Granule cell neurogenesis occurs in adults and can be observed along the hilar border of the granule cell layer (GL) in the hippocampal dentate gyrus [3,6,10,11]. Recent studies have shown that these newly generated granule cells can become integrated into neural circuitry and mature into functional neurons [14]. These neurons were labeled using markers such as bromodeoxyuridine that is incorporated into the DNA of dividing cells. Other markers have been used for labeling the cytoplasm of newly generated neurons, such as doublecortin, TUC-4, β-III-tubulin, Prox-1, and CRMP-4 [7,9]. Unlike bromodeoxyuridine, which labels DNA of dividing cells, the latter markers label the perikaryal cytoplasm, dendrites, and axons of newborn neurons [12].

Granule cells located in the GL near the border with the hilus show atypical morphological characteristics. Using biocytin, we have previously demonstrated basal dendrites arising from the base of normal cell bodies and then curving toward the molecular layer and named them recurrent basal dendrites [4,13,16]. Their frequency was greatest at the hilar border and decreased with the distance away from the hilus [4,13]. Interestingly, epileptic rats displayed granule cells at the hilar border with basal dendrites that entered the hilus and are postsynaptic to mossy fibers [13]. It is possible that the appearance of granule cells with basal dendrites mainly located at the hilar border suggest that they might be newly generated cells.

In the present study, we used immunocytochemistry for doublecortin to label newly generated granule cells in normal rats to determine whether they have basal dendrites. Doublecortin is a protein associated with microtubules within growth cones of newborn neurons [5]. Thus, we also investigated the morphology of dendrites of newly generated granule cells in the adult, and in doing so, we discovered growth cones on these processes.

Adult male, Sprague–Dawley rats (n = 5; 165–275 g; Simonsen, Gilroy, CA) were used in this study. All protocols were approved in advance by the Institutional Animal Care and Use Committee at the University of California at Irvine. Rats were anesthetized with an overdose of Nembutal (pentobarbital sodium; 50 mg/kg, i.p.) and then perfused intracardially with 150 ml of saline...
followed by 200–300 ml of 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). Brains were sectioned at 50 µm with a Vibratome. After rinsing in 0.1 M PBS for 30 min, sections of hippocampus were incubated in 0.5%, 1.0%, and 0.5% PBS buffered H2O2 for 30, 60, and 30 min, respectively. Sections were then rinsed in PBS for 90 min and incubated overnight at 4 °C in anti-doublecortin (1:500, goat polyclonal antibody in 5% normal horse serum, Santa Cruz Bio Tech). The sections were subsequently washed in 0.05% polyoxyethylene-sorbitan monolaurate (Tween 20-PBS) for 15 min and incubated for 60 min in secondary antibody (1:500, biotinylated anti-goat IgG, raised in rabbit, in 5% normal horse serum, Vector Labs). Sections were rinsed again in Tween 20-PBS for 15 min and then incubated 30 min in avidin–biotin horseradish peroxidase solution (Vectastain Elite ABC Kit, Vector Labs). Finally, they were washed 15 min in 0.05% Tween 20-PBS followed by 10 min in PBS and incubated 5 min in 0.025% diaminobenzidine, 0.002% hydrogen peroxide, and nickel ammonium sulfate. Sections were washed with distilled water, mounted onto glass slides, counterstained with thionin, dehydrated, and then coverslips were applied.

Doublecortin-immunolabeled sections were viewed with a Zeiss Axiosplan light microscope, and images were captured with an Axiocam digital camera and prepared with Adobe Photoshop software. Every section processed for immunocytochemistry showed many labeled cells at the hilar border of the GL in the dentate gyrus. The total number of cells per section was determined by counting only those immunolabeled cells that had a distinctly stained, perikaryal cytoplasm (d=6–12 µm) surrounding a translucent nucleus. In addition, the frequency of growth cones found on the apical or basal dendrites of labeled cells was quantified by counting labeled growth cones that appeared to have filopodia or lamellipodia [2,15] at the tips of dendritic processes. Axons were distinguished from dendrites by their having a thinner diameter and projecting into the hilus. Recurrent basal dendrites were identified using previous criteria [4,13,16].

The distribution of doublecortin-immunolabeled granule cells in the dentate gyrus was similar to that previously described, and it corresponded to the distribution of newborn neurons in this brain region using other markers for newly generated neurons [1,7,9,12]. Doublecortin-positive cells were found both in the subgranular zone of the hilus and in the GL at the hilar border (Figs. 1 and 2). Because the cells in the two locations had different morphologies, they will be described separately.

Most of the immunolabeled granule cells in the subgranular zone were found within 50 µm of the GL (Fig. 2E). They typically had a fusiform cell body with its long axis oriented parallel to the GL (Fig. 1A, C, and D). These cells appeared as bipolar cells with two processes, one extending from each end. Most processes either stayed in the sub-
granular zone or curved into and entered the GL as described for recurrent basal dendrites (Fig. 1B and C). Some of the processes were thick and could be identified as dendrites. However, others matched the morphological characteristics of growth cones because they exhibited lamellipodia and filopodia (Fig. 1A and C). The growth cones were often found at the ends of thick processes which rarely branched (Fig. 1B and D).

Fig. 2. Doublecortin-immunolabeled cells in the GL at the hilar border. (A) A large labeled cell body with an apical dendrite (arrowhead) and a recurrent basal dendrite (diamond head arrow). Both dendrites have lamellipodia (asterisks) and filopodia (arrows). (B) Another example of a labeled cell with both apical (arrowhead) and recurrent basal (diamond head arrow) dendrites. Note that the apical dendrite has a lamellipodium with a long filopodium about 10 μm away from the cell body. (C) Two labeled cells with their apical dendrites (arrowheads) in focus. They display both lamellipodia (asterisks) and filopodia (arrows). Note that the cell on the right has a branching apical dendrite, and one of the branches has trifurcating filopodia (top three arrows). (D) Two labeled cells. The one on the right has two basal processes, and one of these is a recurrent basal dendrite (diamond head arrow) that has a lamellipodium (asterisk) and filopodium (arrow) at its tip. (E) A low magnification photomicrograph of doublecortin-labeled cells at the base of the GL and in the SGZ. Several granule cells are shown with dendrites, and the left and right boxes indicate the location of the cells found in panels C and D, respectively. Scale bar = 8 μm for panels A–D and 50 μm for panel E.
Doublecortin-positive cells in the GL had a different morphology from those in the subgranular zone. These cells had either fusiform or round cell bodies with their long axis either perpendicular or at an angle to the GL (Fig. 2). Most of these immunolabeled cells had apical dendrites, and many of them had bifurcating processes. Lamellipodia and filopodia were found along the length (Fig. 2B) and at the growing tips (Fig. 2A and C) of apical dendrites. One apical dendrite showed trifurcating filopodial processes (Fig. 2C). Many of the labeled cells in the GL had basal dendrites (Fig. 2A, B, and D). Some of these dendrites extended horizontally for 20–50 μm and then curved toward the molecular layer (Fig. 2B). Others would arise from the base of the soma and then immediately project toward the molecular layer (Fig. 2D). Both types of recurrent basal dendrites were described previously [16].

The quantitative data were based on 20 sections obtained from three rats that had the best immunostaining. The mean number of doublecortin-positive cells per section of the dentate gyrus was 52.8. The mean percentage of immunolabeled granule cells with growth cones was 45.0%. Not every dendrite of each labeled cell had a growth cone. In fact, only 23.6% of dendrites had growth cones. The mean percentage of granule cells with recurrent basal dendrites was 55.2%, indicating that more than half of all doublecortin-labeled granule cells display this type of dendrite. There were about 1.8 (± 0.89) dendritic processes per each labeled cell (this number reflects both apical and basal dendrites).

The major finding of this report is that about half of all doublecortin-positive granule cells in the adult dentate gyrus have dendrites with growth cones. This result is based on the fact that immunolabeling for doublecortin yields excellent morphological details of neuronal processes in contrast to other markers of newborn neurons that only label nuclei, such as bromodeoxyuridine. Although a previous study described doublecortin-positive growth cones on axons of granule cells [12], the present study is the first to show growth cones on dendrites of dentate granule cells in the adult. This finding suggests that dendrites in the adult dentate gyrus may grow in the same way as those in the immature brain [8] even though the neuropil is denser in the adult with more synaptic connections already established. The present study is significant because it provides new data on how newborn neurons grow dendrites in the adult and is thus pertinent to investigations using stem cells to treat neurological diseases. Thus, the new granule cells may contain a developmental program that is the same as the embryonic and perinatal generated granule cells.

Doublecortin is a known neuronal marker for newborn neurons in the dentate gyrus [7,12]. Although doublecortin was reported to label mature neurons in some brain regions [12], it labels only recently generated neurons in the dentate gyrus according to previous studies using double-labeling methods for other markers of newborn neurons [1,7,9,12]. In fact, a recent study indicated that doublecortin may provide an alternative to labeling with bromodeoxyuridine [1]. Therefore, the present data indicate that doublecortininmunolabeled newborn neurons in the adult have dendritic growth cones.

Another finding of our study is that more than half of the doublecortin-immunolabeled granule cells have recurrent basal dendrites. A previous study indicated that basal dendrites are a normal morphology for newborn granule cells [12]. If doublecortin only labels newborn neurons in the dentate gyrus, then the current data suggest the idea that the newly generated granule cells are pre-programmed to send dendritic processes into both the hilus and molecular layer. It should be noted that the actual numbers of recurrent basal dendrites are probably underestimated in these preparations because of sectioning bias. Based on previous data that doublecortin labels newborn neurons in the dentate gyrus, our results suggest that newly generated granule cells in the adult rat exhibit both dendritic growth cones and recurrent basal dendrites that are typical features of dentate granule cells generated during neurogenesis in the developing rat brain [8]. Therefore, the vast majority of newly generated granule cells are programmed to extend processes initially into the hilus and later into the molecular layer assuming that granule cells are migrating from the subgranular zone into the granule cell layer.

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