The mouse hippocampus is an attractive model system in which to study patterning of a cortical structure. Ongoing studies indicate that hippocampal areas or fields are specified many days before birth – possibly involving signals from within the cortical mantle. Although the hippocampal CA fields are distinguished by cytoarchitecture only after birth, molecular differences between fields appear by late gestation. Moreover, these embryonic fields are already specified to develop additional features that characterize the mature fields. The basic division of the hippocampus into fields may be specified still earlier. Thus, if medial cortical neuropil is isolated in vitro early in hippocampal neurogenesis, it can autonomously generate features of a patterned hippocampus. In vivo, the spatial progression of regional fields and environmental cues suggest that signals regulating growth and patterning could arise from sources close to the hippocampal poles. Observations of mouse mutants indicate that the cortical hem, an embryonic structure close to one pole of the hippocampus, is a source of such regulatory signals.

Introduction

How the developing cerebral cortex is patterned into functionally specialized areas has been primarily studied in neocortex (Rakic, 1988; Grove, 1993; Levitt et al., 1993; O’Leary et al., 1994; Gotz, 1995; McConnell, 1995; Levitt et al., 1997). We have focused instead on the patterning of another part of the cortical mantle, the archicortex or hippocampus (Grove et al., 1992; Tole et al., 1997; Tole and Grove, 1998). The hippocampus and neocortex are anatomically different types of cortex, but both are subdivided into areas or fields (Nauta and Feirtag, 1986), and, in the hippocampus, the normal development and function of each field may be critical to the overall role of the hippocampus in memory (Squire, 1987; Tsien et al., 1996).

Questions we are addressing include how the embryonic cortex is initially divided into neocortex and archicortex, and whether distinct mechanisms control the growth of the two types of cortex. When and how is the developing hippocampus subdivided into fields, and are the underlying mechanisms the same as those that regulate area division in the neocortex? Answers to these questions may reveal differences between hippocampus and neocortex, but may also disclose general principles underlying the assembly of any type of cortex.

The hippocampus is an attractive system for an investigation of patterning. First, the anatomy of the rat and mouse hippocampus is comparatively simple (Fig. 1). The single pyramidal cell layer of the hippocampus is divided into only two major fields, CA1 and CA3, and a small transitional field, CA2. The CA fields are capped by the dentate gyrus, the third major field of the hippocampus. Second, for modern studies of embryonic pattern formation, molecular markers of specific regional cell types have been essential (Lumsden and Krumlauf, 1996; Tanabe and Jessell, 1996), and these are now available for the hippocampus. The hippocampal fields were defined in classical studies by differences in cytoarchitecture and connections (Lorente de No, 1934; Blackstad, 1956; Swanson and Cowan, 1977; Swanson et al., 1978), but the adult rodent CA fields and dentate gyrus also differentially express numerous macromolecules (Frantz et al., 1994; He et al., 1989; Woodhams et al., 1989; Motro et al., 1991; Wisden and Seeburg, 1993; Herms et al., 1994; Breder et al., 1995; Schlingen et al., 1995; Ulrich et al., 1995). Some of these macromolecules can also be used as markers of specific regions in the developing mouse hippocampus (Tole et al., 1997) (S. Tole and E.A. Grove, in preparation). This panel of molecular markers makes possible a range of experiments that would be impractical if regional cell type was to be identified solely on the basis of morphological and connectional features. Molecular markers can be used to determine when hippocampal cells first show features of a CA1, CA3 or dentate cell identity, establishing an upper limit in terms of when field identity must be specified. Markers can also be used to determine the field identity of cells in tissue culture experiments designed to investigate the mechanisms of specification. Finally, markers can be used in studies of mouse mutants to analyze defects in hippocampal patterning, growth and differentiation that result from the loss of function of particular genes of interest.

At several sites in the embryonic central nervous system (CNS) signaling centers regulate the patterning and growth of neighboring tissue via secreted signaling molecules (Lumsden and Krumlauf, 1996; Tanabe and Jessell, 1996). To determine if this could be a mechanism underlying cortical patterning, a search was made for signaling centers in the embryonic mouse telencephalon (Grove et al., 1998b). A candidate signaling center was revealed, adjacent to the hippocampus, at the edge or ‘hem’ of the embryonic cerebral cortex (Grove et al., 1998b). The cortical hem expresses several members of the Wnt (Wingless/Int) and Bmp (Bone Morphogenetic Protein) gene families (Furuta et al., 1997; Grove et al., 1998b), which encode secreted signaling proteins implicated in patterning and growth in many embryonic tissues (Graff, 1997; Parr and McMahon, 1994). Thus, the cortical hem could be the source of signals that regulate the development of the hippocampus. To test this hypothesis, we have begun to analyze hippocampal development in mutant mouse lines in which BMP or WNT signaling is affected at the cortical hem (Grove et al., 1998a) (S.M. Lee, S. Tole, A.P. McMahon and E.A. Grove, in preparation).

Materials and Methods

Descriptions of the experimental methods have been given previously (Grove et al., 1992, 1998b; Tole et al., 1997). For all mouse strains examined, midday of the day of vaginal plug discovery was considered embryonic day (E) 0.5. Mice were born at E19.5/P0. For rat strains examined, the day of vaginal plug discovery was termed E0; rats were born at E21.
A Panel of Molecular Markers Distinguishes the Adult CA Fields

A first approach to the problem of how the hippocampus is patterned into different fields is to determine when the fields first begin to appear. Classic morphological differences between CA fields are evident only after birth in the rodent (Zimmer and Haug, 1978). To determine if molecular differences appear earlier, we identified complementary molecular markers that distinguish CA1 and CA3 in the adult mouse. Candidate markers previously reported to be regionally expressed in the rat or mouse hippocampus (He et al., 1989; Woodhams et al., 1989; Motro et al., 1991; Wisden and Seeburg, 1993; Frantz et al., 1994; Herms et al., 1994; Breder et al., 1995) were screened using in situ hybridization and immunohistochemistry. Marker-defined boundaries were compared with boundaries defined by classic cytoarchitectonic criteria (Lorente de No, 1934; Blackstad, 1956; Zimmer and Haug, 1978; Woodhams et al., 1993).

Expression of five molecules, the Py antigen, and KA1, SCIP, NGFIA and Tyro3 mRNA, distinguish unambiguously between adult CA1 and CA3 (Table 1) (Woodhams et al., 1989; Frantz et al., 1994; Lai et al., 1994). All, or virtually all, CA1 pyramidal cells express SCIP (Fig. 2A), NGFIA and Tyro3 (Table 1). All CA3 pyramidal cells express KA1 (Fig. 2B) and are Py-immunoreactive (Py-IR) (Table 1). CA1 and CA3 markers each label a subpopulation of cells in CA2, consistent with the mixing of the CA1 and CA3 cell classes in this transitional field (Woodhams et al., 1993). The mixing of SCIP and KA1-expressing cells in CA2 becomes evident in the first week after birth (Fig. 3B,C). Of the five markers, only Py marks interneurons in addition to CA field pyramidal neurons. Py, moreover, labels interneurons indiscriminately in all CA fields (Woodhams et al., 1989; Tole et al., 1997). Thus, the panel of five markers distinguishes between different CA fields with respect to pyramidal neurons, but not interneurons.

Molecular Markers Identify Embryonic CA Fields

Three of the five CA field markers appear postnatally, even later than morphological differences between the fields can be discerned (Table 2). However, two markers can be detected at E15.5, 4 days before birth, when the hippocampus can be distinguished from adjacent cortex by a morphological landmark (see Fig. 2). By E15.5, SCIP mRNA expression appears in presumptive CA1 (Fig. 2D) and KA1 expression appears in presumptive CA3 (Fig. 2E). Both SCIP and KA1 are expressed by migrating cells in the intermediate zone (IZ), as well as settled cells in the hippocampal cortical plate (Fig. 2D-E). SCIP, but not KA1, is also expressed by scattered cells close to the ventricular surface. The latter SCIP-expressing cells may be postmitotic cells at the start of migration, or precursor cells that are still dividing. If the latter, SCIP is expressed only in a minority of precursor cells in presumptive CA1. SCIP and KA1 thus appear to mark different populations of postmitotic cells, rather than indicating divisions within the germinal layer of the hippocampus. Nonetheless, expression of SCIP and KA1 shows that hippocampal regionalization arises early, both in terms of overall hippocampal development and in the development of individual cells. The hippocampus is divided into presumptive CA fields during the period of neurogenesis, and individual neurons begin to express these markers as they migrate. It is worth noting, too, that the neat register of SCIP and KA1 labeling in the hippocampal cortical plate with labeling in the underlying IZ (dotted lines in Fig. 2D-E) suggests an orderly, predominantly radial movement of pyramidal cells from the ventricular zone (VZ) to their final position.

Like the two embryonic CA fields, the dentate gyrus, not specifically examined by Tole et al. (Tole et al., 1997), can be identified by molecular markers at E15.5 (Tole and Grove, 1998) (S. Tole and E.A. Grove, in preparation). Indeed, all the region-specific molecular markers of the hippocampus we have examined are first detected at about E15.5, suggesting that various regional cell types in the hippocampus begin to differentiate roughly simultaneously at this age (S. Tole and E.A. Grove, in preparation).

None of the hippocampal markers we have examined pick out interneurons, above or below the pyramidal cell layer, in a field-specific manner. Although new markers may be identified that do distinguish interneurons in this way, it may be that non-pyramidal interneurons do not initially differ between hippocampal fields. Their region-specific functions in the mature brain may be shaped by later anatomical context. Such a hypothesis would be consistent with recent observations that non-pyramidal cells disperse broadly through the cortical sheet, and even migrate in from outside the cortex (Soriano et al., 1995; Anderson et al., 1997; Tan et al., 1998).

Embryonic CA Fields Are Specified to Develop Complex, Mature CA Field Identities

The cell populations that make up the presumptive CA fields can be distinguished in the embryo by their expression of SCIP and KA1. Do these cell populations also already differ in their potential to develop further features of CA field identity? To address this question, hippocampal slices were prepared from

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### Table 1

<table>
<thead>
<tr>
<th>Feature</th>
<th>Function</th>
<th>CA1</th>
<th>CA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCIP</td>
<td>POU-domain transcription factor</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>NGFIA</td>
<td>immediate early gene</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Tyro3</td>
<td>receptor tyrosine kinase</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>KA1</td>
<td>glutamate receptor subunit</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Py</td>
<td>undefined antigen</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>
E17.5 mouse brains and presumptive CA1 and CA3 were subdissected out and cultured separately in organotypic explant culture. After 5–21 days in vitro (DIV), explant cultures were examined for continued expression of the early field markers SCIP and KA1, and for the development of three late-appearing features: Tyro3 expression, Py-immunoreactivity, and a difference in cell body size between CA1 and CA3 neurons (see Table 2). Explants of presumptive CA1 and CA3 not only maintained their expression of the early markers, but also developed, appropriately, the late-appearing features of field identity (Fig. 4). Similar field development occurred in cultures prepared at either E17.5 (Fig. 4) or E15.5 (data not shown), the earliest age at which the two presumptive fields can be distinguished. As might be expected, however, not all field-specific features develop in embryonic hippocampal cultures. For example, NGFIA expression, a marker of CA1, did not develop a region-specific pattern, and may therefore depend on cues provided in a normal anatomical context. The striking finding, however, is that the embryonic hippocampal fields that can be identified during neurogenesis are already specified to develop features of a complex, mature CA field identity.

**By E10.5–E12.5 the Medial Telencephalon Can Autonomously Generate a Patterned Hippocampal Primordium**

Although hippocampal field differentiation begins at about E15.5, ongoing studies (Tole and Grove, 1998) (S. Tole and E.A. Grove, in preparation) suggest that field patterning is specified...
earlier. In these experiments, SCIP, KA1 and additional molecular markers are employed to distinguish particular subregions of the embryonic hippocampus (Tole and Grove, 1998; S. Tole and E.A. Grove, in preparation). Explants of the medial telencephalic wall are harvested at E12.5, when none of the regional markers is yet expressed, and the boundaries of the hippocampal primordium cannot be identified by morphology. After 3 DIV, the medial wall explants upregulated expression of hippocampal markers in a region-specific manner that indicates that embryonic CA1, CA3 and dentate gyrus fields have developed in vitro (Fig. 5) (S. Tole and E.A. Grove, in preparation). For many markers, including SCIP and KA1, expression in the cultured E12.5 explants is almost indistinguishable from normal expression in the medial telencephalic wall at E15.5 (compare Fig. 5A,B with C,D). Preliminary observations suggest that hippocampal markers are upregulated in a patterned manner in explants harvested still earlier, at E10.5 (S. Tole and E.A. Grove, in preparation).

Table 2

<table>
<thead>
<tr>
<th>Feature</th>
<th>First field-specific appearance</th>
<th>Expression at birth</th>
<th>Expression in the adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>KA1</td>
<td>E14.5</td>
<td>CA3</td>
<td>CA3</td>
</tr>
<tr>
<td>SCIP</td>
<td>E15.5</td>
<td>CA1</td>
<td>CA1</td>
</tr>
<tr>
<td>Pyramidal cell size</td>
<td>P3</td>
<td>no difference</td>
<td>CA3 &gt; CA1</td>
</tr>
<tr>
<td>Py</td>
<td>P5*</td>
<td>no expression</td>
<td>CA3</td>
</tr>
<tr>
<td>tyro3</td>
<td>P110</td>
<td>no expression</td>
<td>CA1</td>
</tr>
<tr>
<td>NGFIA</td>
<td>P14</td>
<td>no expression</td>
<td>CA1</td>
</tr>
</tbody>
</table>

*In rat (Woodhams et al., 1989).

Figure 4. Embryonic hippocampal fields develop mature CA1 and CA3 identities in vitro. Isolated explants of E17.5 presumptive CA1 (Pca1) and CA3 (Pca3), maintained for 5–21 DIV, display complementary expression of SCIP, KA1, Tyro3 and Py-IR. Camera lucida drawings show that, as in vivo, CA3 neurons in vitro develop a larger cell body size than CA1 neurons. Scale bar (Pca3/Py) = 300 µm for all photomicrographs.

Figure 5. Explants of the E12.5 medial telencephalic wall generate a patterned hippocampal primordium in vitro. (A–D) Medial telencephalic wall explants. The lower boundary of the explant is the free medial edge of the embryonic cortex. At the upper boundary of the explant, the medial wall has been dissected away from adjacent embryonic neocortex. (A,B) Medial wall explants harvested at E15.5 and processed without culturing to show SCIP and KA1 expression in embryonic CA1 and CA3 respectively. (C,D) Medial wall explants harvested at E12.5 and processed after 3 DIV to show SCIP and KA1 expression. E12.5 explants have upregulated SCIP and KA1 expression in longitudinal bands, indicating the development of embryonic CA1 and CA3 fields.
preparation), a surprising finding given that, at E10.5, the medial wall of the telencephalon has only just begun to form.

The E12.5 explants consist of only medial telencephalic neuroepithelium, with all other tissues removed. Thus, the medial telencephalic wall at E12.5 appears either to be prespecified to develop features of a patterned hippocampus, or the signals required for such specification are present in the medial wall itself.

Hippocampal Fields Are Not Defined by Cell Lineage Restriction Boundaries

Accumulating evidence points to an early division of the hippocampus into embryonic fields. Could the hippocampal fields be defined by cell lineage restriction boundaries set up during neurogenesis? According to this hypothesis, each progenitor cell would generate a clone of committed daughter cells that would disperse within a single field. Such a hypothesis would fit both with the early expression of field-specific markers by newborn neurons, and with the apparently orderly migration of \( \text{SCP} \) or \( \text{KA1} \)-expressing neurons towards the hippocampal cortical plate. One way to test this hypothesis directly is to label individual progenitor cells in the embryonic hippocampus with a heritable marker. The daughter cells generated by a given progenitor cell can then be identified later and their dispersion compared with identified hippocampal field boundaries.

In a set of previous experiments (Grove et al., 1992), the BAG virus, which carries the marker gene lacZ, was injected into the cerebral vesicle of rat embryos at the peak of hippocampal neurogenesis in the rat. Individual progenitor cells in the VZ were infected by the virus and transmitted the lacZ gene to their daughters. Brains of injected animals were analyzed during the third postnatal week when hippocampal field boundaries are morphologically mature. Cells that had inherited the lacZ gene were identified using a histochemical stain for \( \beta \)-galactosidase, and labeled neurons were distinguished from glia by their staining patterns, a method of cell type identification that was verified by intracellular Lucifer yellow injections. Lucifer yellow filled the cells completely and revealed such typical neuronal features as axons and dendritic arbors (Grove et al., 1992).

In brains infected with a low titer of BAG retrovirus, labeled hippocampal neurons appeared in small, widely separated groups of 2–6 neurons. Each labeled group was presumed to be a group of sister cells derived from an individual progenitor cell (supporting statistical analysis has been given previously (Grove et al., 1992)). Almost all of the labeled sister cell groups appeared in the pyramidal cell layer of the hippocampus, and Lucifer yellow staining confirmed that most labeled cells were pyramidal cells, rather than interneurons. This predominance of pyramidal cells was expected given that most hippocampal interneurons are generated before the date of retroviral injection (Altman and Bayer, 1990b).

A striking finding was that sister pyramidal neurons appeared in tight clusters, with neighboring sister cells only 100–200 \( \mu \)m apart in the mature hippocampus (Fig. 6A–D). Examination of brains from litters harvested at E19 indicated a migration pattern consistent with such a finding. At E19, a typical labeled sister cell...
group formed a roughly radial array as the cells migrated from the VZ to the hippocampal cortical plate (Grove et al., 1992). These findings fit with those reported in a variety of studies utilizing electron microscopy, transgene expression and mouse chimera analysis, all indicating a predominantly radial migration pattern for hippocampal pyramidal cells (Nowakowski and Rakic, 1979; Goldowitz, 1989; Soriano et al., 1995).

Surprisingly, given the tight clustering of labeled sister cells, their dispersion was not confined by hippocampal field boundaries (Fig. 6A–D). Figure 6D shows in composite form the subset of labeled sister cell groups, derived from 49 hippocampi, that lay close to field borders. Out of 25 sister cell groups, 10 crossed borders between CA3, CA2 and CA1, or between CA1 and the subiculum (Fig. 6D). Sister cell groups that crossed between CA fields could contain both large CA3-type pyramidal neurons, and small CA1-type pyramidal cells (Fig. 6A).

A statistical analysis tested the possibility that the border-crossing cell groups in the sample were actually composites of two different sister cell groups each of which respected the border. Because the surface area covered by each sister cell group (<0.3 mm²) is so small compared with the available surface area of the hippocampus (estimated at 40 mm²), and because the brains analyzed contained an average of fewer than three such labeled sister cell groups per hippocampus, the probability that the sample of border-crossing groups was made up of such composites was infinitesimally small (Grove et al., 1992). These findings indicate that hippocampal progenitor cells can generate neurons for more than one hippocampal field, and, therefore, that the borders between fields are not defined by cell lineage.

Use of multiple retroviruses with distinguishable tags has since shown that at least some clones containing hippocampal cells spread more widely in the cortical sheet (Reid et al., 1995). Most dramatically, clones are observed that cross from the hippocampus into the neocortex (Reid et al., 1995). These clones could include non-pyramidal interneurons, which appear to disperse more broadly across the cortex than most pyramidal cells (Anderson et al., 1997; Tan et al., 1998). Whatever the composition of such clones, however, their existence indicates that progenitors are not restricted even in generating cells fated for archicortex rather than neocortex.

**Initial Field Patterning Is Not Established by Hippocampal Afferents**

By analogy with a patterning mechanism proposed for neocortex (Allenboer and Shatz, 1994; O’Leary et al., 1994; McConnell, 1995), the hippocampus might receive field patterning cues from extrinsic innervation. However, the major hippocampal afferents arrive too late, at least for the initial subdivision of the hippocampus into fields. Afferents from the septum arrive in the mouse hippocampus at E17.5, and afferents from entorhinal cortex reach the dentate gyrus at E19.5 (Super and Soriano, 1994). Although some entorhinal afferents reach CA1 earlier, at E16.5 (Super and Soriano, 1994), this is still 1 day after embryonic CA1 cells begin to express SCIP, and many days after the isolated telencephalic medial wall is able to develop a patterned hippocampal primordium.

**Nested Expression of Emx Genes Is Not Required for Hippocampal Field Patterning**

In the hindbrain, the nested expression of HOX gene family members reflects the role of these genes in regulating rhombo-meric identity (Lumsden and Krumlauf, 1996). It is therefore intriguing that the vertebrate homologs of the *Drosophila* head gap gene *empty spiracles*, Emx1 and Emx2, are expressed in a nested pattern that defines the most medial part of the embryonic mouse cerebral cortex (Simeone et al., 1992a,b). This pattern suggests that Emx genes function in subdividing the developing medial cortex. Directly supporting such a hypothesis, the loss of functional Emx2 expression results in the apparent specific deletion of the dentate gyrus, as judged by morphology (Pellegrini et al., 1996; Yoshida et al., 1997). A possible explanation is that the region of the neuroepithelium that generates the dentate gyrus is defined by the nested expression of Emx genes, and that in the absence of Emx2, the dentate gyrus is never specified (Yoshida et al., 1997).

In a collaborative study with Peter Gruss and Guy Goudreau (Max Planck Institute, Göttingen, Germany), we analyzed the Emx2 mutant further, using molecular markers to ascertain if hippocampal field patterning is generally disrupted. Surprisingly, with respect to molecular markers, hippocampal field patterning is preserved in Emx2 homozygote mutants. In particular, a dentate gyrus cell population is identifiable by molecular markers, even though no morphological dentate gyrus is seen (S. Tole, G. Goudreau, S. Assimacopoulos and E.A. Grove, in preparation). The hippocampus in the mutant is much smaller than in wildtype mice, reflecting an overall shrinkage and apparent developmental delay in the cortex (Pellegrini et al., 1996; Yoshida et al., 1997) (S. Tole, G. Goudreau, S. Assimacopoulos and E.A. Grove, in preparation). The lack of a morphological dentate gyrus in the Emx2 mutant may therefore be due to an immaturity of the mutant cortex, rather than to a failure to specify dentate gyrus cells.

These findings underscore the need to analyze hippocampal defects in mutant mice with a range of tissue-specific markers. Particular care may be needed in establishing the loss, rather than delayed appearance, of the dentate gyrus. Dentate gyrus neurons continue to be generated many days, or even weeks after neurogenesis in the other hippocampal fields is over (Altman and Bayer, 1990a). Thus, a mutation that induces a general developmental delay in the cortex is likely to have a greater apparent impact on the dentate gyrus than on any other hippocampal field.

**The Pattern of Early CA Field Differentiation Suggests a Source of Specifying Signals**

Expression patterns of SCIP and KAI indicate that early hippocampal field differentiation progresses from the ends, or poles of the hippocampus inwards (Tole et al., 1997). Initially, cells near the dentate and subicular poles of the hippocampus express KAI and SCIP respectively, but a broad stretch of the hippocampus in between expresses neither marker (Figs 2D,E,3A). As development proceeds, the two fronts of SCIP and KAI expression advance inwards (Fig. 3B,C). This marked poles-inward progression appears unique to early field-specific differentiation, and is not seen with more general features of hippocampal development (Tole et al., 1997), suggesting the hypothesis that signals that specify field identity also move inwards from the hippocampal poles.

**Signals from the Cortical Hem Regulate Hippocampal Development**

Signaling centers at tissue boundaries provide growth and patterning cues in the embryonic CNS (Lumsden and Krumlauf, 1996; Tanabe and Jessell, 1996). The early embryonic patterning of the hippocampal anlage suggests that similar patterning mechanisms could be at work in the medial telencephalon, at
least as early as E12.5, and that a source of patterning cues might be positioned near one pole of the hippocampus. A directed search for signaling centers in the embryonic telencephalon has revealed a candidate that is adjacent to the dentate pole of the hippocampus, the cortical hem (Grove et al., 1998b).

To identify potential telencephalic signaling centers, we used as a clue the characteristic expression of members of the Wnt gene family at signaling centers in the neural tube (McMahon and Bradley, 1990; Parr et al., 1993; Parr and McMahon, 1994; Bally-Cuif et al., 1995). A polymerase chain reaction procedure (Gavin et al., 1990) was employed to isolate several members of the Wnt gene family from cDNA derived from E12.5 telencephalon. Three Wnt genes, Wnt2b, Wnt3a and Wnt5a, were found to be expressed selectively in a longitudinal band at the medial margin of the E12.5 telencephalon (Fig. 7A, C) (Grove et al., 1998b). This Wnt-rich zone is composed of cortical neuroepithelium, and thus represents the edge, or ‘hem’ of the embryonic cerebral cortex (Grove et al., 1998b). A second family of powerful developmental control genes, the Bmp gene family, is also represented in the cortical hem, which expresses Bmp2, 4, 6 and 7 (Furuta et al., 1997; Grove et al., 1998b) (Fig. 7B, D).

The cortical hem is first identifiable by Wnt3a expression at E9.5 when the telencephalon is a single vesicle. At this stage, the cortical hem, a single strip of Wnt3a-expressing tissue, lies at the dorsal midline of the telencephalon in a position analogous to that of the roofplate in the spinal cord and brainstem. By E10.5, the dorsal midline begins to invaginate to form the two medial walls of the telencephalic hemispheres, and the cortical hem is split into two and pushed ventrally (Fig. 7G). By E11.5, the cortical hem in each hemisphere is marked by expression of three Wnt genes, Wnt2b, 3a and 5a, and telencephalic choroid plexus epithelium (CPE) begins to differentiate below the cortical hem. After about E12.5, the cortical hem clearly forms a boundary between the embryonic hippocampus and the telencephalic CPE, with the hippocampus developing dorsal to the cortical hem, and the CPE differentiating ventral to the hem (Fig. 7E, F).

By its position and expression of members of the Wnt and Bmp gene families, the cortical hem therefore deserves investigation as a potential source of signals that direct development of the hippocampus. Observations of two mutant mouse lines support such a role.

The extra-toes/3 mutation is a spontaneously occurring mutation that causes defects in both neural and limb development (Hui and Joyner, 1993). Mice that are homozygous for the extra-toes/3 mutation are deficient in both Wnt and Bmp expression in the dorsomedial telencephalon, so that no cortical hem can be identified (Grove et al., 1998a,b). The loss of an identifiable cortical hem is accompanied by a disruption in the development of both flanking structures. Neither the telencephalic CPE nor the hippocampus is detectable by molecular or morphological markers in homozygote extra-toes/3 mutants (Franz, 1994; Grove et al., 1998a,b).

The extra-toes/3 mutant mouse carries a mutation in Gli3, a vertebrate homolog of the Drosophila gene cubitus interruptus (ci) (Schimmang et al., 1992; Hui and Joyner, 1993). In Drosophila embryonic pattern formation, ci activates expression of decapentaplegic and wingless, Drosophila members of the Bmp and Wnt gene families respectively (Kapshing and Kunes, 1994; Von Ohlen et al., 1997). Observations of the extra-toes/3 mutant suggests that Gli3, likewise, regulates expression of Wnt and Bmp genes in the mouse dorsal telencephalon (Grove et al., 1998a,b).

Because the extra-toes/3 mutant mouse shows deficiencies in the expression of several Wnt and Bmp genes in the dorsal telencephalon, it is not possible to ascribe the apparent loss of the hippocampus to any particular gene defect. Moreover, it is possible that the disruption of hippocampal and CPE development is due directly to the loss of Gli3 expression, rather than to abnormalities at the cortical hem. However, a more selective gene expression deficiency at the cortical hem has also been found to disrupt hippocampal development (S.M. Lec, S. Tole, E.A. Grove and A.P. McMahon, in preparation). In a mouse line that lacks functional expression of Wnt3a (Takada et al., 1994), the earliest identified Wnt gene to be expressed selectively at the cortical hem, the developing hippocampus appears by morphology to be truncated. Molecular markers, too, indicate that the hippocampal CA fields and dentate gyrus are absent in Wnt3a homozygote mutants, or are represented by tiny populations of residual cells. By contrast, the neighboring neocortex and telencephalic CPE appear to be relatively unaffected.
Functional Wnt3a expression at the cortical hem therefore appears to be required for hippocampal development.

The deletion of flanking structures around a defective cortical hem is reminiscent of the deletions of brainstem structures seen in mice with signaling defects at the isthmus (McMahon and Bradley, 1990). The isthmus is a well-characterized signaling center at the boundary between the midbrain and the hindbrain that directs both brainstem growth and patterning of the midbrain along the anterior–posterior axis (McMahon and Bradley, 1990; Bally-Cuif et al., 1992; Crossley et al., 1996). A challenge now will be to determine if the cortical hem plays an analogous role in the telencephalon to that of the isthmus in the brainstem, and if so, which signaling components may direct polarity and patterning of the hippocampus.

Discussion

Our findings indicate that the mouse hippocampus begins to differentiate into embryonic fields during the peak of hippocampal neurogenesis (Fig. 8). At this stage, the CA1 and CA3 fields begin to express characteristic molecular features that continue to define these fields into adulthood. The ability of embryonic CA1 and CA3 to develop additional field-specific features in culture indicates, further, that the embryonic fields are already specified to acquire complex, mature field identities.

The initial specification of CA1 and CA3 may begin as early as E10.5–E12.5 (Fig. 8). When isolated at this stage, the beginning of hippocampal neurogenesis, the medial wall of the telencephalon can autonomously generate features of a patterned hippocampus. However, whether the medial wall explants are already patterned when they are placed into culture, or whether specifying cell-cell interactions occur in vitro is not yet clear. Patterning signals could be provided, for example, by the cortical hem, which is difficult to remove without also damaging adjacent tissue.

The ability of medial telencephalic explants to generate an intrinsically patterned hippocampal primordium supports an emerging theme: a surprising degree of autonomy in hippocampal development. Ongoing cues from outside the medial telencephalon, such as those supplied by ingrowing afferent axons (Fig. 8), appear not to be required for the initial division of the hippocampal primordium into fields, for the early differentiation of the fields, or even for the appearance of much later features of field identity. The early autonomy of hippocampal development does not seem to include, however, an early commitment of hippocampal precursor cells to generating neurons for particular fields. Hippocampal precursor cells can generate neurons for more than one field (Grove et al., 1992), and perhaps for neocortical areas as well (Reid et al., 1995). Environmental cues appear to be needed to direct development of field identity during the period of hippocampal neurogenesis. One possible source of such cues is the cortical hem, which lies next to the embryonic hippocampus.

Analysis of the Wnt3a mutant mouse provides strong evidence that cortical hem signaling is required for hippocampal development, and observations of the extra-toes Lhx5−/− mutant provide supportive evidence. Do BMP and WNT signals from the cortical hem regulate hippocampal growth or patterning or both? That the hippocampus in Wnt3a mutant mice is severely shrunken or missing, rather than transformed into a different tissue, may suggest a requirement for tissue growth. However, it is possible that Wnt3a is involved in assigning cells to hippocampal cell fates, and that without correct specification, cells die or fail to proliferate, resulting in a grossly shrunken hippocampus. BMP and WNT signaling have been reported to regulate cell type specification, regional patterning and tissue growth in different developmental contexts (Parr and McMahon, 1994; Thelessl et al., 1995; Fan et al., 1997; Graff, 1997; Ikeya et al., 1997; Liem et al., 1997; Marcell et al., 1997). Further experiments are underway to resolve these issues.

Findings from the Wnt3a-deficient mouse suggest that WNT signaling is needed very early in telencephalic development to establish or maintain the hippocampal primordium. Wnt genes show functional redundancy at several sites in the developing embryo (McMahon and Bradley, 1990; Ikeya et al., 1997); thus, the functional loss of one Wnt gene from the cortical hem, where multiple Wnt genes are expressed, might be expected to have little effect. A possible explanation for the dramatic hippocampal defect seen in the Wnt3a-deficient mouse is that Wnt3a is expressed selectively at the cortical hem before other Wnt genes. Thus, WNT signaling may be crucial for hippocampal development in a narrow time window between E9.5, when Wnt3a is first detected at the cortical hem, and E11.5, when other Wnt genes are expressed. An implication is that the hippocampal primordium may begin to form as early as E9.5 (S.M. Lee, S. Tole, E.A. Grove and A.P. McMahon, in preparation).

A final interesting feature of the Wnt3a-deficient mouse is that the hippocampus is missing or severely shrunken, whereas the immediately adjacent medial neocortex is almost normal in size. Archicortex and neocortex, therefore appear to come under the control of partially different developmental mechanisms. Wnt3a is required for development of archicortex, but not neocortex (S.M. Lee, S. Tole, E.A. Grove and A.P. McMahon, in preparation).

In other parts of the developing CNS, differential expression of homeobox genes indicates the involvement of these transcription factors in early patterning (Tanabe and Jessell, 1996). Emx genes show an intriguing differential expression pattern in the medial telencephalon, but a specific role for nested Emx expression in hippocampal patterning is not supported by our findings. Nonetheless, the early specification and patterning of the hippocampus prompts a renewed search for transcription factors that are differentially expressed in the neuropil of the medial telencephalon. One such transcription factor, the LIM homeobox gene Lhx5, is expressed selectively in the cortical hem and in the likely hippocampal primordium at early stages of hippocampal neurogenesis (Zhao et al., 1999). Disruption of Lhx5 function produces striking defects on either side of the cortical hem: telencephalic CPe fails to develop and hippocampal differentiation is disrupted (Zhao et al., 1999).
These findings suggest that Lhx5 plays a role in the hippocampus and CPe that may be similar to the role played by other LIM homeobox genes in directing differentiation of cell types in the spinal cord and pituitary (Zhao et al., 1999). Of great interest will be the results of ectopically expressing Lhx5 to determine if this alone can direct cells to differentiate as specific hippocampal or CPe cell types. Meanwhile, a directed search for additional transcription factors selectively expressed in the medial telencephalon should continue to shed light on the molecular mechanisms of hippocampal patterning, growth and differentiation.

To date, our findings place constraints on possible models of hippocampal patterning, but do not yet strongly support a particular model. Indications that the hippocampus is patterned in early neurogenesis suggest that specifying cues act on dividing progenitor cells, which then confer particular field identities on their daughters. Certainly, newborn hippocampal neurons appear to know their destination area as they migrate. Moreover, the register of SCIP and KA1 labeling in the early hippocampal cortical plate with labeling in the underlying IZ suggests that most field-specified neurons move in an orderly, radial manner towards their final positions. Thus, positional identity is specified early in the life history of the hippocampal neuron, and apparently maintained in many cells as they migrate.

It is not yet clear how to reconcile these findings with the ability of hippocampal progenitor cells, like neocortical progenitor cells, to generate neurons for more than one area (Grove et al., 1992; Walsh and Cepko, 1992; Reid et al., 1995). In particular, a model of hippocampal patterning in which the daughter cells of lineage-committed progenitor cells faithfully carry positional information by migrating in strict radial arrays from the VZ to the cortical plate is not supported by our findings. Retroviral labeling indicates that sister hippocampal cells that migrate close together in near-radial arrays can still cross hippocampal field boundaries (Grove et al., 1992). However, field boundaries were identified in previous studies by morphology alone. New methods are now available both for identifying hippocampal boundaries with molecular markers (Tole et al., 1997), and for following the dispersion of clonally related cells (Tan et al., 1998). It will be useful to analyze with these new methods the spread of hippocampal clones with respect to field divisions defined by molecular markers.

Meanwhile, several models of hippocampal development can be proposed that are consistent with findings summarized here. Specification signals may act both on dividing progenitor cells and on postmitotic neurons to shape the final field identity of hippocampal neurons. In one scenario, hippocampal neurons are already specified to a particular field identity when they are born, but some mixing of different cell types initially occurs between fields. Regulatory signals then act on postmitotic neurons to refine the field boundaries. Perhaps reflecting such developmental processes, narrow transitional fields are retained in the adult hippocampus. CA1 and CA3 are separated by CA2, which contains a mixture of CA1- and CA3-type pyramidal cells, and CA1 is separated from the subiculum by another narrow transitional field, the prosubiculum (Grove et al., 1992). Whether boundary sharpening actually occurs in the developing hippocampus is unlikely to be established by in situ hybridization or other static cell labeling methods. In future experiments, cells may need to be followed in real time as they maintain or alter their field-specific identities.

Does the early field patterning of the hippocampus occur as part of a general division of the early embryonic cortex into cytoarchitectonic areas? Suggesting that this might be so, hippocampal fields resemble neocortical areas in that both are defined by cytoarchitectonic and connectional criteria (Nauta and Feirtag, 1986). However, mature neocortical areas and hippocampal fields also differ in that neocortical areas form a patchwork pattern, whereas hippocampal fields form roughly longitudinal bands along the anterior–posterior axis of the cortex. This organization is apparent in flattened maps of the mature hippocampus (Swanson et al., 1978) (Fig. 6D), and in the embryo (Fig. 5A,B, in which the hippocampus has not yet rolled up. Thus, hippocampal field specification might not occur as part of a general division of the cortex into cytoarchitectonic areas. Rather, hippocampal patterning may be part of an early division of the cortical sheet into strips that run along the anterior–posterior axis, and could include the division of neo- from archicortex.

Future investigation of hippocampal patterning may reveal general principles of cortical development, or simply elucidate how the hippocampus itself is assembled. In either case, the hippocampus is increasingly the focus of studies of the development of pattern and connectivity in a cortical structure (Grove et al., 1992; Pellegrini et al., 1996; Del Rio et al., 1997; Tole et al., 1997; Yoshida et al., 1997; Cedental et al., 1998; Zhao et al., 1999). An underlying theme of these studies is the application of new genetic and molecular biological tools, brought to bear on a system whose anatomy and functions have been clarified by decades of classic studies.

References


