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Rapid Communication between Neurons and Astrocytes in Primary Cortical Cultures

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The identification of neurotransmitter receptors and voltage-sensitive ion channels on astrocytes (reviewed by Barres, 1991) has renewed interest in how these cells respond to neuronal activity. To investigate the physiology of neuron–astrocyte signaling, we have employed primary cortical cultures that contain both neuronal and glial cells. As the neurons in these cultures exhibit synchronous spontaneous synaptic activity, we have used both calcium imaging and whole-cell recording techniques to identify physiological activity in astrocytes related to neuronal activity. Whole-cell voltage-clamp records from astrocytes revealed rapid inward currents that coincide with bursts of electrical activity in neighboring neurons. Calcium imaging studies demonstrate that these currents in astrocytes are not always associated with slowly propagating calcium waves. Inclusion of the dye Lucifer yellow within patch pipettes confirmed that astrocytes are extensively coupled to each other but not to adjacent neurons, indicating that the currents observed are not due to gap junction connections between these cell types. These currents do not reflect widespread diffusion of glutamate or potassium released during neuronal activity since a population of small, round, multipolar presumed glial cells that are not dye coupled to adjacent cells did not display electrical currents coincident with neuronal firing, even though they respond to locally applied glutamate and potassium. These findings indicate that, in addition to the relatively slow signaling conveyed by calcium waves, astrocytes also display rapid electrical responses to neuronal activity.

[Key words: calcium, glutamate, glia, synchrony, oscillations, gap junctions]

The recent demonstration that astrocytes possess an array of ion channels (reviewed by Barres, 1991) and transmitter receptors (Bormann and Kettenmann, 1988; Sontheimer et al., 1988; Usowicz et al., 1989; Glaum et al., 1990; Wyllie et al., 1991) has renewed interest in understanding how glia respond to neuronal activity. Although the biochemical and electrophysiological properties of isolated astrocytes have been studied extensively, much less is known about how these cells communicate with neurons *in situ*. Seminal studies by Kuffler and others showed that presumed glial cells exhibit slow changes in membrane potential in response to neuronal activity (for review, see Kuffler and Nicholls, 1976). These effects on astrocyte membrane potential were thought to reflect the release and accumulation of potassium in the extracellular space during neuronal activity. However, the ability of glutamate to initiate calcium waves that can be propagated through astrocyte syncytia suggests that neurotransmitters may also convey signals from neurons to glia (Cornell-Bell et al., 1990, 1991; Dani et al., 1992).

To investigate the physiology of neuron–astrocyte signaling, we have employed primary cortical cultures containing both neurons and glia. Several features of this preparation make it well suited for these studies. Neurons and astrocytes can be identified with a high degree of certainty on morphological grounds, so patch-clamp recordings can be readily performed on cells of either type. Furthermore, after physiological recordings, the position of individual cells can be marked and their identity confirmed with standard immunohistochemical procedures. In addition, both optical recording of calcium transients and whole-cell recordings can be performed together, making it convenient to monitor activity simultaneously in neurons and astrocytes. The results obtained with this approach provide evidence that in addition to the relatively slow signaling conveyed by calcium waves astrocytes also display rapid electrical signals in response to neuronal activity.

Materials and Methods

Cell culture and media. Cell cultures were prepared from day 18 gestation Sprague–Dawley rat fetal cerebral cortex, using a papain (EC 3.4.22.2) dissociation method (Murphy and Baraban, 1990). Cultures were allowed to mature for at least 3 weeks for all experiments. The dissociated cells were resuspended at a density of 1.2×10^6 cells/ml in minimal essential medium (MEM) supplemented with 5.5 gm/liter glucose, 2 mM glutamine, 200 μM cystine, 10% fetal calf serum, 5% heat-inactivated horse serum, 50 U/ml penicillin, and 0.05 mg/ml streptomycin; plated onto polylysine-coated (10 μg/ml) 35 mm culture dishes in 1.5–2 ml of medium, or 12-well dishes (1 ml of medium); and placed in a 37°C CO_2-buffered incubator. For imaging experiments, cells were plated on polylysine-coated coverslips and placed within six-well plates. The cultures were fed by addition of MEM with 5.5 gm/liter glucose, 5% heat-inactivated horse serum, and 2 mM glutamine, after about 4–5, 9–10, 12–14, 16–17, and 19–21 d in culture, by removal and replacement of approximately 60% of the medium. Mitotic inhibitors were not added, as glial cell growth was arrested by confluence, and the paucity of growth factors in the medium used for feeding.

Immunostaining. Glial fibrillary acidic protein (GFAP) immunostaining was performed using a polyclonal rabbit antiserum obtained

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from Dako Ltd. at 1:5000 dilution. Anti-A2B5 and galactocerebroside monoclonal antibodies (Boehringer Mannheim) were used to identify type 2 astrocytes, and oligodendrocytes. Immunostaining was performed using a Vectastain kit (Vector) as described (Murphy et al., 1991).

Electrophysiology. For electrophysiological measurements, cells were switched to a Hank's balanced salt solution (by triple exchange) that contained (in mM) 137 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 1.0 $MgSO_4$, 0.44 KH_2PO_4, 0.34 $Na_2HPO_4(7\ H_2O)$, 10 Na^+ HEPES, 1 $NaHCO_3$, 5 glucose, and 10 μM picrotoxin (pH 7.4 and 340 mOsm). All recordings were made at room temperature using the whole-cell variant of the patch-clamp technique (Hamill et al., 1981) and an Axopatch 1C amplifier as described previously (Murphy and Baraban, 1990). Patch-clamp electrodes, pulled from 1B120 F4 glass (World Precision Instruments), had resistances in the range of 4–8 MΩ, as measured in the pipette solution containing (in mM) 140 K^+ methyl sulfate, 2 $CaCl_2$, 11 K^+ EGTA, and 10 K^+ HEPES. In some experiments, the following pipette solution was used in an attempt to achieve better voltage clamping of astrocytes (in mM): 120 CsCl, 20 tetraethylammonium Cl , 2 $MgCl_2$, 1 $CaCl_2$, 2.0 EGTA, 10 HEPES–NaOH. Despite use of solutions with K^+ channel blockers, the cells could not be effectively voltage clamped and were held at −60 mV for all experiments. Spontaneous astrocyte electrical activity persisted with the CsCl filling solution, and these data were combined with that obtained using the $KMeSO_4$ solution. Lucifer yellow (K^+ salt from Sigma) was added to the pipette solution as indicated at 2–4 mg/ml. Some cells were also filled with biocytin (1%) and visualized using peroxidase reagents (Vectastain). Recordings were made in a static bath (~1–2 ml) within a 35 mm tissue culture dish. Agonists and antagonists, diluted into bathing medium, were applied either by pressure ejection (15–20 psi for 0.5–5 sec) from 2–6 μm tip diameter glass pipettes positioned 250–500 μm from the cell of interest (previous experiments indicate that dilution can be up to fivefold), or by addition directly into the bathing medium at 100-fold concentration dissolved in bathing medium. Direct addition of bathing medium or water alone (20 μl to a 2 ml bath) failed to affect ongoing electrical activity or cell stability.

Photomultiplier tube measurement of fluo-3 fluorescence. Changes in cell calcium were estimated using the fluorescent probe fluo-3 (Minta et al., 1989). Fluo-3 undergoes a 40-fold increase in fluorescence upon binding calcium (400 nM K_d). Fluo-3 acetoxymethyl ester (Molecular Probes, Eugene, OR) was dissolved in dimethyl sulfoxide (5 μg/μl) and further diluted into Hank's balanced salt solution (10 μg/ml) in the presence of 0.25% Pluronic F-127. Cortical cultures were incubated with this solution for 1 hr at room temperature. Cells were then washed two times with Hanks's balanced salt solution and observed under epifluorescence (490 nm excitation) at room temperature. Fluorescence was quantitated using a Nikon photomultiplier tube (P1) and displayed on a strip chart recorder. An adjustable mask was placed between the cells and the photomultiplier to reduce the field illuminating the photomultiplier to one neuronal cell body. A neutral density filter was added to reduce photobleaching, and the fluorescence field diaphragm was lowered allowing fluorescence illumination of only the cell imaged by the photomultiplier. To prevent further photobleaching, a shutter was added to the fluorescence lamp. The shutter was typically open for 30 msec every second, which accounts for the broken lines present in the indicated records. Background fluorescence was determined in areas of the culture lacking neurons and subtracted from the indicated records. Results in Figure 1 are expressed in units of $\delta F/F$, where F = baseline fluorescence in the presence of neurons minus non-neuronal background fluorescence. Therefore, a $\delta F/F$ value of 1 would correspond to a 100% increase in baseline fluorescence. For simultaneous records of neuronal calcium transients and glial currents, the shuttering protocol was not used because of the extreme sensitivity of glial recordings to vibration caused by the shutter. As continuous recording was used, signal amplitude gradually faded, as would be expected due to photobleaching. Nevertheless, these records provide a reliable temporal measure of neuronal bursting.

Imaging of fluo-3 fluorescence. The experimental procedures for digital imaging of $[Ca^{2+}]_i$ with fluorescent intracellular indicators has been previously described in detail (Blatter and Wier, 1990; Wier and Blatter, 1991). Briefly, the main components of the system are a Nikon Diaphot inverted microscope, a charge-coupled device camera fiber optically coupled to a microchannel plate intensifier, and a real-time image processor (series 151, Imaging Technology, Inc., Woburn, MA) under the control of a microcomputer. Images obtained at video frame rate (30 Hz) were stored during the experiment on a real-time video disk storage system (model 8300 RTD, Applied Memory Technology, Tustin, CA). Computer programs for data acquisition and analysis were written using the programming language C and the library of subroutines from the series 151, ITEX 151. Changes in $[Ca^{2+}]_i$ measured with the indicator fluo-3 are expressed as % $(F - F_o)/F_o$, where F refers to the fluo-3 fluorescence measured from the cell and F_o represents the fluorescence of the cells presumably at rest. Dividing the F images by an F_o image provides correction for differences in path length, shading, and fluo-3 concentration. Since the cultures show some asynchronous spontaneous activity, it was not possible to record a F_o image in which all cells were simultaneously at rest. Therefore, we chose to create a synthetic F_o image from the lowest fluorescence values at any given image coordinate within an experiment. $[Ca^{2+}]_i$ is expressed as the percentage change of fluo-3 fluorescence as compared to the lowest value measured during an experiment at any particular location in the image. To improve the signal-to-noise ratio, images were spatially filtered by averaging a matrix of 2 × 2 pixels, and then by averaging four successive video frames.

Electrical stimulation. Cultures were stimulated with a bipolar tungsten electrode placed near, but not in contact with, a group of neurons ≈1000 μm away from the cells under study (Murphy et al., 1991). Stimulation parameters were typically 20–200 μsec in duration and 40–90 V.

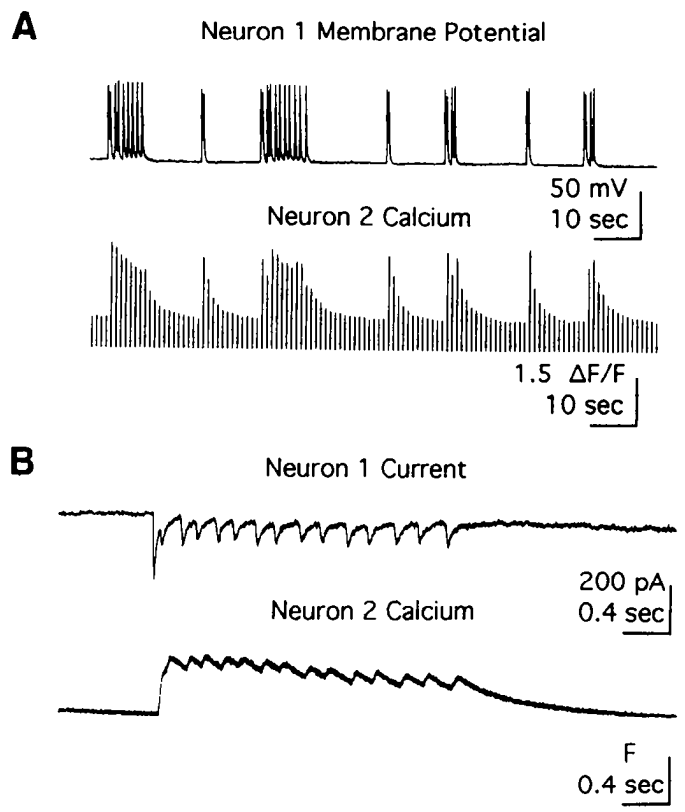

Figure 1. Spontaneous synchronous synaptic activity in cortical neurons in mixed neuron–glia cultures. *A*, Records of membrane potential in one neuron (−65 mV resting membrane potential) and fluo-3 calcium-induced fluorescence in its neighbor (≈100 μm away). Fluo-3 calcium-induced fluorescence was measured using a photomultiplier tube and is expressed as a change in fluorescence over basal levels. To prevent photobleaching of the calcium probe, the fluorescent lamp has been shuttered (see Materials and Methods for details). Under the conditions used, spontaneous calcium and membrane potential transients were always synchronous between pairs of neurons. *B*, In the same cell, spontaneous activity also occurs under voltage clamp. The holding potential was −60 mV. Because of the short duration of this record, a continuous record of fluorescence is shown. Due to photobleaching, this record of calcium-induced fluorescence is expressed in arbitrary units of F. Although the amplitude of the transients is affected by bleaching, temporal information should be unaffected.

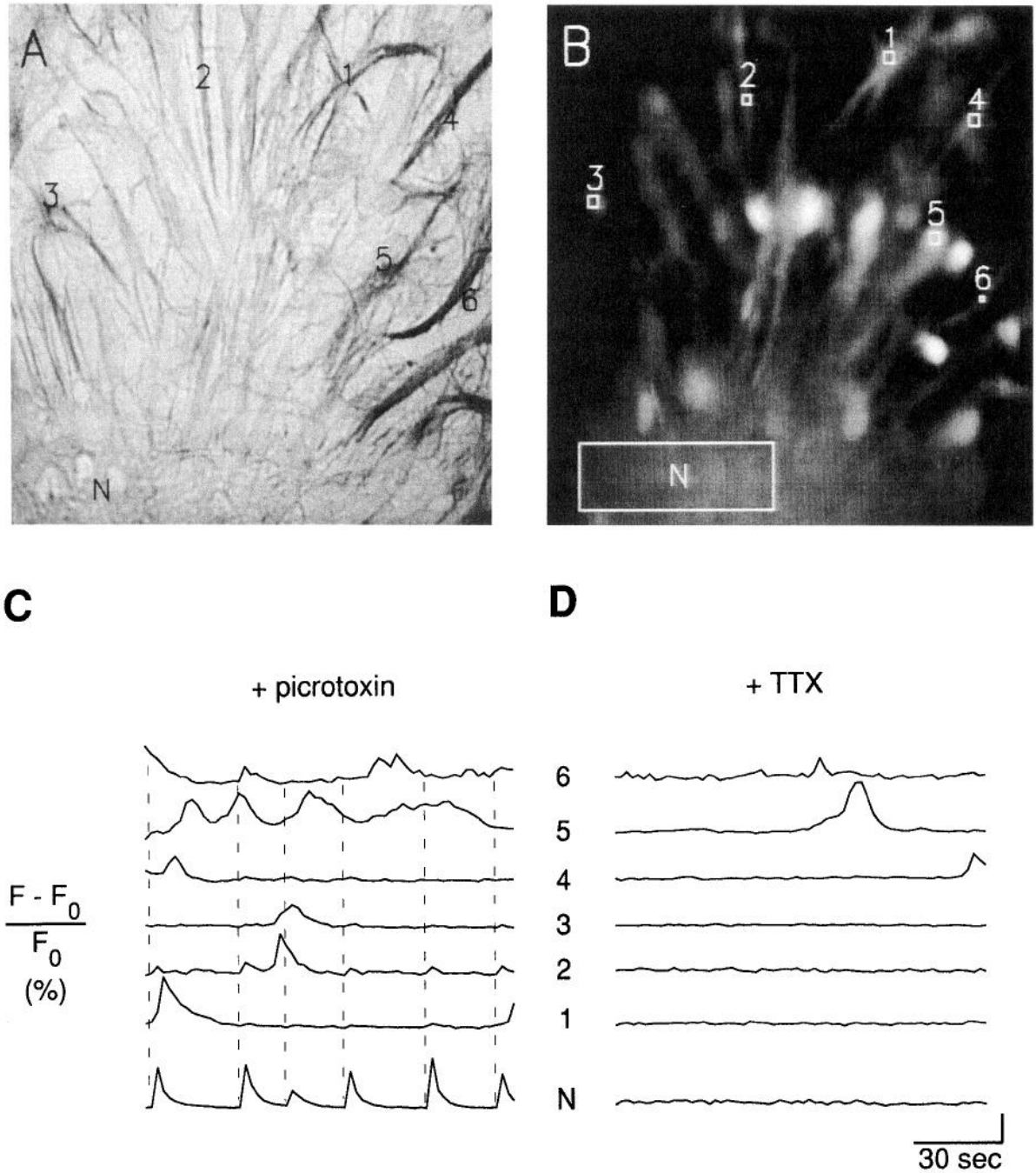

Figure 2. Noncoincident calcium transients in neurons and glia. *A*, Image of a group of glial cells in coculture with neurons visualized with bright-field illumination after the culture has been stained with GFAP antiserum to identify astrocytes. *B*, Fluo-3 fluorescence image that has been enhanced for display purposes. *C* and *D*, $[Ca^{2+}]_i$ transients measured from six individual astrocytes (cells *1–6*) and a clump of neurons (*N*) in the presence of picrotoxin (*C*) and after addition of TTX (*D*). The change in $[Ca^{2+}]_i$ is expressed as % $F - F_o/F_o$. $[Ca^{2+}]_i$ was sampled at 2 sec intervals. $[Ca^{2+}]_i$ was measured from single astrocytes in the area indicated by the *boxes* in *B*. Calibration: *horizontal*, 30 sec; *vertical*, 200% change of $F - F_o/F_o$ for glia cells *1–6* and 100% for the neuronal cluster (*N*), respectively. The dimensions of the *box marked N* are 90 × 23 μm.

Results

In initial experiments, we used calcium imaging to monitor spontaneous calcium transients in cultures containing both neurons and astrocytes to look for calcium transients in astrocytes related to neuronal activity. For these studies, we took advantage of the ability of picrotoxin to synchronize spontaneous calcium transients in neurons located in these cultures (Murphy et al., 1992). Picrotoxin-treated neurons exhibited regular synchronous bursts of calcium transients every 10–25 sec that correspond to underlying bursts of synaptic activity (Fig. 1). After recording several bursts of synchronous activity, cultures were processed for GFAP immunostaining to identify astrocytes (Bignami et al., 1972). Calcium images were then analyzed to determine whether calcium transients displayed by identified astrocytes were related to neuronal activity. We did not detect calcium transients in astrocytes that were temporally related to bursts of neuronal activity. Instead, a subset of astrocytes (36

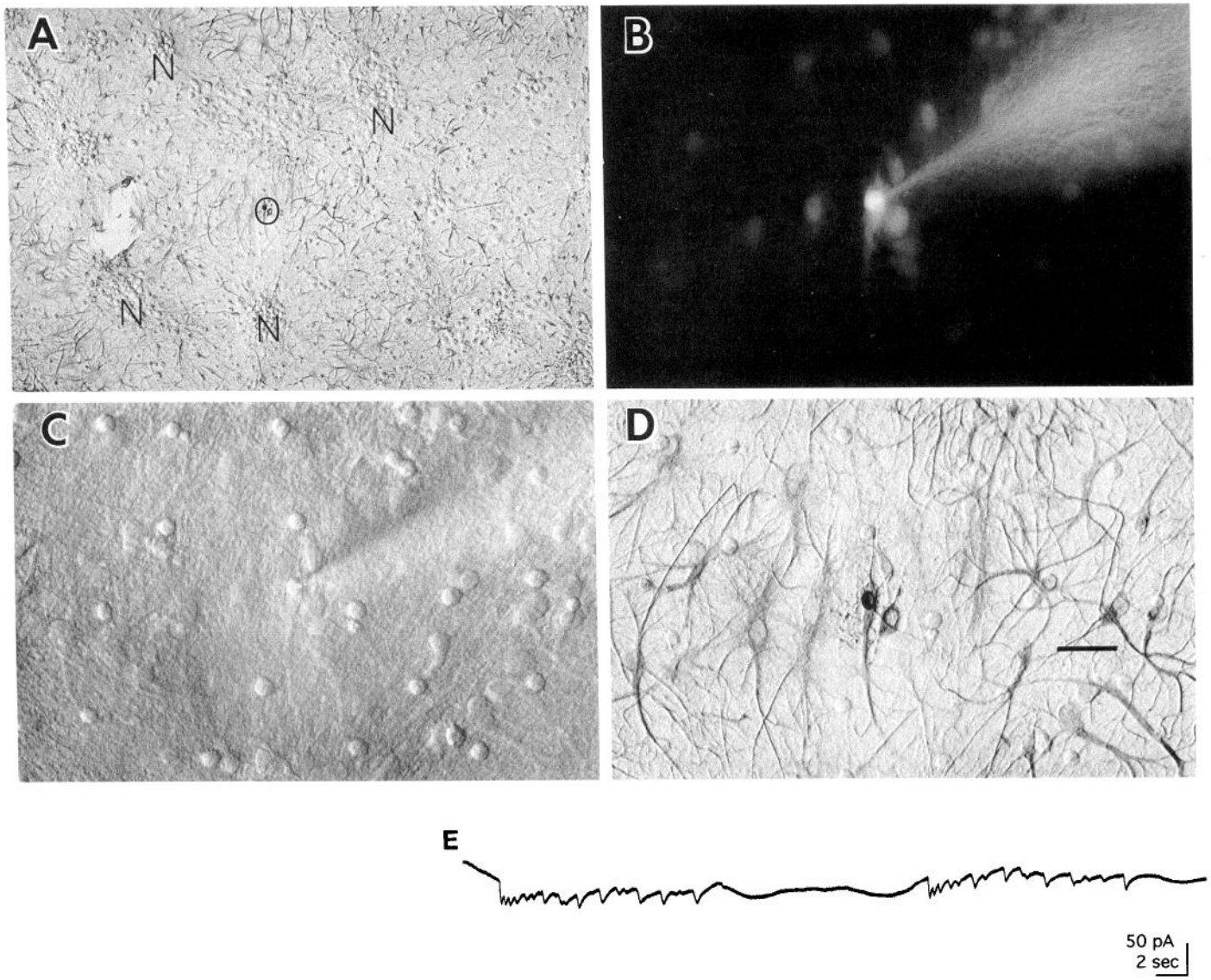

Figure 3. Spontaneous electrical activity in astrocytes. *A*, Low-power photomicrograph of a GFAP-stained culture of mixed neurons and glia. Clumps of neurons are indicated by *N*. *O* marks the location of the astrocyte shown in the subsequent photomicrographs. *B*, Fluorescence photomicrograph during a whole-cell recording. Lucifer yellow, 2 mg/ml, was added to the pipette solution to visualize glial morphology and to assess coupling to other cells. *C*, Photomicrograph of the same field prior to fixation and processing for immunocytochemistry. *D*, High-power photomicrograph of GFAP immunostaining. This photomicrograph demonstrates that the cell being recorded from is a GFAP-positive astrocyte that is dye coupled to several other GFAP-positive cells. The nuclear GFAP staining observed in the cell being recorded from is not usually present in intact astrocytes and is attributed to seal formation and subsequent removal of the patch pipette. *E*, Record of spontaneous currents measured under whole-cell voltage clamp from the cell with the attached pipette in *B* and *C*. Shown are two bursts of spontaneous currents. The time course of these currents closely resembles that previously observed in spontaneously active neurons. The holding potential was −60 mV and the record was filtered at 500 Hz. The bursts of spontaneous currents are superimposed on slow oscillations in membrane current that are seen in most astrocyte records. Scale bar: 120 μm for *A*; 30 μm for *B–D*.

of 48 examined) displayed calcium transients that were not coincident with neuronal activity and exhibited slower kinetics (Fig. 2). The addition of TTX to cultures, which silences neuronal activity, completely blocked the spontaneous calcium transients in 6 of 16 astrocytes examined from three separate experiments. However, in 10 of 16 astrocytes, spontaneous calcium transients were readily detected in TTX-treated cultures. The observation that TTX partially suppresses spontaneous astrocyte calcium transients suggests that neuronal activity contributes to generating astrocyte calcium transients.

To examine whether astrocytes display electrical activity that is related to activity in nearby neurons, we employed whole-cell patch clamping of presumed astrocytes. Although gigaohm seals were made with astrocyte membranes, in whole-cell mode the input resistance of the astrocytes proved to be quite low (41 ± 6 MΩ; $n = 19$ cells) when compared to that of neurons (typically 300–600 MΩ). The low input resistance of the astrocytes did not appear to be due to poor cell viability or seal formation since in current-clamp mode resting membrane potentials averaged −67 ± 3 mV ($n = 5$ cells). This resting membrane potential is close to the expected value for glial membrane potential with an extracellular [K^+] of 5.4 mM, assuming that glial membrane potential is largely determined by the K^+ equilibrium potential (Kuffler and Nicholls, 1976). As expected, depolariza-

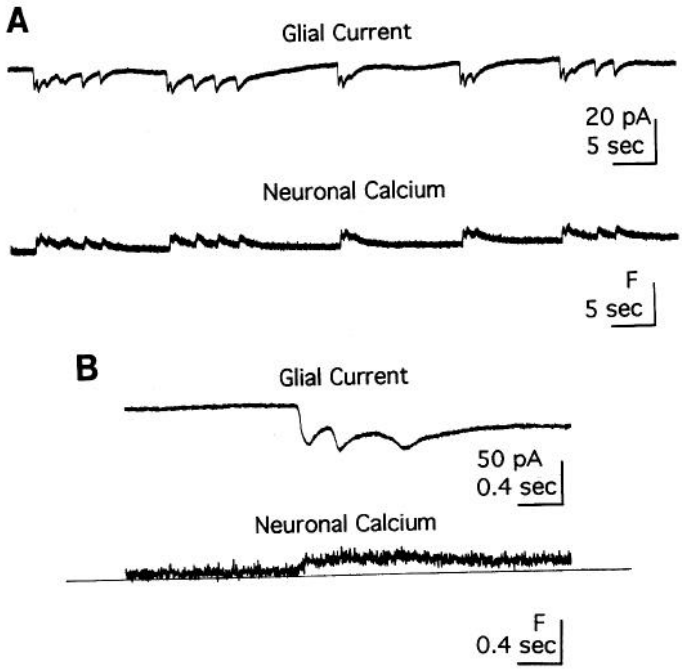

Figure 4. Coincidence of neuronal and glial activity. *A*, A record of spontaneous membrane currents in an identified GFAP-positive astrocyte recorded by the whole-cell method (−60 mV holding potential; 500 Hz filter). In a neuron ≈200 μm away, fluo-3 calcium-induced fluorescence was measured as an indicator of activity. Calcium transients always coincided with bursts of action potentials in picrotoxin-treated neurons (see Fig. 1). The calcium-induced fluorescence trace is of low amplitude due to photobleaching and serves to show the coincidence of neuronal and glial activity. Because of the instability of astrocyte electrical recordings, it was not possible to shutter the fluorescent lamp to prevent photobleaching. To prevent possible photodamage to astrocytes, the fluorescence field diaphragm was lowered, permitting only illumination of the neuron under study. *B*, A record showing coincident neuron–glial activity from another identified astrocyte neuron pair with higher time resolution. This cell pair was approximately 200 μm apart, and the astrocyte was voltage clamped at −60 mV.

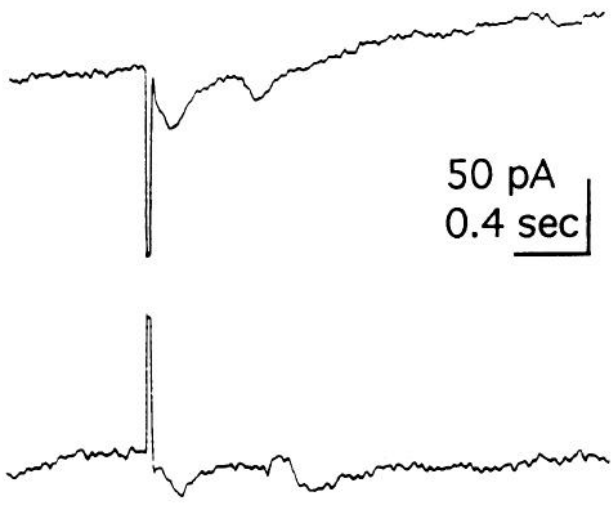

Figure 5. Currents in an identified astrocyte produced by electrical stimulation. A bipolar stimulating electrode was placed over a cluster of neurons ≈500 μm from the astrocyte recorded from, and stimulation at either polarity (±70 V, 90 μsec) resulted in slow inward currents in the astrocyte. The holding potential was −60 mV and the record was filtered at 2 kHz.

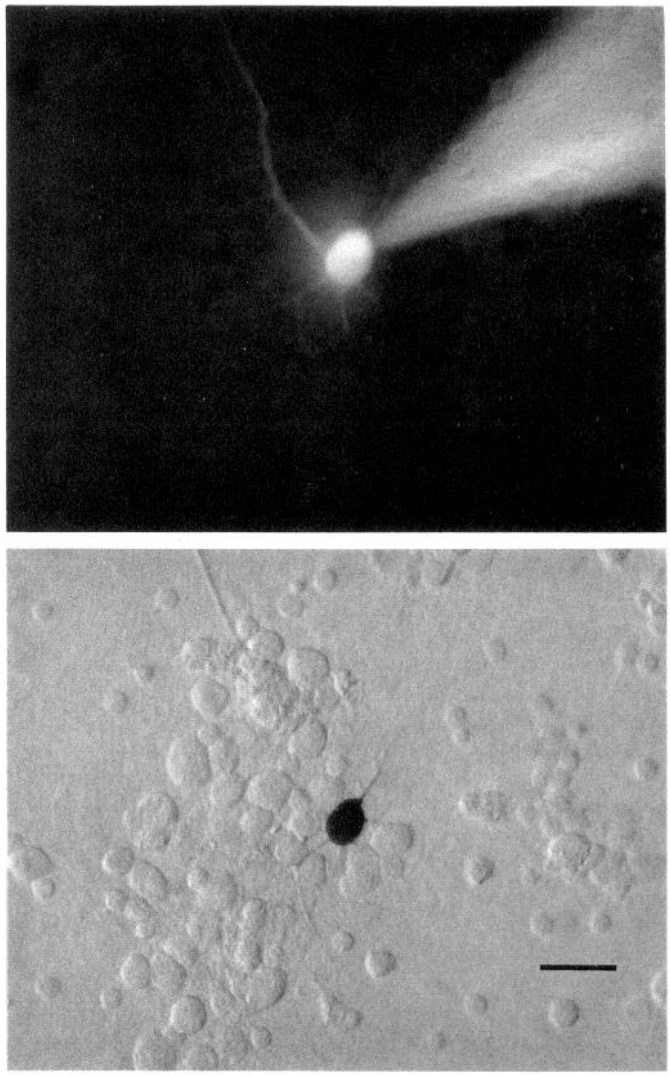

Figure 6. Neurons and astrocytes are not coupled by gap junctions. *Top panel* shows a fluorescence photomicrograph of a live neuron that has been filled with Lucifer yellow and biocytin. After dye loading, the neuron was fixed and processed with avidin-linked peroxidase reagents to reveal possible cell coupling using the biocytin method (*bottom panel*). Neither method indicated coupling of neurons to other neurons or astrocytes that form a near confluent layer around and beneath the cluster of neurons. Scale bar, 30 μm.

tion of presumed astrocytes in current clamp did not result in fast spiking potentials, and under voltage clamp only slowly activating currents could be seen (data not shown). Following whole-cell recording, the location of the presumed astrocyte was documented photographically and cultures were processed for GFAP immunocytochemistry. Conspicuous in almost all recordings made from GFAP-positive astrocytes (15 of 17 records) were bursts of spontaneous inward currents that occurred at regular intervals (Fig. 3).

Since the timing of the spontaneous astrocyte currents resembled that displayed by neuronal activity, we examined whether the events were coincident. In previous studies, we established that neuronal calcium transients coincide with neuronal electrical activity (Murphy et al., 1992). Therefore, we employed simultaneous optical recording of neuronal calcium transients and whole-cell recording from astrocytes (Fig. 4). In all astro-

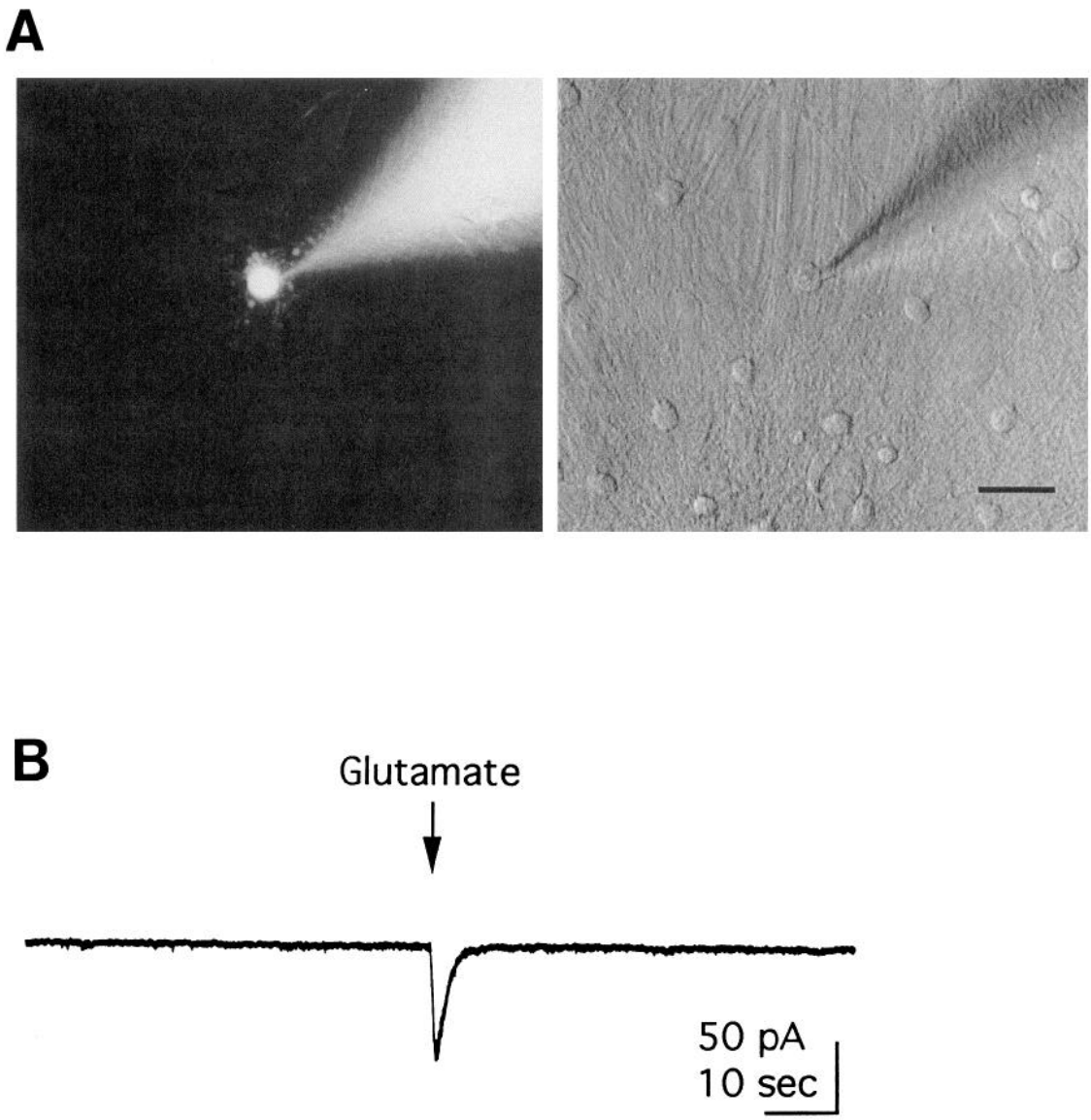

Figure 7. Small oligodendrocyte-like cells are not spontaneously active. *A*, Within mixed neuron–glia cortical cultures a distinct population of small oligodendrocyte-like cells exists. An example of one of these cells filled with Lucifer yellow during a whole-cell recording is shown. Scale bar, 30 μm. *B*, When voltage clamped (a cell similar to the one shown in *A*), these cells did not exhibit any spontaneous activity (12 of 12 cells), although they did have responses to application of glutamate (500 μM) or KCl (20 mM) by pressure ejection. In the presence of extracellular 1 mM $MgSO_4$ this cell exhibited a glutamate response with a linear current–voltage relationship that reversed near 0 mV, consistent with activation of non-NMDA-type glutamate receptors.

cytes identified with GFAP immunocytochemistry ($n = 6$ cells), there was complete correspondence between the time course of calcium transients in neurons and spontaneous inward currents in astrocytes. Examination of astrocyte–neuronal synchrony with faster time resolution did not reveal any significant delay (within 50 msec confidence limits) between these responses. Addition of TTX completely blocks electrical activity and calcium transients in neurons. Under these conditions, no spontaneous electrical activity was apparent in astrocytes ($n = 11$ cells), consistent with activity in neurons leading to activation of astrocytes.

In previous studies, we have used electrical stimulation to trigger synaptically mediated neuronal activity (Murphy et al., 1991). Electrical stimulation (70 V, 90 μsec) delivered by a bipolar stimulating electrode placed near a group of neurons resulted in rapid inward currents in neighboring immunocytochemically identified astrocytes. These currents were inward regardless of stimulus polarity (Fig. 5).

Several observations argue against the possibility that the inward currents recorded in astrocytes reflect extracellular currents produced by active neurons (Jefferys, 1981). Spontaneous currents were not detectable above background noise (≈3 pA) when patch electrodes were placed in close proximity (<10 μm) to neuronal or astrocyte cell bodies ($n = 10$). Astrocytes located at least 100 μm from neuronal cell bodies were typically used for recordings. In addition, currents were not detected by forming a gigaohm seal with astrocyte membranes prior to establishing the whole-cell recording configuration ($n = 4$ seals). Furthermore, spontaneous whole-cell currents in neurons in the presence of picrotoxin were always inward at −60 mV holding potential. In a similar manner, all glial currents were inward

and no inversion of sign was ever observed at −60 mV holding potential. In contrast, excitatory currents propagated extracellularly would be expected to be of either sign depending on the distance to their source.

In some experiments, the fluorescent dye Lucifer yellow was included in the patch pipette to assess whether the synchronous currents observed may reflect gap junction connections between neurons and astrocytes. In experiments performed with pipettes containing Lucifer yellow, astrocytes that were electrically active and GFAP positive were always extensively dye coupled (n = 11 recordings) to surrounding astrocytes, but never to cells with neuronal morphology. In two experiments, astrocytes that were not dye coupled lacked these spontaneous inward currents, suggesting that open gap junctions are necessary for current spread into the astrocyte being monitored. When neurons were filled with Lucifer yellow (n = 19) or biocytin (n = 4), in no case were other neurons or astrocytes labeled, indicating that astrocyte–neuronal synchrony is not due to electrical continuity between these distinct cell types (Fig. 6).

To address whether inward currents were displayed by other non-neuronal cells in the vicinity of astrocytes, we recorded from another class of presumed non-neuronal cell. These cells were small (<15 μm diameter), round, multipolar cells that were GFAP negative and galactocerebroside negative (oligodendrocyte marker; Raff, 1989) (Fig. 7). Staining with antibodies to the A2B5 surface antigen (Raff, 1989) suggested that cells resembling the one in Figure 7 might be oligodendrocyte progenitor cells; however, in preliminary experiments this class of cells did not stain for the A2B5 surface antigen after whole-cell patch clamping. Possibly, antigenicity is destroyed by the whole-cell procedure or the cells recorded from belong to another class of non-neuronal cells. Inclusion of the dye Lucifer yellow in patch pipettes indicated that these cells were not coupled via gap junctions to other cells within the culture (n = 9 cells). Unlike astrocytes, this class of cell had an extremely high input resistance (>1 GΩ), and no spontaneous neuron-like synaptic currents (see Fig. 1) in 12 of 12 cells. Although these cells did not display spontaneous neuron-like currents, they did possess responses (20–80 pA) to exogenously applied glutamate (1 mM; n = 3 cells) or 20 mM KCl (n = 3). In the presence of physiological Mg^{2+}, the glutamate-activated current showed a linear current–voltage relationship and reversed in sign near O mV, indicating that it resulted largely from the activation of non-NMDA-type glutamate receptors. The absence of synchronous activity in this class of glutamate-responsive cells provides evidence that the responses displayed by astrocytes are not the result of extensive release of glutamate or potassium into the bathing medium during neuronal activity, but may be due to a local interaction of these or other substances released from neurons with astrocytes.

Discussion

In whole-cell recordings from identified astrocytes, we have obtained evidence that these cells respond rapidly to neuronal activity. By simultaneously monitoring neuronal activity and astrocyte currents, we have found that these cells display inward currents that coincide with neuronal activity. Characterization of these cells established that these fibrous astrocytes are extensively dye coupled and express GFAP but not the A2B5 surface antigen (data not shown). Accordingly, these cells appear to correspond to type 1 astrocytes (Raff, 1989). Although astrocyte currents were well correlated with neuronal activity, calcium transients in these cells exhibited slower kinetics and were not always coincident with neuronal activity. In some astrocytes (Fig. 2), slow changes in intracellular calcium were observed that appeared to follow neuronal activity and were suppressed by TTX. Consistent with this observation, Dani et al. (1992) have observed propagating waves of calcium in response to tetanic stimulation in hippocampal slice cultures. Perhaps the slow propagation of Ca^{2+} waves through the glial syncytia (10–30 μm/sec; Dani et al., 1992) accounts for the poor correlation we observe between activity in neurons and Ca^{2+} transients in glia. In contrast, propagation of electrical potential would not be subject to the same diffusion constraints, and would be expected to be better correlated with neuronal activity. The presence of both calcium waves and electrical potentials in astrocytes suggests that multiple mechanisms may be involved in integrating astrocyte–neuronal communication.

Intercellular communication between astrocytes is thought to occur through extensive gap junction channels that permit the passage of low-molecular-weight substances from one cell to another (Brightman and Reese, 1969; Dermietzel, 1973, 1974; Bennett and Goodenough, 1978; Bennett and Spray, 1985; Massa and Mugnaini, 1985; Finkbeiner, 1992). A possible mechanism for neuron–astrocyte coincident activity might be gap junctions between neurons and astrocytes. However, the absence of Lucifer yellow dye coupling between neurons and astrocytes in this preparation suggests neuron–astrocyte communication involves diffusible factors detected by astrocytes such as glutamate or potassium. The absence of inward currents in nonastrocytic presumed glial cells (Fig. 7) suggests that the changes in glial membrane potential cannot be accounted for by widespread alterations in extracellular potassium or glutamate. Possibly, local rises in these factors might trigger inward currents in astrocytes in the vicinity of synapses selectively and not affect other classes of glial cells that may not form such an intimate relationship with neurons. These currents could spread via gap junctions producing long-range astrocyte–neuronal synchrony. To address the role of gap junctions experimentally, we tried to block gap junction conductances selectively by using long chain alcohols such as heptanol (Dermietzel et al., 1991; Finkbeiner, 1992). However, these compounds also effectively blocked neuronal activity, making them unsuitable probes to study astrocyte–neuron synchrony.

Astrocytes express functional AMPA-type glutamate receptor channels (Usowicz et al., 1989; Wyllie et al., 1991), raising the possibility that these receptors may mediate the inward currents observed. Alternatively, these inward currents could reflect electrogenic transport of glutamate (Szatkowski et al., 1990). We have confirmed that in this culture system, GFAP-positive astrocytes exhibit inward currents activated by the selective glutamate agonist kainate (data not shown). However, in this preparation, antagonists of AMPA-type glutamate receptors suppress spontaneous or electrically evoked synaptic activity (which likely results from polysynaptic activity), precluding use of these agents in assessing involvement of glial glutamate receptors in mediating these currents. Since astrocytes appear to express a unique complement of glutamate receptor subunits (Keinanen et al., 1990; Burnashev et al., 1992; T. H. Murphy and J. M. Baraban, unpublished observations), perhaps antagonists may be developed to target these receptors selectively and thereby directly test their role in mediating the glial response to neuronal activity.

Previous *in vivo* and brain slice studies have reported activity in nonspiking cells (Ransom, 1974; Casullo and Krnjevic, 1987; Sastry et al., 1988) that was in synchrony with adjacent neurons. These nonspiking cells were assumed to be glia, even though in most of these studies it was not possible to identify them definitively as astrocytes. Work by Gutnick et al. (1981) helped to establish that dye-coupled cells of astrocyte morphology exhibited coincident activity with neurons. Our *in vitro* studies show that GFAP-positive astrocytes exhibit coincident activity with neurons. Since astrocyte properties may change in culture (Barres, 1991), it will be important to perform similar experiments in intact systems, such as acute brain slice preparations. As astrocytes play a key role in regulating many aspects of neuronal excitability, the rapid response of astrocytes to neuronal activity may allow them to alter their metabolic activity quickly. The ability of neurotransmitters to modulate the conductance of gap junctions (Bennett et al., 1991; Enkvist and McCarthy, 1992) may allow them to regulate the spatial extent of rapid signal propagation through the astrocyte syncytia.

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