

**BRIEF  
COMMUNICATIONS**

**HIPPOCAMPAL NEUROGENESIS  
IN CHILDREN**

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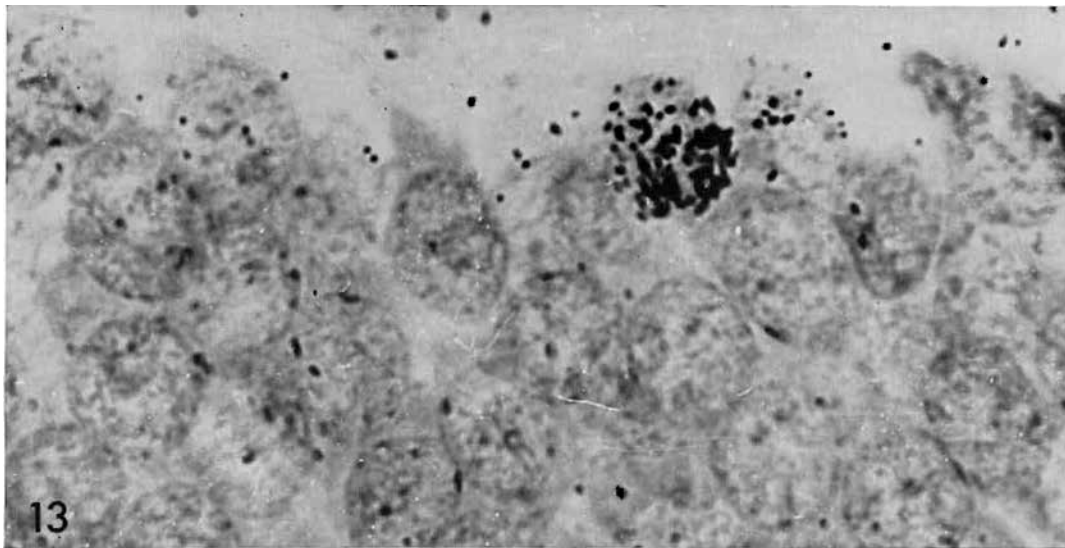
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## ABSTRACT

This paper briefly reviews our findings in the rat nervous system that the majority of the hippocampal dentate granule cells are generated postnatally from precursors that initially populate a large part of the hilus. As development proceeds, the primitive precursor cells settle in a layer that forms a subgranular zone at the base of the dentate granular layer. Neurons continue to be generated by these precursors throughout the adult period in rats. Data showing that guinea pigs and cats also have prominent subgranular zones in the dentate gyrus--even more prominent than in rats--is also briefly reviewed.

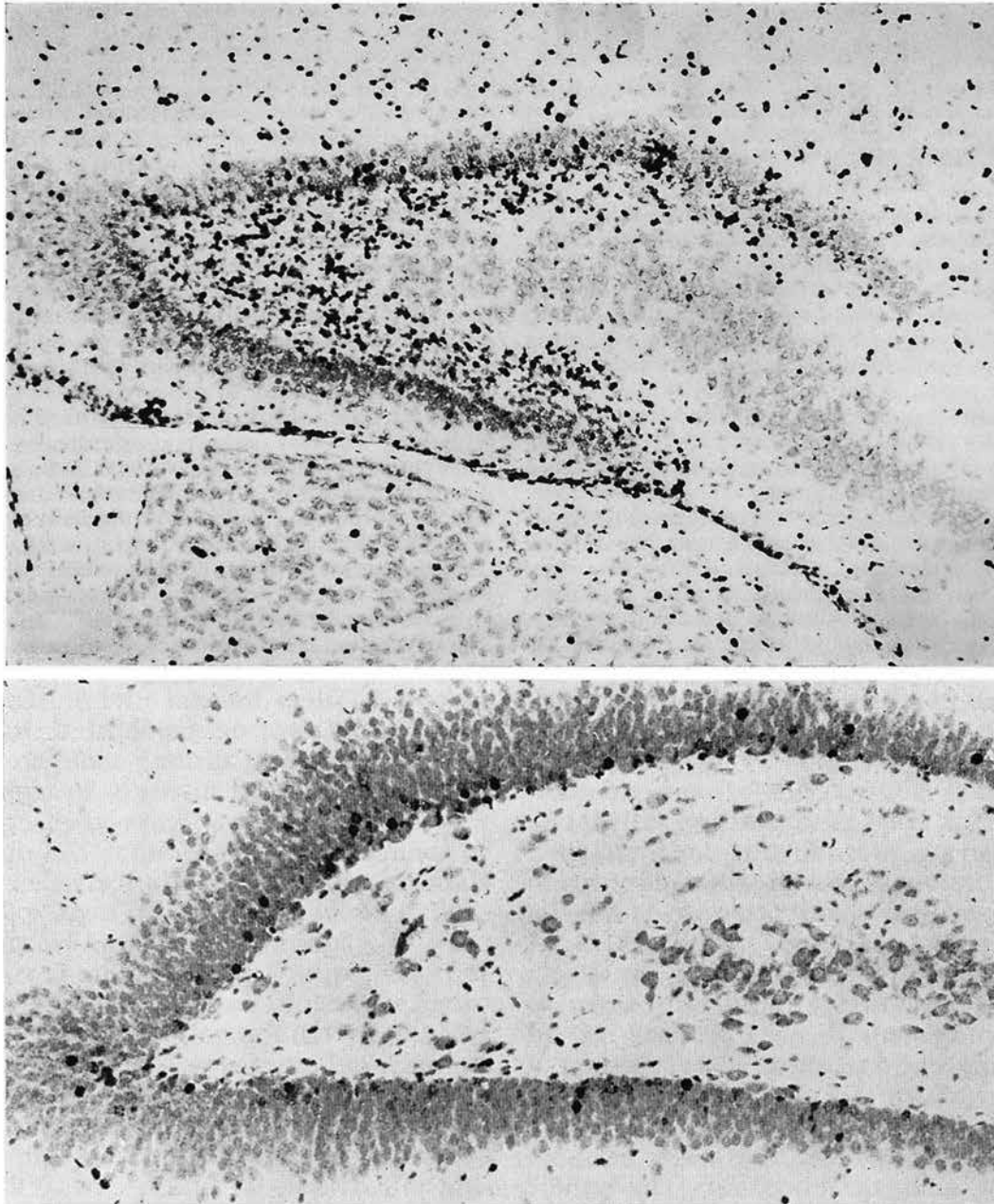
The main purpose of this paper is to point out for the first time that a subgranular zone exists in humans from ages 1 month to 6 years. The subgranular zone was identified after a careful re-examination of Conel's 8-volume work (1939-1967) on postnatal development of the human cerebral cortex. This histological evidence strongly supports the hypothesis that the human dentate granular layer shows the same developmental patterns that exist in other mammals. The subgranular zone is the most likely source of adult-generated granule cells in humans reported by Eriksson *et al.* in 1998.

We found in an early autoradiographic investigation (Altman, 1963) that 2 weeks after adult rats were injected intraperitoneally with a radioactively-tagged specific precursor of DNA ( $^3\text{H}$ -thymidine), the nuclei of a few granular neurons were labeled in the dentate gyrus of the hippocampus (Fig. 1). Since, DNA molecules are metabolically stable, we interpreted that labeling as indicative of adult neurogenesis in the dentate granular layer.



**Figure 1.** The cell with heavy black dots over the nucleus is a granule cell in the hippocampal dentate gyrus of an *adult* rat that was labeled within 2 weeks after an intraperitoneal injection of  $^3\text{H}$ -thymidine. This is one of the first photographs to show adult neurogenesis. (Reproduction of Figure 13 in Altman, 1963).

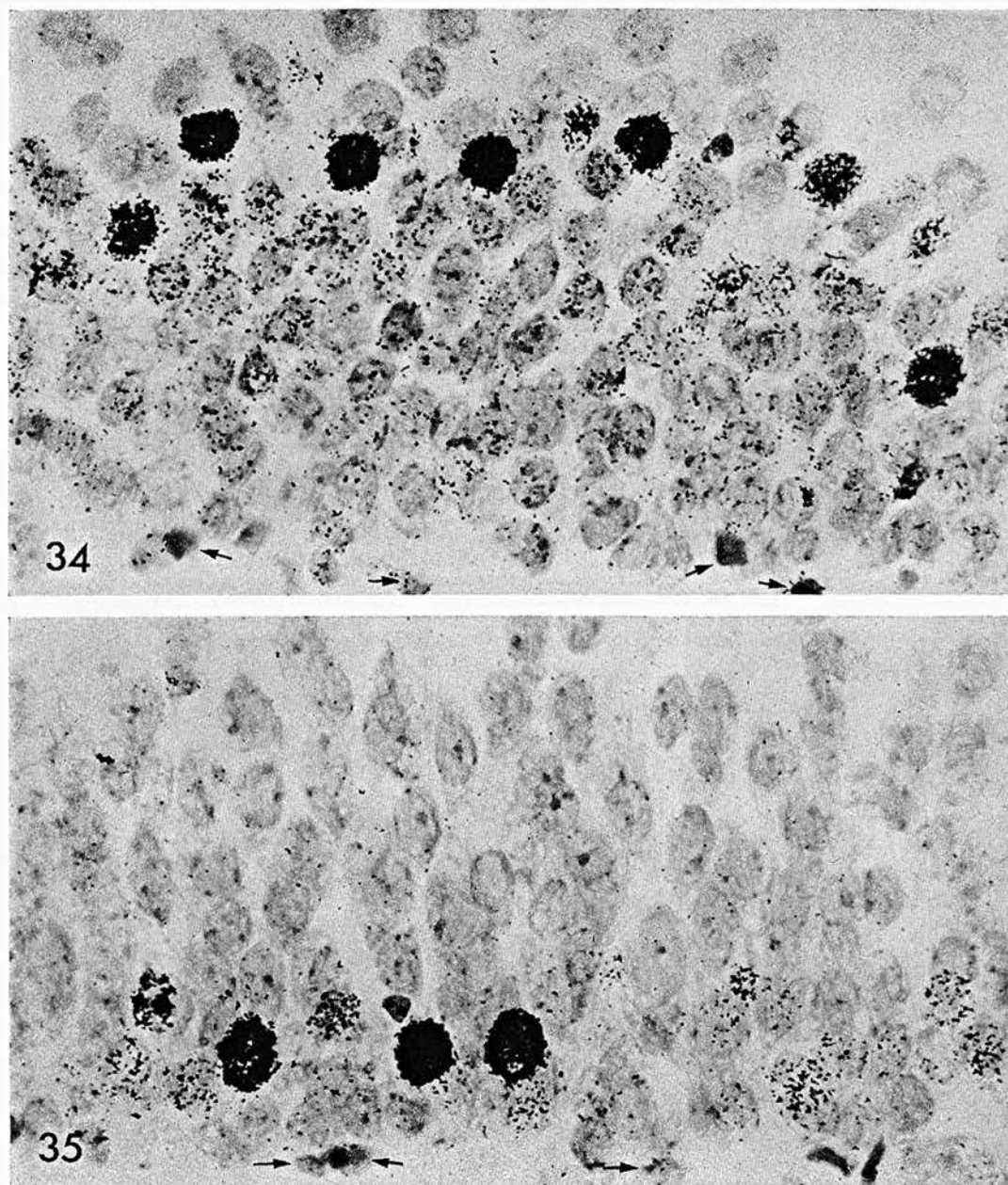
In a subsequent autoradiographic investigation (Altman and Das, 1965), we injected rats of different ages with  $^3\text{H}$ -thymidine and killed them at different intervals thereafter. We found that rat pups killed shortly after multiple injections have a broad band of labeled primitive cells located throughout the hilus of the dentate gyrus (Fig. 2 *top*). Animals killed 2 months after an injection had labeled differentiated granular neurons (Fig. 2 *bottom*). *We postulated that the primitive cells located in what we have later called the **dentate subgranular zone** were the proliferative precursors of the differentiated granular neurons that are generated after birth.* Indeed, the subgranular zone in rats becomes progressively more thin during later development. In adult rats, the zone is fragmented into pockets of immature cells scattered at the base of the granular layer (Fig. 2 *bottom*). The primitive precursor cells were shown to generate adult neurons like the one pictured in Fig. 1 (Bayer, 1982).



**Figure 2.** *Top.* An autoradiogram of labeled cells in the hippocampal subgranular zone and hilus of a rat pup injected with  $^3\text{H}$ -thymidine on postnatal days 5, 6, 7, and 8 (cumulative labeling) and killed on the 9th day. The dentate granular layer is thin at this age with the unlabeled granular cells (those generated before postnatal day 5) making up the outer rim. Many primitive cells are labeled in the hilus and constitute a subgranular zone. (Reproduction of Figure 21 in Altman, 1966)

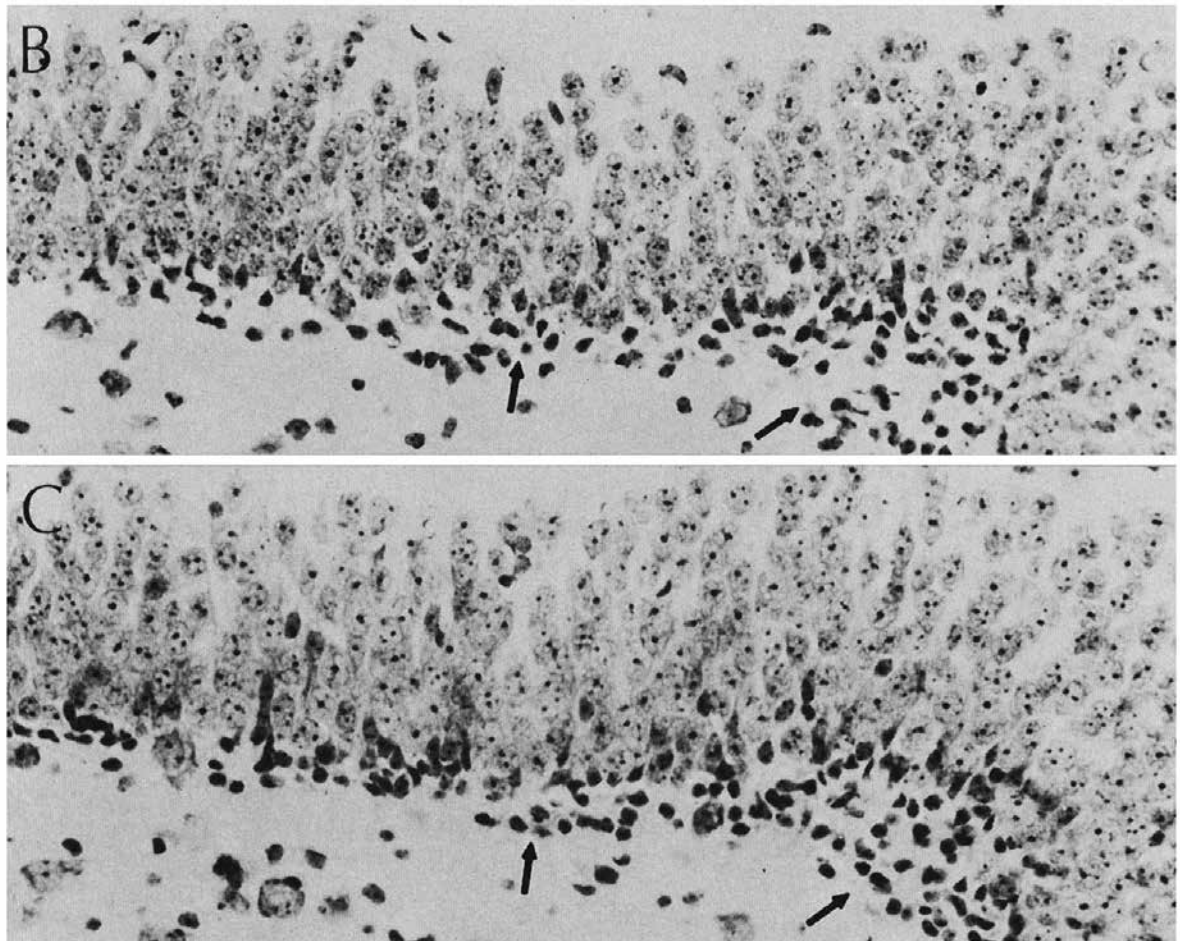
*Bottom.* Autoradiogram of labeled “deep” granule cells (near the hilus) in a rat injected on postnatal day 10 (flash labeling) and killed two months later. The unlabeled granule cells (most generated before postnatal day 10) are in a superficial position. The subgranular zone is now a fragmented scattering of primitive cells at the base of the granular layer. (Reproduction of Figure 1A in Altman and Das, 1965).

We also demonstrated that in the expanding dentate gyrus, the early differentiating neurons (those heavily labeled) tend to settle in the outer rim of the granular layer, and the later differentiating neurons (the lightly labeled progeny of the proliferative cells) tend to settle beneath them in an outside-in pattern (Figure 3).



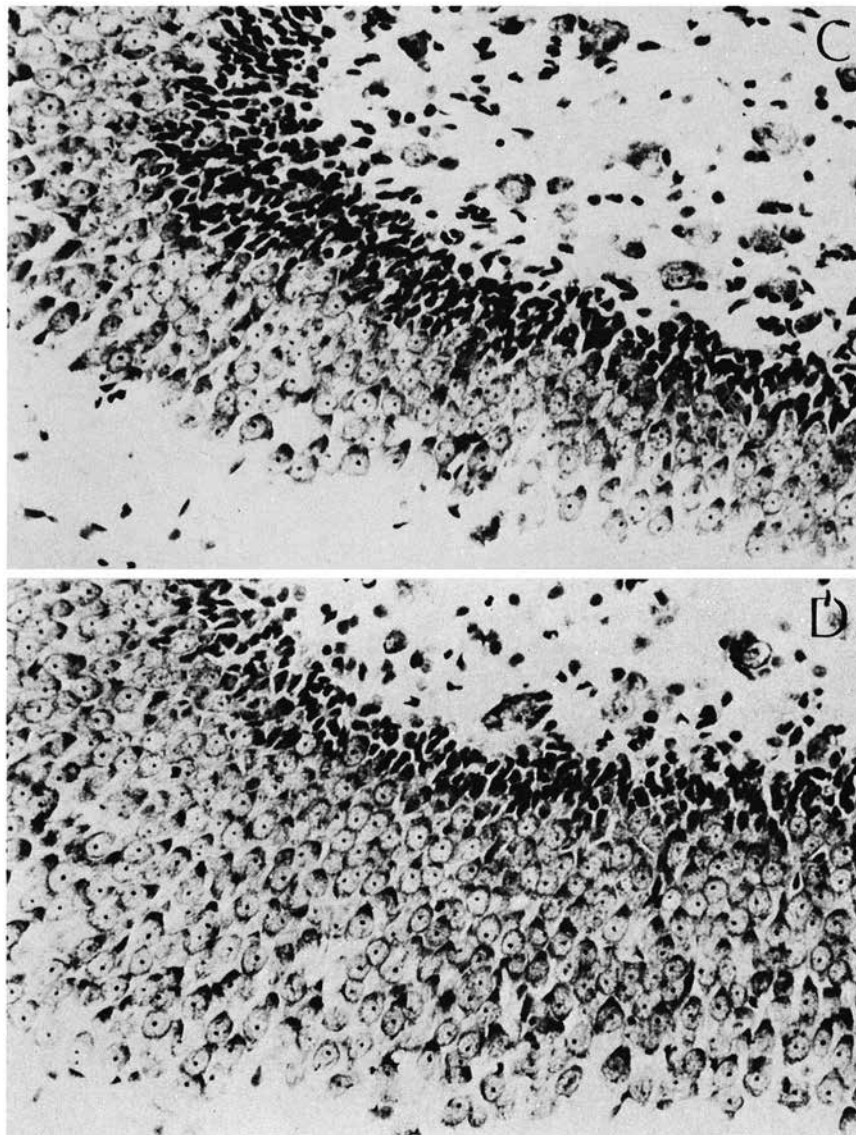
**Figure 3.** Autoradiograms of a 60-day-old rat showing the superficial (older) to deep (younger) pattern of labeled cells in the hippocampal dentate gyrus. *Top.*  $^3\text{H}$ -thymidine injected on postnatal day 2 heavily labels a band of cells just below the few unlabeled cells that formed *before* day 2; lightly labeled and nearly unlabeled cells pile up below. *Bottom.*  $^3\text{H}$ -thymidine injected on postnatal day 13 only labels cells settling deep in the granular layer. *Small arrows* in both photos indicate primitive cells in the subgranular zone. (Reproduction of Figure 4 in Altman and Bayer, 1975).

Having obtained evidence in rats that the primitive precursor cells in the subgranular zone are the source of the postnatally generated hippocampal granular neurons, we inquired into the generality of this phenomenon. Rats are altricial rodents born after a short gestational period (21 days) without fur and with closed eyes. Guinea pigs are precocial rodents born as miniature adults after a long gestation period (about 65 days) with fur, open eyes, and are ready to run. Do guinea pigs have a hippocampal subgranular zone? Indeed, the guinea pig dentate gyrus has a distinct and more prominent subgranular zone (Figure. 4) than rats have. Guinea pigs surviving shortly after being injected postnatally with  $^3\text{H}$ -thymidine have many primitive precursor cells labeled in the subgranular zone, but differentiated cells in the granular layer are only labeled after longer survival times (Altman and Das, 1967).



**Figure 4.** The small dark primitive cells (*arrows*) form a prominent subgranular zone at the base of the granular layer in guinea pigs aged 6 days old (*top*) and 18 days old (*bottom*). (Reproduction of Figure 7B and C in Altman and Bayer, 1975).

But postnatal hippocampal neurogenesis may only be limited to primitive mammals, like rodents. What about more complex mammals with a larger and gyrated cerebral cortex? Consequently, we also examined the dentate gyrus in kittens of different ages (Altman and Bayer, 1975). As illustrated in Figure 5, kittens aged 30 and 60 days have a prominent subgranular zone. It is highly possible that many of the granule cells in cats are generated postnatally by precursors in the subgranular zone, and the continued presence of such a zone in maturing cats suggests that these precursors could continue to generate neurons in the adult period.



**Figure 5.** The small dark primitive cells form a prominent subgranular zone at the base of the granular layer in cats aged 30 days old (*top*) and 60 days old (*bottom*). (Reproduction of Figure 8C and D in Altman and Bayer, 1975).

The question still remained as to whether or not humans, with the largest neocortex and a prominent hippocampus, have a subgranular zone in the dentate gyrus. While preparing a new book, entitled *Development of the Human Neocortex: A Review of the Histological Record*, we re-examined the contributions made by early investigators on the subject. Perusing LeRoy Conel's richly illustrated 8-volume work on the postnatally developing human cerebral cortex (Conel, 1939-1967), we came across his photomicrographs of the developing human hippocampus that were stained with Cajal's gold method. This technique visualizes not only the cell bodies of neurons but also the initial portion of their axons and dendrites; that pattern is clearly shown in the staining of the pyramidal layer of the hippocampus. However, the staining pattern of Cajal's gold method in the dentate granular layer is different. Mature cells are faintly stained, and there is a prominent band of densely-stained immature cells at the base of the granular layer. The position of this densely-staining layer is exactly homologous to the position of the subgranular zones in guinea pigs (Fig. 4) and cats (Fig. 5). The four micrographs that we reproduce in Figures 6 and 7 on the following pages *clearly establish the presence of a pronounced subgranular zone in the brains of human children from 1 month to 6 years age. The histological evidence here presented suggests that the origin of dentate granule cells in humans may be the same as in other mammals.* The generation of hippocampal granular neurons has been demonstrated in adult humans (Eriksson et al., 1998). However, the presence of a subgranular zone in the adult human dentate gyrus and its changes during the adult period remain to be determined.

ON THE FOLLOWING PAGES:

**Figure 6A.** Human dentate gyrus and pyramidal layer at 1 month. (Conel, Vol.2, Fig.202)

**Figure 6B.** Human dentate gyrus and pyramidal layer at 2 years. (Conel, Vol.6, Fig.193)

**Figure 7A.** Human dentate gyrus and pyramidal layer at 4 years. (Conel, Vol.7, Fig.193)

**Figure 7B.** Human dentate gyrus and pyramidal layer at 6 years. (Conel, Vol.8, Fig.193)

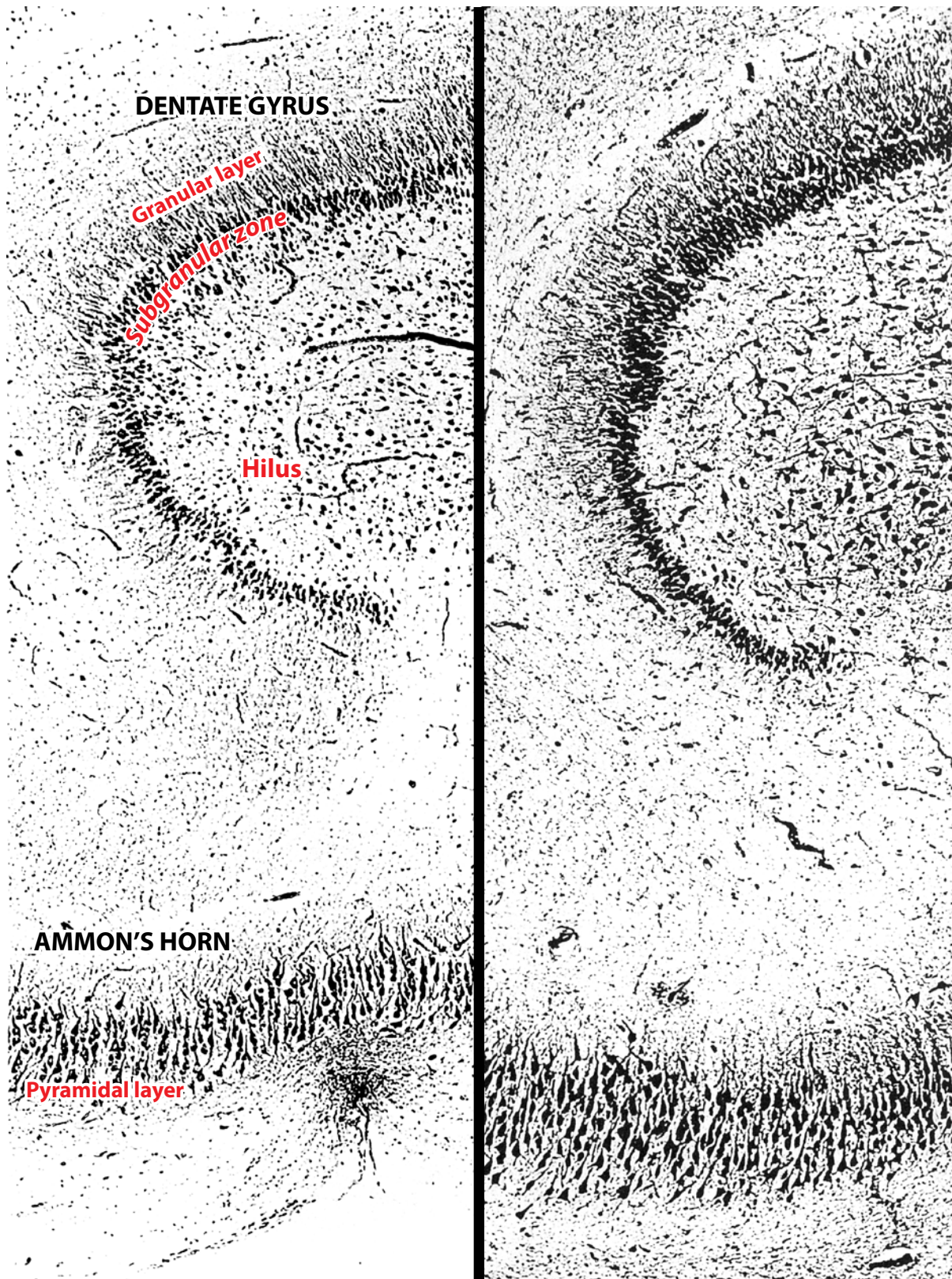
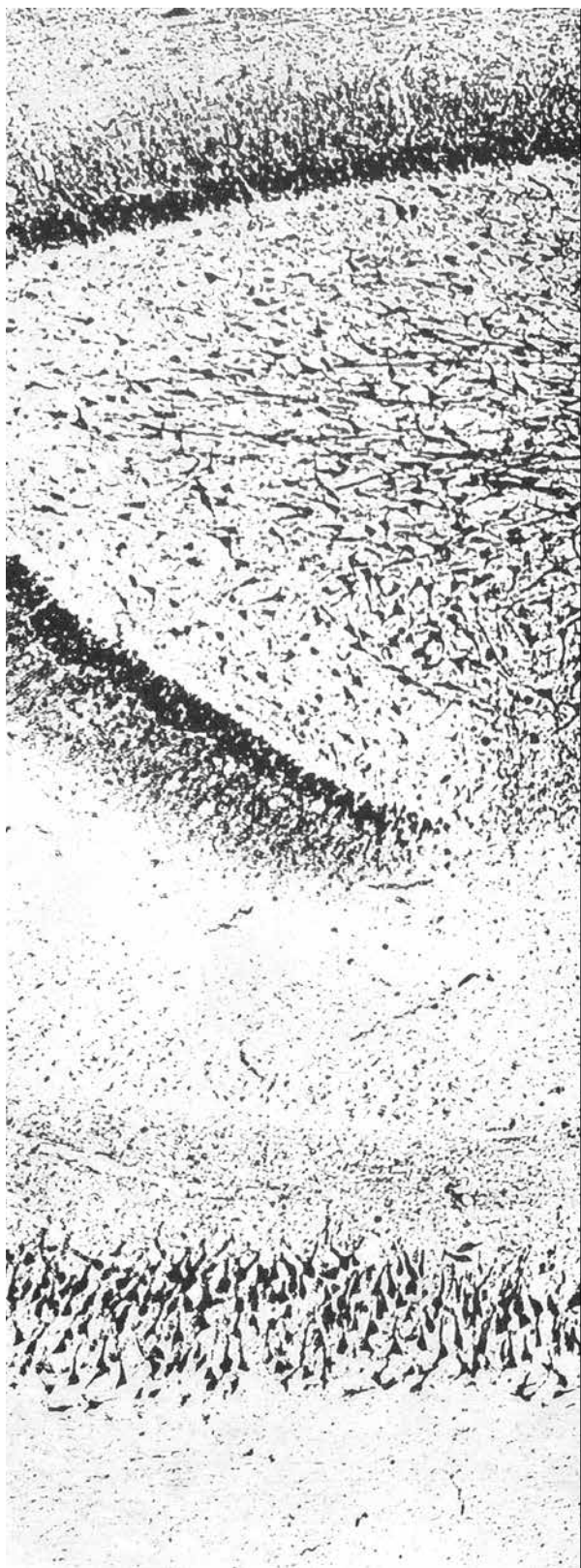
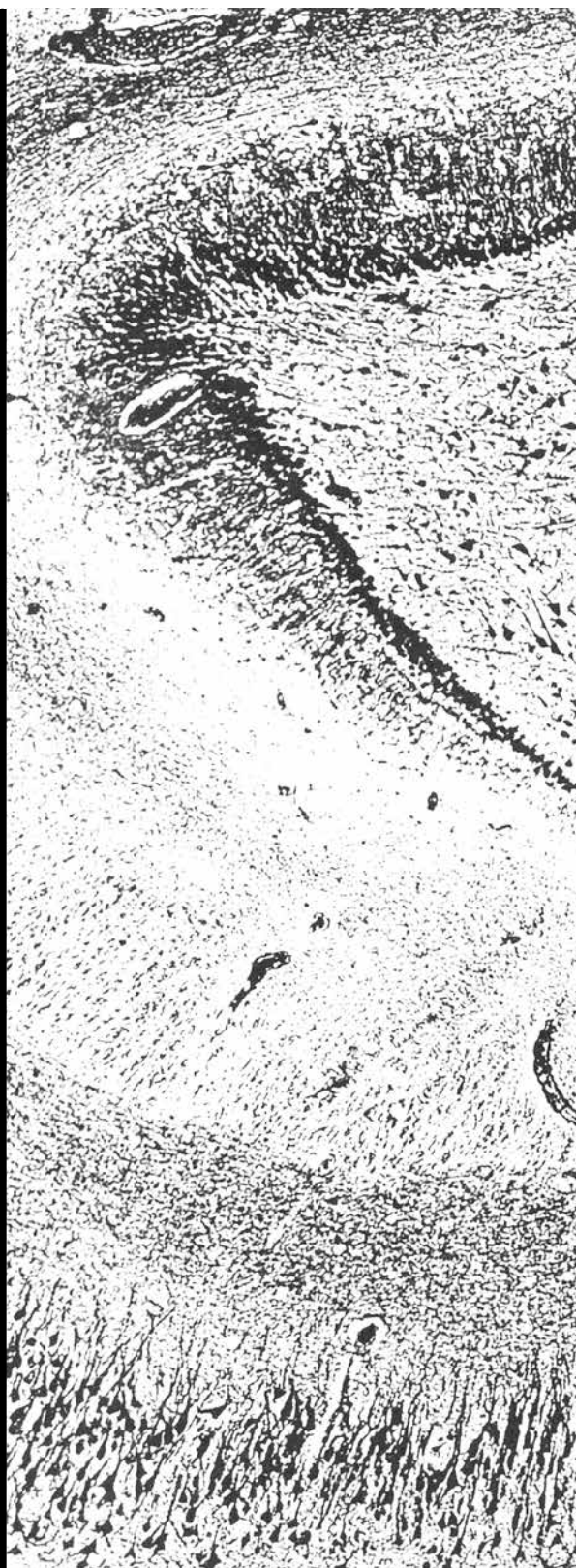


Figure 6A

Figure 6B



**Figure 7A**



**Figure 7B**

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