Dispersion of neural progenitors within the germinal zones of the forebrain

Gord Fishell*, Carol A. Mason & Mary E. Hatten*

Department of Pathology, Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, New York 10032, USA
* Present address: The Rockefeller University, 1230 York Avenue, New York, New York 10021-6399, USA

ONE of the early events in the establishment of regional diversity in brain is the subdivision of the forebrain into the cerebral cortex[1–7] and underlying basal ganglia[8]. This subdivision is of special interest, owing to the striking difference in cellular patterning in these two regions. Whereas the dorsal aspect of the telencephalon gives rise to the laminar, cortical regions of brain, the basal aspect gives rise to nuclear, subcortical regions. To examine early events in the regionalization of the forebrain, we visualized cell movement within the ventricular zones of the dorsal and basal regions of the E15 murine telencephalon. Over an 8–24-hour observation period, labelled cells moved extensively in the plane of the cortical ventricular zone. Cell dispersion was restricted, however, at the border between the cortical ventricular zone and the lateral ganglionic eminence, the basal telencephalic ventricular zone. We suggest that this restriction of cell movements establishes a regional pattern of neurogenesis in the developing brain.

To visualize living cells in the ventricular zone (VZ)[7] of the developing murine forebrain, the lateral wall of the telencephalic vesicle was removed on embryonic day 15 (Fig. 1) and cells were labelled with the lipophilic dye DiI[9]. Confocal imaging demonstrated that DiI-labelled cells along the ventricular surface had the small size and bipolar morphology characteristic of neural precursors in the VZ[1–6] (Fig. 2*a*), expressed cellular antigen markers for VZ cells, including nestin[10] and RC2 (ref. 11) (not shown), and incorporated bromodeoxyuridine (BrdU) (Fig. 2*b*, *c*). This suggests that the explants maintained the pattern of cellular organization and proliferation seen *in situ*[2,3,7].

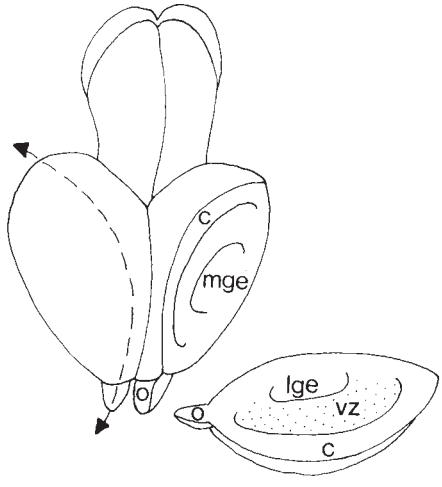

FIG. 1 Preparation of explants of the lateral wall of the telencephalon. Explants of the lateral wall of the E15 mouse telecephalon were prepared by making an incision across the dorsomedial surface of the brain, indicated by the dotted line drawn across the left side of the illustration. The wall of the telencephalic vesicle was subsequently removed by an incision between the lateral and medial ganglionic eminence. The exposed ventricular surface of the explant has a roughly semi-circular shape with a raised central region (the lateral ganglionic eminence) surrounded by a flatter outer region (the cortical ventricular zone). DiI-labelled cells were imaged in the plane indicated by the stippling. The olfactory bulb provided a rostral landmark for the explant. Cortex, C; lateral ganglionic eminence, lge; medial ganglionic eminence, mge; olfactory bulb, o; ventricular zone, vz.

To examine the dispersion of neural progenitor cells in the dorsal and basal telencephalic VZs, DiI-labelled cells were imaged by time-lapse video fluorescence microscopy[12,13]. Within the dorsal (cortical) telencephalic VZ, labelled cells moved in short bursts, reaching rates of 10–100 μm per hour (Figs 3, 4). Motile cells rapidly extended and retracted thin lamellar processes, 5–20 μm in length, after which the cell body moved in the direction of lamellar extension. Within the basal telencephalic VZ, the lateral ganglionic eminence[8], cells moved at comparable rates and frequencies (not shown). Thus neural progenitor cells disperse laterally within the germinal zones of the forebrain.

To evaluate whether cells moved within the cortical VZ in a 'random walk', the mean-square displacement of 21 DiI-labelled cells was plotted as a function of time[14]. The linearity of these plots indicated that the cells moved in a diffusion mode. The duration of cell movement near the ventricular surface was quite variable, with some cells moving for 8–24 h and other cells exiting the plane of focus soon after cellular division. It was not possible to determine whether the cells that did leave the plane of focus became post-mitotic and attached to radial glial cells, or moved to the apical VZ to re-enter S-phase of the cell cycle. In a few cases ($n = 7$), we imaged labelled cells undergoing cell division. The labelled daughter cells appeared to move independently, taking different trajectories within the VZ and exiting to deeper zones out of the plane of focus at different times.

The dispersion of neural progenitor cells within the telencephalic VZ, as seen here, is consistent with recent reports on the dispersion of retrovirally labelled cells in the ventricular zone of the rat cortex[15]. Although initial retroviral marking experiments provided evidence for clustering[16,17] of clonally related cells in cortex, recently it has been shown that some clonally related cells disperse widely during cortical

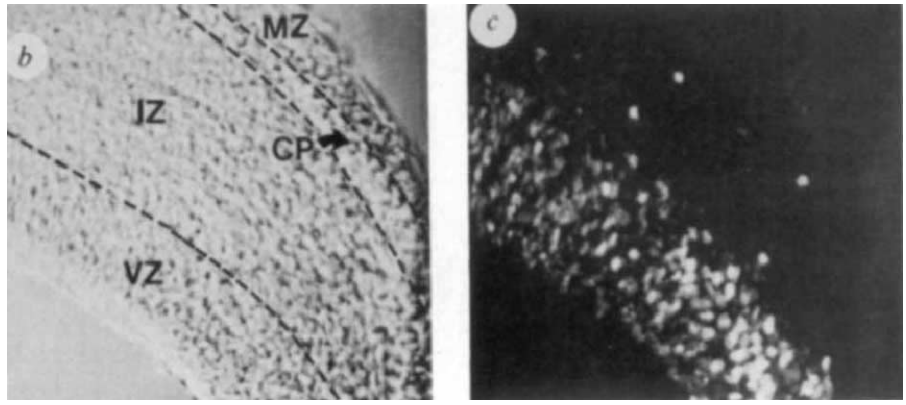

FIG. 2 Dil-labelled cells and BrdU incorporation in E15 cortex explants. To examine the morphology of Dil-labelled cells and determine whether cells continued to undergo cell division *in vitro*, we imaged labelled cells and analysed the pattern of BrdU incorporation in coronal sections of the explants. *a*, Optical sectioning by confocal imaging of Dil-labelled cells within an explant of E15 mouse telencephalon shows labelled cells in the ventricular zone. Ventricular cells can be identified by their position, small size and characteristic, bipolar, apical and ventricular projecting processes. Scale bar, 25 μm. *b*, By differential interference contrast (DIC) imaging, the VZ, intermediate zone (IZ), cortical plate (CP) and marginal zone (MZ) of the explanted tissue are evident. *c*, BrdU labelling of the explant for 8 h reveals a dense band of proliferating cells in the VZ. Scale bar, 25 μm.

METHODS. The E15 mouse telencephalic explant was washed in CMF-PBS (4 °C), transferred to DMEM:F12 medium (supplemented with 10% horse serum and 5% fetal calf serum) and incubated in culture medium containing (0.01-0.02%) Dil (1,1'-dioctadecyl 3,3,3',3'-tetramethyl indocarbocyanine perchlorate; Molecular Probes) at 35 °C for 35'. After washing with fresh medium (35 °C with 5% CO_2), the explant was inverted on a glass coverslip microculture[12] with the ventricular surface facing downwards, overlayered with collagen for 30 min at 35 °C and incubated in fresh medium (35 °C, 5% CO_2) for 8 h. The explant was placed on the preheated (35 °C) stage of a Zeiss Axiovert 35 microscope and time-lapse fluorescent images were acquired as described for Fig. 3. After video imaging, the explant was washed briefly in ice-cold CMF-PBS, fixed in paraformaldehyde (4% in PBS for 4h), washed 2 times in PBS, removed from the collagen, embedded in agarose and sectioned in the coronal plane with a vibratome. Cells were imaged with a Zeiss Axiovert microscope fitted with Zeiss Plan-Neofluor 20×/0.5 or Zeiss Plan-Neofluor 40×/0.85 infinity-corrected objectives and a Bio-Rad MRC-600 scan head with 25 mW Argon ion multi-line laser. A high-sensitivity, green excitation, single-channel filter set was used to acquire images of Dil-labelled cells. A Nomarski image (*b*) was obtained by transmitting the laser light through an optic fibre cable to the scan head before computer acquisition. For BrdU labelling (*c*), BrdU (0.01 mM; 1:100; Amersham, cell proliferation kit) was added to the culture medium at the start of video microscopic observation. To localize BrdU-labelled cells, tissue was treated with 2 M HCl at 50 °C for 1 h, neutralized by washing in PBS (10× and 1×), treated with 10% normal goat serum (30 min) and stained with anti-BrdU antibody (Becton Dickinson; 1:100 in PBS containing 0.02% Triton-X100) overnight (4 °C). After staining, sections were washed in PBS and incubated in fluorescein isothiocyanate-conjugated goat anti-mouse secondary antibody (1:100 for 1 h). Anti-BrdU positive cells (*c*) were imaged with a Zeiss Axiovert microscope fitted with Zeiss Plan-Neofluor 20×/0.5 or Zeiss Plan-Neofluor 40×/0.85 infinity-corrected objectives, using a Bio-Rad MRC-600 scan head with 25 mW Argon multiline ion laser with blue excitation filter set (BHS).

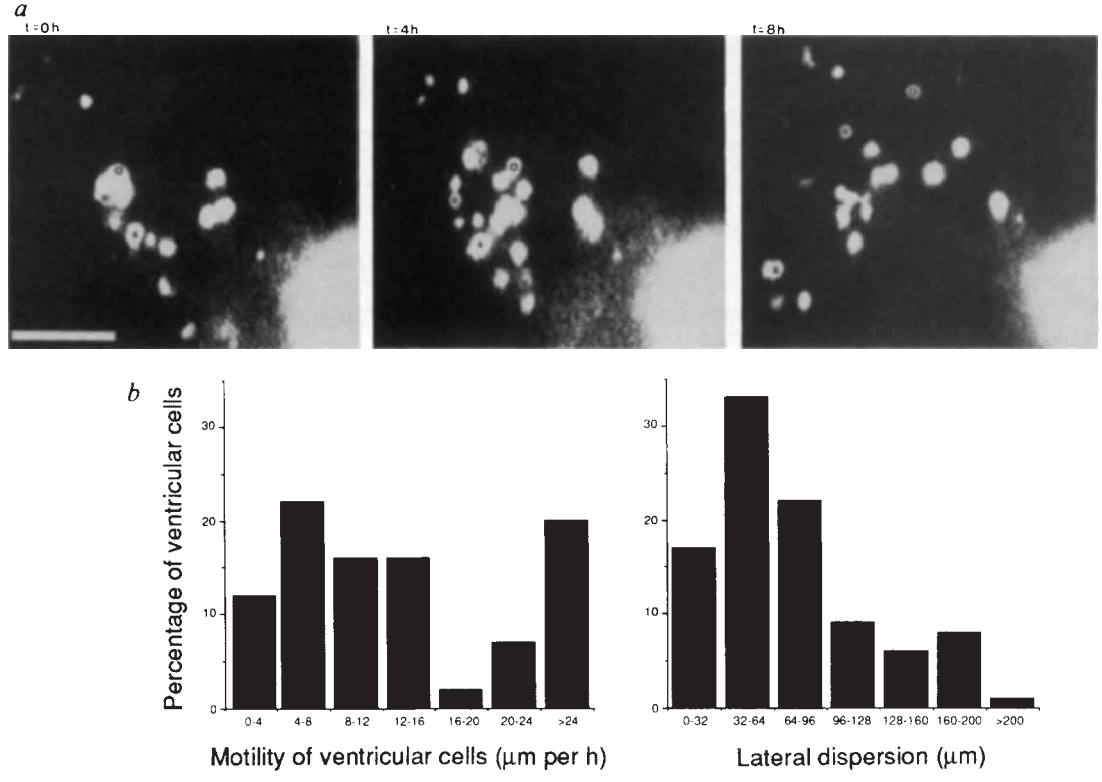

FIG. 3 The motility and extent of lateral dispersion of cortical ventricular cells. *a*, Time-lapse imaging reveals that ventricular cells disperse in all directions across the cortical VZ. After 8 h, the tight cluster of labelled cells has dispersed over a 200 μm^2 area. Positions of three labelled cells (*, ○, △) are shown at 4-h intervals. The Dil crystal in the right of the photomicrograph provides a landmark. The tissue is oriented with rostral, top; caudal, bottom; ventrolateral, left; dorsomedial, right. *b*, Cells move at speeds between 10 and 100 μm per h and disperse up to 200 μm over an 8-h observation period. Left, the speed of movement of 100 Dil-labelled cells over a 1-h period indicates varying rates of cell movement. Given that individual cells move in sudden bursts, speeds measured over a 1-h period underestimate the actual speed of motile cells; right, dispersion of the same population of 100 Dil-labelled cells over an 8-h period.

METHODS. Explants were prepared and labelled with Dil as described for Fig. 2. For time-lapse video imaging, explants were placed on the preheated (Pierce Reactitherm heating module and Lakewood Heating Fan controlled by a YSI temperature controller) stage of a Zeiss Axiovert 35 microscope, with Zeiss Plan-Neofluor 20×/0.5 or Zeiss Plan-Neofluor 40×/0.85 infinity-corrected objectives, phase-contrast and epifluorescent optics and dual ports for video imaging. Images were acquired with a Hamamatstu intensified charge-coupled device (I-CCD) 2400-50/80 camera head and controller, using a Panasonic time-date generator, Uniblitz shutter and D122 shutter drivers with external source signal input controlled by an Image-1 Image Analysis System (Universal Imaging). Epifluorescent excitation/emission was at 510/550 nm. Neutral density filters were added to reduce the excitation light level by 99%. For time-lapse recordings, one image (100-ms exposure) was acquired every 5 min. No cellular phototoxicity was evident for up to 24 h.

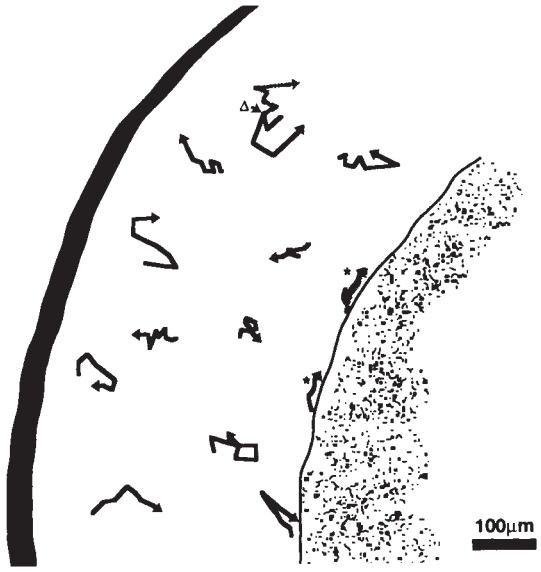

FIG. 4 Path of dispersion of ventricular cells observed over an eight-hour period. The position of DiI-labelled cells in the VZ was recorded by time-lapse video microscopy as described for Fig. 3. A computer reconstruction of the path of dispersion of a sample of these cells, imaged over an 8-h period and compiled from 5 separate preparations, illustrates the movements of a sample of cells. To evaluate whether cells moved in a 'random walk', the mean-square displacement of 21 DiI-labelled cells was plotted as a function of time[14]. The linearity of these plots (not shown) indicated that the cells moved in a diffusion mode. The △ symbol marks the movement of two daughter cells after division of a cell in the plane of focus. The asterisk indicates cells in the border region between the cortical and basal telencephalic germinal zones. In total, cells in 25 explants were imaged, of which 29 DiI-labelled cells were tracked in the border region.

development[18], even crossing functional boundaries[19]. Studies on the dispersion of retrovirally labelled progenitor cells[20,21], or of dye-labelled cells in living tissue slices[22], indicates that although most cells migrate along the radial glial system, some move tangentially within the intermediate zone after leaving the VZ. Together these results suggest that the migratory pathways of immature neural cells are not restricted to the radial plane of the neuroaxis in the early stages of cortical histogenesis.

To determine whether cells move between cortical and basal telencephalic VZs, we imaged DiI-labelled cells along the interface between these regions. When labelled cortical precursor cells entered the boundary region, they moved either in a rostral or caudal direction (Fig. 4), indicating that precursor cells do not cross the border between these two telencephalic VZs. The restriction of cell movement between the dorsal and basal telencephalon seen here is consistent with results showing restricted patterns of gene expression in developing forebrain. Vertebrate homologues of several classes of *Drosophila* genes, including the homeotic gene *forked-head* (*BF-1*; ref. 23), *distal less* (*Dlx* (ref. 24), *Tes-1* (ref. 25)) *achaete scute* (*MASH*[25]) and *empty spiracles* (*EMX-1*; ref. 27) show patterns of expression that are restricted to either the developing cortex or lateral ganglionic eminence. The segregation of precursor cells into two large domains, the cortical VZ and basal telencephalic VZ, provides a mechanism for the subdivision of the forebrain into two major regions. Although these results superficially resemble the development of the hindbrain, where segmental units (the rhombomeres[28]) show restricted patterns of lineage, cell patterning and gene expression, there are several distinctions between forebrain and hindbrain development. The zones defined here are much larger than the developing rhombomeres. In addition, the dorsal aspect of the forebrain does not show the segmental patterning or expression of the segment-specific homeotic genes seen in the developing hindbrain[29].

The dispersion of neural progenitors within germinal zones of forebrain, observed by imaging dye-labelled cells, supports the general conclusion that cell movements are not rigidly restricted during cortical histogenesis. As the surface area of the VZ is quite small compared with that of the overlying cortical plate, the lateral movement of progenitor cells within the VZ provides a simple mechanism for widespread dispersion of neural progenitor cells in the developing forebrain. Identification of the molecular and cellular signals that establish the borders between cortical and basal telencephalic germinal zones should provide further insight into the mechanisms of pattern formation in the forebrain. □

Received 14 December 1992; accepted 4 February 1993.

1. Ramon Cajal, S. *Histologie du Système Nerveux de l'Homme et des Vertebres* Vol. II (Maloine, Paris, 1911).
2. Sauer, F. C. *J. comp. Neurol.* **63,** 13-23 (1935).
3. Sauer, M. E. & Chittenden, A. C. *Proc. Soc. exp. Biol. Med.* **101,** 557-560 (1959).
4. Rakic, P. *J. comp. Neurol.* **141,** 283-312 (1971).
5. Sidman, R. L. & Rakic, P. *Brain Res.* **62,** 1-35 (1973).
6. Berry, M. & Rogers, A. W. *J. Anat.* **99,** 691-709 (1965).
7. The Boulder Committee *Anat. Rec.* **166,** 257-262 (1970).
8. Smart, I. H. M. & Sturrock, R. R. *Ontogeny of the Neostriatum* **23,** 127-146 (1979).
9. Honig, M. C. & Hume, R. I. *Trends Neurosci.* **12,** 333-341 (1989).
10. Friederikson, K. & McKay, R. *J. Neurosci.* **58,** 815-821 (1988).
11. Mission, J.-P., Takahashi, R. & Caviness, V. S. *Glia* **4,** 138-148 (1991).
12. Edmondson, J. C. & Hatten, M. E. *J. Neurosci.* **7,** 1928-1934 (1987).
13. Godemont, P., Salaun, J. & Mason, C. A. *Neuron* **5,** 173-186 (1990).
14. Gruler, H. & Bultmann, B. D. *Blood Cells* **10,** 61-77 (1984).
15. Walsh, C. & Cepko, C. L. *Soc. Neurosci. Abstr.* **18,** 925 (1992).
16. Luskin, M. B., Pearlman, A. L. & Sanes, J. R. *Neuron* **1,** 635-647 (1988).
17. Price, J. & Thurlow, L. *Development* **104,** 473-482 (1988).
18. Walsh, C. & Cepko, C. L. *Science* **241,** 1342-1345 (1988).
19. Walsh, C. & Cepko, C. L. *Science* **255,** 434-440 (1992).
20. Misson, J. P., Austin, C. P., Takahashi, T., Cepko, C. L. & Caviness, V. S. *Cerebral Cortex* **1,** 221-234 (1991).
21. Gray, G. E. & Sanes, J. R. *Neuron* **6,** 211-225 (1992).
22. O'Rourke, N. A., Dailey, M. E., Smith, S. J. & McConnell, S. K. *Science* **258,** 299-302 (1992).
23. Porteus, M. H., Bulfone, A., Ciarnello, R. D. & Rubenstein, J. L. R. *Neuron* **7,** 221-229 (1991).
24. Tao, W. & Lai, E. *Neuron* **8,** 957-966 (1992).
25. Price, M., Lemaistre, M., Pischetola, M., Di Lauro, R. & Duboule, D. *Nature* **351,** 748-751 (1991).
26. Lo, L.-C. *et al. Genes Dev.* **5,** 1524-1537 (1991).
27. Simeone, A. *et al. Nature* **358,** 687-690 (1992).
28. Fraser, S., Keynes, R. & Lumsden, A. *Nature* **344,** 431-435 (1990).
29. McGinnis, W. & Krumlauf, R. *Cell* **68,** 283-302 (1992).

ACKNOWLEDGEMENTS. We thank T. M. Jessell, M. Placzek and J. Dodd for comments and critical reading of the manuscript, and C. Walsh, C. L. Cepko and S. K. McConnell for communication of results before publication. Supported by an NIH grant (M.E.H.), an NSF FAW award (M.E.H.) and an MRC of Canada scholarship (G.F.).

Radial mosaicism and tangential cell dispersion both contribute to mouse neocortical development

Seong-Seng Tan & Sibilah Breen

Embryology Laboratory, Department of Anatomy & Cell Biology, The University of Melbourne, Parkville 3052, Australia

THE mammalian neocortex is generated by waves of migrating cells originating from the ventricular zone[1,2]. Radial migration[3] along radial glia[4,5] has been proposed as the dominant mechanism for this process. The radial unit hypothesis[3] is poorly supported by retroviral lineage studies, however, and although some clones show limited radial organization[6], the emphasis appears to be on widespread tangential dispersion[7-10]. Here we investigate the pattern of cortical cell dispersion using transgenic mice in which roughly half of the brain cells are coloured by a transgene[11]. We find that the neocortex is randomly divided into diffused bands, the majority of cells within each band have the same colour, and their radial orientation suggests radial dispersion. Superimposed upon this was a significant contribution by tangentially dispersed cells that did not respect clonal borders. These observations indicate that cortical specification is not dependent upon a single