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# The Extending Astroglial Process: Development of Glial Cell Shape, the Growing Tip, and Interactions with Neurons

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**To analyze how astroglial cells attain the complex shapes that support neuronal migration and positioning *in vitro* (Hatten et al., 1984; Hatten 1985), early postnatal mouse cerebellar cells were plated in microcultures, and glial process outgrowth was monitored by high-resolution time-lapse video microscopy combined with immunocytochemical localization of antisera to glial filament protein (GFP), and by electron microscopy.**

**The 2 principal astroglial forms seen in these cultures, stellate and Bergmann-like (Hatten et al., 1984), begin to develop their distinctive shapes by the outgrowth of processes in the first 8 hr after the cells are plated. Glial process extension is most vigorous in this period, resulting predominantly in stellate forms. A second population of glial cells, having fewer, longer processes reminiscent of Bergmann glia *in vivo,* first appears about 5 hr after plating. During the next 16–24 hr, while the stellate cells only slightly increase their process length, the bipolar cells double their length.**

**The most striking feature of the elongating glial process is its highly motile tip, which rapidly extends microspikes and lamellopodia. Unlike the neuronal growth cone, which is the expanded terminal of a thin neurite shaft, the glial growing tip forms the end of a wide, paddle-like process that is filled with motile mitochondria and masses of glial filaments, and is bordered by an undulating lamella fringed by microspikes.**

**Soon after the emergence of glial processes, cell–cell interactions between the growing glial process tip and granule neurons occur. Within minutes of an initial encounter between the glial process and the neuron, contact relationships that are stable during the observation period form between the cells. Subsequently, many neurons extend a small neurite onto the glial process, and astroglial process extension continues by the movement of the glial growing tip out beyond the neuron.**

**Thus, cerebellar astroglia *in vitro* develop complex shapes in the same fashion as do neurons: the outgrowth of processes tipped by a motile ending. The growing tips of astroglial processes interact with neurons, resulting in the stable association of neurons and glia.**

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The different forms of astroglia found in the CNS appear to be highly correlated with specific neuronal behavior and positioning. Elongated forms, such as radial glia in embryonic brain or Bergmann glia in postnatal cerebellum, guide migration of postmitotic neurons (Rakic, 1971; Sidman and Rakic, 1973). The multipolar stellate forms found in white and gray matter of the mature nervous system harbor neuronal perikarya, axons, and synaptic arrangements (Palay and Chan-Palay, 1974). While the outgrowth of neuritic processes has been studied extensively, especially *in vitro,* little is known about astroglial process formation and the role of the growing process in the development of glial shape. One question pertains to the form of the elongating glial process—in particular, whether this resembles the well-described neuronal growth cone, the motile probing end of neuronal axons and dendrites that mediate axonal elongation and cell–cell interactions.

An *in vitro* microculture system has been developed that promotes both astroglial differentiation and specific neuron–glial interactions (Hatten and Liem, 1981; Hatten et al., 1984). Several features of this culture system make it ideal for studies of extension of glial processes and evolution of complex glial forms. In the first hours after plating the cells are randomly positioned, few neurons and glia have processes, and the neuron–glial assemblies that are typical of established cultures have not yet developed. The different astroglial forms seen in the intact brain, especially in the cerebellum (Bovolenta et al., 1984), develop after 1 d *in vitro,* and neurons associate with them and display behaviors on them appropriate to their relationships *in situ*: stellate forms of astroglia anchor resting neurons, while elongated Bergmann-like forms support neuronal migration (Hatten et al., 1984). By observing these cultures periodically in the first several hours after plating, the extension of glial processes and the onset of interactions between neurons and glia can be examined in detail.

Evidence from other experiments using this model system demonstrate that glia organize neuronal positioning over the first 24 hr and that, conversely, neurons influence glial differentiation (Hatten and Liem, 1981; Hatten, 1985, 1987). What is not known is how astroglial cells attain complex shapes: whether their morphogenesis occurs, as in neurons, by process extension, and how cell–cell interactions with neurons influence glial process outgrowth.

In the present study, the progression of astroglial forms and the cytology of the growing glial process are charted during the first 24 hr after plating in cultures of dissociated cerebellum, both by immunocytochemistry with antisera against glial filament protein (GFP) and by transmission electron microscopy. In addition, the behavior of the growing glial process and growing tip, and the interaction of neurons and glia during glial process outgrowth, are observed directly with video time-lapse microscopy, which allows high-resolution observation of cellular activities in real time (Edmondson and Hatten, 1987).

## Materials and Methods

*Cerebellar cultures.* C57BL/6J mouse cerebellar cells from animals at postnatal day 5–7 were dissociated into a single-cell suspension and plated at a cell density of 1.5–2.0 × $10^6$ cells/ml in glass coverslip microcultures (50 μl) as described by Hatten et al., (1984). The culture surface was pretreated with 50 μg/ml polylysine (Sigma, St. Louis, MO). All cultures were maintained in Eagle's basal medium with Earle's salts (Gibco Laboratories, Grand Island, NY) supplemented with glutamine (4 mM), penicillin–streptomycin (20 U/ml), glucose (6 mg/ml), and horse serum (10%; Gibco).

After 0.5, 1, 3, 5, 8, or 24 hr *in vitro,* astroglial process extension and the association of neurons with glia were observed by one of 3 methods:

*Immunocytochemistry: antisera.* Antisera against the major GFP (AbGF; GFP, 51,000 Da) in bovine brain were raised in rabbits kindly provided by our colleague, Dr. R. Liem, as described (Liem, 1982). In some experiments the antiserum was preabsorbed with purified GFP.

The peroxidase–antiperoxidase (PAP) method was used for all experiments, as described (Hatten et al., 1984). After 0.5, 1, 3, 5, 8, and 24 hr *in vitro,* cultures were fixed with 4% paraformaldehyde in 0.1 M Sorenson's phosphate buffer for 30 min at room temperature, and washed 3 times with PBS. After incubation with normal goat serum (10% in PBS containing 0.05% Triton X-100) for 30 min, primary antibody (1:100 or 1:500 in PBS containing 1% normal goat serum and 0.05% Triton X-100) was added and the cultures incubated for 1 hr at room temperature. They were then rinsed 3 times with PBS before the addition of peroxidase-conjugated goat-anti-rabbit IgG (1:100 in PBS containing 0.05% Triton X-100) for 30 min at room temperature. After washing with Tris buffer (0.05 M, pH 7.6), cultures were incubated in diaminobenzidine (10 mg, dissolved in 10 ml 0.05 M Tris buffer, with 0.003% $H_2O_2$ added just prior to use) for 5–10 min at room temperature, and rinsed extensively with PBS.

The coverslip was removed from the bottom of the dish with a razor blade and mounted with PBS–glycerol without dehydration. Preparations were studied with both phase microscopy and differential interference contrast (DIC) optics. Photographs were taken of immunostained cultures using video-enhanced DIC optics, as described below.

*Electron microscopy.* After 0.5–24 hr *in vitro,* microwell cultures were fixed with 3% glutaraldehyde in 0.1 M Sorenson's phosphate buffer for 30 min at room temperature. After 3 additional washes with phosphate buffer, the cultures were postfixed in 1% osmium tetroxide for 30 min, washed in 0.9% NaCl for 30 min, stained *en bloc* with 0.5% uranyl magnesium acetate in 0.9% NaCl for 1 hr in the dark at 4°C, and washed in saline for an additional 30 min. After dehydration in ethanols, cultures were embedded in Epox (E. F. Fullam, Schenectady, NY) or Epon 812. Blocks of microwells were removed and glued to Epon stubs. Thin sections were stained with uranyl acetate and lead citrate and were examined in a JEOL 100S electron microscope.

*Time-lapse video microscopy.* The details of high-resolution video microscopy of the cultures have been described (Edmondson and Hatten, 1987). In brief, at various times (0.5, 1, 3, 5, or 8 hr) after plating, cultures were removed from the incubator, sealed with a coverslip, and placed on a Zeiss IM inverted microscope equipped for DIC optics. Images from a Zeiss 63×/1.4 N.A. planapochromat oil-immersion objective were projected via a 20× eyepiece onto a Hamamatsu C1965-01 chalnicon video camera. The video signal was sent through a time-date generator (MSI Video Systems) and was recorded onto a time-lapse optical memory disc recorder (Panasonic) at the rate of 1 frame/4 sec.

Most video sequences were made over a 60–90 min period, regardless of the period of time the cells had been in culture. Photographs were taken directly off the video monitor (Sony PVM-122) onto Kodak Technical Pan Film with a Nikon 35 mm camera set at ASA 80.

*Identification of cell types.* By comparing the patterns of cells in immunostained cultures to the fields visible with DIC optics or in the electron microscope, neurons and glia could be easily identified. In the video studies, astroglia were further identified by their large size and flatness on the dish, and glial processes by their characteristic organellar arrangements, both obvious with DIC optics. In the electron microscope, astroglia had pale cytoplasm and were the only cell type that contained intermediate filaments throughout the time course studied. After 3 hr, astroglia were recognizable as the central cell in a cluster, especially in sections close to the surface of the culture dish.

## Results

In the first hour after plating (Figs. 1, *a, b*; 2*a*), astroglia attached to the culture substratum and extended processes. Neurons were evenly dispersed, and the cell surface was relatively inactive during this period. Over the next 1–8 hr (Figs. 1, *c–e*; 2*a*), rapid astroglial process extension continued. The growing astroglial processes formed numerous contacts with neurons during this initial period of outgrowth.

Glial process outgrowth resulted in the development of at least 2 major glial forms, each developing by simple radial extension of processes (Figs. 1, 2). Up to 5 hr, nearly all glia expressed stellate forms. However, from 3 to 5 hr, a web extended between processes of some glial cells (Figs. 2*b,* 3). Such webs consisted either of a fanlike array of GFP-positive spokes, connected by unstained areas of cytoplasm, or of a single, flattened process containing a meshwork of GFP-positive bundles of filaments (Fig. 3, *b, c*). This variation on the stellate form was apparently transitional, since it was rarely seen after 5 hr.

From 5 to 8 hr, it was possible to recognize a subpopulation of astroglial cells that had one or 2 longer processes emerging from opposite ends of the soma, in a bipolar or tripolar fashion; these cells appeared to progress to forms resembling the cerebellar Bergmann glial fibers (Figs. 1*f,* 2*c*). Whereas the processes of stellate astrocytes had attained their full length, 40–60 μm, by about 8 hr *in vitro,* the Bergmann-like cells continued to elongate their processes over the next 12–16 hr, until these were 100–150 μm in length. In some cases, one or 2 processes on stellate cells had elongated much further than the other branched stellate processes, producing shapes intermediate between stellate and Bergmann forms (Fig. 2*b*), as described in our previous studies (Hatten et al., 1984).

Branching of processes occurred by at least 2 means. In some cells, both secondary and tertiary branches arose *de novo* from the tips of processes. A second means consisted of cavitation, or "etching," of the webbed extensions described above. As seen with video microscopy, this was preceded by reorganization of organelles from a disorganized mesh to radial spokes within the web, then a shrinking of the flattened cytoplasmic lamella around these spokes (not shown). However, the processes resulting from this type of moulding did not appear to grow beyond the former border of the web.

To analyze the activity of the growing tips of the processes, we used a combination of high-resolution time-lapse video (Edmondson and Hatten, 1987) and electron microscopy. Within 30 min after plating, the soma of astroglial cells gave rise to numerous microspikes (Fig. 4*b*). The diameter of the microspikes was 0.5–1 μm. By electron microscopy, they were seen to contain flocculent material that resembled microfilaments (Fig. 4*c*). The microspikes protruded either directly from the cell soma or from thin skirts of cytoplasm that extended onto the surrounding substratum (Fig. 5, *a, c*). Thicker, fingerlike

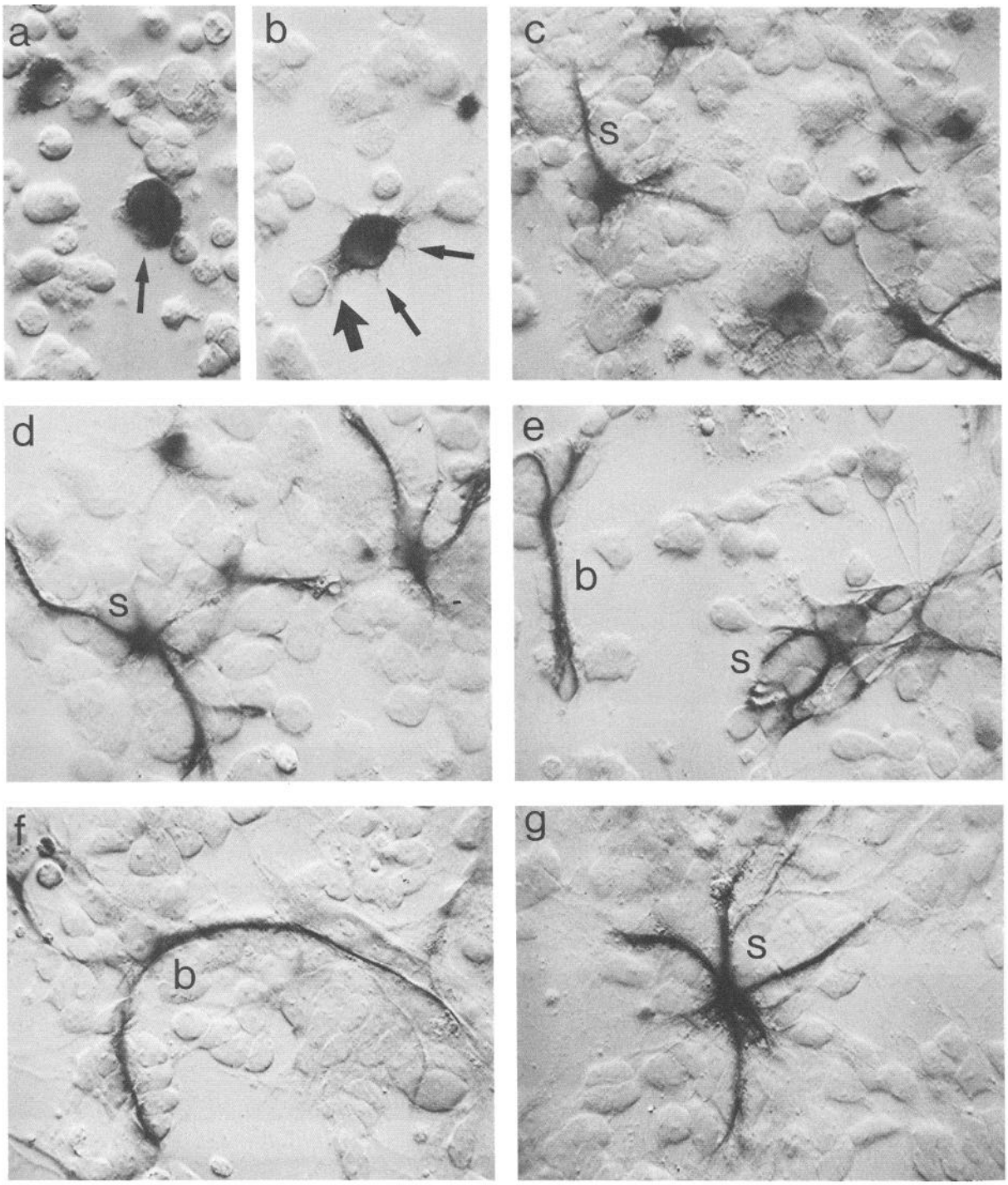


*Figure 1.* Time course of astroglial process outgrowth and association of neurons with astroglia, in cultures immunostained with antisera to GFP (*arrow, a*), at various times after plating. *a,* 30 min; *b,* 1 hr; *c,* 3 hr; *d,* 5 hr; *e,* 8 hr; *f, g,* 24 hr. In the first hour, astroglia settle on the dish and extend microspikes (*thin arrows, b*) and wide, paddlelike processes (*thick arrow, b*). Glial process extension is rapid in the next several hours, resulting in stellate forms (*s* in *c–e*). By 8 hr (*e*), 2 forms, stellate and bipolar (*b*), are evident, and stellate forms nearly reach their maximum size. At 24 hr (*f, g*), highly elongated Bergmann-like glia (*b* in *f*) are prominent, coexisting with stellate forms (*s* in *g*). Video-enhanced DIC optics.

processes and buds of cytoplasm also formed on the soma and contained large electron-lucent vesicles (Fig. 4*c*).

Glial processes originated as a paddle of cytoplasm projecting microspikes, which slowly spread out as a broad flange from the soma (Figs. 1*b*; 5, *c, d*; 6). The processes gradually became flattened arms of cytoplasm, approximately 10 $\mu$m in width, which adhered closely to the substratum. In these processes, a 2–3-$\mu$m-thick, organelle-filled central region was flanked by a thinner peripheral lamella that ruffled actively (Fig. 7).

Visible with video microscopy within the center of the extending processes were several radially oriented tubular structures, 1–2 $\mu$m in diameter (Figs. 5, *c, d*; 7, *a–c*; 8). By staining with rhodamine 1-2-3 (not shown), and by electron microscopy of individual identified glial cells, these structures were identi-

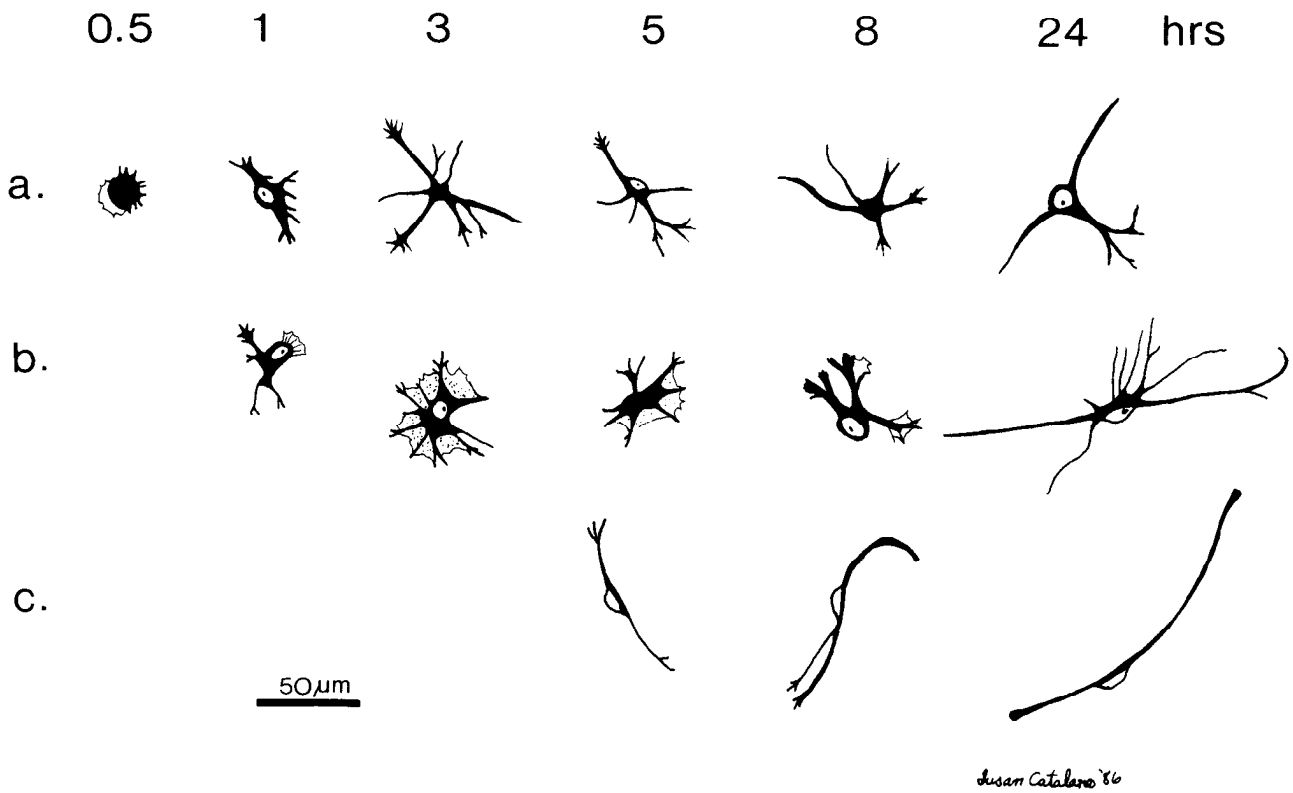


*Figure 2.* Schematic table of the different forms of astroglia prominent at various times after plating. All forms begin as spherical cells with microspikes. Broad paddles tipped with active growing tips emerge in the first hour. Distinct stellate forms are seen from 3 hr onward (*a*), nearly reaching their maximal size at 8 hr. Webbed variations of the stellate forms are present transiently (3–5 hr, *b*). Bi- or tripolar cells are evident only after 5 hr (*c*), and elongate primarily from 8 to 24 hr. Intermediate forms have features of both stellate forms (multiple branches) and bipolar forms (2–3 longer processes; 24 hr, *b*). Bar, 50 μm.

fied as mitochondria (Fig. 6*c*). The mitochondria within the central core of the process displayed saltatory movements back and forth into the leading growing edge of the glial process, and extended into and retracted from individual microspikes at the growing tip. The movement of the mitochondria was usually limited to the axial direction within the process.

In the first 5–8 hr after plating, the peripheral lamella was 3–5 μm wide (Figs. 5, 7, 8). This lamella was often expanded in width at the leading edge of the growing process (Fig. 8*a*). Short microspikes emerged in all directions from the lamellar border. The lamella slowly and regularly advanced and receded on the culture substrate, occasionally lifting from the substrate and undulating. The microspikes on the edges of the lamella along the length of glial processes were less motile than those at the growing tip, and appeared to tether the process.

By electron microscopy, the organelles within the glial growing tip were seen to include accumulations of electron-lucent vesicles, both large and small (Fig. 7*d*). These vesicles were most often in clumps at the leading end of the growing process, but single, large vesicles also occurred in the shaft. Networks of smooth endoplasmic reticulum were seen extending into the terminus of the growing process. Both immunostaining and electron microscopy showed abundant, but disorganized, intermediate (glial) filaments within the growing glial process (Fig. 6). The glial filaments were found in the central core of the process, but did not extend into the leading edge or the peripheral lamella (Fig. 7*d*).

As the early phase of process extension progressed, there was variation from cell to cell in the extent of process development, some cells still being essentially spherical with microspikes, others having extended long, flattened processes. Although process outgrowth was initiated on several points around the perimeter of the cell soma, one process often appeared to extend in advance of the others, and was usually richer in mitochondria (Fig. 5, *c*, *d*). The processes extended steadily, moving at an average rate of approximately 10 μm/hr, always in a radial direction from the soma. The slow, steady outgrowth was occasionally punctuated by periods of quiescence.

From 5 hr onward, elongation of glial processes was accompanied by a thinning and rounding up of the initially broad and flat glial processes, and narrowing of the peripheral lamella, which resulted, by 24 hr, in 3–5 μm-wide processes that were tipped by a somewhat expanded ending. The direction of outgrowth of emerging processes seemed random and appeared to bear no relationship to the location of other cells.

The video time-lapse sequences revealed a particularly striking behavior of the glial process when it encountered neurons. High-power video microscopic fields were chosen in which neurons were evidently in the path of, but not yet in contact with, an extending glial process. These neurons had a spherical cell body, sometimes rimmed by a small skirt of flattened cytoplasm resting on the substratum, but lacked neurites (Fig. 8).

The neuron extended and retracted microspikes, as did the glial process while it advanced, until contact was made either via microspikes or the lamella of both cell types or by glial contact directly on the neuronal cell soma. These points of con-

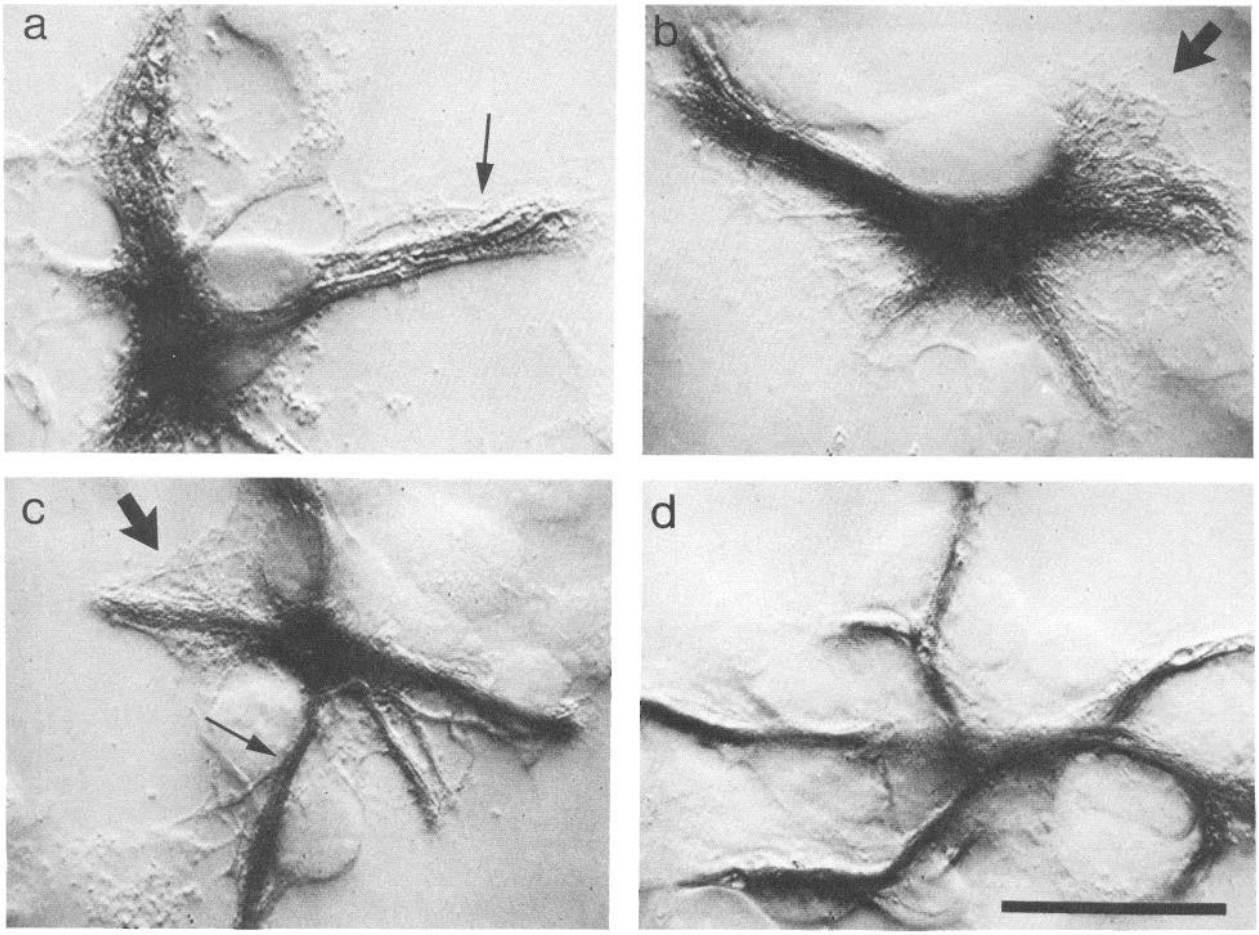


*Figure 3.* Astroglial cells in various stages of differentiation at 3 (*a*) and 5 (*b–d*) hr *in vitro*; cultures stained with AbGFP, DIC optics. *a–c,* Astroglial cells have distinct, GFP-positive processes (*thin arrow, a, c*), but also have webs that join these processes. Some webs have dispersed filaments (*arrow, b*) and other webs lack GFP (*thick arrow, c*). *d,* This cell is typical of the stellate forms seen from 5 hr onward, and lacks webs. Bar, 20 μm.

tact served as the origin of a rapidly expanding zone of cell surface apposition that served to join the cells (Fig. 8, *b, c*). The initial connection invariably progressed within 5 min to a region of contact several microns wide that did not separate for as long as the cells were observed (Table 1). In some cases, the neuron subsequently extended a short neurite which grew onto the surface of the glial cell (Table 1, Fig. 8*d*).

Before and during such an interaction, the glial process proximal to the growing tip was relatively quiescent. At the tip, the lamella undulated occasionally as microspikes slowly extended and withdrew. In the area of contact by neuronal microspikes, the ruffling activity of the glial growing tip did not dramatically increase or decrease as formation of the stable neuron–glial contact proceeded. There was never any visible change in the behavior of the glial process immediately prior to contact by neuronal microspikes.

During the time that neurons associated with glial cells in the first 8 hr after plating, the ultrastructure of neuron–glial appositions revealed several types of cell–cell interactions. Prominent among them was a highly irregular appearance of the cell surface of both cell types (Fig. 9). As we observed in neurons and glia in 24 hr cultures, and between granule neurons in the resting phase of migration and the glial processes on which they move (Hatten et al., 1984; Gregory et al., 1988), small, adherent junctions also marked the appositions of neurons with glia (Figs. 7*d,* 9).

## Discussion

The present experiments suggest that astroglial cells attain complex morphologies in much the same fashion as do neurons, by the outgrowth of processes tipped by a motile ending. Glial process extension begins prior to cell–cell contacts with neurons, but continues in concert with the establishment of contact relationships with neurons. The combination of glial process extension and neuronal binding to glia generates a highly nonrandom distribution of neurons relative to glia after 24 hr.

One striking observation of this study was that astroglial cells do not enter a flat cell stage prior to emergence of these differ-

**Table 1. Results of encounters between neurons and glia[a]**

| Variable | Observed value |
|---|---|
| Astroglial cells observed with video time-lapse | 15 |
| Encounters between neurons and glia | 12 |
| Stable contacts formed[b] | 12 |
| Granule neurons extending processes on glia | 4 |

[a] Cells were observed on the average for 60 min; encounters usually occurred within the first 15 min.

[b] See text for explanation of "stable contacts."

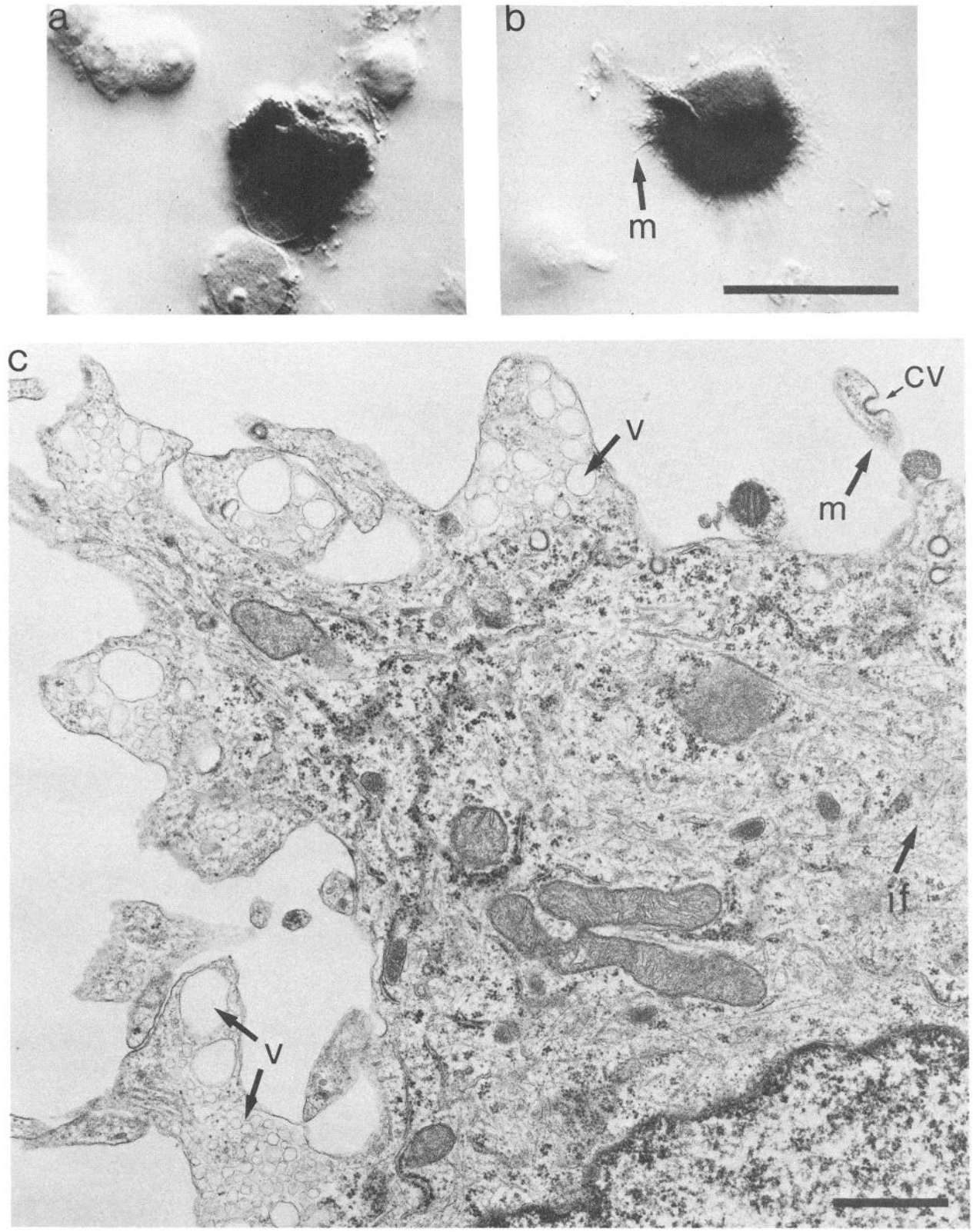


*Figure 4.* Surface features of astroglial cells in the first hour in culture. *a,* At 30 min, the glial cell (stained with AbGFP as in Fig. 1) has a lobulated surface and the soma is full of GFP. *b,* At 1 hr, microspikes (*m*) have emerged from this glial cell, as it begins to flatten on the culture substratum. *c,* Electron micrograph of a glial cell in the first hour after plating, showing buds of cytoplasm containing electron-lucent vesicles of various diameters (*v*). Numerous disoriented intermediate filaments (*if*) fill the cytoplasm. Microspikes (*m*) contain microfilaments. A coated vesicle (*cv*) has formed in this microspike. Bar in *b*: 20 μm (*a, b*); 1 μm (*c*).

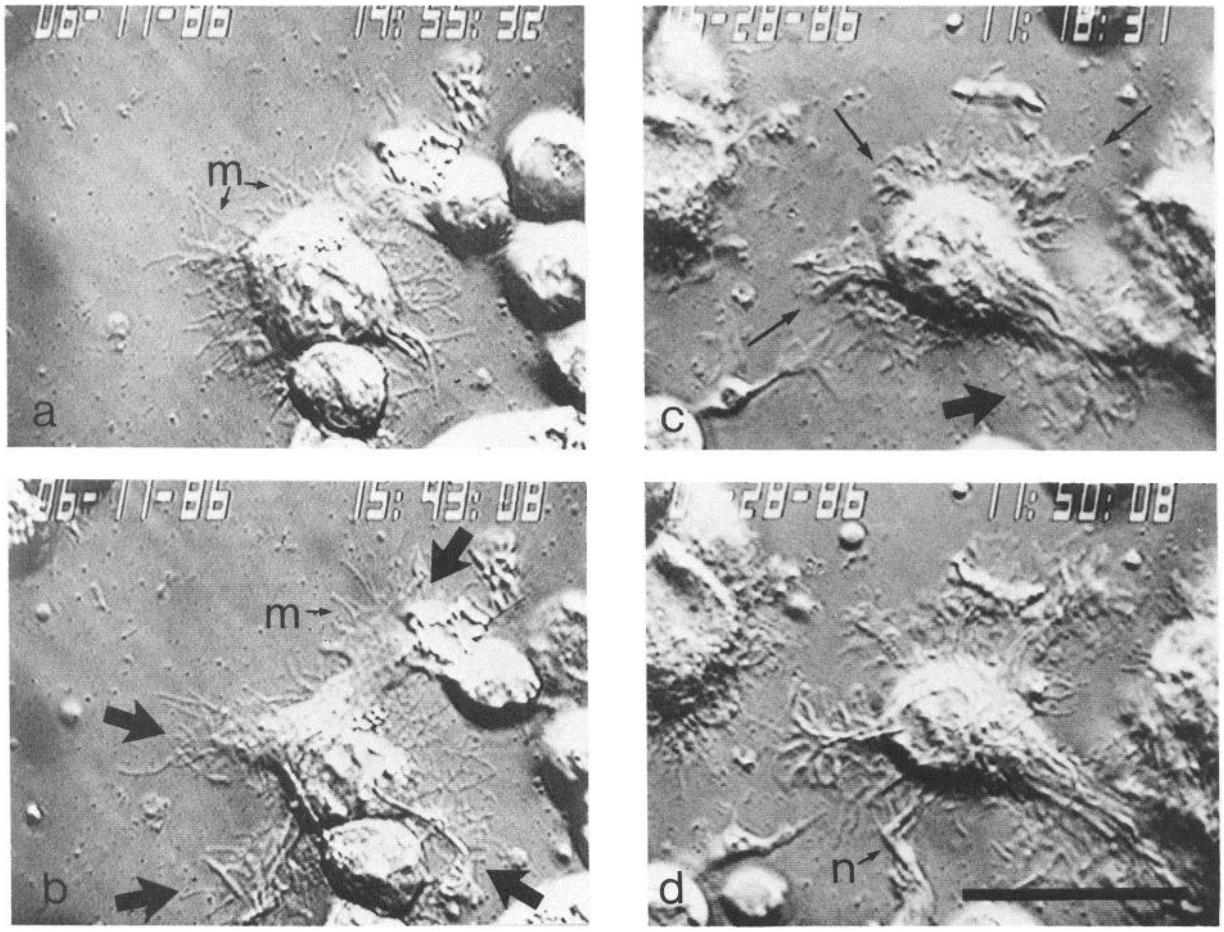


*Figure 5.* Video microscopy of 2 living astroglial cells in the early phases of glial process outgrowth. *a,* After 30 min *in vitro,* this cell bears numerous microspikes (*m*), many of which are apposed to the substrate. *b,* Fifty minutes later, process outgrowth has begun on 4 points of the soma (*arrows*). Processes originate as broad, flattened paddles fringed with microspikes (*m*). *c,* After 50 minutes *in vitro,* this astroglial cell already bears a broad process (*thick arrow*), and initiates growth of at least 3 more (*thin arrows*). *d,* Thirty-two minutes later, these 3 processes are more well-defined. Note the prominent rod-shaped mitochondria in the core of the processes. Interaction with short projections of neurons (*n, arrow*) and the peripheral lamella of the glial cell have begun. Bar, 20 μm.

entiated shapes, as they do when cultured in the absence of neurons (Hatten, 1987) or in long-term cultures in which neurons have been selectively killed (McCarthy and DeVellis, 1980; Trimmer et al., 1982). The 2 glial forms seen in cerebellum—stellate and elongated Bergmann-like glia—develop directly from the rounded cells that settle on the culture substratum soon after plating.

The interaction of individual neurons with glial processes is remarkable for its rapid rate of progression to a stable apposition. Initial contact, via microspikes and lamellar protrusions, progresses into appositions of larger areas of membrane as microspikes merge and widen, often culminating in the extension of a short process by the neuron onto the glial process.

The extension and resorption of microspikes and lamellopodia on glial growing tips is reminiscent of the activity of the neuronal growth cone. Studies of the role of protrusive elements in neurite extension have focused on growth cone preference for different substrates (Hammarback and Letourneau, 1986), different classes of neurites (Kapfhammer and Raper, 1987), or guidepost neurons *in situ* (Bastiani and Goodman, 1984; Bentley and Toroian-Raymond, 1984). The present study demonstrates an important new role for the glial growing tip and its appendages: interaction with neurons, and the resulting binding of the 2 cells, as a prelude to a continuing association of neurons with glial cells.

A striking feature of initial neuron–glial contacts was that there was little "trial and error" in the joining of the cells. The apparently avid neuronal affinity for glia has been observed in other settings. Neurons added to isolated glia bind rapidly to them, inducing glial shape transformation (Hatten, 1987). Growing neurites *in vitro* greatly prefer astroglia to fibroblasts (Fallon, 1985). What is not known is whether the rapid binding of granule neurons to stellate astroglia observed in this *in vitro* system occurs *in vivo* when granule neurons descend into the internal granule layer, rich in stellate astroglia.

Growing glial processes share features with the 2 classic examples of motile cellular structures, the leading edge of fibroblasts and neuronal growth cones (Bunge 1977; Abercrombie, 1980; Letourneau, 1985; Wessells, 1982). All 3 structures display ruffling movements or rapid undulations of membrane at cellular borders. In addition, each structure advances along the substrate at a rate of 10–50 μm/hr.

The major distinctions among the 3 cell types are morphology, the mode of locomotion, and the disposition and function of appendages such as microspikes. The initially broad astroglial process expresses a combination of both fibroblastic and neu-

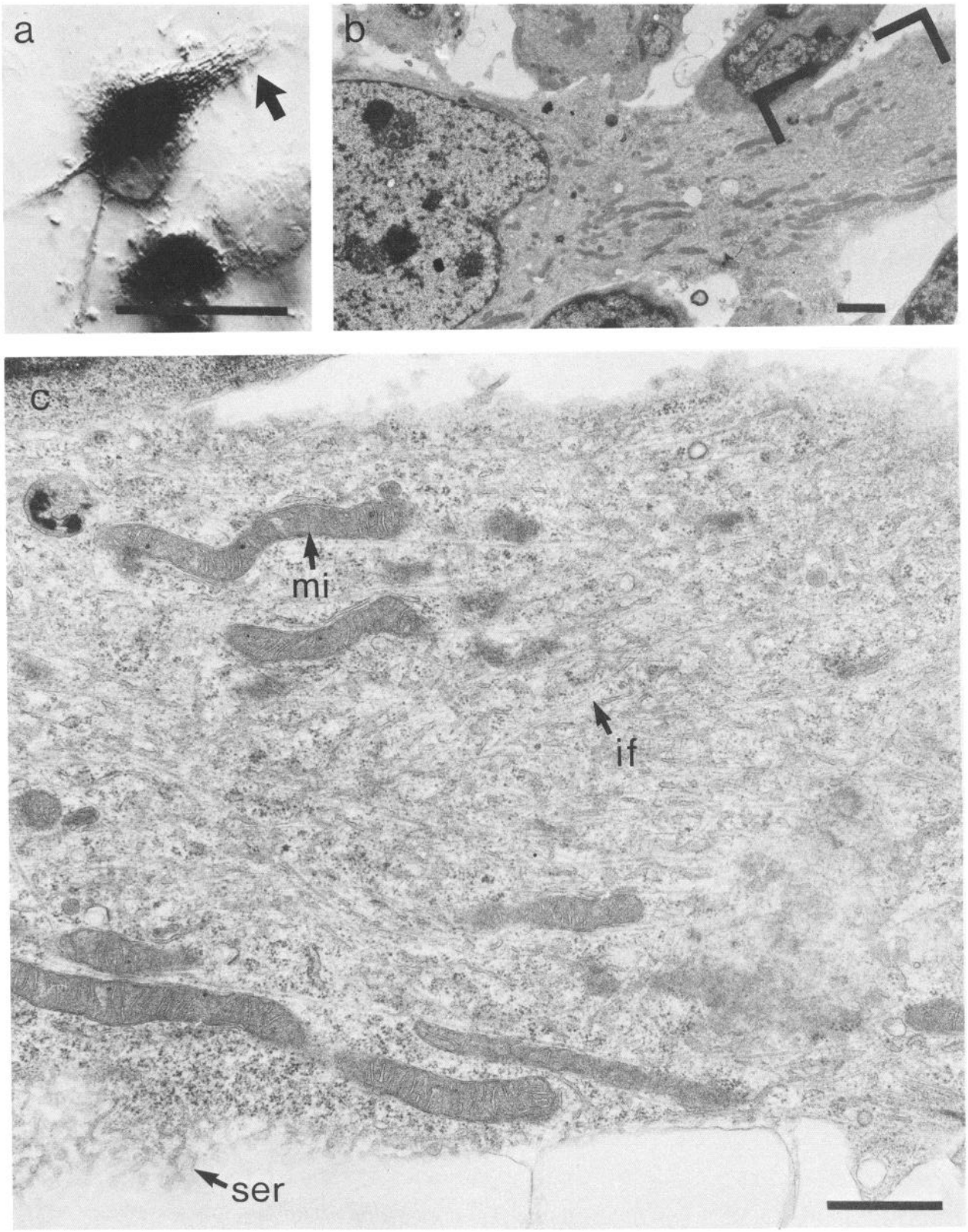


*Figure 6.* Features of the growing glial process in the "paddle" phase. *a,* At 3 hr, this cell (stained with AbGFP, as in Fig. 1) bears 2 thin processes and one broad process (*thick arrow*) rich in GFP. *b, c,* Low- and high-magnification views of a glial cell in the paddle phase, showing elongated mitochondria (*mi*) and masses of intermediate filaments (*if*) within the broad process. Smooth endoplasmic reticulum (*ser*) is apparent at the edges of the process. Bars: 20 μm (*a*); 2 μm (*b*); 1 μm (*c*).

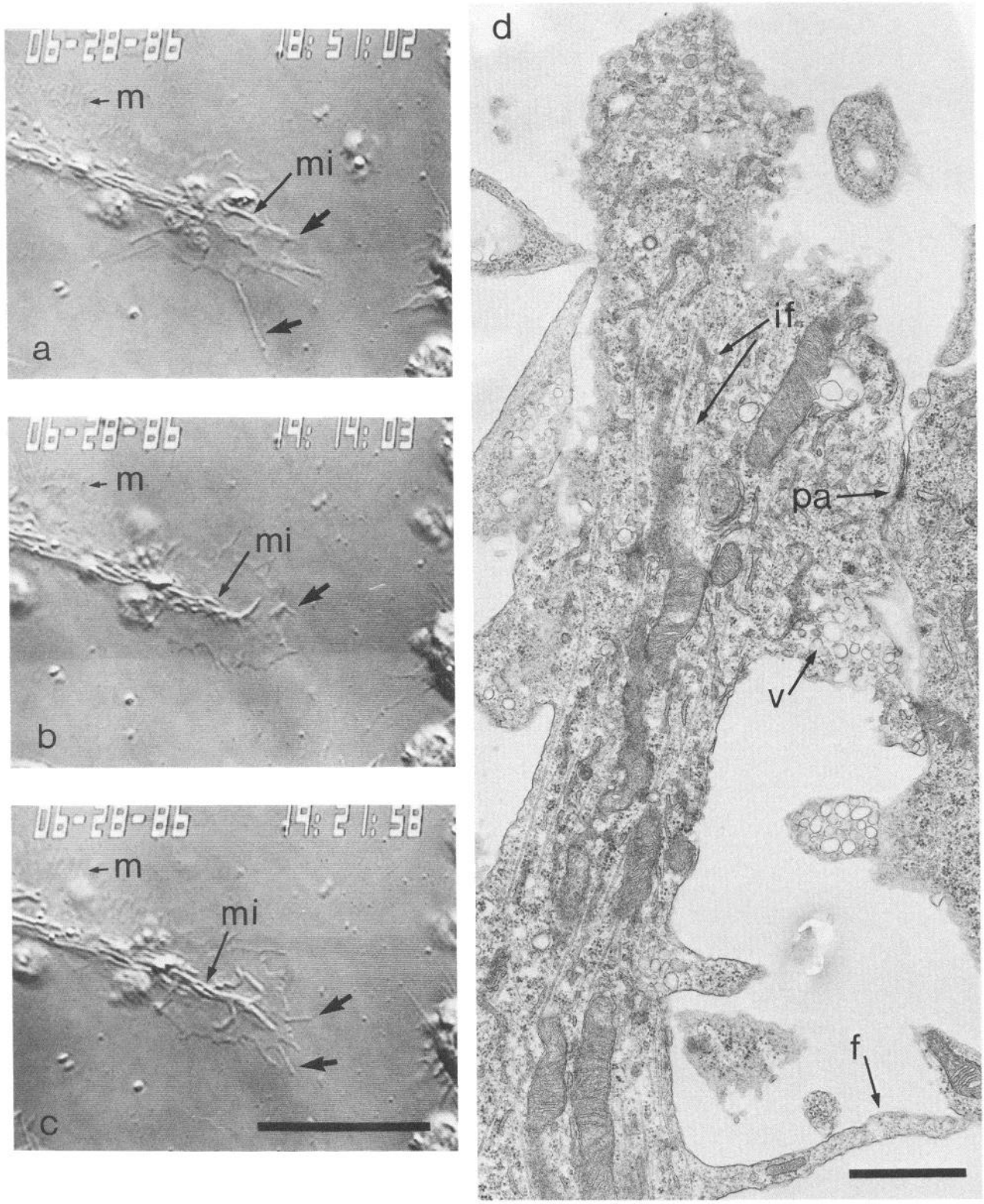


*Figure 7.* Features of the glial growing tip. *a–c,* Video microscopy of a living glial process with an extended, thinned process, 8 hr after plating. Although the process does not elongate during the 30 min observation period, microspikes (*arrows*) withdraw and extend, and mitochondria (*mi*) move in and out of the central core of the process and spread portions of the growing tip. Note the peripheral lamella flattened on the substrate and the relatively immotile microspikes (*m*) that fringe the lamella. *d,* Cytology of the growing tip on an elongated glial process, 5 hr after plating. Microtubules extend through the central core of the process, while disoriented intermediate filaments (*if*) are prominent in the process but do not extend to the edges of the growing tip. A system of smooth endoplasmic reticulum is evident. *v,* vesicles; *f,* filopodium. A punctate density (*pa*) marks the apposition of glial process and neuron. Bars: In *c,* 20 μm (*a–c*); in *d,* 1 μm.

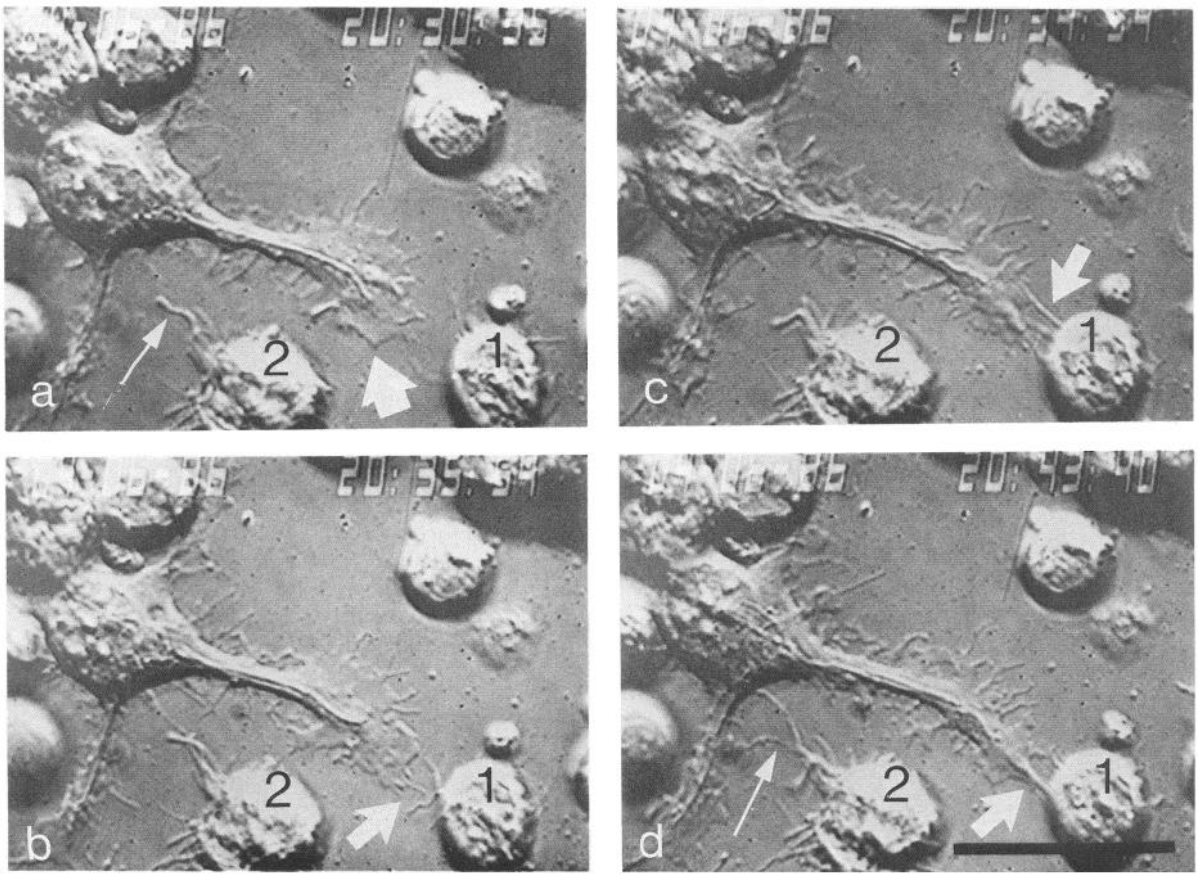


*Figure 8.* Video microscopy of a living culture, beginning 90 min after plating, showing interactions between glial growing tip and neurons. Judging from their size, the neurons are granule cells. Sequence begins 90 min after plating. *a,* The undulating lamella of the glial cell (*thick arrow*) advances towards neuron *1*. *b,* Five minutes later, 3 neuronal protrusions (*thick arrow*) extend toward the lamella of the glial cell. *c,* Upon contact, these coalesce to form a stable bridge (*thick arrow*) between neuron and glial process. *d,* Within a further 5 min, the neuron begins to project a process (*thick arrow*) onto the glial process tip. In *a* and *d,* note that neuron *2* also projects a motile microspike toward the glial cell soma (*thin arrow*). Bar, 20 μm.

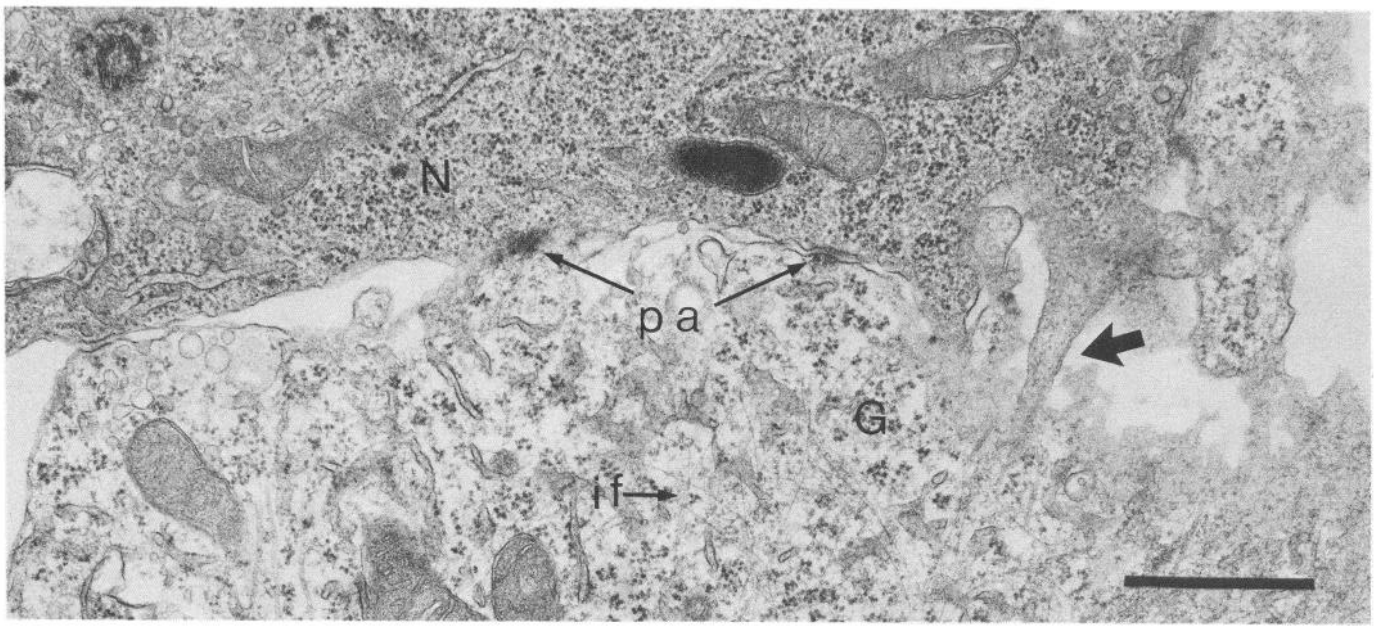


*Figure 9.* A common type of cell–cell interaction between neurons (*N*) and astroglia (*G*), showing a neuronal microspike touching the highly irregular glial cell surface (*arrow*). Puncta adherentia (*pa*) are common where neurons contact glia. *if,* Intermediate filaments. Bar, 1 μm.

ronal shapes and behaviors. Fibroblasts are extensively spread and usually lack long, forward-moving processes, moving instead by extension of a broad, leading lamella that forms adhesion sites with the substrate (Abercrombie, 1980). Only a trailing process is attached behind, becoming stretched as the fibroblast moves. In contrast, neurons have a rounded cell soma that is generally immotile, and thin, extended dendritic or axonal processes tipped with small expansions or growth cones that mediate elongation.

Cytological similarities to the neuronal growth cone include the networks of smooth endoplasmic reticulum (Bunge, 1977) and large, electron-lucent vesicles (Rees and Reese, 1981; Cheng and Reese, 1985). One distinction between the growing glial and neuronal processes is the abundance of intermediate filaments in the extending glial process. In retinal ganglion cell axons, neurofilaments are not assembled *en masse* in neurites until after neurite extension (Pachter and Liem, 1984).

Previous *in vitro* studies on astroglial differentiation have not demonstrated process outgrowth per se. Cytochalasin or dibutryl cyclic AMP induces astroglia to transform from flattened shapes to process-bearing ones (Trimmer et al., 1982; Federoff et al., 1984; Goldman and Chiu, 1984). Cavitation of flattened processes, accompanied by reorganization of cytoplasm, has been proposed as the mechanism for this phenomenon. As we observed in some video sequences, cavitation or collapse of a fan-like expanse of cytoplasm can result in the formation of processes, but in the astroglia in our cultures, and in those used for the studies on db cAMP, these processes were only as long as the initial borders of the cell.

The vast majority of astroglia in our cultures extended processes by outgrowth directly from the cell soma as opposed to cavitation. During this outgrowth interactions between neurons and the growing glial process occurred. One interpretation of this observation is that cell–cell contacts with neurons are needed for glial process outgrowth to occur. While both modes of morphogenesis lead to stellate glial cell shapes, and cAMP induces cytoskeletal changes that lead to cellular shape modifications, only cell–cell contacts with neurons accompany true outgrowth of glial processes in this system.

The present data are consistent with results from our previous studies, showing that neurons induce glial differentiation by a membrane-mediated mechanism (Hatten 1985, 1987), that glial differentiation is perturbed in the neurological mouse mutant weaver because of a failure of granule neurons to bind to glia (Hatten et al., 1986; Edmondson et al., 1988), and, in studies with anti-astrotactin antisera, that glial process extension is perturbed in concert with the blocking of neuron–glial contacts (Edmondson et al., 1988). The question remains as to how the neuron–glia interactions observed in the present study direct glial process extension and, especially, how the morphological and molecular composition of neuron–glial contacts influences cytoskeletal reorganization and motility of the glial process.

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