantia nigra and adjacent ventral tegmental area project to a dorsomedial strip of the caudoputamen complex, while the dorsolateral substantia nigra projects to the more lateral and ventral areas (Beckstead et al., 1979; Carter and Fibiger, 1977; Van Der Kooy, 1979; Veening et al., 1980). The neurogenetic gradients in both the substantia nigra (Altman and Bayer, 1981) and caudoputamen complex (Bayer, 1984) can be correlated with this topographic projection. The oldest substantia nigra neurons are located in the dorsolateral part (generated before E15), while the younger neurons (generated after E15) are located in the ventromedial part (large outlined arrows, Fig. 12). The axonal projections are arranged so that the oldest nigral neurons project to the region of the neostriatum containing the oldest neurons, while the youngest nigra neurons project to the youngest neostriatal neurons.

There is equally strong anatomical evidence, demonstrated by a variety of methods, that the striatum projects back to the substantia nigra (Cowan and Powell, 1966; Gerfen et al.,
NEUROGENETIC GRADIENTS AND ANATOMICAL CONNECTIONS

Fig. 12. Correlations between neurogenetic gradients and anatomical connections in the nigrostriatal system. Axons of early originating nigral neurons (solid lines) project via the medial forebrain bundle (MFB) to early-originating cells in the ventrolateral part of the caudoputamen complex. To complete the circuit, axons from superficial neurons in the lateral part of the complex (solid lines) project via the internal capsule (IC) to the dorsolateral substantia nigra. Thus early originating cells in both structures are interconnected. The same relationship holds for those neurons intermediate in age (axons are represented as dashed lines), and late in age (axons are represented as dotted lines). It should be noted that the ventromedial nigra projects throughout the medial tier of the caudoputamen, not just to the ventral part as shown in the diagram (from Bayer, 1984).

1982; Grofova, 1975; Grofova and Rinvik, 1970; Hattori et al., 1975; Kemp, 1970; Moore et al., 1971; Nagy et al., 1978; Nauta and Mehler, 1966; Niimi et al., 1970; Szabo, 1967, 1970; Voneida, 1960). Some of the more recent studies have demonstrated a topographic relationship in the rat so that the ventrolateral striatal cells project to the dorsolateral substantia nigra, while the dorsomedial striatal cells project to the ventromedial substantia nigra (Bunney and Aghajanian, 1976; Domesick, 1977). This projection also correlates with neurogenetic gradients (Fig. 12) so that older striatal neurons project to older nigral targets while younger neurons project to younger targets.

The dopamine axons growing into the striatum may arrive at a time when striatal neurons are sufficiently mature to receive them. Tennyson et al., (1972) found that one of the first areas to develop dopamine fluorescence in the fetal rabbit was a region in the ventral putamen in the neighborhood of the temporal limb of the anterior commissure. In the rat, this area contains the earliest generated medium-sized cells. Tennyson also reported two stages of axonal growth into the striatum. The first preferentially arborized in the morphogenetically more mature putamen, while the second group of axons arborized in the caudate, then extended later into the putamen. Perhaps the shifts in the superficial to deep gradients are related to this two-stage growth of monoamine axons.
3.3.1.3. **Neurogenetic gradients in the nucleus accumbens**

The nucleus accumbens is a ventromedial extension of the striatum which bulges into the septal area and extends laterally beneath the temporal limb of the anterior commissure. Figure 13 shows the time of origin of nucleus accumbens neurons. For purposes of quantification, the nucleus was initially divided into medial, intermediate, and lateral strips at levels A9.4 and A9.0; only the medial strip extends to level A8.6. Each strip was further subdivided into dorsal and ventral parts. The proportion of labeled cells was determined in each subdivision of each strip. Statistical evaluation (sign test) showed no significant differences between subdivisions along the rostrocaudal axis and between the dorsal parts of both medial and intermediate strips; consequently, these data were combined. Figure 13 shows the proportion of cells arising over two day periods in five significantly different segments of the nucleus. Neurogenesis in ventral parts significantly precedes that in dorsal parts ($p < 0.0001$). Within both ventral and dorsal parts, neurogenesis in lateral areas significantly leads that in medial areas ($p < 0.025$). In all parts of the nucleus, the bulk neurons are produced from E17 through E22. Approximately 10% (dorsomedial part) or less (remaining parts) of the neurons form during the first three days of postnatal life. Neurogenesis is essentially complete by P4. The time span for neurogenesis in the nucleus accumbens is essentially the same as for medial parts of the caudate nucleus, indicating that the two structures are part of the same complex.

The anatomical connections of the nucleus accumbens include both limbic and striatal characteristics. Limbic inputs arise in the septum (Powell, 1963; Siegel and Tassoni, 1971b;
Swanson and Cowan, 1976), hippocampus (DeFrance et al., 1980; DeFrance and Yoshihara, 1975; Meibach and Siegel, 1977; Siegel et al., 1974; Siegel and Tassoni, 1971; Swanson and Cowan, 1976), amygdala (DeFrance et al., 1980; DeFrance and Yoshihara, 1975; Gloor, 1960; Ito et al., 1974; Krettek and Price, 1978a; Price and Powell, 1970), and from limbic thalamic nuclei (Chung et al., 1976; Heimer, 1972; Herkenham, 1978; Swanson and Cowan, 1975b). In return, the nucleus accumbens projects to the septum (Conrad and Pfaff, 1976; Powell and Leman, 1976; Williams et al., 1977), hippocampus (Conrad and Pfaff, 1976; Powell and Leman, 1976), amygdala (Nauta et al., 1978), limbic thalamic nuclei (Conrad and Pfaff, 1976; Nauta et al., 1978; Powell and Leman, 1976; Williams et al., 1977) and hypothalamus (Conrad and Pfaff, 1976; Nauta et al., 1978; Powell and Leman, 1976; Scott and Leonard, 1971; Swanson and Cowan, 1975b). The striatal character of accumbens is shown by its projections to the globus pallidus (Conrad and Pfaff, 1976; Jones and Mogenson, 1980; Nauta et al., 1978; Swanson and Cowan, 1975b; Williams et al., 1977) and ventral caudoputamen complex (Conrad and Pfaff, 1976; Powell and Leman, 1976) and by being reciprocally connected to the monoamine centers in the ventral tegmental area (Carter and Fibiger, 1977; Chronister et al., 1980; Nauta et al., 1978; Simon et al., 1976; Troiano and Siegel, 1978) and the substantia nigra (Carter and Fibiger, 1977; Conrad and Pfaff, 1976; Fallon and Moore, 1978; Moore, 1978; Nauta et al., 1978; Powell, 1963; Swanson and Cowan, 1975b; Troiano and Siegel, 1978; Williams et al., 1977). Taking note of these anatomical connections, Heimer (1972, 1978) placed the nucleus accumbens into the “ventral striatum” which has strong limbic-olfactory ties. Some of these anatomical relationships correlate with both the lateral-to-medial and ventral-to-dorsal patterns of neurogenesis. Nauta et al. (1978) found that anatomical relationships became progressively more limbic as one proceeded from lateral to medial along the ventral striatum. The older anterior part of the basolateral nucleus of the amygdala projects to older lateral parts of accumbens, while the younger posterior basolateral nucleus projects to younger medial parts of accumbens (Krettek and Price, 1978a).

3.3.1.4. The olfactory tubercle

In macrosomatic animals, such as the rat, the olfactory tubercle is a prominent structure in the rostral basal telencephalon. One of its distinguishing features is the presence of dense clusters of granule cells, the islands of Calleja. The areas surrounding these islands have a laminated appearance and have been traditionally divided into three layers: (I) a cell-sparse superficial plexiform layer, (II) a layer of medium to small sized neurons packed 6–8 deep, and (III) a deep layer. Layer I receives direct input from the main olfactory bulb (Cragg, 1961; Heimer, 1968; Powell et al., 1965; Price, 1973; Scalia and Winans, 1975; White, 1965). For this reason, the olfactory tubercle has generally been considered to be part of the primary olfactory cortex (Fox, 1940; Gurdjian, 1925; Lauer, 1945; Lohman, 1963, White, 1965). There is recent anatomical evidence which challenges this classification. The neurons in layer II have traditionally been called pyramidal cells, but Golgi impregnations show them to have an atypical and irregular structure different from pyramidal cells in cortical areas (Millhouse and Heimer, 1984). Layer III contains two intermingled cell populations. There are scattered medium to small sized cells resembling the neurons in the striatum. These cells are often arranged in bridge-like structures extending from the striatum toward layer II (Heimer, 1978). There are also sparse large cells, which Golgi impregnations show to resemble neurons in the globus pallidus and substantia innominata (Millhouse and Heimer, 1984). There is histochemical evidence of continuity between the olfactory tubercle and the striatum since these areas are among the structures most intensely stained for acetylcholinesterase and for choline acetyltransferase (see Fallon et al., 1983 and Krieger, 1981 for reviews). There is also ample histochemical evidence to include large cells of the olfactory tubercle in the ventral pallidum (Haber and Nauta, 1983; Switzer et al., 1982). In addition, developmental patterns link the olfactory tubercle to both the striatum and pallidum.
Fig. 14.
Neurogenetic gradients throughout the olfactory tubercle are shown in Fig. 14 (Bayer, 1985a). There is a strong lateral (older) to medial (younger) gradient in all three layers (Fig. 14A, B, C). Even though the olfactory tubercle resembles a cortex, the data in Fig. 14B indicate that the deep to superficial cortical neurogenetic gradient is lacking. The production of medium to small cells throughout all layers and in the striatal bridges is simultaneous. In a true cortex, deep cells, even if they are small, are generated before more superficial neurons. For example, the smaller neurons in layer V of the entorhinal cortex are generated earlier than the larger stellate neurons in layer II (Fig. 2; Bayer, 1980a). The presence of layer I makes the olfactory tubercle look like part of the olfactory cortex, but its neurogenetic patterns indicate that it is a combined striatal/pallidal structure. The large polymorph cells scattered throughout layer III originate early (Fig. 14C) and in patterns resembling neurogenesis in the magnocellular basal telencephalic nuclei (Fig. 15; Bayer, 1985b). The small to medium-sized cells in both layers II and III are produced 4–6 days before birth in the rat (Fig. 14B, C), at a time when most striatal neurons are generated in the caudoputamen complex (Bayer, 1984) and nucleus accumbens (Fig. 13, Bayer, 1981). In spite of these similarities, the presence of direct olfactory input makes the olfactory tubercle a unique component of the basal ganglia.

The strong lateral to medial neurogenetic gradient throughout the olfactory tubercle can be related to the organization of its anatomical input from the olfactory bulb. The lateral (older) part of the tubercle receives olfactory fibers earlier than the medial (younger) part (Leonard, 1975; Schwob and Price, 1984). Also correlating with the relatively late neurogenesis and anterior placement of the olfactory tubercle in the olfactory projection zone (Fig. 8), several reports indicate that some tufted cells (younger output neurons) project to the olfactory tubercle (Haberly and Price, 1977; Schneider and Scott, 1983; Scott, 1981; Scott et al., 1980). The lateral to medial neurogenetic gradient also correlates with other anatomical connections. The lateral part of the tubercle gets input from the anterior piriform cortex, while the medial part gets input from the posterior piriform and entorhinal cortices (Groenewegen et al., 1982; Haberly and Price, 1978a; Newman and Winans, 1980; Sorensen and Witter, 1983). Limbic neocortical areas project more heavily to medial rather than to lateral parts (Newman and Winans, 1980). There are also differential mediolateral projections from the basolateral and basomedial nuclei of the amygdala (Fallon, 1983; Newman and Winans, 1980). Fallon (1983) reported some evidence of a mediolateral topography between the thalamic and hypothalamic inputs. Finally, there are differences in the efferent projections of the olfactory tubercle; lateral parts project to the lateral ventral pallidum while medial parts project to the medial ventral pallidum (Newman and Winans, 1980). In an extensive study of the chemical neuroanatomy of the olfactory tubercle, Fallon et al. (1983) report many differences along the lateral–medial plane. For example, met-enkephalin, substance P and luteinizing hormone releasing hormone (LHRH) are more concentrated in medial parts of the tubercle. Thus, medial and younger parts of the tubercle get a different complex of afferents, project to different targets, and have a unique set of neurochemical characteristics than do more lateral and older parts of the tubercle.

Fig. 14. Neurogenesis of the large and small-to-medium sized cells in the olfactory tubercle. All bar graphs are the proportion of cells originating on the indicated days (dashed line designates the time of birth). All arrows in drawings indicate gradients of neurogenesis. (A) Development of layer II neurons in the pars lateralis (piriform cortex transition area), and in lateral and medial parts of the pars intermedia. (B) Development of the small-to-medium sized cells throughout layers II, III and the striatal bridges at lateral and medial locations in the pars intermedia. There is no gradient in the superficial–deep plane indicating that the olfactory tubercle does not develop like a cortex. (C) Development of the large and small cells in layer III at lateral and medial locations. The early large cells fit into neurogenetic gradients in the pallidum, while the later small cells originate in patterns similar to those found in the striatum. Notice that throughout the olfactory tubercle, regardless of lamina or cell type, lateral locations always originate earlier than medial locations (from Bayer, 1985a).
3.3.2. Neurogenetic gradients in the pallidum

3.3.2.1. Similarities between different pallidal components

The diffuse magnocellular nuclei in the basal telencephalon contain large, isodendritic type neurons and get major input from either the neostriatum (caudate, putamen) or the ventral striatum (nucleus accumbens, olfactory tubercle) (Heimer, 1978; Heimer et al., 1982; Heimer and Wilson, 1975; Mogenson et al., 1983). There are three subgroupings in the magnocellular basal nuclei. (1) the nuclei in the medial septal diagonal band complex, including both vertical and horizontal limbs; (2) the globus pallidus, ventromedial to the postcommissural caudoputamen complex and lateral to the internal capsule; (3) the substantia innominata ventral to the globus pallidus and the striatum, and joined with the diagonal band complex by cellular bridges. Johnston (1923) observed several different mammalian brains and noted that the large neurons scattered beneath the anterior commissure and in the diagonal band of Broca could only be compared with those in the globus pallidus. Obenchain (1925) spoke of a "giant cell stream" throughout the basal telencephalon of marsupials. Later descriptive anatomical studies traced these large cells from the globus pallidus all the way to the olfactory tubercle (Fox, 1940; Humphrey, 1936; Johnson, 1957; Lauer, 1945; Young, 1936). Heimer and coworkers (Heimer, 1978; Heimer et al., 1983; Heimer and Wilson, 1975) have offered the hypothesis that there is an enlarged pallidal system in the mammalian brain which has both dorsal (globus pallidus) and ventral (diagonal band, substantia innominata) components. One of the best examples of continuity between pallidal components is the presence of cholinergic neurons. In primates, the cholinergic cells of the basal nucleus of Meynert and rostral basal forebrain (as indicated by choline acetyltransferase content) do not continue into the globus pallidus (McKinney et al., 1982; Mesulam et al., 1983), although some globus pallidus neurons are acetylcholinesterase positive (Parent et al., 1979). However in rats, the ventromedial globus pallidus contains cholinergic cells (Johnston et al., 1981; Kelly and Moore, 1978; McKinney et al., 1983). Recent advances in histochemical techniques have shown a remarkable similarity between neurochemical characteristics of the globus pallidus and the more rostral basal telencephalic nuclei beneath the anterior commissure. In the rat, for example, both the dorsal and ventral pallidum contain prominent iron deposits and stain intensely for enkephalin (Haber et al., 1985; Haber and Nauta, 1983; Switzer et al., 1982). Thus, two separate lines of evidence (classical histology, neurochemical characteristics) suggest that all neurons of the magnocellular basal telencephalic nuclei constitute a single system. There is also neurogenetic evidence for such a continuity (Bayer, 1985b).

Figure 15 diagrammatically illustrates the three major neurogenetic gradients seen throughout all of the magnocellular basal nuclei except the entopeduncular nucleus (Bayer, 1985b). First, there is a caudal to rostral gradient in the globus pallidus (top plate), substantia innominata (intermediate plate), and diagonal band-medial septal complex which extends into the olfactory tubercle (bottom plate). This is illustrated in Fig. 15 by an increase in shading in areas containing older neurons at the caudal limits of each of the three nuclear complexes, respectively. The visual impression of a rostral to caudal direction is illustrated in Fig. 15 by a narrowing of the structures in the rostral end; in reality, many structures (e.g. globus pallidus) are wider at their anterior ends. Second, there is a lateral-to-medial gradient within the diagonal band-medial septal complex, confirming the findings of previous [3H]thymidine autoradiographic studies (Crepes, 1974; ten Donkelaar and Dederen, 1979). This is illustrated by darker lateral shading in Fig. 15. A lateral to medial neurogenetic gradient is also found rostrally in the substantia innominata and globus pallidus (not shown in Fig. 15). Third, there is a superficial to deep gradient between the various basal telencephalic nuclei so that at any one rostrocaudal level, more dorsal (deep) cells are generated later than ventral (superficial) cells. This is shown by an increase in shading in ventral areas along a given vertical dimension in Fig. 15 (note that the dorsal part of the globus pallidus is not illustrated). It is striking that neurons of the magnocellular basal nuclei, scattered throughout such a large expanse of the telencephalon, should show this uniformity of neurogenetic gradients.
3.3.2.2. Neurogenetic gradients in the magnocellular basal nuclei correlated with their anatomical connections to the cerebral cortex

A host of experimental anatomical studies have demonstrated that the entire cerebral cortex receives input from the magnocellular basal nuclei. The experimental evidence is accumulating that this projection is primarily cholinergic (Hartgraves et al., 1982; Johnston et al., 1981; Kelly and Moore, 1978; Lehmann et al., 1980; McKinney et al., 1983, 1982; Mesulam et al., 1983). In the rat, cells in the globus pallidus project to widespread neocortical areas (Finch et al., 1984; Johnston et al., 1981; Kelly and Moore, 1978; Lamour et al., 1982; Lehmann et al., 1980; McKinney et al., 1983; Saper, 1984). The substantia innominata projects mainly to the neocortex (Divac, 1975; Divac et al., 1978; Finch et al., 1984; Johnston et al., 1981; Kelly and Moore, 1978; Lamour et al., 1982; Lehmann et al., 1980; Price and Stern, 1983; Saper, 1984), as well as to the piriform cortex (Haberly and Price, 1978). The horizontal limb of the diagonal band projects to the neocortex (Divac, 1975; Divac et al., 1978; Domesick, 1976; Lamour et al., 1982; Lehmann et al., 1980; Price and Stern, 1983; Saper, 1984) as well as to the piriform cortex (Saper, 1984). On the other hand, the vertical limb of the diagonal band–medial septal complex projects mainly to the entorhinal cortex and hippocampal region (Beckstead, 1978; Domesick, 1976; Hartgraves et al., 1982; McKinney et al., 1983; Meibach and Siegel, 1977; Saper, 1984; Swanson and Cowan, 1976) and to the cingulate cortex (Fallon, 1983).

Some of the more recent studies have reported that the magnocellular basal nuclear projection to the cortical mantle is topographic (Lamour et al., 1982; Price and Stern, 1983;
Saper, 1984). Many of the connections mapped by Saper (1984) can be correlated with neurogenetic patterns. Figure 16 shows the broad outlines of the correlation between neurogenetic gradients in the cortex, neurogenetic gradients in the magnocellular nuclei, and their anatomical connections. Throughout the entire length of the cortex, lateral parts near the rhinal sulcus originate before medial parts (Fernandez and Bravo, 1974; Woodhams et al., 1981; Altman and Bayer, unpublished data). Within the magnocellular basal nuclei, the older neurons are located laterally and ventrally, while younger neurons are situated medially and dorsally. The large open arrows in Fig. 16 point in the direction of the youngest neurons in the cortex and magnocellular basal nuclei, respectively. To give some specific examples of these correlations (based on Saper, 1984), the older neurons in the ventral parts of the globus pallidus project to the early developing granular insular cortex near the rhinal sulcus, while progressively younger neurons in the dorsal globus pallidus project to later developing dorsomedial cortical areas such as the retrosplenial and visual cortices. Within the diagonal band-medial septal complex, the older lateral cells in the horizontal limb project to the laterally placed and early developing piriform cortex while younger medial cells in the vertical limb and medial septal nucleus project to the late developing hippocampal region along the medial cortical edge, again maintaining the older to older (Dashed lines in Fig. 16) and younger to younger relationship (dotted lines in Fig. 16). Within the ventral part of the rostral globus pallidus, the older neurons in lateral parts project to earlier developing lateral frontal cortex while younger neurons in medial parts project to a later developing prelimbic cortex along the medial cortical wall.

4. Mechanisms of Temporal Ordering of Anatomical Connections

The preceding review gives evidence that even though each structural complex in the telencephalon has a unique chronology of neurogenesis, patterns of anatomical interconnections between them can be positively related to temporal patterns of neurogenesis. This is best shown by the hippocampal pyramidal cells where all of their major afferent and efferent connections are relatable to the chronology of neurogenesis. The neurogenetic
hypothesis postulates that these relationships are not coincidental. The remainder of this paper will examine what mechanisms might be used by the nervous system as complex developmental events are translated into a mature structure characterized by precise anatomical interconnections.

4.1. CELL LINES IN THE NEUROEPITHELIUM

The neuroepithelium is the transient embryonic tissue that produces the entire complement of neurons in the central nervous system. Our observations on the morphogenesis of the mammalian nervous system indicate that before neurons are produced, the germinal matrix undergoes a complex series of configurational transformations that varies greatly regionally and over time. We postulate that these spatio-temporal changes reflect the formation and dissolution of regional germinal “mosaics”, each consisting of discrete cell lines destined to produce neurons either for different brain structures or for different types of neurons of two heterogeneous brain structures. If this is correct, then the changing anatomy of the embryonic neuroepithelium is the first step in the precise chronological ordering of neuron production, and it is also a long term predictor of the mature anatomy of the nervous system. Two terms are used here to bring out the significance of the heterogeneity of the neuroepithelium, “cell line” and “neuroepithelial zone”. The cell line is a hypothetical construct. It is conceived to be a genetically homogeneous (clonal) population of precursor cells destined to produce a specific population of neurons. The term neuroepithelial zone is an empirical construct. It designates a group of germinal cells in the neuroepithelium that are distinguishable. Some zones can be delineated from others by morphological landmarks, others by differences in spatio-temporal dynamics of cell proliferation. In some zones there is evidence that they are the sources of neurons forming a particular brain structure or different classes of cells within a brain structure. The demonstration that a neuroepithelial zone consists truly of a single cell line would require an in vitro procedure and is not dealt with further in this paper.

4.1.1. Examples of spatial segregation of neuroepithelial zones

The neuroepithelium surrounding the third ventricle produces the neurons of the diencephalon, one of the most anatomically complex brain regions. The diencephalic neuroepithelium contains evaginations and invaginations, reminiscent of the neuromeres of classical neuroembryological studies (Berquist and Kallen, 1954). These structures are the result of variations in mitotic activity between adjacent loci. For example, an active locus surrounded by others less active will often transiently evaginate into the ventricle, presumably making room for the numerical peak in its mitotic population. The ongoing analysis of diencephalic morphogenesis indicates that young neurons generated in spatially distinct neuroepithelial zones are destined to settle in specific diencephalic nuclei (Altman and Bayer, 1978b, 1979c, 1986). Since the diencephalic neuroepithelium shows several spatially segregated cell lines, two examples will be discussed below, along with some preliminary observations of a spatially segregated cell line in the basal telencephalon which generates mitral cells.

4.1.1.1. The subthalamic neuroepithelium

The subthalamic nucleus has traditionally been considered to be part of the subthalamus or ventral thalamus. Our observations on the development of the hypothalamus (Altman and Bayer, 1986) indicate that neurons in the subthalamic nucleus (ST, Fig. 17) derive from a neuroepithelial zone in the anterodorsal region of the mammillary recess (st, Fig. 17C) caudal to the site of origin of the neurons of the premammillary nuclei (pm, Fig. 17B). The mammillary recess is undisputably part of the hypothalamic rudiment as are the other structures originating at this site, the premammillary nuclei and the tuberal magnocellular nucleus. Ontogenetically therefore, the subthalamic nucleus is part of the “hypothalamus proper”. The postmitotic subthalamic neurons take a semicircular route (arrow, Fig. 17C)
as they migrate from their germinal source to their final locations along the lateral wall of the diencephalon just medial to the cerebral peduncle. The cells settle in a modified "outside-in" pattern, the earliest generated neurons becoming situated most rostrally and dorsolaterally and the later generated neurons caudally and ventromedially. This neurogenic gradient correlates with some of the anatomical connections of the subthalamic nucleus. Van Der Kooy and Hattori (1980) found that the medial subthalamic neurons in the rat project mainly to the medial part of the substantia nigra. Since medial neurons are younger in both nuclei, then younger neurons in the subthalamic nucleus establish connections with younger nigral neurons.

4.1.1.2. The reticular neuroepithelium

There is a neuroepithelial zone in the diencephalic neuroepithelium, identifiable as a slight bulge in the ventricular wall on E14 (Fig. 18), that separates the thalamic neuroepithelium from the hypothalamic neuroepithelium. This is called the reticular protuberance (rt, Fig. 18; Altman and Bayer, in preparation) because neurons can be shown to migrate from this zone into the thalamic reticular nucleus. In animals injected with [3H]thymidine on E14 and killed two hours later, the reticular protuberance shows a dense core of intensely labeled cells (Fig. 18B), presumably due to a rapid increase in the number of neuroblasts. Long survival autoradiography indicates that E14 is the peak day for thalamic reticular neurogenesis (Altman and Bayer, 1979a). Zones in the dorsally adjacent thalamic neuroepithelium will generate neurons later; and label uptake is less pronounced in short survival autoradiograms. The migratory stream of heavily labeled young reticular neurons is traced in animals exposed to [3H]thymidine on E14 and killed one (Fig. 19A), two (Fig. 19B) and three (Fig. 19C) days later. By E15 (Fig. 19A) the swelling of the reticular protuberance is reduced or absent, and its heavily labeled progeny enter the reticular migratory stream. The migratory stream lengthens on E16 (Fig. 19B) and reaches the lateral wall of the diencephalon on E17 (Fig. 19C).

4.1.1.3. The mitral cell neuroepithelium

Using the data for the time of origin of mitral cells as a starting point (Fig. 5), the series of short and sequential survival autoradiograms of brains exposed to a single pulse label of [3H]thymidine on days when mitral cells are being generated were examined. The following brief account gives preliminary observations on mitral cell development. The oldest mitral cells are heavily labeled in the day E13 and E14 injection series. The majority of mitral cells originate on day E15. Sequential survival times after an E15 injection proved to be best for locating the neuroepithelial source of the mitral cells and for delineating their migratory route. On E15, the presumptive olfactory bulb is a slight bulge in the anterior part of the telencephalon. The methacrylate embedded series indicates that this site is already contacted by early fibers of the olfactory nerve. Mitral cells are being generated in the neuroepithelium ventrally adjacent to this region. One day after an E15 injection (E16), many heavily labeled neurons are apparently streaming out of this neuroepithelial zone heading toward the ventral surface of the brain where olfactory nerve fibers accumulate and where the olfactory bulb is evaginating. Observations in animals with appropriate survival times in the E16 and E17 injection series indicate that mitral cells continue to be generated by a neuroepithelial zone that lies behind the olfactory evagination. After a pause of approximately 24 hr at the ventral surface of the brain, young mitral cells migrate into the bulb. They first occupy a transition zone just outside of the neuroepithelium before moving into the formative mitral cell layer. The neuroepithelium that evaginates to produce the olfactory bulb is not the source of the mitral cells and presumably contains cell lines that will give rise to other neuronal populations in the olfactory bulb.

On E18, the mitral cell layer is composed of neurons heavily labeled by thymidine injections given on days E13 and E14. Neurons labeled on E15 are located in the transition zone. By E20, neurons heavily labeled on E15 are just arriving at the base of the mitral
Fig. 17. Coronal thymidine radiograms, from rostral (A) to caudal (C), from a rat labeled with [H]thymidine on day E15 and killed two days later. The migration of neurons (arrow in C) from the subthalamic neuroepithelium (st) to the subthalamic nucleus (ST) is indicated. The active subthalamic neuroepithelium is situated behind the declining premammillary neuroepithelium (pm) and above the very active mammillary neuroepithelium (ma). The lateral mammillary nucleus (ML) is composed mostly of unlabeled cells and the premammillary nucleus (PM) of labeled cells. (Scale bar = 200 μm).
Fig. 18. A. The reticular protuberance (rt) between the thalamic (th) and hypothalamic (hy) neuroepithelium in an autoradiogram from a rat labeled with [3H]thymidine on day E13 and killed on day E14. B. Enlargement of the reticular protuberance. v3d, dorsal third ventricle; v3v, ventral third ventricle (scale bar in A = 100 μm, in B = 50 μm).
FIG. 19. A. An autoradiogram from a rat administered [3H]thymidine on day E14 and killed on day E15. The reticular protuberance beneath the thalamic neuroepithelium has receded and migrating cells appear outside the neuroepithelium. This migratory stream is identified as the reticular nucleus migration (rtm). It can be seen extending laterally beneath the formative thalamus (TH) in a rat injected with [3H]thymidine on day E14 and killed on day E17 (C) (scale bar = 100 µm).
cell layer, forming a sublayer extending along its entire length. At the same time, neurons heavily labeled with an E16 injection are scattered throughout the transition zone. By E22 (the day before birth) the mitral cell layer is composed of neurons originating on or before E15. The mitral cells generated on E16 and E17 are spread out along the base of the layer just as neurons generated on E15 were two days before. In the adult, mitral cells of all ages are scattered throughout the layer. However, the developmental observations indicate that in spite of the random pattern, their time of arrival is strictly according to age.

4.1.2. Successive transformations of a germinal zone: observations in the neocortex

In contrast to the spatially segregated pattern, various neurons in the neocortex are generated by a relatively homogeneous neuroepithelium that undergoes successive transformations (Fig. 20; Altman and Bayer, unpublished observations). In the earliest phase of cortical development (days E13 to E14), the entire neuroepithelium in the roof of the telencephalon constitutes a uniform proliferative matrix (UN, Fig. 20) where most mitoses occur at the ventricular surface. By E15, the oldest cortical neurons have been produced by this neuroepithelium and are migrating into the primordial plexiform layer (Marin Padilla, 1970, 1971, 1972, 1978 and 1983); they reach their destinations on E16 and E17. After E16 the uniform neuroepithelium becomes divided horizontally into periventricular and subventricular components. Some of the proliferative cells migrate out and continue to undergo mitosis in a new germinal zone located just outside of the original undivided neuroepithelium. This region has been called the subependymal layer (Allen, 1912; Kershman, 1938; Smart, 1961; Altman, 1966; Privat and Leblond, 1972). However, ependymal cells do not appear until approximately E19 (Altman and Bayer, unpublished).

![Diagram of cortical neurogenesis](image)

**Fig. 20.** Summary diagram of the proposed major events in cortical neurogenesis. The unsegregated, primitive neuroepithelium (UN) produces the neurons of the molecular (mo) and fusiform (fu) layers on day E15, and these reach the developing cortex on days E16–17. Then the neuroepithelium divides into the periventricular (PN) and subventricular (SN) neuroepithelia. During its peak of activity on days E16–E17, the periventricular neuroepithelium produces the neurons of the projection zone (PZ) of the cortical plate (CP) which reach their locations (layers V and IV) between days E17–E21. On day E19, the transformed periventricular neuroepithelium (TN) begins to produce radial glia and ependymal cells (EP). The neurons produced on days E19–E21 in the subventricular neuroepithelium reach the association zone (AZ) of the cortical plate (layers III and II) on days E21–P3. The white matter (WH) begins to form on day E18; the corpus callosum (CC) on days E20–E21. Transitory zones are omitted from this diagram.
The name subventricular neuroepithelium (SN, Fig. 20) is more appropriate to distinguish it from the periventricular neuroepithelium (PN, Fig. 20) remaining at the ventricular surface. On the E16 the periventricular neuroepithelium undergoes its first transformation. It continues to actively produce neurons from E16 to E18, but these are a different type than those generated a few days earlier. These younger cells migrate into the cortical plate (CP, Fig. 20) between E17 and E21, and eventually differentiate into the projection zone neurons (PZ, Fig. 20) in the deeper layers of the cortex. On E19 a second and final transformation changes the periventricular neuroepithelium to the primitive ependyma (TN, Fig. 20). Mitotic activity becomes much reduced and neurons are no longer produced. Ependymal cells begin to appear along the ventricular surface. Between E19 and E21, the subventricular neuroepithelium actively produces neurons which migrate into the cortical plate between E21 and P3. These youngest cells eventually differentiate into the association neurons (AZ, Fig. 20) in the superficial layers of the cortex. Thus, all of the neocortical neurons are ultimately derived from the same neuroepithelium, but each class of cells is generated at a different time and from a different derivative of the original stem cell population.

4.1.3. Neuroepithelial structure and the neurogenetic hypothesis

The preceding examples drawn from our ongoing studies of early embryonic development indicate that the mammalian neuroepithelium, although apparently composed of undifferentiated cells that look much alike with standard histological techniques, is actually a composite of distinct zones. Short survival [3H]thymidine autoradiography shows that the neuroepithelium is a mosaic. Patches with high mitotic activity (high label uptake) are adjacent to patches of low mitotic activity (lower uptake) in spatially segregated zones. This spatial segregation is prominent throughout the brainstem (Altman and Bayer, 1982) and spinal cord (Altman and Bayer, 1984) where neuronal populations are generated rapidly, usually within two to three days, by their respective cell lines. Then they migrate, some via circuitous routes, to settle in nonlaminated ganglionic (nuclear) structures. In structures such as the neocortex and cerebellar cortex, different populations of neurons are segregated in regularly arranged laminae extending throughout a large expanse of tissue. Neuroepithelia producing cortical structures generate neurons in succession over a longer period of time (weeks rather than days) in temporally segregated zones. For example, the external germinal layer producing the microneurons in the cerebellar cortex generates predominantly basket cells between P4 and P7, predominantly stellate cells between P8 and P12 and predominantly granule cells between P13 and P21 (Altman, 1969).

One can ask the question: To what extent is the heterogeneity of the mature nervous system “preformed” in the heterogeneous proliferative matrix of the primary and secondary neuroepithelia? The neurogenetic hypothesis is that each zone is composed of a different cell line predestined to generate specific populations of neurons. The proper assembly of the central nervous system begins with the activation of discrete neuroepithelial zones in a predetermined sequence. The activity of several neuroepithelial zones is integrated into neuroepithelial fields which generate neurons for a set of related brain structures, such as the limbic system, so that each neuronal population is produced at just the right time for it to reach its final location, grow its axons to the proper targets and elaborate its dendrites to receive a specific set of afferent inputs.

4.2. DIRECT CORRELATIONS BETWEEN TIME OF NEURON ORIGIN AND PATTERNS OF ANATOMICAL CONNECTIONS

4.2.1. Using time of neuron origin to code for mature anatomical connections: HRP and [3H]thymidine double labeling studies

4.2.1.1. Methodological considerations

The cumulative data on precise patterns of neurogenesis has provided an impetus to design experiments to directly test whether the sequential production of neurons might be
a factor in facilitating the establishment of orderly connections within and between nerve structures. A double labeling method, using both \(^{3}H\)thymidine autoradiography and HRP histochemistry, has been developed. The basic strategy is to expose animals to \(^{3}H\)thymidine at various times during embryonic life so that a particular neuronal population is differentially labeled: older cells are unlabeled and younger cells are labeled. Since the \(^{3}H\)thymidine label is retained permanently in the neuronal nucleus, anatomical tracing experiments can be performed in the adult brain. We typically inject horseradish peroxidase (HRP) into a known efferent target of a particular neuronal population in adults that have been exposed to \(^{3}H\)thymidine at a particular stage of development to differentially label the younger neurons of a selected population. After 24 hr survival following the HRP injection, the animals are killed and the brains are processed to reveal both HRP retrograde transport as well as \(^{3}H\)thymidine labeling in the same tissue section. In this way, the time of origin of an individual neuron can be related to the position of some of its axon terminals. Our first experiments investigated the projections of the olfactory bulb mitral cells. This population provides an especially rigorous test of the temporal hypothesis since the cell bodies are not arranged according to their time of origin in the mitral cell layer. Early generated neurons lie adjacent to those generated two to three days later in an apparently random pattern. This is also true for all populations of tufted cells. It is not surprising that neuroanatomists find that mitral and tufted cell projections are basically non-topographic (Haberly and Price, 1977; Scott et al., 1980; Luskin and Price, 1982). The aim of the thymidine/HRP double labeling studies is to see if a “hidden temporal order” exists in the projections of the olfactory output neurons. Might mitral cells of different ages project to different targets? Consequently, some \(^{3}H\)thymidine injections were timed to isolate only the oldest mitral cells as unlabeled, while others were timed to isolate the youngest mitral cells as labeled.

4.2.1.2. Preliminary results

The results from the first experiments are illustrated in Fig. 8 and are discussed in terms of their correlations with neurogenetic gradients in Section 3.2.4 above. In spite of the fact that mitral cells from all parts of the bulb project in a diffuse manner to their target structures in the basal telencephalon, the axons of older mitral cells terminate in different targets than do axons of younger cells. This is the first demonstration that any order exists in the olfactory projection. Some observations of the embryonic development of the basal forebrain provide a clue as to how mitral cell axons may become temporally organized while growing toward their targets. As mitral cells move away from their source neuroepithelial zone, they pause at the ventral surface of the brain (see Section 4.1.1.3) for approximately one day. During this pause, the mitral cells may start to grow their axons. The oldest cells (generated on E13 and E14) start out closer to the posterior targets in the olfactory projection zone. There is a maximum distance of 1 mm between the neuroepithelium that generates the mitral cells and the amygdala and posterior piriform cortex. Due to rapid growth of the telencephalon, mitral cells generated on E16 and E17 are over twice as far away from these posterior targets. Thus, early mitral cells not only start out closer but also have more time to grow their axons to the posterior telencephalon. Younger mitral cells are at a disadvantage to establish connections with posterior target sites. Their axons (if they ever reach these levels) would arrive later and thereby may compete less successfully for synaptic sites.

We also have done some preliminary double labeling experiments in the lumbar spinal cord (Altman et al., in preparation). The results indicate that motor neurons innervating flexors of the hindlimb originate earlier than the motor neurons innervating hindlimb extensors. These two double labeling experiments, performed at opposite poles of the neuraxis, indicate that in a variety of systems (sensory as well as motor) the time of origin of a neuron is an important factor in specifying the position of its axon terminals.
4.2.2. Characteristics of immature axonal projections: examples drawn from developing neocortical connections

On the one hand, the chronologies of neurogenesis in the developing brain and patterned nerve connections in the mature brain are extremely precise and well ordered. On the other hand, there is a growing literature indicating that axonal projections from young neocortical pyramidal cells develop in two stages: initial diffusion followed by elimination of some collaterals.

1. Axons from pyramidal cells in the neocortex begin to grow to their targets before these cells have reached their final locations in a specific cortical lamina (Fisher et al., 1983; Reh and Kalil, 1981; Wise and Jones, 1976; Wise et al., 1979). For example, Wise and his coworkers (Wise and Jones, 1976; Wise et al., 1979) found that pyramidal cells with axons extending into the corpus callosum during the early postnatal period are situated in the deep layers of the cortical plate and below it in the white matter. During the next few weeks, the cells migrate upward to reach their final locations in laminae V (fewer cells) and III (more cells). Reh and Kalil (1981) found that pyramidal cell bodies contributing axons to the corticospinal tract in hamsters do not reach their final locations in the neocortex until after their axons have grown into the spinal cord. Fisher et al. (1983) reported essentially the same finding in cats for pyramidal cells projecting to the caudate nucleus. Thus, neocortical pyramidal cells have extensive axonal projections long before their dendrites and somata reach a mature state.

2. Axons will grow toward a target but may sit in a fiber tract for several days before invading the gray matter where terminations will be established (Donatelle, 1977; Wise et al., 1979; Martin et al., 1980; Reh and Kalil, 1981). Some of the best documentation for this finding comes from the work of Reh and Kalil (1981) on the development of the corticospinal tract in the hamster. Axons reach cervical levels of the spinal cord by P4, but the first penetration of axonal terminal branches into the intermediate horn does not take place until P6. Wise and Jones (1976) found essentially the same in the rat with regard to axons in the corpus callosum. Interestingly, during the time that the axon terminals are invading their target sites, the dendrites and cell bodies in the cortex are undergoing rapid differentiation to the adult stage (Wise and Jones, 1976), and behavioral maturation associated with these terminations is being expressed (Donatelle, 1977). Even though the axon has precocious growth, it appears that the formation of actual synapses in a target is delayed until the afferent input to the presynaptic neuron is becoming established.

3. Experimental manipulations can induce growing axons to take aberrant pathways toward their targets (Castro, 1975; Bernstein and Stelzner, 1983). Bernstein and Stelzner (1983) showed that early bilateral destruction of the spinal dorsal columns induces the growing corticospinal fibers to bypass the lesioned area, take an aberrant route in the lateral funiculus, and still reach their appropriate targets in the intermediate horn. Furthermore, their experiments indicated that if corticospinal neurons in one hemisphere are lesioned at an early stage, the remaining corticospinal neurons will terminate bilaterally in the intermediate horn instead of the normal pattern of contralateral termination. Thus, if the appropriate axon terminals are missing at a critical developmental stage, inappropriate (or less appropriate) axons may grow into the target structure and establish connections.

4. Initially, axonal branches are sent for long distances to both appropriate and inappropriate targets. The documentation for this finding is based on several studies of two major pathways, the corticospinal tract and the corpus callosum. During the first postnatal week, HRP injections into the rat corticospinal tract will backfill neurons throughout the motor and sensory areas of the neocortex (Wise et al., 1979; Leong, 1983; Bates and Killackey, 1984) including visual cortex (Stanfield et al., 1982). HRP injections during the third postnatal week backfills pyramidal cells only in the motor areas of the neocortex. This is in agreement with Reh and Kalil's (1982) finding in the hamster that the number of axons in the pyramidal tract decreases during the time that the mature projection is being established. Essentially the same finding has been made in a study of
callosal connections. In rat somatosensory cortex, pyramidal cells associated with the barrel subfields do not contribute axons to the corpus callosum (Wise and Jones, 1976). However, injections of retrograde anatomical tracers during the first postnatal week into contralateral cortex will label pyramidal cells in the barrel subfields (Ivy and Killackey, 1981, 1982; Ivy et al., 1984; O'Leary et al., 1981). Innocenti (1981) found a wider than normal distribution of callosal neurons in the visual cortex of early postnatal kittens. Many of these studies have further established that the decrease in the number of projecting cells is not associated with cell death, but rather with an elimination of axon collaterals (O'Leary et al., 1981; Ivy and Killackey, 1982; Innocenti, 1981; Stanfield et al., 1982).

In a well designed double labeling experiment in cats, Innocenti (1981) found that when one tracer is injected early (fast blue, first postnatal week) and another tracer is injected later (nuclear yellow, fourth postnatal week) into the same area of visual cortex, pyramidal cells in the contralateral visual cortex are either single or double labeled. Cells single labeled with fast blue are located in medial area 17, while lateral area 17 contains cells that are double labeled. This indicates that the single labeled cells in medial area 17 have lost their axon collateral in the corpus callosum but do not die. Ivy and Killackey (1982) showed further that some neurons in the somatosensory cortex which initially project to both the contralateral cortex and to the ipsilateral motor cortex retain the ipsilateral connection and lose the contralateral connection.

4.2.3. Possible mechanisms of temporal ordering of mature contacts

The literature reviewed above indicates that during stage one of axonal growth, neocortical neurons grow axons into several fiber tracts typically taken by other cortico-fugal axons; the trajectory does not appear to be rigidly specified. During the second stage, there is selective elimination of some axon collaterals. We hypothesize that the neurogenetic specification of connections also follows a two stage process. First, the loose specification of the axonal trajectory; second, the precise specification of synaptic contacts, as indicated by the preliminary double labeling experiments on olfactory bulb mitral cells and spinal cord somatic motor neurons (Section 4.2.1). In the second stage, the axon collaterals retained in the adult brain can be related to time of neuron origin.

What mechanisms are involved in the expression of these specifications? There is a growing literature that during neuronal development, glycoprotein coats on the cell membrane are involved in the recognition of a particular environment (Moscona, 1980). Research on the cell surfaces of axons indicate that growth cones isolated from various neuronal populations in rats have different cell surface properties (Pfenninger et al., 1984). Subsets of growing axons in the leech are preferentially stained by antibodies against specific cell surface antigens (McKay et al., 1983). Thus, there is heterogeneity in the expression of the cell surface coat. These surface molecules are also temporally coded. Critical periods in the appearance of cell recognition molecules have been found in chick optic tectal and retinal cells (Merrell et al., 1976). Certain cell surface antigens have a transient appearance in spinal nerve ventral roots just as axons are growing out to make their connections (Tanaka and Obata, 1984). In the more rigid developmental program of the invertebrate nervous system, cell surface antigens appear to be involved in directing the trajectory of axon growth. For example, axons with different surface antigens will fasciculate along different pathways in the developing grasshopper nervous system (Goodman et al., 1984). In the more plastic mammalian nervous system, the surface molecules may code more specifically for synaptic contact rather than for fiber trajectory. For example, it has been shown morphologically that endocytoptic vesicles on Purkinje cell dendrites contain small evaginations of parallel fiber axons (Altman, 1982). Dontenwill et al. (1983) found Purkinje cells to engulf parallel fiber surface glycoproteins during synaptogenesis.

The neurogenetic hypothesis would argue that as the various neuronal populations are generated by unique neuroepithelial cell lines in discrete neuroepithelial zones, there is also temporal specification of a unique complex of recognition molecules. For example, axons
growing from older mitral cells may have different cell surface antigens than those of younger mitral cells or tufted cells. Even though diffuse axon growth may occur initially, the recognition molecules on the growth cones of the inappropriate collaterals may not be "right" for establishing synaptic contact with neurons in a specific target structure. Consequently, only the appropriate collaterals make contact and insure their continued sustenance. The neurogenic hypothesis postulates that temporal coding is important in determining which collaterals will be retained. In the adult brain, one can no longer test whether the recognition molecules are different in older vs younger mitral cells since the unique morphogenetic environment is no longer present. However, thymidine labeling in the nucleus is retained throughout life, and gives us a "clue" as to the developmental differences that once may have existed within a neuronal population.

5. Concluding Remarks

In this review we have outlined the evidence that many of the major anatomical interconnections of the telencephalon can be related to the order of neurogenesis and to neurogenic gradients of either the source neurons (entorhinal cortex, olfactory bulb mitral cells), receiving neurons (lateral septal nucleus, cortical nuclei of the amygdala, anterior olfactory nucleus, primary olfactory cortex) or both (magnocellular basal nuclei, striatum). These findings have been discussed in light of broader principles of neural development. The hypothesis has been offered that there is considerable "preformation" in the proliferative matrix of the neuroepithelium which is assumed to be composed of a multiplicity of cloned cell lines, each of them generating a different neural population. These heterogeneous germinal cell lines may be identified in some regions with simple autoradiographic techniques as "patches" or "mosaics" in the neuroepithelium each having its unique spatio-temporal course of cell proliferation, each generating young neurons that migrate by a common route to a common destination. Examples are shown for the subthalamic nucleus, the thalamic reticular nucleus, and the mitral cells of the olfactory bulb. Larger, more homogeneous germinal matrices generate neurons for the neocortex in temporally segregated zones. Apparently, the dynamic spatio-temporal heterogeneity of the germinal matrix antedates, and presumably is a precondition, of the cytoarchitectonic heterogeneity of the adult nervous system and the specific interconnections of its components. The identification of these cell lines and an analysis of their properties is an important task of current developmental neurobiology. Work in progress will thoroughly map the neuroepithelial zones and cell lines throughout the entire telencephalon. Finally, HRP and [3H]thymidine double labeling studies indicate that axonal terminations of neurons are specified by their time of origin and a possible mechanism whereby axons can be temporally ordered during development was explained.

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