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Central nervous system neurons migrate on astroglial fibers from heterotypic brain regions *in vitro*

(cell movement/cell–cell interaction/development)

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ABSTRACT **In different regions of the developing mammalian brain, neurons follow the processes of radial glial cells over very different trajectories to reach their destinations in specific neuronal layers. To investigate whether the movement of neurons along glial fibers is specified by glia in a given region or whether glia provide a permissive substrate for migration in different brain regions, we purified neurons and astroglial cells from developing cerebellum and hippocampus and analyzed neuronal migration on heterotypic glial fibers with time-lapse, video-enhanced differential interference microscopy *in vitro*. Granule neurons purified from early postnatal rat cerebellum migrated on astroglial processes of glia purified from late embryonic or early postnatal rat hippocampus with a cytology, neuron–glial relationship, and dynamics of movement that were indistinguishable from those of mouse granule cells migrating on cerebellar astroglial processes *in vitro* [Edmondson, J. C. & Hatten, M. E. (1987) *J. Neurosci.* 7, 1928–1934]. In the reciprocal combination, hippocampal neurons migrated on cerebellar glial processes in a manner that was also remarkably similar to migration along homotypic, hippocampal glial fibers [Gasser, U. E. & Hatten, M. E. (1990) *J. Neurosci.* 10, 1276–1285]. In all cases, migrating neurons had a characteristic appearance, apposing their cell soma against the glial fiber and extending in the direction of migration a motile, leading process that enfolded the glial fiber with short filopodia and lamellipodia. As seen by video microscopy, neurons moved along homotypic and heterotypic glial processes by translocation of the soma and were not "pulled" forward by the leading process. As the neuron moved, the nucleus remained in the posterior portion of the cell and cytoplasmic vesicles moved forward from the soma into the leading process. The dynamics of the movement of neurons along heterotypic glial substrates, including the speed and periodicity of motion, was identical to that of neurons migrating along homotypic glial substrates. These experiments suggest that the mechanism of movement of neurons along glial fibers is conserved in these two brain regions during development.**

In the developing mammalian brain, the radially oriented processes of astroglial cells provide the primary pathway for the migration of young neurons from their site of genesis in ventricular zones to their position in the cortical plate (1–3). Differences in the cytoarchitecture of different cortical regions (3) and variations in cell-surface and extracellular matrix antigens (4–6) have suggested that neuron–glial relationships of migrating cells might differ among brain regions. This view is supported by studies on neurological mutant mice showing that the weaver and reeler genes perturb glial-guided migration of selected neurons in cerebellar cortex and cerebral cortex, respectively, without affecting migration in the other region (7–12).

Previous studies used a microculture system to analyze the dynamics of neuronal migration by living cells along glial fibers. In studies on granule neurons purified from early postnatal mouse cerebellum (13, 14), and more recently in studies on rat hippocampal neurons (15), migration was shown to occur on highly elongated glial processes (13) *in vitro*. With time-lapse, video-enhanced differential interference contrast microscopy, it was possible to resolve the cytological features of migrating cells and to correlate these features with the dynamics of motion of the neuron along the glial guide (14). Cells from the cerebellum and hippocampus showed remarkable similarity in the cytology, neuron–glial relationship, and dynamics of movement of neurons along glial guides. Migrating cells had a unique morphology, elongating into a bipolar form, apposing their cell soma against the glial arm, and extending a leading process as they moved. With correlated electron microscopy, a specialized "interstitial" junction was observed between the neuronal soma and the glial guide (16). This structure is currently a candidate for the site at which the force for neuronal movement is generated, since in the video recordings of migrating cells it appears that the cells maintain the apposition beneath the neuronal cell soma as they move (13–15). Another distinctive feature of migrating cells, reported in detail for cerebellar neurons (14), was the saltatory motion of the neuron. Neurons moved for 4–5 min, paused for an approximately equivalent period, and then moved again with a periodic rhythm. Thus the cytology, neuron–glial relationship, and dynamics of movement of neurons along cerebellar and hippocampal glial fibers appeared to be strikingly similar.

To test the regional specificity of glial-guided migration, we "mixed and matched" neurons purified from cerebellum and hippocampus with astroglial cells of the same or other region and analyzed the cytology and neuron–glial apposition of migrating neurons in heterotypic cocultures with time-lapse, video-enhanced differential interference contrast microscopy (14). We chose these two regions because their cytoarchitecture and synaptic connections are well described and the time course of their development is known. In both regions, glial-guided neuronal migration establishes the pattern of neuronal layers and the bulk of this migration occurs in the late embryonic and early postnatal periods (1, 17–21), the time at which we harvested the cells for *in vitro* studies.

MATERIALS AND METHODS

Purification of Neurons and Astroglia from Cerebellum and Hippocampus. Cerebellar granule cells and astroglia were purified from neonatal Sprague–Dawley rats on postnatal days 0–3 (P0–P3) as described previously for mouse cells (22, 23). In brief, a single-cell suspension was separated into a large- and a small-cell fraction by a two-step Percoll step gradient, yielding neuronal populations of 95–98% purity. Pure granule

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Abbreviations: E*n*, embryonic day *n*; P*n*, postnatal day *n*.
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neuron and astroglial cell fractions were generated by subsequent plating (45–60 min) on a culture surface treated with poly(L-lysine) (22). For the present experiments, neuronal preparations were made with three serial preplating steps.

Hippocampal tissue was harvested from Sprague–Dawley rats at embryonic day 20 (E20) or P0–P3. A single-cell suspension was prepared as described (15), after which the suspension was passed through a monofilament polyester screen (33-μm mesh size; Tetko, Elmsford, NY) to remove cellular aggregates before plating on a surface treated with polylysine (25 μg/ml) for 30–50 min, after which the neurons were dislodged by sharply rapping the dish against the lab bench and removed by washing. The purity of the resulting neuronal and astroglial cell populations was confirmed as described previously by immunostaining with antisera against the glial filament protein, a marker for astroglial cells, and against NILE (nerve growth factor-inducible large external) protein, a marker for neurons (13, 22–24).

Homotypic and Heterotypic Recombination Microcultures of Neurons and Glia from Cerebellum and Hippocampus. For homotypic cultures, neurons and glia from either cerebellum or hippocampus were recombined at ratios between 4:1 and 10:1 in a final volume of 50 μl in glass-coverslip microcultures (25) in Eagle's basal medium with Earle's salts (BME) supplemented with 10% horse serum, 4 mM glutamine (13, 14), 8 mM glucose, and a penicillin/streptomycin mixture at 20 units/ml (all medium components except glucose were from GIBCO). Prior to the addition of the cells, the culture surface was pretreated with Matrigel (Collaborative Research; dilution 1:25–1:100, 45 min at 35.5°C). Subsequently the dishes were washed three times with H_2O prior to the addition of medium containing serum. After 1 hr, the medium was exchanged for serum-free medium (N2), and a 25-mm glass coverslip was placed over the culture well. In all experiments, the final plating density was 1–2 × 10^6 per ml; plating efficiency was 90–95%.

For heterotypic cocultures, granule neurons purified from P0–P3 rat cerebellum were added to hippocampal astroglial cells purified either from the same animal or from E20 rat hippocampus, at a neuron/glial cell ratio of 10:1, and plated in medium as described above. In reciprocal recombinations, neurons purified from E20 rat hippocampus, were cocultured with astroglia purified from the same tissue or with glia purified from P0–P3 rat cerebellum. Twelve to 36 hr after the cells were plated, we examined neuronal behavior on glial processes in real time by video-enhanced differential interference contrast microscopy (14).

Video-Enhanced Differential Interference Contrast Microscopy. For video microscopy, the medium was changed to L15 medium supplemented with 10% horse serum and 8 mM glucose, and the chamber was sealed with a second coverslip as described (14). The behavior of bipolar granule neurons that were closely apposed to glial fibers was observed with differential interference contrast microscopy (14). The image was recorded on a Panasonic memory disk recorder with a Hamamatsu Chalnicon video camera mounted on a Zeiss Axiovert microscope fitted for Nomarski optics. A Zeiss Plan-Neofluor ×100/n.a. 1.3 oil-immersion objective was used. An Image-1 computer system was used to enhance and pair the images for still photography of selected frames, as well as to drive the memory disk recorder at a speed of one frame per second. As described previously (13–15), after imaging neuronal migration, we scribed the field and immunostained with antibodies against the glial filament protein (24) to confirm that the process on which the cells migrated was glial in origin. In the present study, we analyzed more than 300 heterotypic recombination cultures.

RESULTS

In cocultures of cerebellar granule cells with hippocampal glia, extensive neuron–glial interactions were seen (13, 24) and approximately 10–20% of the neurons had the extended, bipolar shape of migrating neurons (13, 14). Time-lapse, video-enhanced differential interference contrast microscopy of the behavior of 66 migrating neurons revealed that cerebellar neurons migrated along heterotypic glial substrates, and provided detailed information on the cytology of migrating neurons and the dynamics of their movement along glial fibers.

In cocultures of cerebellar granule cells with hippocampal glia, migrating granule neurons closely apposed the cell soma against the glial arm, forming a site of cell–cell contact along the length of the neural cell soma and extending a leading process along the glial fiber in the direction of migration. Both lamellipodial and filopodial extensions were common along the surface of the leading process, the latter being relatively short (1–5 μm) when compared with filopodia seen on neuronal growth cones. In all of the cases we observed, the nucleus remained in the posterior portion of the neuronal soma and intracellular vesicles flowed from the area just forward of the nucleus down into the leading process as the neuron moved along the glial fiber. The cytology of the

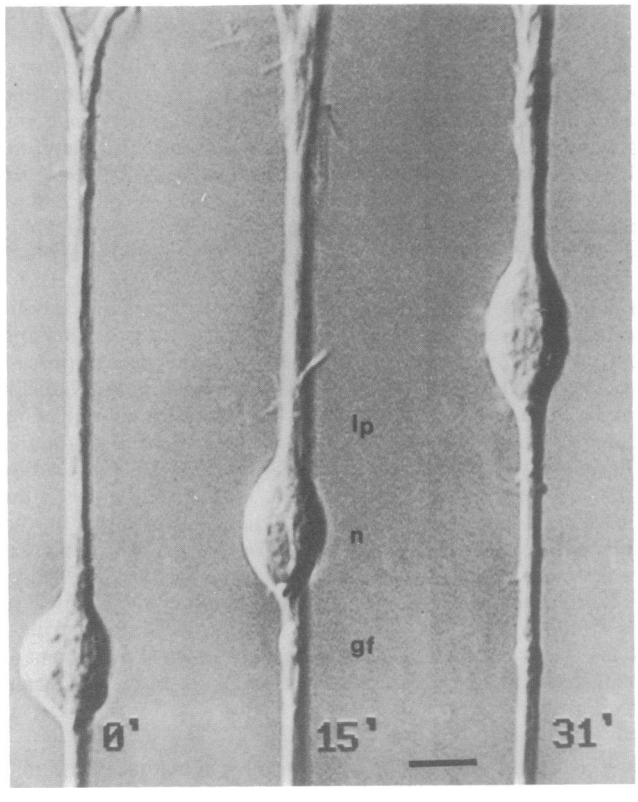

FIG. 1. Rat cerebellar granule neurons migrate on rat hippocampal glial processes *in vitro*. Cerebellar granule cells were purified from neonatal rats on P4 and added to hippocampal astroglial cells purified from embryonic rat hippocampus harvested on E20, at a ratio of 10:1. After 24 hr *in vitro*, the behavior of bipolar granule neurons that were closely apposed to glial fibers was observed with differential interference microscopy (13). The image was recorded on a Panasonic memory disk recorder with a Hamamatsu Chalnicon video camera mounted on a Zeiss Axiovert microscope fitted for Nomarski optics. A Plan-Neofluor ×100/n.a. 1.3 oil-immersion objective was used. The neuron (n), visible in the plane above the glial fiber (gf), moves along the glial process by extending its cell soma along the glial fiber and extending a leading process (lp) in the direction of migration. Short filopodia (1–5 μm) are seen along the leading process. Time elapsed in real time was 0, 15, and 31 min. (Video-enhanced differential interference contrast microscopy; bar = 5 μm.)

migrating granule cell and the dynamics of its movement along hippocampal glial processes closely paralleled results reported previously for mouse granule cell migration on cerebellar astroglial fibers (13, 14). Fig. 1 shows the migration of a rat cerebellar granule neuron on a rat hippocampal glial fiber.

In reciprocal cocultures of rat hippocampal neurons with rat cerebellar glia, similar results occurred (Fig. 2). The hippocampal neuron moved along the cerebellar glial process by apposing its cell soma along the glial fiber. As seen for hippocampal neurons migrating along homotypic, hippocampal glial fibers (15), as the hippocampal neuron moved, the nucleus remained in the posterior portion of the cell soma, and movement of cytoplasmic organelles was evident forward of the nucleus into the leading process. Identical results were seen for large (6–10 μm) and small (4–6 μm) hippocampal neurons.

The cytology, mode of movement, and speed of migration of hippocampal neurons on cerebellar astroglial processes closely resembled that described for migrating cerebellar granule cells on hippocampal glia. In both cases, migrating neurons assumed a stereotyped posture on the glial arm, forming a site of cell–cell contact along the length of the cell soma and extending a thin leading process with lamellopodia and very short (1–5 μm) filopodia (Fig. 3).

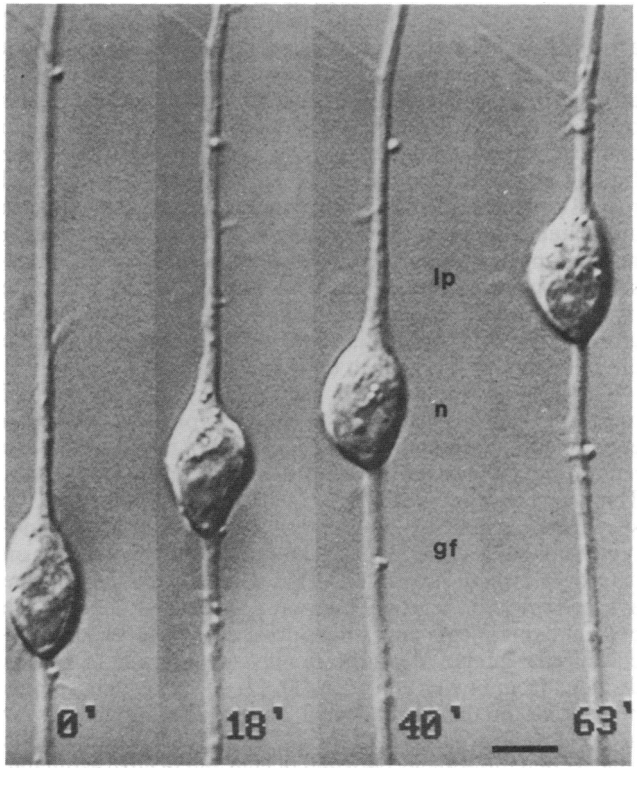

FIG. 2. Mode of migration of a rat hippocampal granule cell along a rat cerebellar astroglial fiber *in vitro*. The hippocampal granule neuron (n), purified from rat hippocampus on E20 (21), moves along a cerebellar glial fiber (gf), purified on P5 (19), by arching its cell body along the glial guide. The nucleus remains in the posterior portion of the cell, and extensive movement of cytoplasmic organelles into the leading process (lp) is seen. The leading process is a highly active structure with numerous lamellipodial and filopodial extensions. Filopodia tend to be short (1–5 μm) in comparison with filopodia commonly seen on neuronal growth cones. Although it is not evident in the planes photographed here, as the neuron moves, an extensive site of cell–cell contact between the migrating neuron and the glial fiber is maintained along the length of the neuronal cell body. Time elapsed in real time was 0, 18, 48, and 63 min. (Video-enhanced differential interference contrast microscopy; bar = 5 μm.)

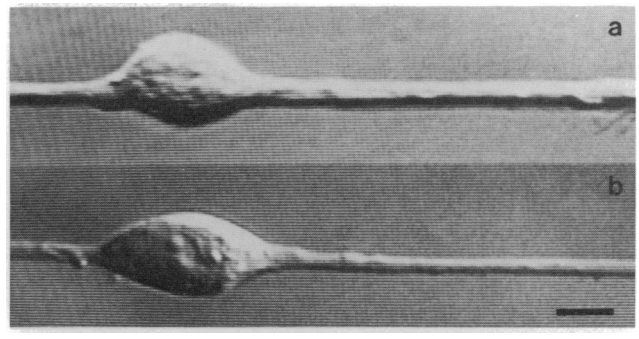

FIG. 3. Cytology of cerebellar neuron migrating on a hippocampal glial fiber and hippocampal neuron migrating on a cerebellar glial fiber. In heterotypic cocultures, migrating neurons have a stereotyped cytology, extending their cell soma along the glial guide and extending a leading process in the direction of migration. (*a*) A cerebellar granule cell (P4) migrating along a hippocampal glial fiber (E20). (*b*) A hippocampal granule neuron (E20) migrating along a cerebellar glial process (P5). (Video-enhanced differential interference contrast microscopy; bar = 5 μm.)

On either homotypic or heterotypic glial substrates, cerebellar granule neurons or hippocampal neurons moved at speeds between 15 and 60 μm/hr. In Table 1, the speed of migration of six neurons is given. In all of the combinations we studied, as measured previously for cerebellar granule neurons (14), neuronal migration was saltatory, with a motion phase of 4–6 min, followed by a resting phase of approximately the same duration. During the motion phase, cells moved at 50–60 μm/hr. As seen for cerebellar neurons, migrating neurons moved with this periodicity for 30–120 min, before stopping for indeterminate periods and then moving again. The speed of migration of individual cells at any given time point was therefore quite variable, ranging between 0 and 60 μm/hr, when cells were videotaped for short periods of time. However, when the cells were followed for 2 hr or more, the speed of migration averaged to 10–20 μm/hr.

As seen for cerebellar glial-guided migration (13, 14), we often observed several neurons simultaneously migrating on the same glial fiber (Fig. 4). This was common both in cultures of cerebellar neurons with hippocampal astroglia and in the reciprocal combination. In each of these cases, when only one neuron was present on the glial arm, migration was bidirectional. When two more neurons occupied the fiber, both neurons moved in the same direction.

Unlike the cerebellar granule cell preparation, which was uniform, the hippocampal neuron preparation was a mixture

Table 1. Speed of neuronal migration in homotypic and heterotypic cocultures of neurons and glia from cerebellum and hippocampus

Neuron source	Astroglial cell source	Average speed of migration, μm/hr
Cerebellum	Cerebellum	25.5 (85.0)
Cerebellum	Hippocampus	27.6 (90.0)
		55.8 (47.0)
Hippocampus	Hippocampus	54.6 (45.8)
		36.0 (50.0)
Hippocampus	Cerebellum	25.8 (100)

Homotypic or heterotypic cocultures of rat cerebellar cells and rat hippocampal cells were prepared as described in Figs. 1 and 2, and the movement of neurons along astroglial processes was measured by differential interference microscopy with a Universal Imaging "Image 1/AT" morphometric analysis system. Each value is the average speed determined for a single neuron. Numbers in parentheses are the times of observation (minutes) from which the values were calculated.

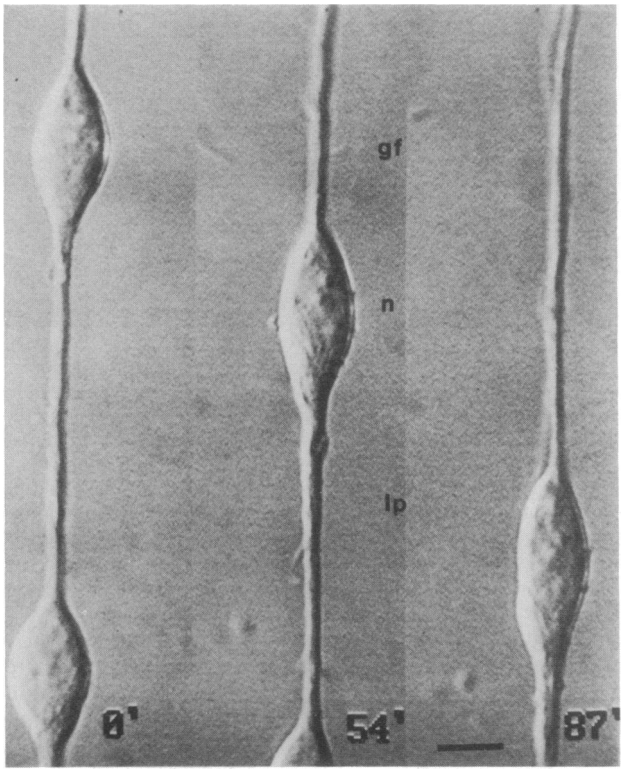

FIG. 4. Migration of two hippocampal neurons on a single cerebellar astroglial process. Neurons were purified from E20 rat hippocampus and added to astroglial cells purified from P5 rat cerebellar tissue. In the frame at 0 min, two neurons are seen migrating along the same glial process. After 54 min, the neuron (n) seen at the top of the frame at 0 min migrates along the glial fiber (gf), extending a leading process (lp) in the direction of migration, while the neuron seen at the bottom of the frame at 0 min moves along the glial process to a position off of the screen in the frame at right (87 min). The two neurons are similar in appearance as they migrate along the glial fiber, and they move in the same direction. (Video-enhanced differential interference contrast microscopy; bar = 5 μm.)

of hippocampal granule cells and larger neurons (25, 26). Elongated, bipolar profiles were seen for both the small granule neurons and for larger hippocampal neurons, even though, as in the reciprocal coculture or in homotypic cocultures of either region, migrating neurons were a minority of the total neuronal population.

To examine the role of the leading process in the forward motion of the neuronal soma of neurons moving along heterotypic glial substrates, we examined the synchrony of the forward movement of the neuronal soma with the extension of filopodia and lamellopodia along the leading process by a frame-by-frame analysis of the video recordings of 16 migrating neurons. As reported previously for cerebellar neurons on homotypic glial substrates (14), in all of the cases we examined, such measurements revealed that neuronal movement was not synchronized with the extension of lamellopodia or filopodia along the leading process (data not shown).

DISCUSSION

The features of migrating neurons seen in the present studies on heterotypic recombinations of neurons and glia purified from rat cerebellum and hippocampus *in vitro* were remarkably similar to results of previous studies on glial-guided migration of mouse cerebellar granule neurons on cerebellar glial substrates and of rat hippocampal neurons on hippocampal substrates *in vitro* (14, 15). The common features of the migrating neuron on either homotypic or heterotypic glial fibers were the apposition of the neuronal cell body against the glial fiber, the extension of a leading process in the direction of migration, the movement of organelles from the nucleus into the cytoplasm, and the saltatory periodicity of motion. In all of the cases we observed, movement occurred by the translocation of the soma along the glial fiber. The major variable among types of neurons in either region or among neurons from different regions was the size of the neuronal soma. These results suggest that the cytology, neuron–glial relationship, and mode of movement of migrating neurons are conserved in these two regions of the developing brain.

The present experiments show, as seen previously for cerebellar cells (13, 14) and for hippocampal neurons (15) *in vitro*, that the leading process is a highly active structure that exhibits extensive membrane activity, including the extension of short filopodia and lamellipodia. The tip of the leading process rapidly extends and retracts as the neuron migrates along either homotypic or heterotypic glial fibers, but neither these motions nor those of filopodia or lamellopodia are synchronized with the motions of the cell soma (14). Thus the leading process does not appear to "pull" or "push" the neuron forward. Instead movement appears to be generated along the apposition of the neuronal soma to the glial fiber. A candidate structure in the mediation of migration is the "migration junction," a specialized junction between the migrating neuron and the glial fiber, seen with correlated video and electron microscopy (16).

Although the leading process resembles the growth cone of a neurite in its motility, the present study suggests that it differs in several aspects. First, whereas the growth cone is an expansive ending of a thin neurite, the leading process is contoured to the dimensions of the glial fiber. Second, video and electron microscopic analyses (13–16) suggest that the leading process of the migrating neuron is simply a tapered rostral portion of the cell, rather than a site at which growth and extension occur. Third, the cell soma of the migrating neuron appears to be the site of adhesion to the glial substrate. In growth-cone locomotion, the growth cone is the site of adhesion to the substrate. A common feature shared by the growth cone and the leading process is that both guide the directionality of the neuron (14).

The striking similarities in the cytology and behavior of migrating neurons, seen in the heterotypic recombination experiments, suggest that the mechanism of movement of the neuron along the glial fiber is conserved among brain regions. This interpretation is supported by the observation that the speed and periodicity of movement of neurons along homotypic glial guides (13–15) are remarkably similar to those on heterotypic glial substrates. The values obtained for the average speed of neuronal migration along glial fibers in the present experiments correspond to speeds observed for cerebellar glial-guided migration *in vitro* (13, 14) and to speeds deduced from *in vivo* analyses (20, 21, 27), including studies of neurons in the ferret visual cortex after isochronic or heterochronic transplantation (28, 29). The present results suggest that differences between *in vivo* and *in vitro* estimates of the speed of migration relate to the duration of time over which the cells are followed, with actual speeds of 50–60 μm/hr averaging to slower speeds of 10–20 μm over observation periods of hours or days. The finding that the periodicities of motion *in vitro* and the average speeds of migration for cells in various cortical regions *in vivo*, in species ranging from rodents to humans, are so similar suggests a common mechanism of movement by neurons along glial guides.

These experiments are consistent with the interpretation that the mechanism of glial support of neuronal migration is conserved across brain regions, and suggest that astroglial fibers provide a permissive pathway for neuronal migration in the developing brain. The regulation of the distances traveled along radial glial fibers and the termination of migration, seen

to vary so greatly during the establishment of neuronal layers in various cortical regions (3), are therefore likely to be mediated by hierarchical neuron–neuron interactions rather than neuron–glial interactions.

In this model, neurons would migrate along glial fibers until cell–cell interactions with neurons diverted the neuron from the glial guide into a neuronal layer. This view is supported by studies on the formation of cortical layers, suggesting that many central nervous system neurons are committed to a particular laminar fate prior to their migration along radial glia. Studies by Caviness and coworkers (11, 12, 30–32) and by Lemmon and Pearlman (33) on the neurological mutant mouse reeler, an animal in which neuronal migration along glial fibers in the cerebral cortex is prematurely arrested, stranding immature neurons in sites deeper than the layers in which they would normally reside, have shown that many reeler neurons manage to form appropriate connections in spite of their premature detachment from glial guides. In addition, ablation experiments by Jones and coworkers (34) and by Jensen and Killackey (35) have suggested that ectopic neocortical neurons can form appropriate subcortical projections, and transplantation experiments by McConnell (28, 29) have indicated that subpopulations of presumptive deep-layer primary cortical neurons can migrate into the appropriate neuronal layer after isochronic or heterochronic transplantation.

Our *in vitro* experiments suggest that glial fibers provide a permissive substrate for neuronal movement from ventricular zones into neuronal layers, a substrate that restricts and directs the movement of young neurons in a radial direction but does not guide the cells into a particular layer. These experiments further suggest that the "stop signals" for neuronal migration, signals that direct a neuron into a particular layer of the cortex, arise from neuron–neuron interactions. Although this model suggests that the glial fiber is a passive partner in neuronal migration, it does not diminish the importance of glial-supported neuronal migration to neuronal survival and differentiation. This is evidenced in the neurological mutant mouse weaver, where cerebellar granule neurons, a primary site of action of the weaver gene (10), die in ectopic positions (8, 9) after failing to attach to the glial fiber and migrate into the internal granule cell layer.

The details of the mechanism of the movement of neurons along glial fibers remain to be described. In particular, the role of the geometry of the glial fiber, the contractile elements of the migrating neuron, the relationship of neuron–glial adhesion ligands to cytoskeletal elements, and the interaction of the leading process of the migrating neuron with growth cones of neurons already settled in layers the neuron is traversing all require further analysis.

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