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The Time of Origin of Neurons in Ammon's Horn and the Associated Retrohippocampal Fields*

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Summary. The time of origin of the neurons in the hilus of the dentate gyrus, in the *regio superior* and *regio inferior* of Ammon's horn, and in the following retrohippocampal fields – the subiculum, presubiculum, parasubiculum, medial and lateral entorhinal areas and the perirhinal cortex, has been determined in the rat, by the technique of ^{3}H-thymidine autoradiography. In each field the cells are generated over a limited period of about five days, with the majority being formed in a 24–48 h period. As in the neocortex, the cells in the various hippocampal fields are generated in a distinct "inside-out" sequence with respect to the ependymal zone in which they arise. In addition there are two distinct gradients along the transverse, or dentato-rhinal axis, of the formation. Thus the neurons in the hilar region of the dentate gyrus (including field CA_4) tend to arise earlier than the pyramidal cells in the *regio inferior,* and these in turn, are on average, generated earlier than those in the *regio superior* or in the *subiculum.* In the retrohippocampal region there is a comparable gradient extending medially from the perirhinal cortex to the presubiculum, the cells in the lateral entorhinal area being formed, on average, earlier than those in the medial entorhinal cortex, and these, in turn, are generated earlier than those in the para- or presubiculum. There is no evidence for a dorso-ventral (or septo-temporal gradient) in any part of the hippocampal formation like that found in the dentate gyrus. Cell counts indicate that there are over 320,000 pyramidal cells in the *regio superior* (field CA_1) and about 150,000 in the *regio inferior* (fields CA_2 and CA_3).

Key words: Ammon's horn – ^{3}H-thymidine autoradiography – Proliferative gradients – "Inside-out" sequence – Retrohippocampal fields.

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Introduction

In a previous study (Schlessinger et al., 1975) we described the sequence of neurogenesis in the *stratum granulosum* of the dentate gyrus of the rat. Here we report the sequence of neuronal proliferation in the hilus of the fascia dentata, in the two principal cytoarchitectonic fields of Ammon's horn, often referred to as the *regio inferior* and the *regio superior,* and in the various retrohippocampal cortical areas associated with the subicular complex (the parasubiculum, subiculum and presubiculum) and the entorhinal and perirhinal areas. Although to a considerable extent this analysis repeats the classical ^{3}H-thymidine autoradiographic study of Angevine (1965) on the mouse hippocampus, three considerations have prompted us to place our observations on record.

First, the majority of recent experimental studies on the morphology and connectivity of the hippocampal region, and its capacity for synaptic rearrangement following partial deafferentation (see Swanson and Cowan, 1977; Swanson et al., 1978, for references), have been carried out in the rat, rather than the mouse. Second, Angevine's study focussed specifically on the time of origin of cells in the temporal or caudal third of the hippocampus. While this was adequate for the identification of the "inside-out" gradient in the formation of the neurons in all parts of the hippocampal region (except the dentate gyrus in which the granule cells are generated in an "outside-in", sequence), it was unsuitable for determining whether or not there are comparable gradients in neurogenesis along the temporo-septal axis of the hippocampal formation. Our recent observations on the associational and commissural connections of the dentate gyrus (in which there is a clear temporo-septal gradient in the time of cell origin) have served to emphasize that an understanding of the relevant developmental sequences may throw considerable light on certain patterns of connectivity (Gottlieb and Cowan, 1972, Fricke and Cowan, 1978). Third, it is a matter of some interest to know whether in a closely inter-related system of connections such as exists in the hippocampal formation (see Blackstad, 1956; Raisman et al., 1965; Gottlieb and Cowan, 1973; Swanson and Cowan, 1977; Swanson et al., 1978) there is some recognizable sequence in the time of cell genesis, or in the general pattern of cell proliferation.

Materials and Methods

The majority of the brains used for this study were from the series described previously (Schlessinger et al., 1975) and also used for our analysis of the time of neuron origin in the septal region (Swanson and Cowan, 1976). Pregnant albino rats were given a single injection of tritiated thymidine (^{3}H-thymidine, specific activity 6 μCi/mMol, Schwarz Biochemicals) in a dose equivalent to 5 μCi/gm body weight, at different gestational stages from the 12th day until just before birth (on day 20/21). Most of the offspring were sacrificed towards the end of the third postnatal week, by vascular perfusion with 10% formalin, and their brains removed and prepared for paraffin embedding. Serial sections were cut at 10 μm in the horizontal and frontal planes and a 1-in-10 series of sections from each brain was processed for autoradiography in the manner described by Cowan et al. (1972). Since the appearance of the entire hippocampal region is most clearly displayed in horizontal sections, the systematic analysis of the location of ^{3}H-labeled neurons was carried out on the horizontal sections. The location of every *heavily labeled neuron* (characterized by 15 or more silver grains over the nucleus) was marked on outline drawings of the relevant region in every tenth section throughout the entire dorso-ventral extent of the

hippocampus. The outline drawings were made with a Leitz projection apparatus and the position of the labeled cells determined with a high power objective, using readily identifiable landmarks for the precise location of each neuron. For the sake of clarity, in the accompanying figures the heavily labeled cells are marked by filled circles which are appreciably larger than the actual cells but their relative positions are accurately represented.

As in our previous study of the dentate gyrus, we have thought it of interest to estimate the numbers of pyramidal cells in the *regio superior* and *regio inferior* of the Ammon's Horn. The cell counts were done in two ways. For the first estimate, the volume of the entire *stratum pyramidale* of field CA_1 (which is generally considered equivalent to the *regio superior)* and of fields CA_3 and CA_2 (which together constitute the *regio inferior)* was determined by first computing the area of the entire stratum from a 1-in-10 series of horizontal sections through the hippocampal formation, with the aid of the acoustical data tablet/computer system described by Cowan and Wann (1973) and by measuring the mean thickness of the sections (10.3 μm). The mean density of the cells in the *stratum pyramidale* was then estimated from nucleolar counts, using an oil-immersion objective, and sampling a number of fields proportional to the area of the stratum at each level. Knowing the volume of tissue in each oil immersion field and the total volume of the stratum, the total population of pyramidal cells could be readily computed. The actual counts were then subjected to the Abercrombie (1946) correction [1], using figures of 2.7 μm and 2.2 μm for the mean diameters of the nucleoli in fields CA_3 and CA_1, respectively. For the second estimate (which was done by our colleague, Dr. Gary Banker) each field was treated as if it were of uniform width, so only its linear dimensions were measured on the data tablet, and the mean numbers of neuronal nucleoli/unit length of each field, counted. Since this estimate gave significantly lower figures than that based on areal measurements, a second "areal measurement" was made, using the same mean nucleolar density estimate used for the "linear estimate". Since the results of this and the first count were in good agreement, no further cell counts were attempted.

Results

I. The Organization of the Hippocampal Formation in the Rat

To facilitate the subsequent presentation of our data on the time of origin of neurons in the various cytoarchitectonically defined fields under consideration, it will be helpful to begin with a brief account of the organization of the hippocampal formation in the rat, and the terminology that will be used in this study. For more detailed analyses of the entire region reference should be made to the studies of Blackstad and his colleagues, which are based not only on Nissl-stained preparations but also on a variety of histochemical procedures (Blackstad, 1956; Geneser-Jensen and Blackstad, 1971; Mellgren and Geneser-Jensen, 1972; Haug, 1974, 1976). The subdivisions which we have recognized for the purposes of this study are illustrated in Figure 1, which is a low-power photomicrograph of a horizontal section through the temporal third of the hippocampal formation at the level indicated in the inset diagram.

The area in question extends from the medial border of the cerebral hemisphere marked by the hippocampal fissure, to the rhinal sulcus, which separates the so-called limbic or rhinencephalic cortical structures from the neocortex. Starting at the hippocampal fissure nine readily distinguishable cytoarchitectonic fields can be recognized. These are: the dentate gyrus, the hilar region of the dentate gyrus

[1] As we were only interested in obtaining an approximate figure for the total numbers of neurons in each field, we have not attempted to apply one of the more sophisticated correction factors such as those listed in Konigsmark (1970) or more recently given by Hendry (1976) and Wimer (1977).

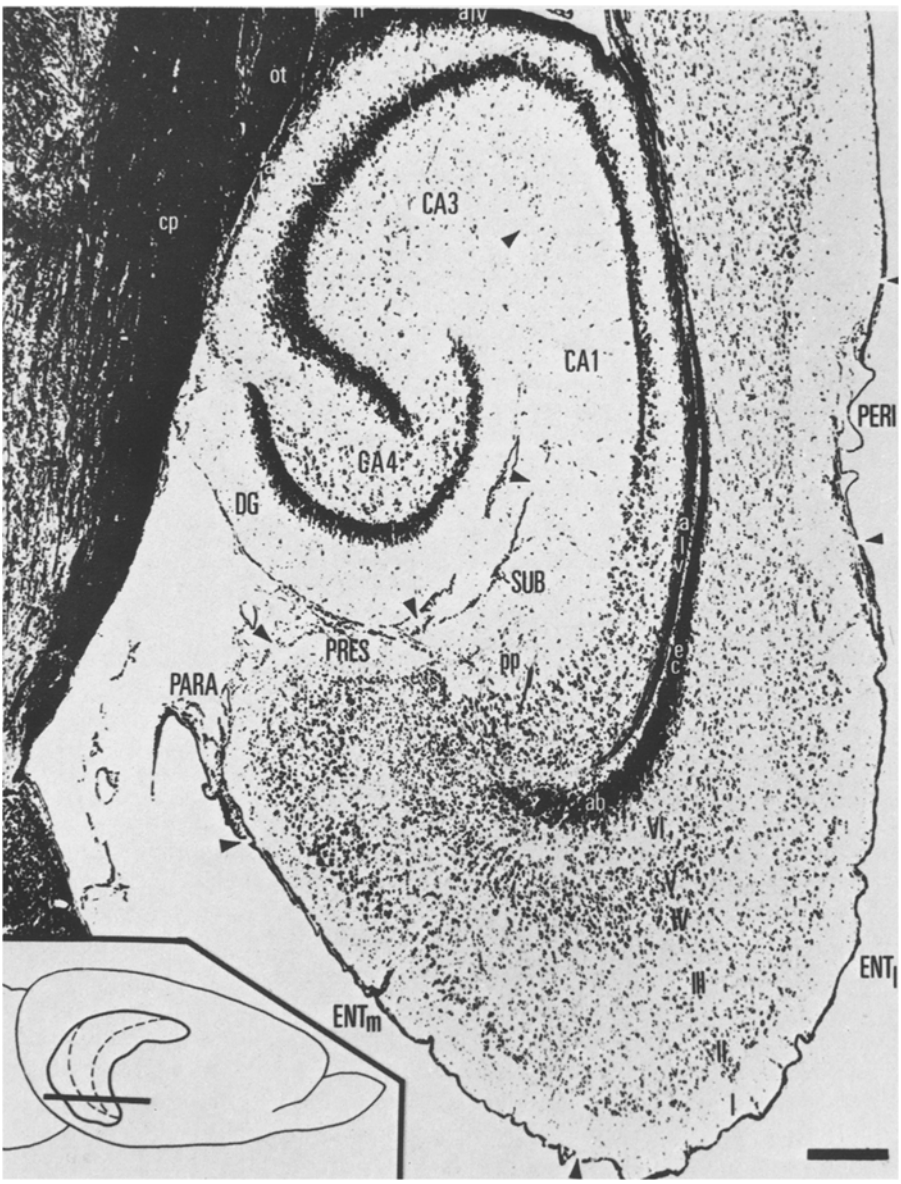

Fig. 1. A low-power photomicrograph of a horizontal section, stained by the Klüver-Barrera method, through the temporal third of the hippocampal formation at the level indicated in the inset diagram. Abbreviations used in this and some of the subsequent illustrations: *ab*: angular bundle; *alv*: alveus; CA_1, CA_3: fields of Ammon's horn; CA_4: hilus of dentate gyrus (including cells of field CA_4); *cp*: cerebral peduncle; *DG* dentate gyrus; *EC*; external capsule; Ent_i, Ent_l, Ent_m: intermediate, lateral and medial parts of the entorhinal cortex; *fi*: fimbria; *ot*: optic tract; *PARA, PRES*: para- and presubiculum; *pp*: perforant path; *SUB*: subiculum. Scale 250 μm. Modified from Swanson and Cowan (1977) with permission

(which for convenience we will refer to simply as field CA_4[2]), the *regio inferior* of Ammon's horn (which we have labeled CA_3 in Figure 1[3]), the *regio superior* (which corresponds to field CA_1 as defined by Lorente de Nó, 1934), the subiculum (including the prosubiculum of Lorente de Nó), the presubiculum (area 27) and parasubiculum (area 49), the medial and lateral parts of the entorhinal cortex (area 28), and the cortex around the rhinal sulcus which we shall refer to as the perirhinal area (area 35). As we have previously dealt with the development of the granule cells of the dentate gyrus (Schlessinger et al., 1975) we shall make no further reference to it here. Finally, since all the illustrations have been derived from the horizontal series of sections the terms dorsal and ventral should be understood to refer only to the location of the sections in the dorso-ventral series; they do not correspond to the terms "dorsal" or "ventral" hippocampus which some authors have used without reference to the cytoarchitectonic distinctions.

II. The Number of Cells in Ammon's Horn

After applying the appropriate correction factor (Abercrombie, 1946) our first count of the numbers of cells in the hippocampus (based on its linear dimensions) gave a figure of 277,000 for the number of pyramidal cells in the *regio superior* and 147,000 for the *regio inferior,* or a total of 422,000 for the entire Ammon's horn (excluding field CA_4). The more careful of the two areal counts gave corrected figures of 328,000 for the *regio superior* and 162,000 for the *regio inferior* (for a total of 490,000 pyramidal cells). The corresponding figures for the second areal count were 312,000 for the *regio superior,* 144,000 for the *regio inferior,* and 456,000 for the total number. Thus although it is customary to think of the *stratum pyramidale* as a uniform layer of cells, some neurons in both CA_1 and CA_3 are loosely scattered superficial and deep to the main body of the lamina, and for this reason the greatest weight should be placed on the areal counts which made allowance for this scatter. The finding that the *regio superior* contains about twice the number of pyramidal neurons as the *regio inferior* may at first be surprising in view of the fact that its total area is only about two-thirds that of the *regio inferior* ($22\,mm^2$ vs $30\,mm^2$ – Swanson et al., 1978). However, the cell density in the *stratum pyramidale* is significantly greater in field CA_1; in our material the density of pyramidal neurons in CA_1 was about 2.8 cells/1000 μm^3, whereas in CA_3 it was only 1.4 cells/1000 μm^3.

[2] There is still a good deal of controversy over the precise identification of the cells which comprise the hilar region of the dentate gyrus. The majority of the cells are certainly intrinsic to the hilar region and should more correctly be referred to either as the polymorphic zone of the dentate gyrus or as the "hilus fasciae dentatae" (Blackstad, 1956). In some mammals the terminal part of Ammon's horn is reflected within the hilar region and has accordingly been designated as field CA_4 by Lorente de Nó (1934). We have elsewhere used the term CA_4 operationally for those neurons which send their axons outside the hilar region to the dentate gyrus of both sides (Swanson, et al., 1978). Since in Nissl preparations the various cell types which comprise the hilar zone cannot be distinguished, we shall here refer to the entire region as the "hilar zone" or as field CA_4; the two terms are used interchangeably and without any special connotation.

[3] The most lateral part of the *regio inferior* is a transition zone between field CA_{3a} of Lorente de Nó (1934) and field CA_1. This narrow transition zone is frequently referred to as field CA_2. In the present analysis, the cells of field CA_2 have been included with those of field CA_3 under the general term *regio inferior*.

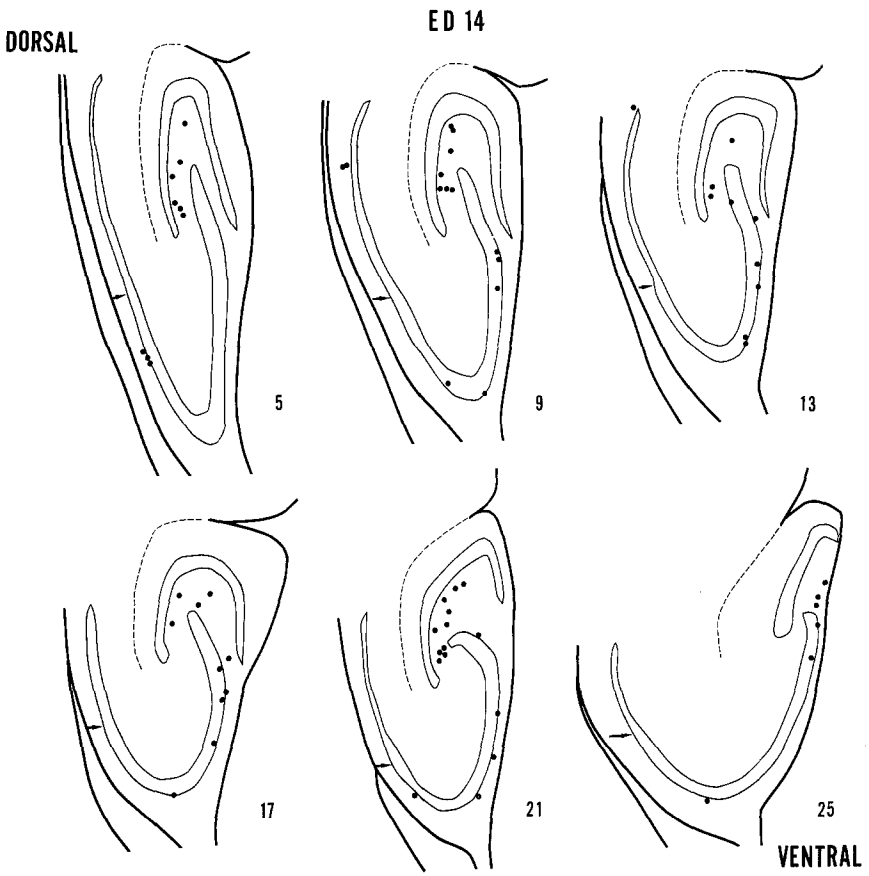

Fig. 2. A series of tracings of horizontal sections through the hippocampus in a 3-week old rat whose mother had received an injection of ^{3}H-thymidine on the 14th day of gestation (ED 14). In this and the subsequent figures the location of every heavily-labeled neuron is indicated by black dots. For the sake of clarity the size of the dots is considerably larger than the actual cells, but their relative locations are correctly indicated. The numbers beside each tracing refer to their positions within the dorso-ventral series.

These figures for packing density correlate well with our measurements of perikaryal diameters in the two regions; the mean diameter of the pyramidal cells in field CA_1 (measured across their short axis) was found to be 11 μm, whereas in field CA_3 it was 19 μm. Our findings on the numbers of cells in the *regio inferior* are in good agreement with those of Gaarskjaer (1978) who has estimated that there are 143,000 cells in the *regio inferior* of the rat and a further 31,900 in the hilar region (which was not included in our counts).

As we have pointed out elsewhere, it is somewhat surprising that the number of dentate granule cells exceeds the number of neurons in the *regio inferior* by such a considerable factor (Schlessinger et al., 1975). Our own earlier estimate of the number of cells in the dentate gyrus suggested that in the rat there are approximately 630,000 granule cells; however, in a more recent study Gaarskjaer

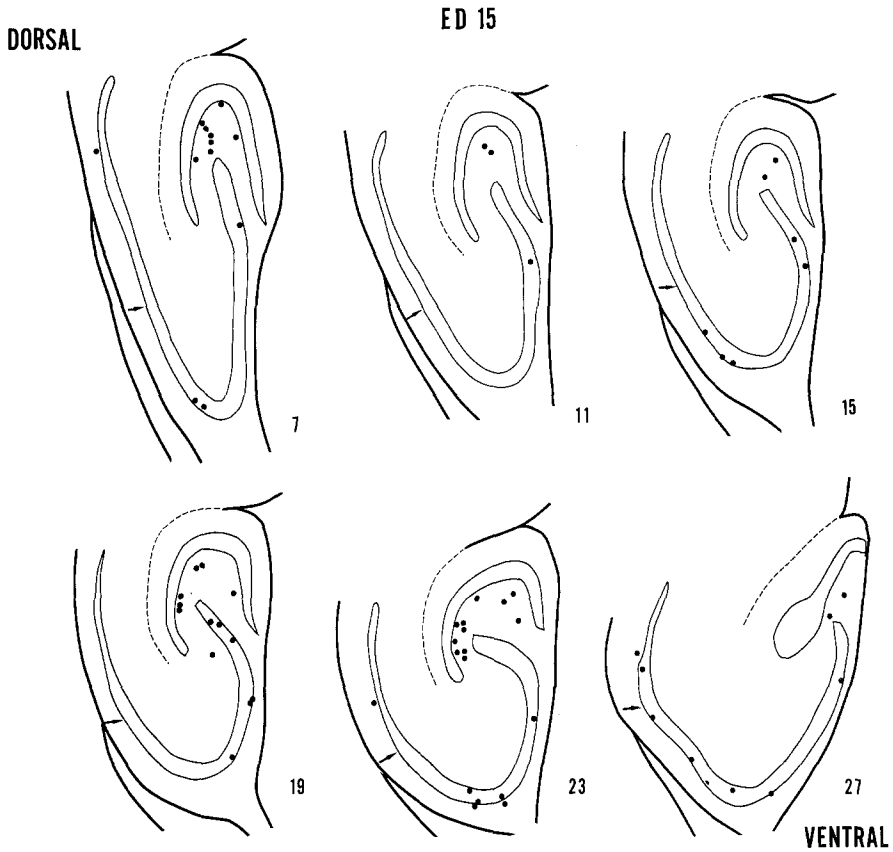

Fig. 3. The distribution of heavily labeled neurons in a rat labeled on embryonic day 15 (ED 15)

(1978) suggests that the number may be as high as 980,000.[4] Thus the total number of granule cells exceeds the number of cells in the *regio inferior* (including those in the hilus) by between 5 and 6 times, but as Gaarskjaer (1978) has pointed out, over the septo-temporal extent of the two structures the relative numbers of the two classes of cells varies quite markedly, the granule cell: pyramidal cells ratio being highest at the septal end and lowest near the temporal end of the hippocampus. It is also noteworthy that the total number of fibers in the fimbria (measured near its septal end) exceeds the total number of pyramidal cells in Ammon's horn by at least a factor of 2 (Wyss et al., 1978). This finding is less surprising in view of the fact that the fimbria is known to be a complex bundle containing, in addition to the axons of the Ammonic pyramids, commissural fibers linking the hippocampal formations of the two sides, septo-hippocampal afferents, and a substantial number of projection fibers from the subicular complex (Swanson and Cowan, 1977).

[4] The discrepancy in the two estimates is probably due to differences in the counting and sampling procedures. Gaarskjaer's estimate was based on nucleolar counts, whereas that of Schlessinger et al., was based on counts of granule cell nuclei. In addition different corrections were applied.

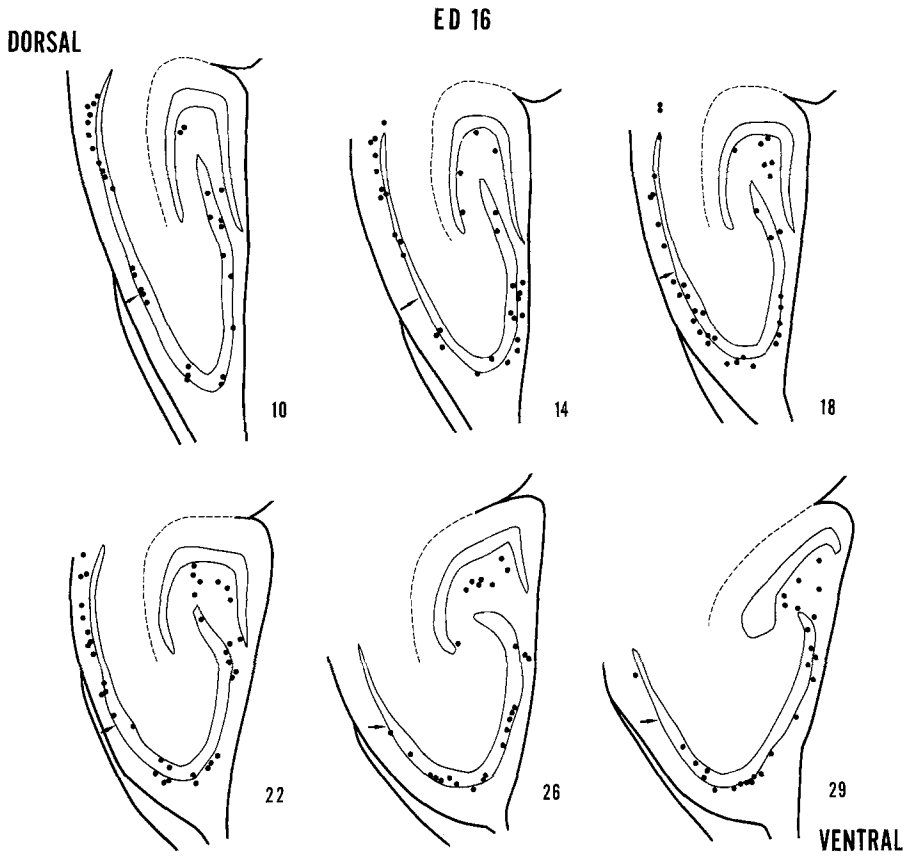

Fig. 4. The distribution of heavily labeled neurons in a rat labeled on embryonic day 16 (ED 16)

III. The Time of Origin of the Cells in Ammon's Horn and in the Hilar Region of the Dentate Gyrus.

For convenience we have separated our analysis of the time of origin of the cells of the hippocampal formation into two parts. Here we shall consider the *regio inferior*, the *regio superior*, and the cells in the hilar region of the dentate gyrus. The locations of the heavily labeled neurons evident on each successive day from the 14th through the 20th days of gestation are shown in figures 2 through 8. From these it is clear that the earliest neurons to be generated (i.e., to complete their last mitotic division) are located in the hilar region on embryonic day 14 (no heavily labeled cells being seen in the brains of animals whose mothers were injected with ^{3}H-thymidine on the 12th or 13th days of gestation. At ED14 relatively few neurons are found elsewhere in Ammon's horn, and it is noteworthy that at this stage most of the heavily labeled

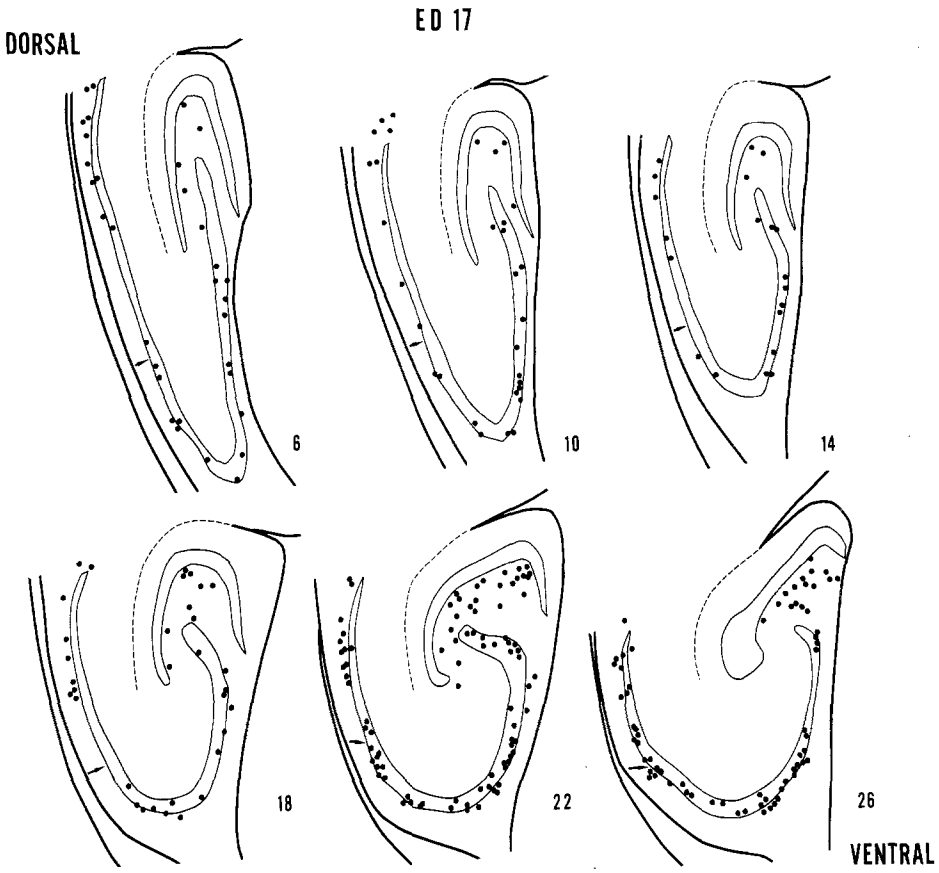

Fig. 5. The distribution of heavily labeled neurons in a rat labeled on embryonic day 17 (ED 17)

cells in the hilar region are related to the inner[5] blade of the dentate gyrus, and that the few heavily labeled neurons seen in Ammon's horn are all located along the deep aspect of the *stratum pyramidale,* where it abuts upon the *stratum oriens.* After injections of ^{3}H-thymidine on ED15 a similar pattern of labeling is seen, but rather more heavily labeled neurons are present throughout Ammon's horn than on ED14. In the animals injected on ED16 there is a significant increase in the proportion of heavily labeled cells found deep to the outer blade of the dentate gyrus, and at this stage the majority of the labeled neurons is in the *regio inferior* of Ammon's horn. However, a significant number of heavily labeled neurons is also seen in the deep part of field CA_1.

[5] In this paper, as in our study of the associational connections of the hippocampal formation (Swanson et al., 1978), we shall refer to the two blades of the dentate gyrus as "inner" and "outer". Thus the *inner* blade corresponds to the "lateral", "suprapyramidal", or "dorsal" blade of other workers. Similarly, the term *outer* blade refers to the "medial", "infrapyramidal", or "ventral" blade.

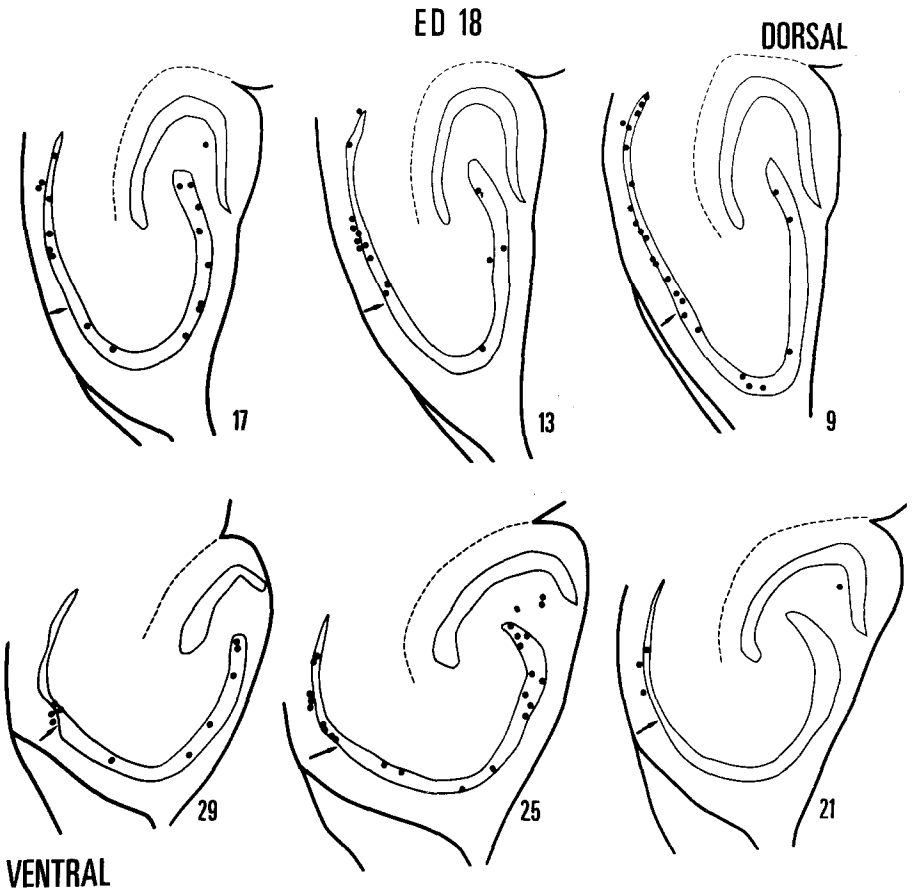

Fig. 6. The distribution of heavily labeled neurons in a rat labeled on embryonic day 18 (ED 18)

Essentially the same labeling pattern is seen in the animals injected on ED17, although at this stage most of the labeled cells in the *regio inferior* are clearly in the deep part of the *stratum pyramidale* and in the adjacent part of the *stratum oriens.* By ED18 the proliferation of neurons destined for the hilar region of the dentate gyrus has essentially ceased, and at this time most of the pyramidal cells in Ammon's horn lie in the midportion of the *stratum pyramidale.* In the animals labeled on ED19 the major focus of proliferative activity can be seen to have moved toward the *regio superior,* but scattered labeled cells are also seen in the *regio inferior* throughout its mediolateral extent. By ED20, which is just before birth, proliferative activity in Ammon's horn is at a low level; only a few heavily labeled neurons are seen in animals injected at this stage, and these are located in subfield CA_{3c} and in field CA_1.

This data can be presented in summary fashion in the form of a graph in which the percentage of the total number of heavily labeled neurons seem in each

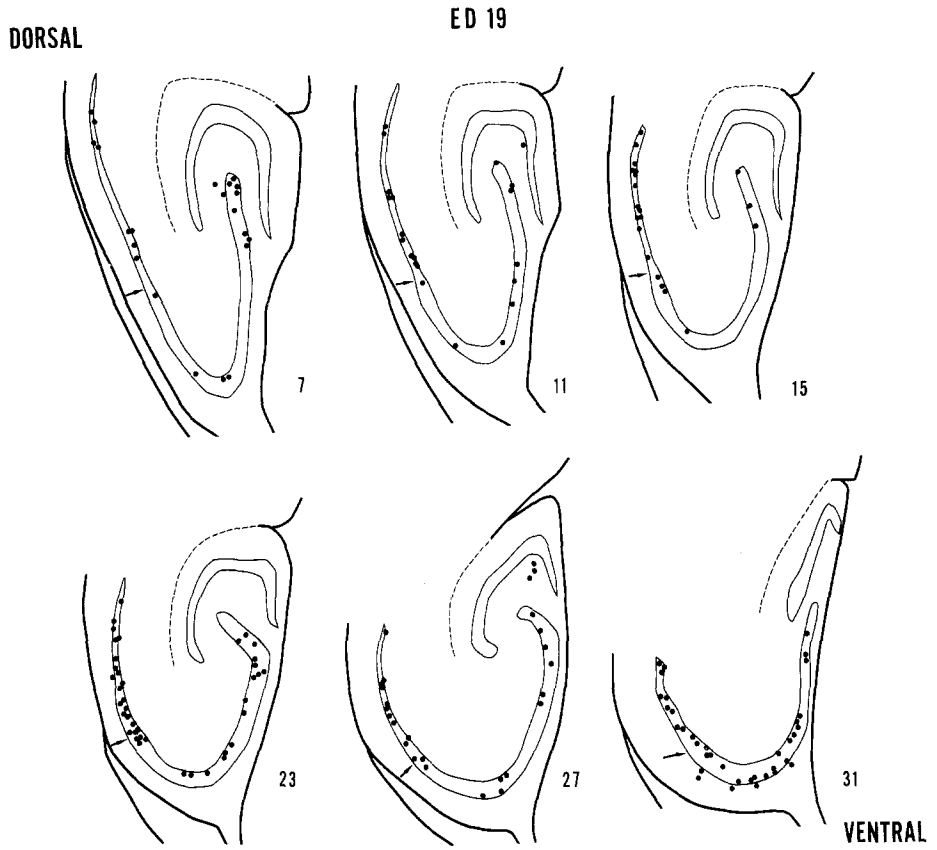

Fig. 7. The distribution of heavily labeled neurons in a rat labeled on embryonic day 19 (ED 19)

cytoarchitectonic field is plotted as a function of the day on which the ^{3}H-thymidine was administered to the pregnant mothers (Fig. 9). From this it is clear that the entire population of neurons in Ammon's horn is generated over a period of seven days, from ED14 through ED20. However, the time span and the rate at which they are produced in each field is not uniform. The cells destined for the hilar region of the dentate gyrus appear to begin proliferation sooner, and also to end their phase of proliferation at an earlier stage, than those destined for Ammon's horn. More than 90% of the neurons in the hilar region are generated by ED17. On the other hand, the *regio inferior* lags behind this by fully 24 h; thus by ED15 less than 5% of the neurons in the *regio inferior* are generated, and it is not until ED19 that 95% of the neurons have become post-mitotic. When Ammon's horn is viewed as a whole it is evident that the cells in the *regio inferior* are generated over an intermediate period, since not only do they lag behind those in the hilar region by about one day, but they also lead those in the *regio superior* by about the same period. In other

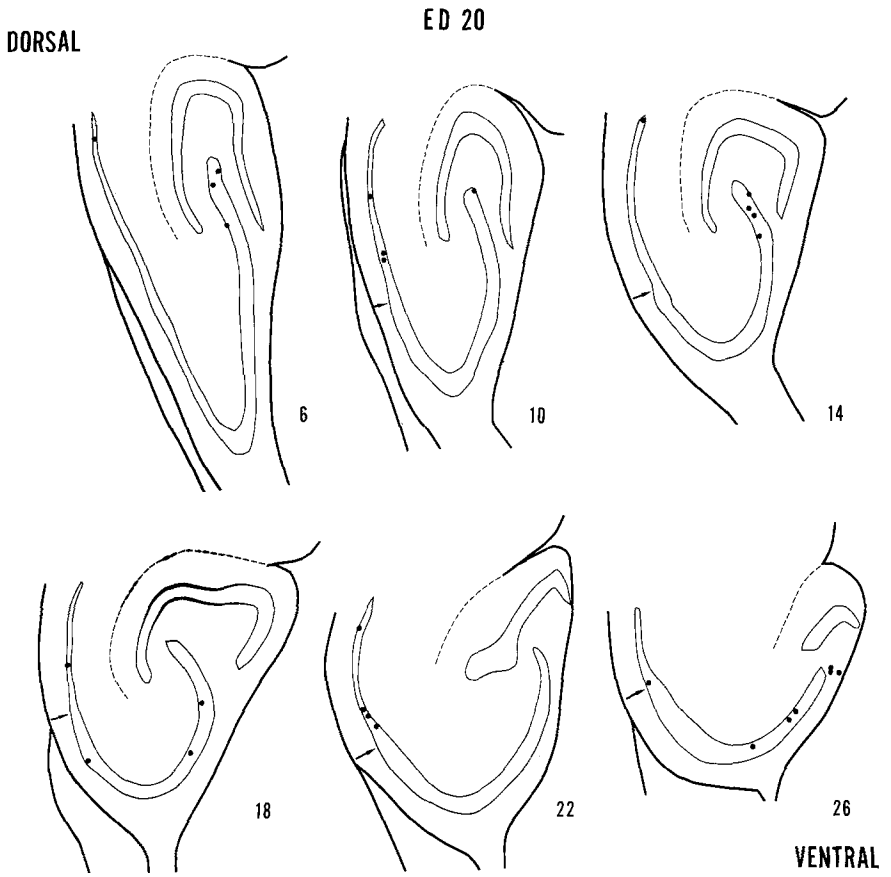

Fig. 8. The distribution of heavily labeled neurons in a rat labeled on embryonic day 20 (ED 20)

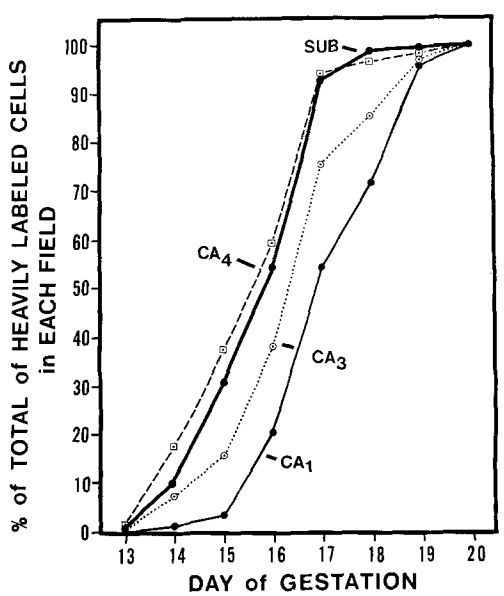

Fig. 9. A series of four curves to show the relative times of origin of cells in the *regio inferior* (CA_3 and CA_2), the *regio superior* (CA_1), the hilus of the dentate gyrus (CA_4) and the subiculum (SUB)

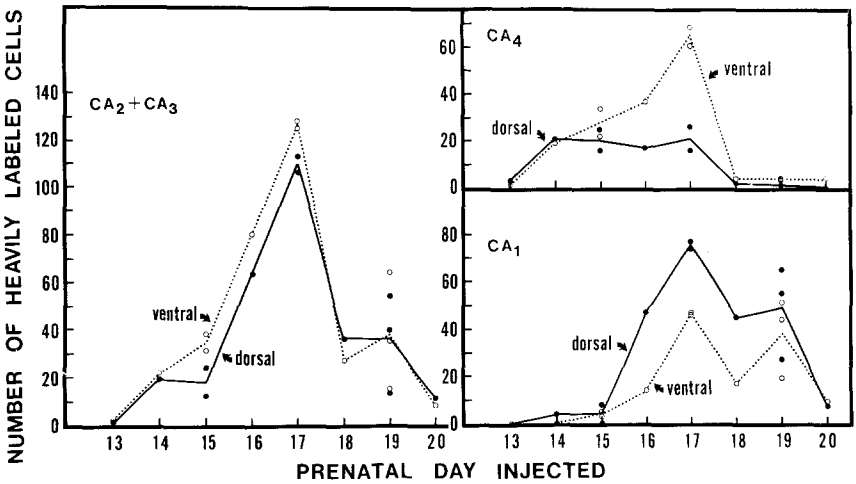

Fig. 10. To show the relative times of origin of pyramidal cells in the dorsal and ventral halves of each of the major fields of the hippocampus. The data are taken from horizontal sections of the brains of animals whose mothers were injected with ^{3}H-thymidine on one or other day between the 13th day of gestation and just before birth

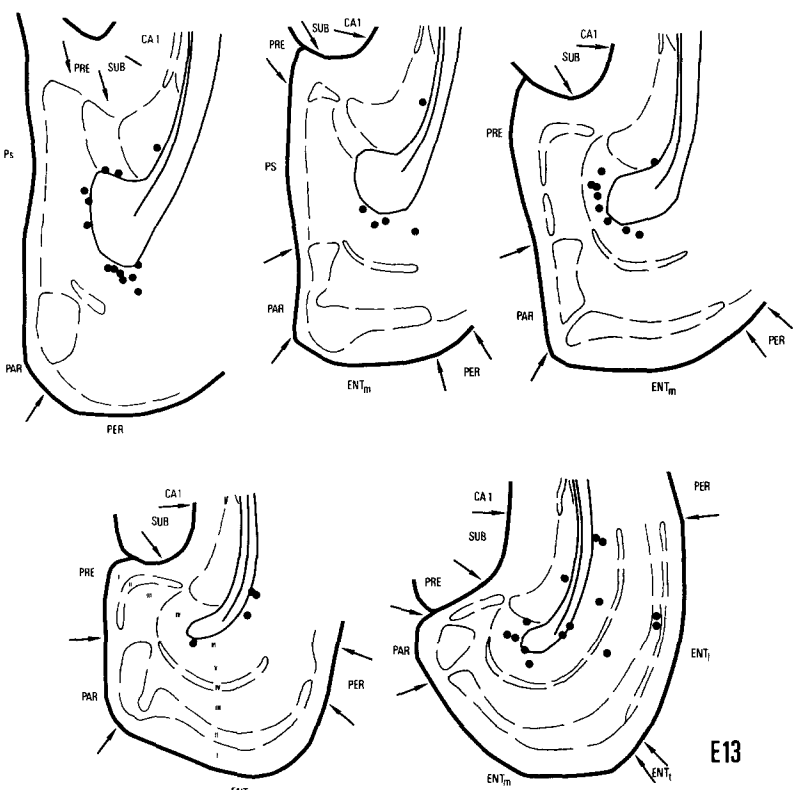

Fig. 11. The location of heavily labeled cells in the retrohippocampal fields of the subiculum (*SUB*), pre- and parasubiculum (*PRE, PARA*), the medial and lateral entorhinal areas (ENT_m, ENT_l) and the perirhinal cortex of an animal labeled with ^{3}H-thymidine on the 13th day of gestation (ED13). The arrows mark the boundaries between adjoining fields, and the numbers alongside each tracing indicate its position within the dorso-ventral series

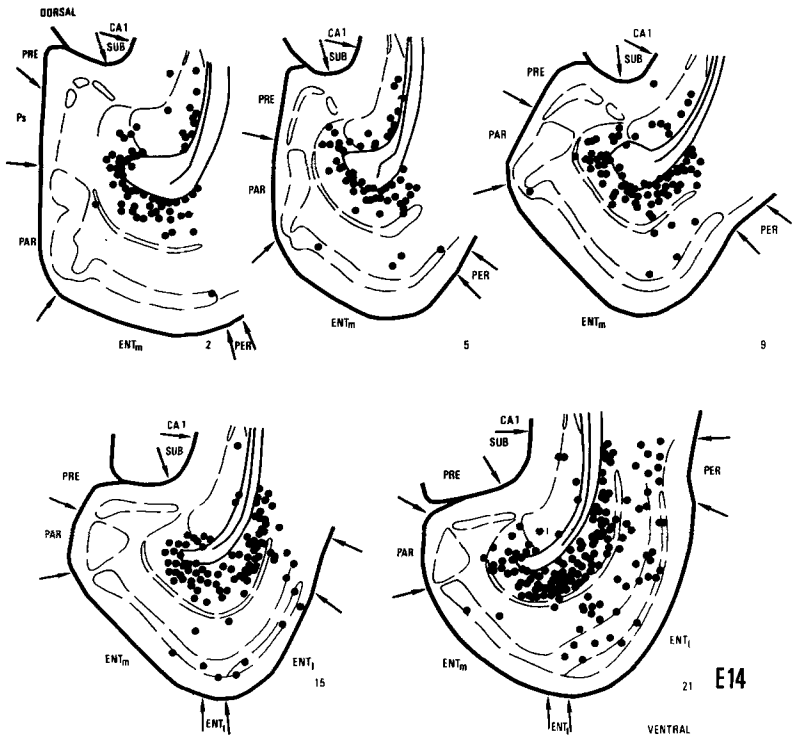

Fig. 12. The position of cells generated on embryonic day 14 (ED14)

words there is a distinct proximo-distal gradient (with respect to the dentate gyrus) in the time of origin of the neurons of Ammon's horn, even though in each field the period of peak proliferation is quite short (of the order of 2, or at the most, 3 days (see Fig. 9).

We have been unable to find evidence for a comparable gradient in the time of neurogenesis along the septo-temporal axis of Ammon's horn. In neither our frontal nor horizontal series of sections, is there any suggestion of a difference in the period of peak neuron proliferation in the septal or temporal thirds of the various cytoarchitectonic fields. In Figure 10 we have plotted the number of heavily labeled neurons observed in the dorsal and ventral halves of the *regio inferior,* the *regio superior,* and the hilar region based on the horizontal series of sections used for Figures 2 through 8. It is clear from these graphs that there are no obvious differences between the dorsal and ventral parts of Ammon's horn (which correspond roughly to the septal and temporal halves along the longitudinal axis of the hippocampus). On the other hand, as we have noted above, there is a clear "inside-out" gradient in the time of origin of the pyramidal cells in both the *regio superior* and *regio inferior* comparable to that which is found in other cortical fields (excluding the dentate gyrus).

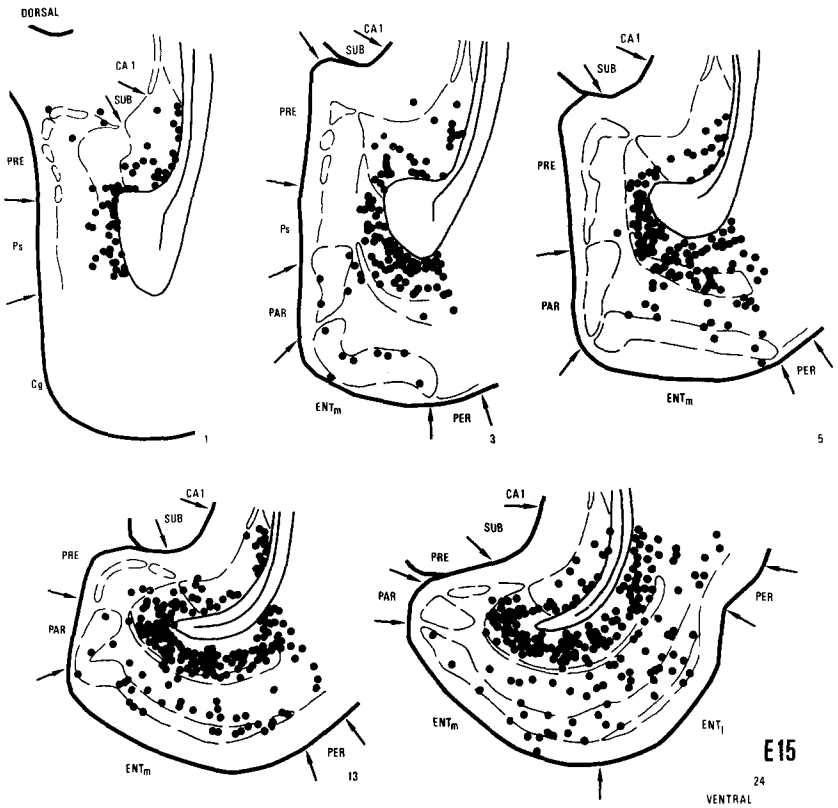

Fig. 13. The position of cells generated on embryonic day 15 (ED15)

IV. The Time of Origin of the Cells in the Retrohippocampal Region

The results of our analysis of the pattern of cell proliferation in the various cortical fields between Ammon's horn and the rhinal sulcus are shown diagrammatically in Figures 11 through 18. From this it is evident that the earliest neurons to be generated in the retrohippocampal region are found in the deepest layers of each of the relevant fields following ^{3}H-thymidine injections as early as the 13th day of gestation (ED13). And interestingly, a few neurons among the more superficial layers (II and III) of the lateral entorhinal area are also labeled at this stage. That the latter finding is not fortuitous is borne out by the observation that in the animals injected on ED14 a considerable number of heavily labeled neurons is found in layers II, III and IV of the lateral entorhinal area, in addition to the large number found in the deeper layers of this and the adjoining fields. The peak period of proliferation for the lateral entorhinal area appears to be around ED15 at which time heavily labeled neurons are found throughout its depth. At this stage some neurons in the intermediate layers (II and III) of the medial entorhinal area also appear heavily labeled, but in the more medial fields (including the para- and presubiculum) the heavily labeled neurons remain mostly confined to the deeper

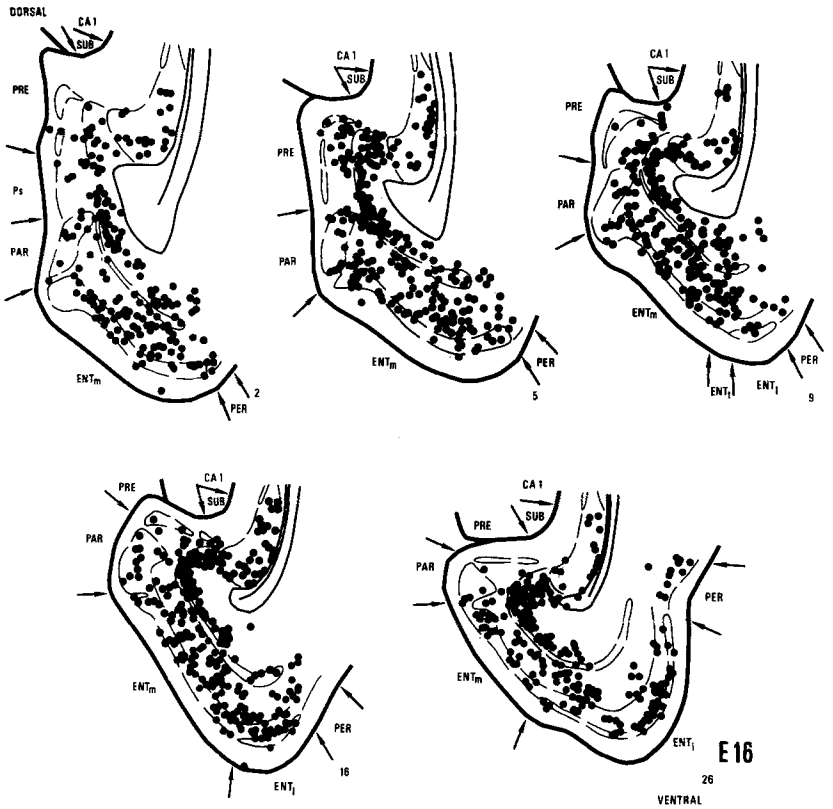

Fig. 14. The position of cells generated on embryonic day 16 (ED16)

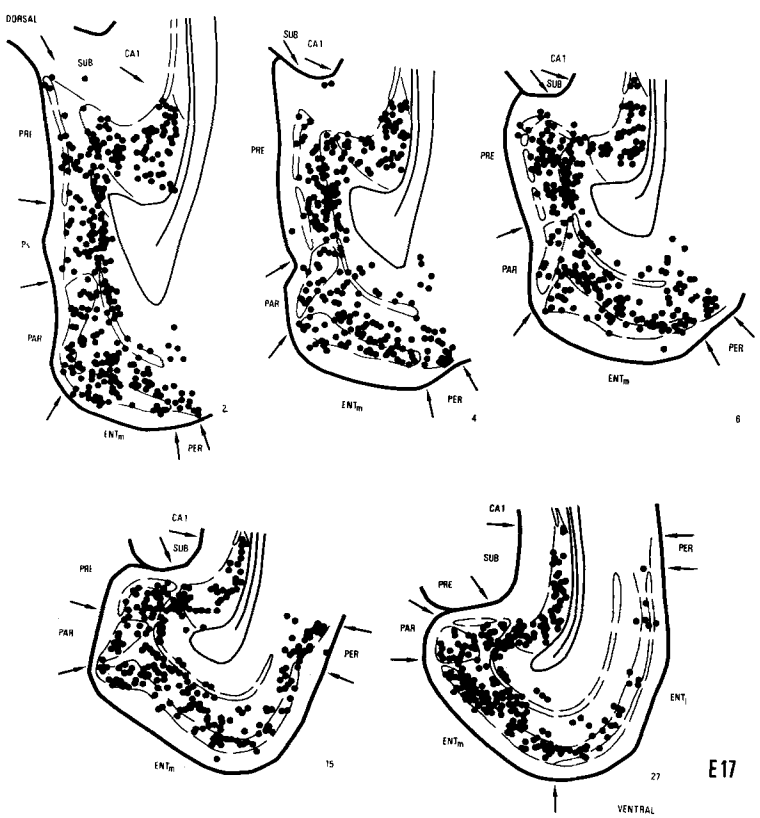

Fig. 15. The position of cells generated on embryonic day 17 (ED17)

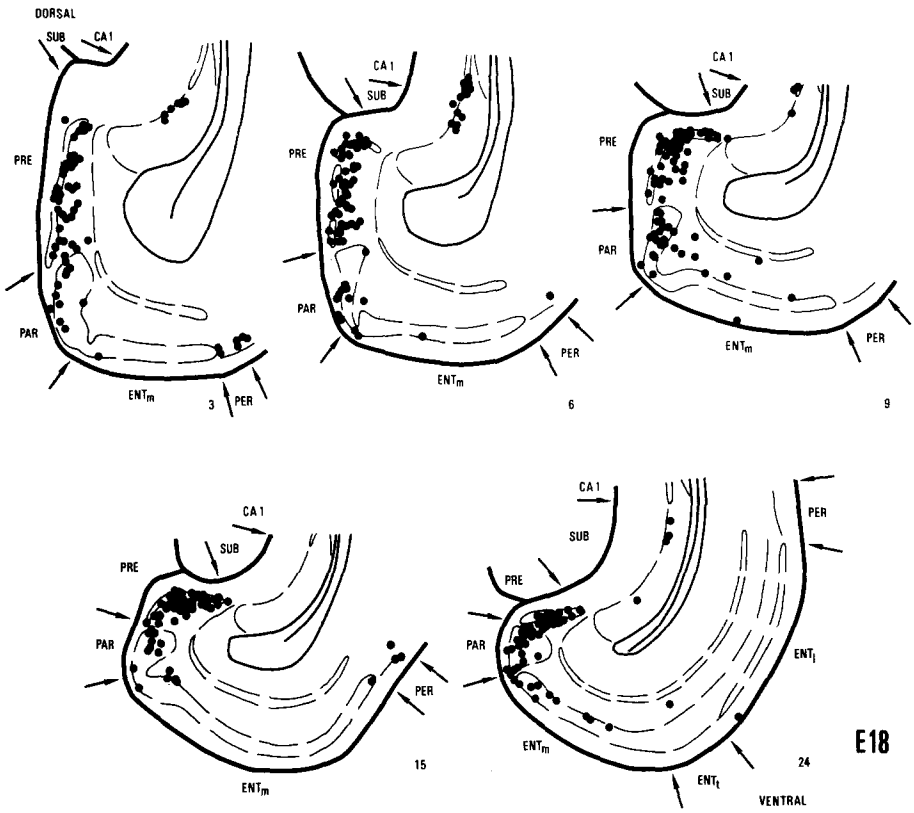

Fig. 16. The position of cells generated on embryonic day 18 (ED18)

layers. By the time that a significant number of heavily labeled neurons is seen in the intermediate cellular layers of the pre- and parasubiculum, cell proliferation is at a relatively low level in the lateral entorhinal area, and the heavily labeled neurons that are seen in this field are mainly in its superficial two layers.

This pattern persists through ED17, and by ED18 essentially no labeled neurons are seen in the lateral entorhinal area and only relatively few are seen in the superficial layers of the medial entorhinal area. At this stage there is still a considerable number of labeled neurons in the pre- and parasubiculum and in the more superficial part of the pyramidal layer of the subiculum. Finally, by ED19, there are relatively few heavily labeled cells in the retrohippocampal region and almost all of these adjoin the relatively cell-free molecular layer of the pre- and parasubiculum. In animals injected on the day before birth only occasional labeled neurons are seen throughout the region.

It is clear from this that there is a gradient of peak neurogenesis from the perirhinal area laterally to the presubiculum medially, with the perirhinal and the lateral part of the entorhinal area leading the pre- and parasubiculum by about 24 to 48 h. The subiculum itself is formed over a more restricted period of time than the pre- and parasubiculum or the *regio superior,* cell proliferation in the subiculum

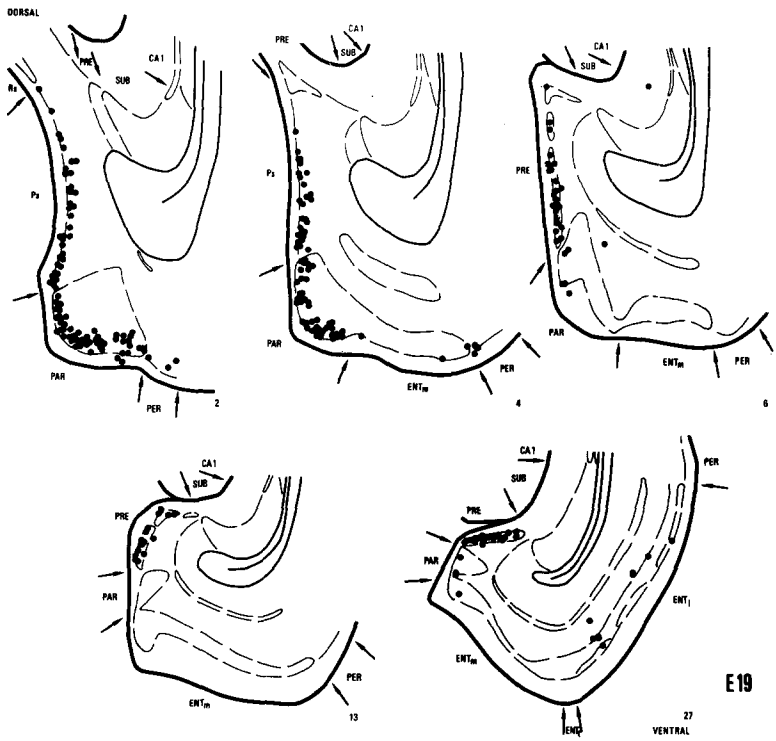

Fig. 17. The position of cells generated on embryonic day 18 (ED19)

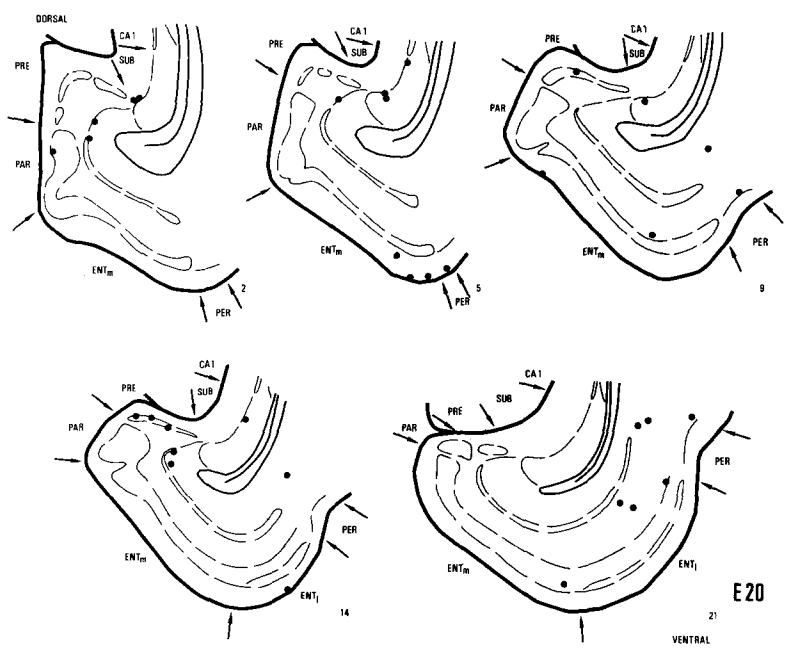

Fig. 18. The position of cells generated on embryonic day 20 (ED20)

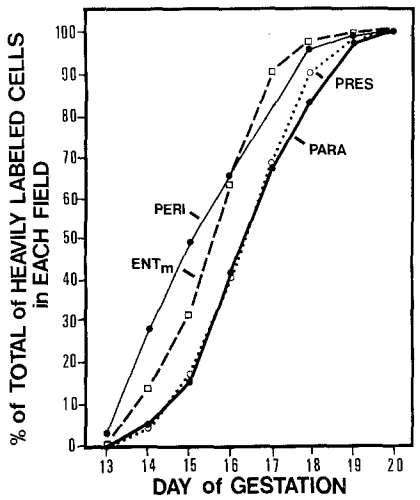

Fig. 19. A graph to show the relative times of origin of neurons in the perirhinal area (*PERI*), the medial entorhinal cortex (ENT_m) and the para- and presubiculum (*PARA, PRE*)

being virtually over by the end of the 17th day of gestation. This is perhaps not unexpected since the subiculum consists essentially of a broad layer of rather large pyramidal cells and lacks the more complex lamination of the adjoining retrohippocampal fields. The progressive shift in the time of neurogenesis in the perirhinal and medial entorhinal areas, and in the para- and presubiculum is graphically shown in Figure 19.

Again, apart from the very clear "inside-out" gradient in cell proliferation and the clear sequence of cell proliferation seen along the lateral to medial dimension, there is no indication in the retrohippocampal fields of a ventral to dorsal gradient in the pattern of cell proliferation comparable to that seen in the dentate gyrus (Schlessinger et al., 1975).

Discussion

Three general conclusions can be drawn from the observations presented above. First, the neurons in each field of Ammon's horn and in each cortical area in the retrohippocampal region, are generated over a limited period of time, usually lasting no more than 5 days; in most cases the majority of the neurons passes through its last phase of DNA synthesis within a period of 24–48 h. In this respect, the hippocampal formation closely resembles other cortical and most subcortical structures in the rat brain, but is strikingly different from the dentate gyrus, in which cell proliferation proceeds at a relatively high level over a period of 3 weeks (from about the 14th day of gestation until the end of the second postnatal week), and some granule cell continue to be generated several months after birth (Kaplan and Hinds, 1977).

Second, two distinct gradients in the time of neuron origin in the hippocampal formation as a whole can be recognized. (1) In each cytoarchitectonic field the deepest neurons in cortical layers are generated first, and subsequently those in the progressively more superficial layers are formed. Again, this pattern is common to all other areas of the cerebral cortex with the notable exception of the dentate gyrus

in which the most superficial granule cells are the first to become post-mitotic (Angevine, 1965; Schlessinger et al., 1975). (2) The earliest areas to be formed are the hilar region of the dentate gyrus (including field CA_4) and the perirhinal area; as one moves towards the subiculum from either of these two fields the time of peak neuron proliferation is found to occur progressively later. Both the inside-out gradient and those across the transverse dimensions of the hippocampus and the retrohippocampal fields are less obvious than those seen in the dentate gyrus, no doubt because of the relatively compressed period of neurogenesis in each of the former cortical fields. Despite careful examination of several series of brains we have not been able to identify a temporal to septal gradient in the time of cell origin in Ammon's horn comparable to that seen in the dentate gyrus, nor is there evidence in our material for a ventral to dorsal gradient in neurogenesis in the retrohippocampal fields.

Third, there appears to be no clear relationship between the time of origin of neurons in the various parts of the hippocampal formation that are known to be interrelated by associational projections. For example, while the neurons in the *regio superior* are generated over more or less the same period as those in the subiculum to which it projects (Hjorth-Simonsen, 1973; Swanson and Cowan, 1977; Swanson et al., 1978), the cells in the entorhinal area which provide the major input to the dentate gyrus have all passed through their final mitosis by about the 18th day of gestation when less than 10% of the neurons in the dentate gyrus have been generated. However, it is perhaps of some interest that the deeper lying neurons (which are the earliest to be generated) contribute principally to the subcortical or extrahippocampal projections, whereas the later-generated, more superficial cells, appear to be mainly concerned with intrahippocampal associational and commissural interconnections (Meibach and Siegel, 1975; Steward and Scoville, 1976; Sikes et al., 1977; Haberly and Price, 1978).

Finally, it should be pointed out that the general pattern of neurogenesis in the hippocampal formation of the rat closely resembles that which Angevine (1965) has reported for the mouse. And while there are some minor differences, the similarities are a great deal more striking. It remains to be determined whether in different strains of rats variations in the pattern of cell generation in the different regions comparable to those reported by Vaughn and his colleagues (1977) in the mouse, also occur.

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