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Mechanisms of glial-guided neuronal migration in vitro and in vivo

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Summary. Our laboratory has developed an in vitro model system in which glial-guided neuronal migration can be observed in real time. Cerebellar granule neurons migrate on astroglial fibers by apposing their cell soma against the glial arm, forming a specialized migration junction, and extending a motile leading process in the direction of migration. In vitro assays indicate that the neuronal antigen astrotactin functions as a neuron-glia ligand, and is likely to play a role in the movement of neurons along glial fibers. In heterotypic recombinations of neurons and glia from mouse cerebellum and rat hippocampus, neurons migrate on heterotypic glial processes with a cytology, speed and mode of movement identical to that of neuronal migration on homotypic glial fibers, suggesting that glial fibers provide a permissive pathway for neuronal migration in developing brain. In vivo analyses of developing cerebellum demonstrate a close coordination of afferent axon ingrowth relative to target cell migration. These studies indicate that climbing fibers contact immature Purkinje neurons during the migration and settling of Purkinje cells, implicating a role for afferents in the termination of migration.
Key words. Neuronal migration; astrotactin; neuronal antigen; heterotypic recombination; glial-guided migration.

Introduction

Neuronal migration has traditionally been viewed as a discrete step in CNS development, separated in time from preceding periods of neuronal proliferation in ventricular zones and from subsequent periods of axon ingrowth into neuronal layers of the cortex [38, 55]. Although the role of astroglia in providing a cellular substrate for migration is well established [8, 10, 12, 19, 45–52, 55], the hierarchical roles of neuron-neuron interactions in terminating glial-guided migration have not been addressed. To examine the mechanism of glial-guided neuronal migration, we have developed an in vitro model system [19]. Initially we focused on the migration of the granule neuron in the developing mouse cerebellum, using in vitro systems to define the mode of neuronal movement along glial fibers [8, 12, 20], the regional specificity of migration [11] and the molecular mechanism of migration [9]. To examine

whether afferent axon ingrowth plays a role in arresting neuronal migration, we have used axonal dye filling methods together with cell-specific antigen markers[30,32,33] to trace the ingrowth of axons into the embryonic and early postnatal cerebellum and define the interactions of growing afferent axons with migrating target neurons.

Glial-guided neuronal migration in vitro

To analyze the migration of living neurons along glial fibers, we developed an in vitro model system for mouse cerebellar cells[16,19]. A key feature of this culture system is the small volume (20–50 µl) in which we culture the cells. In these microcultures, relationships between neurons and glial cells develop that are not readily seen in more conventional, larger cultures, and astroglial cells differentiate into highly elongated, radial forms which resemble the glial forms which have been shown to support neuronal migration in brain[20]. Subsequently numerous migratory profiles are seen along glial fibers in the culture.

With video-enhanced differential contrast (AVEC-DIC) microscopy, we obtained a detailed view of the morphology and behavior of migrating neurons on glial processes, in real time[8] (fig. 1). Although only a small percentage of the cells (10%) move along glial fibers at any given moment, the cytological features of *living*, migrating granule neurons are similar to those described by Rakic[45,51] for cells that had been assumed to be migrating in vivo. Migrating neurons express a highly extended bipolar shape along the glial fiber, forming a close apposition with the glial process along the length of the neuronal cell soma and extending a leading process in the direction of migration (fig. 1). As the neuron moves, the site of contact with the glial fiber, just underneath the soma, is maintained, suggesting that this is the site at which the force for motion is generated. The leading process is a highly motile structure, extending and retracting short filopodia and lamellopodia which enfold the glial fiber. As the neuron moves, organelles flow from the cell soma forward into the leading process. Thus, the migrating neuron appears to move along the glial fiber by forming a 'footpad' beneath the cell soma and extending a tapered, leading process along the glial guide[8] (fig. 1). Our video observations were extended by correlating the behavior of migrating neurons with their cytology, as viewed in the electron microscope[12]. After recording the movement of a migrating neuron on a glial process, we fixed the culture and marked the area of the dish around the cell. In cells that were moving prior to fixation, a specialized migration junction, an 'interstitial junction', was present beneath the neuronal cell soma at the site of apposition with the glial fiber. This junction consisted of a widening of the intercellular space, and filamentous material in this space that spans the cleft and membranes of each cell, contiguous with cytoskeletal elements. The interstitial junctions were seen only in cells that were moving along the glial process. In contrast, in resting cells, puncta adherentia or attachment junctions were found where the neuron apposes the glial fiber, and unlike the migration junction, these small focal densities lacked any obvious connections to the cytoskeleton of the apposing cells[12]. Thus, migrating neurons form a 'migration junction' along the apposition between migrating neuron and glial fiber, the site that by video microscopy appears to be the force-generating locus during forward movement.

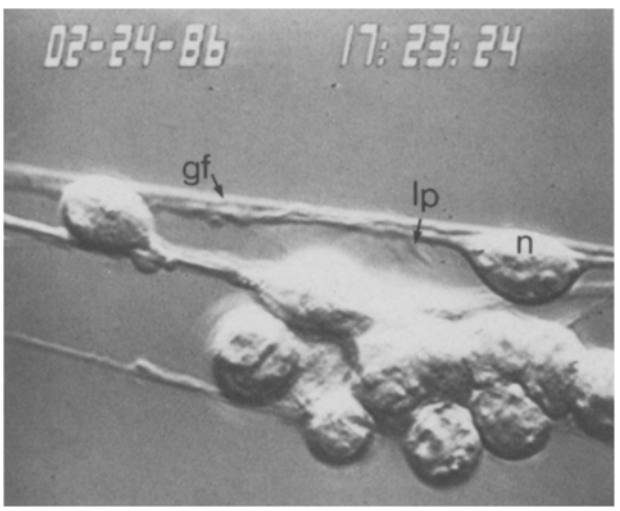

Figure 1. Cytology and neuron-glia relationship of a cerebellar granule cell migrating along an astroglial fiber. Cerebellar cells were dissociated from early postnatal mouse cerebellar tissue and cultured in microwells for 24 h prior to observation with video-enhanced differential interference contrast microscopy. The granule neuron (n) closely apposes the glial fiber (gf) along the length of the neuronal cell soma, and extends a leading process (lp) in the direction of migration along the glial guide. (Reprinted in modified form from Edmondson and Hatten[8]).

To examine whether the behaviors we had observed with cerebellar granule cells were general characteristics of migrating neurons, we developed a microculture system for hippocampal cells[10]. With this culture system, Urs Gasser observed a similar mode of movement and neuron-glia relationship of hippocampal neurons moving along glial guides. Both large hippocampal neurons, possibly representing pyramidal neurons, and small hippocampal neurons, representing hippocampal granule neurons, migrated along glial fibers in vitro and both exhibited the cytological features and dynamics of cerebellar neurons migrating along glial processes[10] (fig. 2).

Weaver cerebellar cells

These observations, although made in a tissue culture system, confirmed that neurons migrated along glial processes in complex cortical regions. The issue then arose of how migration is regulated, by the neuron or the glial cell. The neurological mutant mouse *weaver* provided an experimental paradigm for this issue, because in *weaver* cerebellum, granule cells fail to migrate along the Bergmann glia and die in ectopic positions[4,50,57].

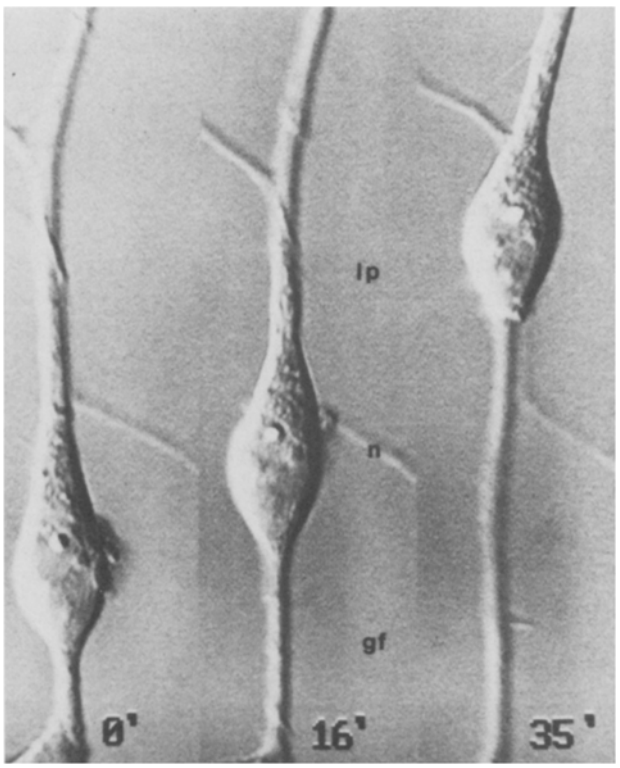

Figure 2. Glial-guided migration of hippocampal neurons in vitro. The neuron (n) contacts the glial process (gf) along the interface with the neuronal cell soma and extends a leading process (lp) in the direction of migration. As the neuron moves, the nucleus remains in the posterior portion of the neuronal soma and cytoplasmic organelles stream forward into the leading process.

Anatomical studies of *weaver* showed defects in the glial cells in *weaver*, Bergmann glia displayed abnormal arbors and were deranged and misaligned, rather than aligned in the radial pattern presumed to support migration [50]. Thus defects were seen in both the neurons and glial fibers of *weaver*, making the issue of which cell type was defective difficult to answer from studies of intact brain.

To analyze the primary site of action of the *weaver* gene, we developed methods to purify neurons and astroglia from normal and *weaver* cerebellum, and recombined them in homotypic and heterotypic co-cultures to analyze migration along glial fibers [21]. These experiments showed that normal neurons would migrate on either normal or *weaver* glia, but *weaver* neurons migrated on neither, suggesting that the granule cell is the site of action of the *weaver* gene.

The in vitro experiments suggested that the defects in the cellular architecture of the *weaver* cerebellum occurred as a cascade of secondary events subsequent to the failure of the *weaver* neuron to bind to astroglial cells. In particular, the defects in glial form and organization were shown, by in vitro analyses, to result from the failure of the neuron to bind to the glial process [21]. This interpretation was supported by other in vitro studies in the laboratory showing that neurons regulate astroglial differentiation by a membrane-mediated mechanism [13, 14]. The role of neurons in regulating astroglial differentiation has been reviewed recently [18].

Regional specificity of glial-guided migration

We have further investigated the regulation of migration by neurons versus glia by combining neurons and glia, each purified from different brain regions, and analyzed migration along heterotypic substrates in vitro [11]. Such experiments provided an experimental analysis of the hypothesis that the navigational instructions for migration are encoded in the neuron. To prepare the co-cultures, we purified neurons and astroglia from the cerebellum and the hippocampus. The hippocampus was of particular interest, because its cytoarchitecture differs markedly from that of cerebellum; glial fibers in the hippocampus are shorter than those in cerebellum and they arc through the hippocampal tissue in an undulating pattern [6, 23, 40, 42, 52]. If migration is regulated in the neuron, we expected neurons from one region to migrate freely on glia from either region. If the information was restricted to the glial fiber, we expected the opposite, namely that neurons would migrate only on homotypic astroglial substrates.

The results of these recombination experiments were striking. First, neurons from cerebellum or hippocampus migrated freely on heterotypic astroglial cells (fig. 3). Second, and more importantly, the cytology of migrating cells, the mode of migration, neuron-glia relationship, dynamics and speed of movement of the neurons on homotypic and heterotypic glial substrates were identical [11] (fig. 4). The finding that the features of migrating cells are so similar in homotypic cultures and in heterotypic recombination experiments suggests that the mechanism of movement of the neuron along the glial fiber is conserved among brain regions, and that astroglial fibers provide a permissive pathway for neuronal migration in the developing brain [11, 17]. The regulation of the timing, extent and termination of migration, seen to vary so greatly during the establishment of neuronal layers in various cortical regions [49, 55], is therefore likely to be mediated by neuron-neuron interactions rather than neuron-glial interactions.

A number of behaviors of migrating neurons were similar in homotypic and heterotypic co-cultures of neurons and glia from cerebellum and hippocampus. First, migration is bi-directional in vitro, when the glial fiber is occupied by a single neuron [8, 11, 20]. In that situation, the single neuron migrates to the end of the glial fiber, pauses and then migrates back in the opposite direction. When two or more cells are migrating along the fiber, migration becomes oriented and cells do not turn back to migrate in the opposite direction when they reach the end of the glial fiber. This suggests that 'traffic' loads, not gradients of molecules along the glial fiber, define the directionality of migration in the living tissue.

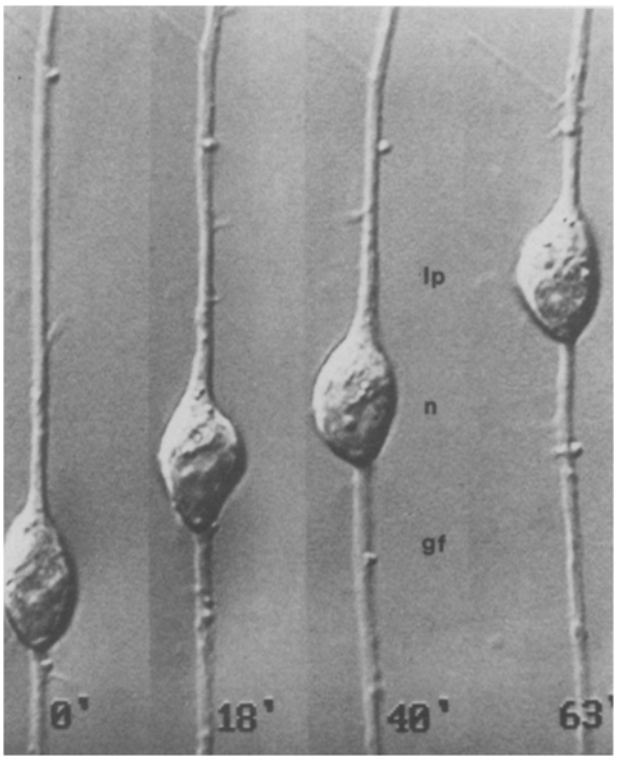

Figure 3. Hippocampal neurons migrate along cerebellar astroglial fibers in vitro. In heterotypic co-cultures of neurons and glia from different brain regions, extensive neuronal migration is seen. The hippocampal neuron moves by arching its cell body along the cerebellar glial guide (gf). As the neuron(s) moves, it arches its cell soma along the glial guide, seen here in the optical plane beneath the neuron. The leading process is a highly active structure with numerous lamellopodial and filopodial extensions. Filopodia tend to be short (1–5 μm) in comparison with filopodia commonly seen on neuronal growth cones. lp, leading process. Time elapsed (min) in real time.

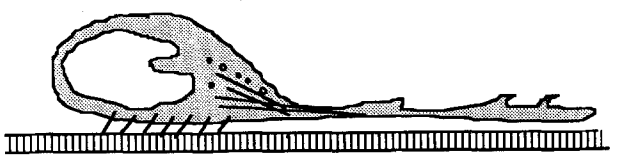

Figure 4. Model for glial-guided neuronal migration. Migrating neurons express a bipolar shape, the cell soma closely adhering to the glial fiber and extending a leading process in the direction of migration. As the neuron moves, a specialized contact, the 'interstitial junction', forms along the somal apposition with the glial fiber, short filopodia and lamellipodia extend from the leading process as it enfolds the glial fiber, and organelles flow from the nucleus forward into the leading process.

Second, in all of the cases we have examined for cerebellar cells and hippocampal neurons, neuronal migration is saltatory[8, 11, 20]. The neuron moves at speeds of 50–60 μm/h and then pauses in cycles lasting 4–6 minutes. Then intermittently, the neuron stops for indeterminate, longer periods of time (minutes to hours) before moving forward again[8]. Over a period of hours in vitro, the speed of glial-guided migration averages 10–20 μm/h, a figure that agrees with most values obtained in vivo studies[8, 11, 20, 35, 40, 47, 48]. Our in vitro studies suggest that slower speeds observed in some in vivo analyses (2–5 μm/h) relate to the long time periods over which the cells were followed, periods during which the neurons probably stopped moving for extended periods.

Third, frame-by-frame analysis of video recordings of migrating cells indicate that the neuron moves by pushing its soma against the glial fiber, rather than as the result of the leading process pulling or pushing the neuron along the glial guide. The tip of the leading process rapidly extends and retracts as the neuron migrates, but neither these motions nor those of filopodia or lamellopodia are synchronized with the motions of the cell soma[8, 11]. One possibility for generating the traction for the cellular movement is the 'interstitial junction', a specialized junction between the migrating neuron and the glial fiber, seen with correlated video and electron microscopy[12]. In this junction, fibrils span the intercellular space and extend into the cytoplasm of both cells, and appear to be contiguous with cytoskeletal elements.

The primary role of the leading process appears to be setting the directionality of migration, by selecting which glial fiber to migrate along. In vitro, we have often observed migrating neurons shifting from one glial fiber to another. This occurs by the translocation of the leading process from one fiber to the other and requires that the two glial fibers be overlapping or close together, as we have not seen cells detach from the fiber and then move alone to another fiber. The finding that neurons shift from one fiber to another is of special interest, given the recent studies of Misson and Caviness[39] showing that glial fibers branch and fasciculate as they course from the ventricular zone out to the cortical plate. Our in vitro studies suggest that the neuron can navigate through complex glial terrain by shifting, in some cases, to other glial fibers[8, 17].

A number of issues remain to be studied for glial-guided neuronal migration. In particular, the role of the geometry of the glial fiber, the contractile elements involved in neuronal motility, the relationship of neuron-glia adhesion ligands to the specialized 'migration junction' and to cytoskeletal elements, and the interaction of the leading process of the migrating neuron with growth cones and cell bodies of neurons already present in layers the neuron is traversing, all require further analysis.

Although our studies of heterotypic recombinations of neurons and glia from several brain regions suggests that the glial fiber is a passive partner in neuronal migration, they do not diminish the importance of glial-guidance to neuronal survival and differentiation, or of neuron-glia binding to neuronal migration. This is evidenced in the neurological mutant mice *weaver* and *reeler*[4]. In *weaver*, cerebellar granule neurons fail to *attach* to the glial fiber or to migrate along Bergmann glia[20, 50] and subsequently die in ectopic positions[57]. In *reeler*, a mutation which affects migration in cortex[2, 3], neurons bind to astroglial fibers and migrate but migration is arrested prematurely,

apparently from a failure of the neurons to *detach* from the glial guide[44].

Molecular basis of migration

A major issue in glial-guided neuronal migration is the molecular mechanism of neuronal movement along glial fibers. In order to define the ligands which bind the migrating neuron to the glial guide, we have used our culture system as a functional assay for immune activities that block neuron-glial interactions. With this approach, we have identified an immune activity, which we named astrotactin, which blocks neuron-glia interactions in vitro[9]. By immunoprecipitation and Western blot analysis, the major activity of the astrotactin antiserum recognizes a neuronal glycoprotein of apparent molecular weight 100 kDa. The band at 100 kDa is not immunoprecipitated in Triton extracts of granule cells from the neurological mutant mouse *weaver*, an animal that suffers defects in neuron-glia interactions including glial-guided migration of the granule neuron, suggesting that the expression of astrotactin is altered in *weaver*[9].

Antibodies against astrotactin block the binding of neuronal membranes to glial cells[59], the establishment of neuron-glia contacts, the organization of neuronal positioning by glial processes[9] and the migration of neurons along glial processes in vitro (Fishell and Hatten, unpublished observations). In contrast, antibodies against the major families of neuron-neuron ligands, N-CAM[61], L1[53], NILE[37], TAG-1[5] and N-cadherin[13], do not block any of these neuron-glia interactions in vitro[9,59]. Our in vitro studies therefore suggest that astrotactin functions as a neuron-glia ligand.

To examine the expression of astrotactin in the developing mouse cerebellum, in particular whether the timing of expression of astrotactin overlaps with the period of granule cell migration along the Bergmann radial glia and assembly into the internal granule cell layer, we immunostained cerebellar cells in vitro and in vivo, in tissue sections from embryonic, postnatal and adult cerebellum, with anti-astrotactin antibodies. We then used Western blot analysis to measure the levels of astrotactin protein at different developmental stages in the mouse cerebellum[60].

In cell culture, anti-astrotactin antibodies label the surfaces of cerebellar neurons, but not astroglial or oligodendroglial cells. The expression of astrotactin by cerebellar granule neurons is developmental stage specific, astrotactin is not expressed by proliferating, embryonic granule cells, but is abundant on postnatal cells. The expression of astrotactin appears to be restricted to CNS neurons, since PNS neurons including sympathetic neurons and the PC 12 cell line do not express astrotactin. In tissue sections, a discrete pattern of staining is seen. Cells in proliferative zones, either the external granule cell layer of the cerebellum or ventricular zones of the developing cerebellar or cerebral cortex, are unstained. Staining is most intense in migrating neurons, coursing through the emerging cortical plate of the cerebellar and cerebral cortex, or migrating along Bergmann glia in the cerebellum. Some neurons in the internal granule cell layer are also stained, suggesting that astrotactin is expressed during periods of neuronal migration and initial assembly into neuronal layers. Axons in the white matter do not express astrotactin. In the adult brain, astrotactin staining is dramatically reduced[60].

Western blot analysis of the levels of astrotactin protein demonstrates that the expression of this protein is developmentally regulated. Low amounts of astrotactin are seen in late embryonic mouse cerebellum, the period just prior to granule cell migration, high amounts are seen in early postnatal cerebellum, the period of granule cell migration and assembly into the internal granule cell layer, and low levels are seen in the adult after migration and assembly have ceased[60]. Thus astrotactin appears to function as a neuron-glial ligand and to be expressed during the periods of neuronal migration along astroglial fibers in the developing brain. Work is in progress to molecularly clone astrotactin in order to define its relationship to other cell adhesion ligands.

Our finding that the neural cell adhesion molecules do not regulate neuron-glial interactions in vitro is consistent with the hypothesis that neurons navigate along glial fibers via neuron-glia ligands and that they detach from the glial guide via neuron-neuron interactions. The termination of migration via neuron-neuron interactions is presumably mediated by neuronal adhesion ligands. This view is supported by studies showing that antibodies against L1 perturb granule cell migration[27] by disrupting parallel fiber interactions, rather than by binding at the neuron-glia interface[43].

Stop signals for migration

Our in vitro studies on migration have suggested that astroglial cells provide a permissive substrate for cell migration, and that the neuron-glia ligand astrotactin functions to bind the neuron to the glial guide. A role of neuron-neuron interactions in arresting migration has been suggested by recent in vitro experiments in our laboratory, where we have observed the interactions of extending growth cones with the leading process of neurons migrating along glial fibers. In studies of hippocampal cells, Urs Gasser found that growth cones temporarily arrest the movement of the neuron along the glial fiber (Gasser and Hatten, unpublished observations). The interaction of the growth cone with the migrating cell is striking, as the tip of the growth cone actually seems to collide with the migrating cell, inhibiting its motion. These observations, have led us to examine whether neuron-neuron interactions provide a stop signal for migration via contact inhibition mechanisms[1].

Thus, in this model, neurons would migrate until cell-cell interactions with other neurons or axons arrest their

movement, providing a 'stop signal'. If neurons do provide the stop signal for migration, it is likely that they do so by one of two broad mechanisms: a) neurons in a given layer of the developing cortex signal the neuron and b) afferents contact migrating neurons along the route of migration and signal them to stop (fig. 5). Both would be expected to operate via hierarchical adhesion mechanisms[25].

In the first case, neurons would migrate along glial fibers until cell-cell interactions with neurons divert the neuron from the glial guide into a neuronal layer. Neurons that had completed migration would signal the migrating neuron to cease moving on the glial fiber, to abandon the glial fibers, and to position in the cellular layer of region (fig. 5a). Such a mechanism is likely to involve concentration effects of cell adhesion receptor systems in layers of cells with similar 'birthdays'. Thus, the neural adhesion molecules expressed by neurons generated at the same time would be similar in a given layer but might differ from layer to layer, since many neural adhesion molecules, e.g. N-CAM, show temporal regulation of their structure[7,25]. Such a scheme could easily apply to the cerebral cortex, where the later generated neurons migrate through layers of earlier generated cells and settle in newly formed layers above them[38,55].

A number of recent studies, including those of Caviness on the *reeler* mouse[2,3,44], transplantation experiments by McConnell[35,36], and ablation experiments by Jones[26] and Jensen and Killackey[24], have suggested that subpopulations of cortical neurons are committed to specific lamina prior to their migration along radial glial fibers. These studies are consistent with a model where glial fibers provide a permissive pathway for neuronal migration[11,17].

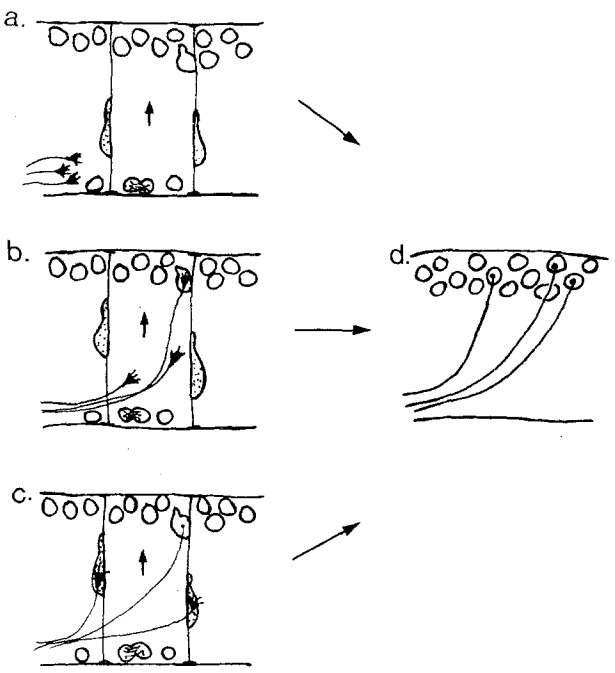

Figure 5. Coordination of afferent ingrowth and neuronal migration. In embryonic cortical regions, neurons migrate along radial glial fibers. Afferents contact target neurons at three possible points in the migration sequence: *a* afferents wait until migration and settling of target neurons in cell-specific layers is completed (*d*) before contacting targets. *b* afferents contact target cells near the end of their migratory trajectory, possibly arresting migration. The neuron would then leave its glial guide. *c* afferents contact targets while targets are migrating, and stay in contact during the remainder of migration and positioning.

Neuron-axon interactions in migration

In considering a possible role for axons in neuronal migration, it is important to recall that the timing and patterns of cell migration and afferent ingrowth vary greatly among brain regions. Traditionally, neuronal migration and positioning have been thought to occur first, well before afferent ingrowth and invasion of the target cell zone. In several brain regions, axons are thought to grow into areas just beneath emerging neuronal layers and to 'wait' there until migration is completed (fig. 5a), before the initiation of cell-cell contacts and synapse formation[56,58,63]. Such scenarios were established by rather low resolution methods, matching thymidine birthdating of neurons in given cortical layers with other preparations, either with Golgi impregnations or anterograde axonal labeling with peroxidase or tritiated amino acids, that provided information on the time of arrival and morphology of afferents.

Based on these analyses, it was concluded that migration and afferent ingrowth and contact with targets were events separated in time. For a given region following this schedule of events, a role for the afferents in modulating migration would have to be discounted. Although this may in fact be the case for some brain regions, for example, the corticopontine projection[41], new axonal labeling methods yielding labeling of fine axonal growing tips show that in complex cortical regions of rodent brains, afferent ingrowth may coincide with, or rapidly follow, target cell migration and settling[54].

To examine the coordination of afferent ingrowth with neuronal migration in the developing cerebellum, we analyzed the time course of Purkinje cell migration and ingrowth of inferior olivary afferents, the climbing fibers, in embryonic mouse cerebellum. Purkinje cells arise in the proliferative zone lining the fourth ventricle between E11 and E13[22,38] and subsequently migrate out into the thickening cerebellar cortical plate. With antibodies against the glial-specific antigen RC2 to visualize radial glia in the embryonic cerebellum[39] and antibodies against the calbindin protein to visualize Purkinje cells[34], our recent studies indicate that Purkinje cells migrate along radial glia between E14 and E17, and settle into a zone just beneath the zone of proliferating granule cells, which lines the roof of the cerebellar anlage at this age (Mason and Misson, unpublished observations).

Climbing fiber afferents were anterogradely labeled by depositing crystals of the fluorescent dye DiI[41] into the inferior olivary nucleus of fixed embryonic brain, during the period of Purkinje cell migration[33]. At E15, imma-

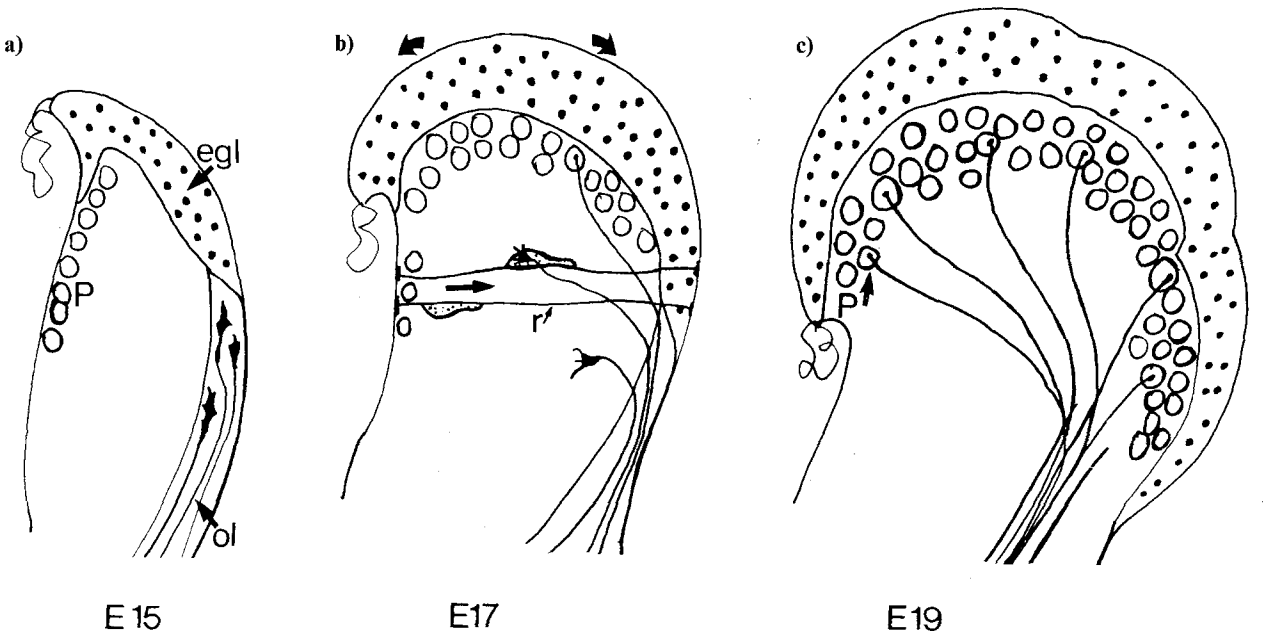

Figure 6. Time course of ingrowth of inferior olivary afferents and migration of target Purkinje cells. At E15, olivary afferents travel in a tract at the anterior cerebellum, and Purkinje cells are several days post-mitotic, but have not yet initiated migration. By E17, olivary afferents invade the cerebellar anlage, and contact Purkinje neurons during their migration from the neuroepithelium to their position beneath the forming external granule cell layer (egl). Other fibers already project into the Purkinje cell layer (P). At E19, olivary axons project into the wide zone of Purkinje cells, yet are relatively simple. A 'waiting' period thus occurs from E17 to P3, after afferent contact with the target and before afferent arborization on target cells.

ture climbing fiber axons run in a broad tract from the lateral brainstem, and have just reached the border of the cerebellar anlage (fig. 6a). By E17, the axons exit this tract into the region that is becoming the immature Purkinje cell settling zone, just beneath the spreading sheet of the external granule layer at the pial surface of the cerebellar anlage. The olivary axons project into this zone, and also project straight ahead into the zone of actively migrating Purkinje cells (fig. 6b). By E18–19, most olivary axons project into the wide band of Purkinje cells, several cells thick, as simple unbranched fibers with small growing tips (fig. 6c).

With double labeling of Purkinje cells and ingrowing olivary axons, we were able to map more precisely the whereabouts of olivary afferents relative to their migrating target cells. At E17, many fibers infiltrated the zones of migrating Purkinje cells and appeared to contact these cells. Others projected into the emerging Purkinje cell layer where Purkinje cells had begun to settle. In a few instances, olivary fibers actually entered the neuroepithelial zone and contacted cells before they had begun to migrate.

This analysis suggests that cerebellar afferents contact target neurons as they migrate (fig. 5c), resulting in the simultaneous segregation of target neurons and afferent axons into an immature Purkinje cell zone in the late embryonic period. This sequence of events stands in contrast to an earlier report of climbing fiber invasion during the first postnatal week of the already formed Purkinje cell monolayer[58]. Because axons were labeled by transported amino acids and detected autoradiographically, the afferents appeared as 'dots', primarily over the white matter tracts, and were thus thought to wait in the zones below the emerging Purkinje cell layer.

The cell-cell contacts formed between ingrowing axons and immature Purkinje cells were of special interest. Within the immature Purkinje cell zone at E18–19, a day or two after the afferents have contacted the Purkinje cells the growing tips of olivary afferents make simple punctum adherens contacts, some of which include small accretions of vesicles and postsynaptic densities, characteristic of immature synapses[29,33]. These contacts generally occur along the Purkinje cell somata, by unbranched olivary axons. This relationship continues from E18 to P3, when olivary axons begin to branch and extensively arborize[34].

Our earlier analysis of morphology and projections of immature mossy fibers[30,32] can be explained in the light of this scenario. In the mouse cerebellum, mossy fibers enter the cerebellum at the onset of granule cell migration. As they project into the internal granule cell layer, the growing tips make contacts with granule neurons that have already migrated inward from the external granule layer. By P3–7, mossy fibers, recognizable by their globular en passant boutons in the internal granule layer, send fine branches upward into the Purkinje cell layer. These fine branches have small growing tips that contact Purkinje cells as well as granule cells that filtrate down between Purkinje cells during migration toward the internal granule layer[30,32]. Thus, both mossy and climbing

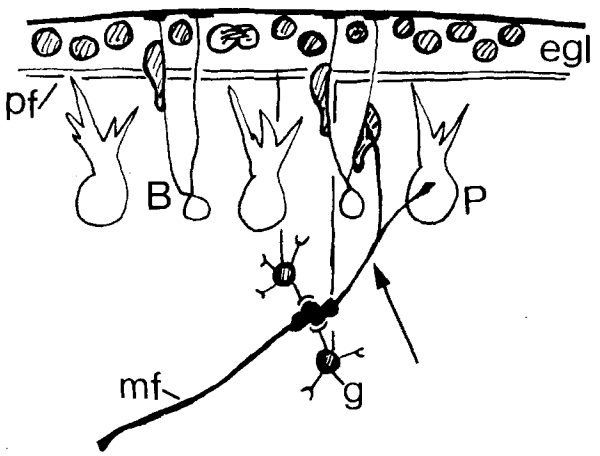

Figure 7. Mossy fibers contact migrating granule cells. A mossy 'combination' fiber has large globular synaptic boutons typical of mossy fibers, that contact post-migratory granule cells. These fibers also send transient fine branches into the Purkinje cell layer. There the small growing tips contact Purkinje cells, an 'aberrant' target for mossy fibers, as well as migrating granule cells, that move inward through the Purkinje cell layer toward the internal granule cell layer below.

afferents might contact targets during migration without a lag between the events of target migration and afferent ingrowth (fig. 7).

These studies on cerebellum suggest that afferent climbing and mossy fiber axons do not 'wait' for target neurons to migrate, settle, and to begin to differentiate (fig. 5a), rather they interact with target neurons immediately upon entry to the target region, during periods of migration of target neurons and assembly of targets into cell layers. Upon contacting the migrating cell, the afferent growth cone might signal the release of the migrating cell from the radial glial guide as well as the cessation of migratory movement (fig. 5b). Alternatively, after contact, the afferent might simply travel with the migrating cell to its final destination (fig. 5c). A 'waiting' period may exist, but at a later time, after the initial contact of olivary afferents with the Purkinje cell at E16–17, and before climbing fiber arborization at P3, a period of 5–6 days. During this time Purkinje cells reorganize into a monolayer and initiate dendritic development, with the olivary afferents all the while contacting their soma.

Thus, the migration of the Purkinje cell and the ingrowth of its afferents are closely orchestrated and overlap temporally and spatially. An important issue is to what extent these factors, relative to putative 'recognition' mechanisms, are crucial for the formation of specific synaptic connections. To address this, we are currently investigating the behavior and cellular interactions of cerebellar afferents and target neurons with a newly devised in vitro model system. In this system, Purkinje cells purified from later embryonic cerebellum or granule neurons purified from early postnatal cerebellum are co-cultured with explants of their appropriate or inappropriate afferents, from the inferior olivary nucleus or pontine nucleus of the brainstem, respectively. By observing the neurites and growth cones emanating from the explants with high-resolution video microscopy, the role of afferents in arresting or modulating target cell migration and the interactions that mediate target cell selection can be directly tested.

Model for neuronal migration

A model which emerges from these studies on developing cerebellum is that the radially aligned fibers of astroglial cells set up the scaffold for neuronal migration from generative zones into the developing anlage of the cerebellar cortex. Neurons migrate along these astroglial fibers, using them as permissive substrates for directed movement. The most prominent neuron-glial ligand during this phase of cerebellar development is the neuronal antigen astrotactin. Other surface ligands, such as integrin, are likely to be involved in the actual movement of the neuron, but astrotactin provides a 'recognition' ligand. Migration would then be arrested by neuron-neuron contacts, either by neurons that had already reached the target cell layer, or by afferents, as in the migration of the Purkinje and granule cell. At this stage, neuron-neuron interactions mediated by neural cell adhesion molecules would then be more prominent and would lure the neuron from the glial fiber into the emerging neuronal cell layer.

Several basic questions about neuronal migration and its termination remain, many of them requiring analysis in experimental systems apart from the intact brain. First, we need to further define the molecular mechanism of glial-guided migration and to begin to establish its genetic regulation. Second, we need to use correlated in vitro and in vivo analyses to examine the role of neuron-neuron interactions and neuron-afferent interactions in arresting neuronal migration and begin to define the molecular mechanism of this process.

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Neuronal growth cone migration

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Summary. The neuronal growth cone is a semi-autonomous portion of the developing neuron that is highly specialized for motile activity. Migrating neurons may share some features with neuronal growth cones. I review some of what has been learned about growth cone initiation, the differentiation of axons and dendrites, the role of the cytoskeleton in motility, the movements of membrane vesicles, the factors regulating the rate and direction of growth cone movement, and the further differentiation of growth cones as they enter the target area and initiate synaptogenesis. Where appropriate, I draw comparisons to what is known about the migration of neurons.
Key words. Growth cones; neural development; cell motility; cytoskeleton; actin; synapse formation.

Introduction

The neuronal growth cone is the tip of an elongating axon or dendrite that is intimately associated with pathway formation and synaptogenesis. While the movement of the cell body determines the position that the neuron will assume, the movements of the growth cones determine the initial morphology of the developing cell. Movements of the growth cones also determine which cells the neuron will have the opportunity to form synapses with.

Growth cones have frequently been compared to motile cells. Ramon y Cajal described the growth cone as 'a concentration of protoplasm of conical form, endowed with ameboid movements'[52]. Speidel, studying the movements of growth cones in vivo, noted the resemblances between growth cones, fibroblasts, and endothelial cells[58]. That the growth cone can indeed be viewed as an independent motile structure is most convincingly demonstrated by severing the growth cone from its cell body in vitro. When this is done, normal movement of the growth cone continues for several hours[55]. Growth cones were recently termed 'leukocytes on a leash', for these reasons, as well as for biochemical similarities in signal transduction pathways between leukocytes and growth cones[51]. An even more felicitous comparison might be made between growth cones and migrating neurons.

I would like to briefly summarize the stages of neuronal migration, highlighting the similarities to growth cone movement. Figure 1 shows the structural similarities be-

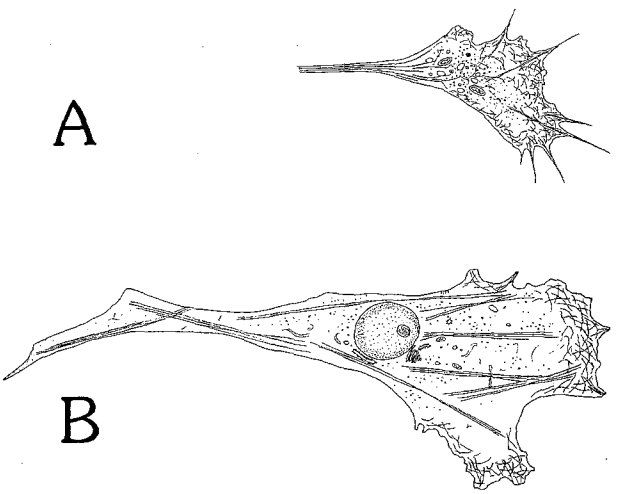

Figure 1. Similarities between growth cones and motile cells.
A Schematic of a growth cone, showing lamellipodia, filopodia, and the distribution of cytoskeleton and vesicles in the growth cone. The lamellipodia are thin, veil-like sheets that extend forward while remaining attached to the substrate. Filopodia are very narrow spikes which extend and retract rapidly, often moving from side to side with no attachment to the substrate, as if probing their environment. Microtubules extend from the neurite only partly into the growth cone, most vesicles are in the growth cone core, and actin filaments fill the peripheral portions of the growth cone. Most of the actin filaments form an apparently random meshwork that fills the lamellipodia, bundles of microfilaments fill the filopodia and extend partly back into the growth cone. This is a composite drawing, reflecting data on the cytoskeleton from mammalian growth cones[41], and data on the vesicles from molluscan growth cones.[24].
B Schematic of a migrating cell. This is of a fibroblast in vitro. Motile fibroblasts have lamellipodia very similar to those in growth cones, also filled with a crosslinked actin meshwork. The fibroblast also has a number of stress fibers, which are bundles of actin filaments that traverse the cell, and usually end before the leading lamellipodia.