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NEURONAL-ASTROCYTIC INTERACTIONS

Implications for Normal and Pathological CNS Function

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CHAPTER 10

Do astrocytes process neural information?

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Introduction

The astrocyte has been a puzzle since this brain cell was first described by nineteenth-century neuroanatomists. While abnormalities of the astrocyte are prominent in many diseases of the central nervous system, a clear picture of the normal function of this cell has been slow to emerge. Recent advances in cellular and molecular neurobiology have provided some new pieces to the puzzle, however, and may be leading to major revisions in our thinking about not only the astrocyte itself but also about the brain as an information-processing organ. Many of the relevant new findings are described in excellent recent reviews (e.g., Kimelberg and Norenberg, 1989; Barres, 1991), which should be consulted for a broader overview.

This chapter will focus on the long-standing but controversial hypothesis that astrocytes play an active role in neural information processing, and especially on the impact of two recent developments. One is the very recent discovery that astrocytes possess a Ca-based form of excitability. This astrocytic property may be functionally analogous to the electrical excitability of neurons even though it is very different in details and in mechanism. The second development is the growing realization that brain function involves neuromodulatory elements that work on very slow time-scales in comparison to axonal conduction and fast synaptic transmission. This realization has broadened our horizons regarding the characteristics of potential neural information-processing elements.

This chapter also includes some original theoretical work addressing previously unexplored modes by which astrocytes might modulate synaptic function.

Background

The brain is composed of two neural cell types: the neurons and the neuroglia. The astrocyte is one type of neuroglial cell. In mammals, neurons and astrocytes are found in roughly equal numbers, intimately intermingled in every part of the central nervous system. Both neurons and astrocytes have elaborately arborized forms (see Fig. 1) and interconnect to form complex networks. Both neuronal and astrocytic networks have complex topologies reminiscent of the circuits of electronic computing machines. The neuronal networks have attracted far more scientific attention than astrocytic networks, however, because the neurons alone possess the special property of electrical excitability.

Electrical excitability allows neurons to generate and transmit signals in the form of action potentials. Since the initial discovery of electrical excitability, this phenomenon has been recognized as a compelling basis for the nervous system's ability to respond rapidly to sensory input, to encode and process information, and to generate the motor outflow. The neuroglial cells, including the astrocytes, have never
Fig. 1. Tracings of a neuron and an astrocyte showing the broad similarity in their arborized forms. Only the initial segment (a) of the neuron’s long axonal process is shown in this drawing. The velate expansions of the astrocytic branches evident here are typical of many, but not all, gray matter astrocytes. (Modified from Peters et al., 1991.)

been shown to exhibit any form of electrical excitability. They have therefore been viewed by the vast majority of neuroscientists as playing only indirect and passive roles in brain function, limited to providing structural, metabolic and trophic support to the neurons.

The astrocyte has distinctive, highly specialized contacts with a variety of neural cell types and other brain structures. Fig. 2 schematizes some of these structural relationships. As indicated in the figure, astrocytes elaborate lamellar expansions that line both the subpial and ependymal surfaces of the brain. Neighboring astrocytic expansions overlap in such a way that both the exterior and ventricular surfaces of the brain are composed entirely of astrocytic lamellae. Neural tissue is also lined by astrocytic projections where it is invaded by blood vessels: neighboring astrocytic expansions again appear to form an essentially continuous lining. Astrocytes also make numerous contacts with other astrocytes, resulting in the elaborate astrocytic networks that pervade all central nervous tissues. Finally, astrocytes make a wide variety of specialized contacts with specific neuronal structures, such as cell bodies, synapses and nodes of Ranvier. For instance, cell bodies within cortical cell layers are often lodged within a continuous, honeycomb-like matrix of astrocytic lamellae, as beautifully illustrated by the high-voltage electron microscopy of Hama and his colleagues (e.g., Kosaka and Hama, 1986). Each of these microanatomical specializations surely has substantial functional significance. While all of these specializations seem consistent enough with the conventional view of the astrocyte as a purely supportive cell, many other possibilities remain to be explored.

The hypothesis that astrocytes participate in neural information processing

In dissent to the supporting-cell view of the astrocyte, a few neuroscientists have entertained the hypothesis that these neuroglial cells play a much more direct and active role in neural computation (e.g., Pomerat, 1952; Galambos; 1961; De Robertis and Gerschenfeld, 1961; Hyden, 1962; Hild and Tasaki, 1962; Hertz, 1965; Kuffler and Nicholls, 1966; Orkand et al., 1966; Trachtenberg and Pollen, 1970; Stewart and Rosenberg, 1979; Vernadakis, 1988; Laming, 1989; Kimelberg and Norenberg, 1989; Teichberg, 1991; Barres, 1991). This hypothesis arises from diverse anatomical, physiological and neurochemical observations.

Electron microscopy and immunohistochemistry have established that the individual astrocytes composing astrocytic networks are interconnected by gap

![Fig. 2. A schematic illustration of some of the distinctive structural relationships between the astrocyte and other brain elements. The diagram should not be taken to imply that any individual astrocyte makes every one of the contacts illustrated, but all of the specializations shown are present in brain tissues in enormous numbers and variety.](image)
junctions — a connection well-suited to the transmission of chemical or electrical signals from cell to cell. This feature suggests that astrocytes, like neurons, might possibly transfer and process information in functional circuits. Electron microscopy has also established that many central nervous system synapses are intimately ensheathed by astrocytic lamellae, as schematized in Figs. 2 and 3. The separation between astrocytic and neuronal membranes is often as little as 10 nm — a distance smaller than the dimensions of many protein molecules. This structural relationship seems ideally suited to allow an astrocyte both to respond to and to modulate synaptic transmission. Some of the possible functional links are depicted in Fig. 3.

Physiological studies have shown that astrocytes can respond electrically to potassium fluxes associated with neuronal electrical activity (for reviews, see Kuffler et al., 1984; Barres, 1991), even though the astrocytes are not themselves electrically excitable. Astrocytic potassium metabolism may, in turn, affect neuronal electrical activity. Astrocytes are known to possess numerous potassium channels (see Barres, 1991, for review), and ionic interactions with neurons may be expected due to the very small volume of the narrow extracellular space shared by the two cell types. In addition, physiological studies have also shown that astrocytes participate in the metabolic cycles that replenish pre-synaptic supplies of neurotransmitter molecules (see Kimelberg and Norenberg, 1989). Finally, there is a large and growing body of work, including both physiological and receptor-binding studies, demonstrating that astrocytes possess a wide variety of neurotransmitter receptors (see Bevan, 1990; Barres, 1991, for reviews). Still, until very recently, there have been no reports of astrocytic excitability or any other form of propagated signal flow through astrocytic networks. This has changed with the introduction by Roger Tsien and his colleagues (Tsien, 1988; Minta et al., 1989) of sensitive new optical methods for the measurement of intracellular calcium (Ca) concentrations.

The astrocytic Ca wave

Cellular signals can take many forms. One is the famous electrical action potential of the neuron, the muscle and many endocrine cells. One of the most ubiquitous, however, is the cytosolic Ca signal (Stryer, 1988; Alberts et al., 1989). Cytosolic Ca signals usually take the form of transient increases in the cytosolic Ca\(^{2+}\) ion concentration. Such Ca signals arise from gated fluxes of these Ca ions across cellular membranes and exert their diverse and potent actions via effects of specific Ca-dependent enzymes within the cytosol. Cytosolic Ca signals are measured using the fluorescent probe...
Fig. 4. Cytosolic Ca signals in cultured rat hippocampal astrocytes exposed to the neurotransmitter glutamate. Cells were loaded with the fluorescent Ca probe fluo-3 and mounted in a perfusion chamber on the stage of a fluorescence microscope equipped for video recording. A–D are images before and after switching perfusate from control saline to a saline containing 100 μM glutamate. Increases in brightness correspond to increases in cytosolic Ca2+ ion concentration. A is the pre-glutamate control, B is 2 sec after the perfusate switch and C is 10 sec after the switch. D is a series of images sampled at 2 sec intervals from the small area indicated by the box in C. The black arrowhead above the top row of D indicates the time of glutamate application. E–H are time-series measurements from the video records of the same experiment, illustrating the range of different response types observed. E is a sustained-oscillator type (the same cell as shown in D). F is another sustained-oscillator type showing a decreasing frequency characteristic (sampled from the cell indicated by the solid, straight arrow in C). G is a damped-oscillator cell (curved arrow in C). H is a step-responder cell (short, open arrow in C) that participates in two long-distance waves late in the experimental run (times indicated by asterisks). (From Cornell-Bell et al., 1990a.)
molecules developed by Tsien and his colleagues. Some of the first indications of astrocytic Ca responsiveness to neurotransmitters came in non-imaging applications of fluorescent Ca probes (Sugino et al., 1984; Enkvist et al., 1988). Calcium probes can also be used in imaging modes, however, and such methodologies can provide many kinds of information that would not be available in other ways. The earliest applications of fluorescence Ca imaging to cultured astrocytes in fact revealed an extremely striking new phenomenon: a cytosolic Ca signal called a Ca wave (Finkbeiner et al., 1989; Cornell-Bell et al., 1990a; Dani et al., 1990, 1991, 1992; Charles et al., 1991; Jensen and Chiu, 1991; Cornell-Bell and Finkbeiner, 1991; Inagaki et al., 1991). Figs. 4 and 5 show examples of Ca waves observed in cultured astrocytes stimulated by the excitatory neurotransmitter glutamate.

Astrocytic Ca waves can be considered to be a form of cellular excitability, formally and perhaps functionally analogous to the electrical excitability of neurons. Like neuronal action potentials, astrocytic Ca waves can propagate over substantial distances without change in velocity or amplitude. This implies that the wave is actively regenerated as it travels (as are neuronal action potentials) by an excitation process that must represent the release of stored cellular energy (see Meyer, 1991; Meyer and Stryer, 1991). In contrast, most other chemical cell signals (including many other Ca signals) grow smaller and slower with increasing distance from their site of origin, reflecting the physics of a passive diffusion process. The active astrocytic Ca wave can even cross via gap junctions from one astrocyte to another — again without decrement in amplitude or velocity. Like neuronal action potential firing,

![Image](https://example.com/image)

**Fig. 5.** A wave of cytosolic Ca increase spreading through a confluent culture of rat hippocampal astrocytes. Procedures are as in Fig. 4, except that the image contrast associated with the Ca transient is increased by subtracting an unstimulated control image from each of the frames shown. A – D are a time series of images collected 6, 12, 18 and 24 sec after the onset of a long-distance wave induced by 100 μM glutamate application. E shows a superimposed series of tracings (corresponding to the images shown in A – D) to illustrate the progression of the wavefront of Ca increase. F is a three-dimensional plot of fluorescence time-courses sampled at eight different points along the axis indicated by a dotted line in E. This plot shows the synchronized response of cells at each point to the initial glutamate application (t = 0 sec) and the spatio-temporal progression of two waves beginning about 200 sec and 250 sec later. (From Cornell-Bell et al., 1990a.)
astrocytic Ca wave responses to steady stimuli are often oscillatory, as exemplified in Fig. 4. Finally, a threshold level of stimulation appears to be necessary to trigger active astrocytic Ca waves (Cornell-Bell et al., 1990a; Cornell-Bell and Finkbeiner, 1991), again in analogy to the threshold electrical stimulation required for the firing of neuronal action potentials. When astrocytic Ca waves are visualized by video playback of time-lapse recordings, the patterns of Ca wave propagation are beautifully intricate and varied. These beautiful wave patterns are compelling if not rigorous indications that these patterns could serve some computational function.

Ca waves similar to those observed in astrocytes have been described in many other cell types. They were first described in eggs as a part of the early response to fertilization (Glikey et al., 1978), but have since been observed in a very wide variety of tissue cells (Meyer and Stryer, 1991; Jaffe, 1991). One property of the Ca wave that appears to be conserved across eggs, astrocytes and many other cell types is velocity, which is usually between 10 and 90 μm/sec. It is possible that the basic mechanism underlying the Ca wave is also conserved across all these diverse cell types. Ca waves often appear to be triggered by products of phospholipid hydrolysis, such as inositol trisphosphate (IP3), and appear to be sustained primarily by an ion-channel-mediated release of Ca ions from the endoplasmic reticulum. The Ca waves triggered in cultured astrocytes by glutamate, for instance, appeared to be triggered primarily by a phospholipase C-coupled glutamate receptor and can be elicited in Ca-free external media. These observations are consistent with an IP3-mediated release of Ca from an intracellular store (such as endoplasmic reticulum). There are also indications in astrocytes (e.g., Cornell-Bell et al., 1990a; Cornell-Bell and Finkbeiner, 1991) and other cells (see Meyer and Stryer, 1991), however, that surface membrane entry of Ca ions may also serve, perhaps in cooperation with the IP3 pathway, to facilitate the intracellular Ca release process and to augment its effectiveness. Fig. 6 schematizes one possible interpretation of the pharmacological results that have been obtained on cultured astrocytes (Pearce et al., 1986; Cornell-Bell et al., 1990a; Jensen and Chiu, 1990, 1991; Glau et al., 1990; Ahmed et al., 1990). There are also astrocytic receptors for agents other than glutamate that may function independently or in conjunction with glutamate receptors to trigger astrocytic Ca waves or other Ca transients (e.g., norepinephrine: Salm and McCarthy, 1990; ATP: Neary and Noreenberg, this volume; see also Dave et al., 1991; McCarthy and Salm, 1991; see Bevan, 1990; Barres 1991, for reviews). The basic mechanisms and cell biology of Ca waves have become a very active research area, but will not be considered here further (see Berridge, 1990; Jacob, 1990; Meyer, 1991; Meyer and Stryer, 1991; Jaffe, 1991, for excellent recent reviews; Bezprozvanny et al., 1991; Finch et al., 1991). We will note in passing, however, that the fact that Ca waves and Ca excitability are not restricted exclusively to astrocytes should not make them any less interesting to neurobiologists: action potentials and electrical excitability are also found in many cells outside the nervous system.

One of the most intriguing aspects of the astrocytic Ca wave is its responsiveness to neuronal activity, as shown by the recent work from my laboratory (Dani et al., 1990, 1991, 1992) and illustrated in Figs. 7 and 8. We showed that stimulation of a de-
Fig. 7. The effect of 50 Hz electrical stimulation of dentate granule cells on cytosolic Ca in neurons and astrocytes in the pyramidal cell layer of region CA3. Neurons respond within milliseconds and astrocytes within 2 sec to such stimulation. The preparation is an organotypically cultured rat hippocampal slice (see Gahwiler, 1988). Cells were stained with the Ca probe fluo-3 and visualized using a laser confocal microscope and video recording procedures. All images represent an optical section taken at a plane approximately midway through the 120 μm thickness of these slices. A is resting fluorescence observed after fluo-3 loading. The orderly array of horizontally oriented fusiform cell bodies are pyramidal cell bodies. Stratum pyramidale lies to the left; stratum lucidum lies to the extreme right. The numbered boxes indicate areas where fluorescence intensity measurements were taken for C. B is a retrospective immunofluorescence image showing the same field as A. Such images were collected to identify specific cells as astrocytes. The arrows and arrowhead mark cell bodies and a process positive for the astrocytic marker glial fibrillary acidic protein (GFAP, Bignami et al., 1972). C shows fluo-3-fluorescence increases measured over both astrocytes (traces 1–5) and a neuron (trace 6) during Ca responses to dentate stimulation. The data were measured at the positions indicated by the correspondingly numbered boxes in A. Traces are shifted arbitrarily along the ordinate axis for clarity. D–F are fluorescence change images calculated at several time-points following electrical stimulation: D is the earliest response to electrical stimulation. The horizontal arrows indicate the horizontal scan line active at the time of stimulus onset (the image was scanned at the rate of 500 lines/sec). Numerous fine processes and pyramidal cell bodies exhibit Ca increases during this first second of stimulation. E was collected after two more seconds of stimulation (t = 4 sec), the pyramidal cell bodies exhibit large cytosolic Ca increases, especially within their nuclei. Many GFAP-positive cell bodies and processes (e.g., arrows and arrowhead, same positions as in B) now also exhibit substantial cytosolic Ca increases. F was collected after 4 sec of stimulation (t = 6 sec), and nearly every astrocyte within the field has responded. Again, the position of the arrow corresponds to the same arrow marking a GFAP-positive cell body in B. Scale bar, 20 μm. (From Dani et al., 1992.)

fined, glutamatergic neuronal tract afferent to region CA3 of cultured hippocampal slices (Gahwiler, 1988) could elicit Ca waves in astrocytes within a brain tissue environment. These astrocytic responses appear to be triggered by synaptic glutamate release, acting via a receptor like that identified in cultured astrocytes. The patterning of astrocytic Ca wave propagation observed in the tissue slice environment is even more intricate and varied than that observed in astrocyte cultures. The existence of richly patterned astrocytic Ca waves in a brain tissue environment and the fact that such waves can be initiated by neuronal activity suggest the possibility that Ca waves could encode and process the information inherent in neuronal activity patterns. This possibility gives new resonance to the “dissenting
minute's" astrocytic information processing hypothesis.

Ca waves as a possible basis for astrocytic information processing

The general hypothesis that astrocytic networks play an active role in neural information processing can be schematized as shown in Fig. 9. The rest of this chapter will explore one particular form of this hypothesis: a form based on the idea that astrocytes encode information by the patterning of their Ca waves. These three propositions articulated in Table I provide a useful framework for thinking about this hypothesis. The three propositions can be mapped onto the schematic of Fig. 9 as indicated by the numerals on that figure. These three propositions could comprise a powerful argument on behalf of astrocytic information processing. If all three are true, the logical conclusion would be that astrocytic network activity plays an active role in shaping neuronal network activity. Since virtually no neuroscientist doubts that neuronal network activity is the primary basis for the brain's computational function, we could restate the implications of our three propositions as follows: the brain processes information through the joint activity of neuronal and astrocytic networks, not by neuronal network activity alone.

How good is the evidence for the three propositions? The first two are supported most directly by the work from our laboratory (Dani et al., 1990, 1991, 1992), but these results are very new and a great deal more work must be done to determine their range of applicability. As a minimum, it is important that our results be confirmed by other laboratories and that similar results be obtained with acute brain slice preparations. It would be more desirable still to replicate these experiments using some whole-animal recording situation and physiological stimuli. Of course it will also be important to learn whether similar neural-astrocytic signaling is observed in other species and brain regions besides the hippocampus of rats. The third proposition, that astrocytic Ca waves affect neuronal function, has no direct experimental support at present, but nonetheless seems quite plausible. We shall explore this third proposition in much more detail below, but first we shall ask whether or not the idea of astrocytic information processing could hold up to any serious scrutiny from the all-important view-
TABLE I

Propositions that may implicate astrocytes in neural information processing

1. Astrocytic Ca waves are initiated in response to neuronal network activity
2. Ca waves propagate actively through astrocytic networks
3. Astrocytic Ca waves feed back to alter activity in neuronal networks

point of a contemporary neurobiologist’s “common sense”.

Ca waves and common conceptions of neural information processing

The complexities of the brain and of animal behavior guarantee that it is at present impossible even to define, much less explain, neural information processing. Even so, it would be unreasonable to deny that neurobiologists do, in fact, share a common set of conceptions on the point. These conceptions of neural information processing are probably based largely on vague analogies with information processing by human-engineered computing machinery. Based on such vague, shared conceptions, we can at least attempt to ask a vague but crucial question: are the specific properties of astrocytic networks and Ca waves, as we now understand them, at all suited to the requirements of neural information processing, as we now understand it? Arguments to the contrary could note that astrocyte networks lack at least two of the most conspicuous attributes commonly associated with neuronal networks: one of these attributes is a high signaling speed, the other is discrete point-to-point circuitry. Astrocytes also lack any structure analogous to the neuron’s long axonal fiber. The range over which an individual astrocyte could transmit a signal is therefore limited to a few tens of micrometers, in contrast to distances of over a meter for certain neurons. Furthermore, while astrocytes seem to interconnect with their nearby neighbors in a diffuse and indiscriminate fashion, neurons are thought to connect only with very specific targets, which can be very far away (although much more often they are very close — within tens of micrometers). In each of these contrasts between astrocytes and neurons, the astrocyte might seem somehow less well suited to an information-processing role. Let us therefore examine these contrasts in more detail.

Is astrocytic signaling fast enough to mediate useful information processing?

Table II compares neuronal action potentials and astrocytic Ca waves in terms of propagation velocities, intrinsic oscillation frequencies and latencies of response to afferent neuronal activity (synaptic delay in the neuronal case). These numbers clearly indicate, at least, that the spatio-temporal province of astrocytic computation (if any) must be quite different from that associated with neuronal networks. Does it make sense to think of anything as slow as an astrocytic network as a part of the brain’s information processing machinery? Maybe. For instance, astrocyte networks might mediate slow modulations of neuronal function, like those underlying arousal, selective attention, mood change or learning (see Müller and Best, 1989, for an intriguing possible instance). We probably would wish to think of any such modulatory agent, however slow, as bona fide active components of the brain’s information processing machinery. There is a precedent for this. Over the last two decades, it has become clear that many neuronal actions, including most of those mediated by neuropeptides and biogenic amines, like norepinephrine, dopamine and serotonin, are just as slow, or even slower than signaling in

TABLE II

Comparison of neuronal and astrocytic signal speeds

<table>
<thead>
<tr>
<th></th>
<th>Neuronal action potential</th>
<th>Astrocytic Ca wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum signal velocity</td>
<td>100 m/sec</td>
<td>0.00002 m/sec</td>
</tr>
<tr>
<td>Maximum oscillation frequency</td>
<td>200 Hz</td>
<td>0.1 Hz</td>
</tr>
<tr>
<td>Minimum response latency</td>
<td>1 msec</td>
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astrocyte networks. In spite of the slow time-courses of neuromodulatory transmitter actions, there is probably no neuroscientist who would wish to exclude the peptidergic and aminergic neurons from consideration as active components of the brain's information processing machinery. Moreover, it appears that the field of neurobiology is entering an era in which people will talk about genes and their control machinery as participating in neural information processing. Astrocytic Ca waves can work very much faster than genes!

Could astrocytes actually form functional circuits?

If they do, these circuits must be very different from those formed by neurons. Neurons seem to form very precise and specific synaptic circuits. Even though we do not know in much detail how any of these neuronal circuits actually work, it is widely assumed that their precise wiring diagrams are somehow fundamental to neuronal network computation. Astrocytes, on the other hand, interconnect with their neighbors through extensive gap-junctional contacts in an apparently non-specific manner. Could a network of nearly syncytial astrocytes possibly compare in computational function to a neuronal network of discrete synaptic circuits?

Probably not. But recall that the modulatory aminergic and peptidergic neurons also appear to act in a spatially diffuse fashion. Perhaps brain function, in all its complexity and glory, requires many different kinds of computation – some discrete, fast, and specific; others slow and diffuse. Until we have a much better grasp of the true rules of neural computation, it is probably inadvisable to eliminate any particular type of circuit from consideration. Furthermore, the patterning of Ca wave propagation evident in hippocampal slices (Dani et al., 1991, 1992) suggests that astrocytic signaling may actually be a great deal more intricate and varied and patterned than the simple anatomy of astrocytic networks might suggest. Such intricacy could arise from the dynamics of the Ca signal propagation (see Winfree, 1980, for discussion of patterning in excitable media) or from the variability and plasticity of gap-junctional coupling (Kettenmann and Ransom, 1988; Dermietzel et al., 1991).

In summary, we can draw two tentative conclusions about astrocytic Ca signaling and neural information processing. (1) Astrocyte Ca waves probably could not mediate the same kinds of functions as neuronal action potentials and fast synaptic transmission: they are far too slow. (2) Astrocyte Ca waves probably could carry out the actions similar to those of the peptide and biogenic amine transmitters released by modulatory neurons: astrocytic networks would not necessarily be either slower or more diffuse in their actions than such neurons. These conclusions must be tentative because we still know so little about exactly how nervous systems encode and process information. It is intriguing to note, however, that by demonstrating certain, simple cellular interactions between astrocytes and neurons, we may be able to prove that astrocytes are actively involved in whatever computation it is that neuronal networks perform long before we understand the computation itself.

Do astrocytic Ca waves affect neuronal function?

Having argued that astrocytes are not absurdly mismatched to a respectable information-processing role, we shall return to our main line of argument in favor of such function. We left off earlier noting that there is now evidence for only two of the three propositions which could logically establish that astrocytic Ca waves process information. The third, unsubstantiated proposition requires that astrocytic Ca waves exert some significant effect on neuronal activity patterns. Why spend time thinking about this idea before it is supported by experimental fact? Are there any good reasons to believe that experimental support might arrive any time soon?

First, how damaging is the present lack of evidence? The earliest reports of astrocytic Ca waves are still quite recent (e.g., Finkbeiner et al., 1989). Thus, the lack of positive evidence in favor of Ca-wave effects cannot be taken to mean that there are none, because there has so far been very little time for anyone to explore the possibility. Nonetheless, one might argue that if astrocytes could affect
neuronal function, their actions should have been obvious long ago to physiologists studying neurons using intracellular recording techniques. It is fair to say that the concept of “astrocytic input” to the neuron is relatively scarce in the lore of neurophysiology (but not non-existent: see Laming, 1989; Sastry et al., 1990). To put this counterargument in perspective, however, it is necessary to understand that much of what neurophysiologists actually see when recording intracellularly in intact central neural tissue never makes it into print. The more intact a piece of neural tissue, the more intracellular recordings are a world of electrical chaos, of unexplained baseline shifts and slow or sudden “spontaneous” changes in synaptic strength, and so on. Most of this chaos is normally and rightfully ignored in the interests of isolating and analyzing particular, identified phenomena of synaptic or neuromodulatory origin. Within this seeming chaos, however, there is still plenty of room for presently undocumented astrocyte actions on neuronal excitability or synaptic transmission. It should be remembered that the phenomena of neuromodulation were essentially overlooked during whole decades of the intracellular analysis of fast synaptic transmission. Thus, it may not be unreasonable to think a little longer about the possible that astrocytes actively shape neuronal network activity.

As noted above, cytosolic Ca signals are ubiquitous and potent regulators of cellular function. Among the cellular functions that are usually regulated by cytosolic Ca are ion channel opening, contractile and shape-determining activities of the cytoskeleton, and production and secretion of messenger substances. There are indications that astrocytic functions in each of these categories are regulated by cytosolic Ca (e.g., Quandt and MacVicar, 1986; Murphy et al., 1988, 1990; Cornell-Bell et al., 1990b, 1992), and, one would therefore expect, by Ca waves. Experimental documentation of physiological Ca wave action remains, however, an experimental challenge for the immediate future. The next step will be to ask whether Ca-dependent astrocyte functions exert significant effects on neuronal activity.

Based on our general body of cell-biological knowledge, we can generate numerous attractive hypotheses about how astrocytic Ca waves might influence neuronal activity. A representative selection of such hypotheses follows.

**Possible links from the astrocytic Ca wave to neuronal activity**

1. As noted above, astrocytes are known to possess Ca-dependent potassium channels, and there is also evidence that glial potassium transport can shape the electrical excitability of nearby neurons. Therefore, *astrocytic Ca waves and Ca-dependent potassium channels may govern neuronal excitability by modulating fluxes of potassium ions into narrow extracellular spaces*. Given the very small volumes of the extracellular spaces in CNS neuropil, these extracellular potassium effects should be very rapid.

2. There is strong evidence that astrocytic ion fluxes drive slowly varying extracellular electrical currents through CNS neuropil; these are detectable as slow field potentials of substantial amplitude (see Laming, 1989). These field potentials are interesting because they probably exert small but significant biasing effects on neuronal excitation. Astrocytic Ca waves and Ca dependent potassium channels provide a very likely explanation for the patterned opening of astrocytic ion channels that must drive the spatially non-uniform and time varying astrocytic ion current underlying such field potentials. Therefore, *astrocytic Ca waves acting via Ca-dependent potassium channels may modulate neuronal excitability by governing the flow of electrical current through narrow extracellular spaces.***

3. The transport of Ca ions across plasma membranes can be influenced profoundly by cytosolic Ca. Numerous sites of Ca action on both passive Ca influx and active Ca extrusion have been documented. Actions on Ca influx include several expressed at the level of voltage-dependent Ca channels, which are found in abundance on astrocytes (Barres, 1991). Probable examples of cytosolic Ca actions on Ca channels include: (a) direct actions of Ca exerted by Ca binding to the actual channel moiety; (b) ac-
Fig. 10. A model for changes in Ca ion concentration within presynaptic extracellular cleft spaces. A. The geometry of an astrocytically-ensheathed synapse (left) is approximated by coaxial cylindrical forms, shown in two orthogonal views (right). Two solid cylinders represent the pre-synaptic (PRE) and post-synaptic (POST) elements, while an annular cylinder represents the ensheathing astrocytic element (Ast). Pre-synaptic Ca channels whose opening constitutes the major forcing function to changes in presynaptic cleft Ca concentrations are arranged in a regular array within a central circular area on the internal PRE cylinder face, labeled as “the active zone”. B. Specific dimensions and numerical parameters used for the present simulations. These dimensions are representative of values determined by electron microscopy. The clustering of the pre-synaptic Ca channels within the active zone, and the value for their density are as determined by a combination of structural and physiological methods (Smith and Augustine, 1988; Roberts et al., 1990; see also Robitaille et al., 1991). C. Time-courses of pre-synaptic Ca influx (upper panel) and extracellular Ca concentrations (lower panel) in response to the transient increase in pre-synaptic Ca permeability shown in the upper panel. This Ca permeability wave form was chosen to simulate the events of the pre-synaptic action potential: (1) Ca channels are opened by the depolarization, but there is little Ca influx until (2) the action potential downstroke suddenly increases the electrochemical driving force on Ca ions, and initiates the major Ca influx, which lasts until (3) Ca channels close in response to the repolarization. At the peak, just over half of the Ca channels are open. Amplitudes of single Ca channel currents were approximated by a direct proportionality to $[\text{Ca}]_{\text{o}}$, set to a value of 0.7 pA at $[\text{Ca}]_{\text{o}}$ =.
tions mediated via Ca-dependent phosphorylation of Ca channels; (c) actions mediated by Ca-dependent Ca channel dephosphorylation; or (d) indirect actions mediated by Ca effects on potassium channels that in turn influence membrane potential (Hess, 1988; Armstrong, 1989; Bean, 1989). Similar phenomena are implicated in regulation of the pumps and exchangers effecting active Ca extrusion (Carafoli, 1991). In addition, Ca efflux responds to cytosolic Ca concentration following simple mass action, since cytosolic Ca ions are, of course substrates for their own active extrusion. Fig. 10 shows results of some preliminary theoretical work which suggests that modest astrocyclic Ca fluxes could have a powerful influence on Ca concentrations in the narrow extracellular spaces of CNS synaptic neuropil. Since many aspects of neuronal function, including synaptic transmission, are highly dependent on extracellular Ca, regulation of Ca-ion fluxes at the astrocyclic plasma membrane provides another possible link between the astrocyclic Ca wave and neuronal function. Theoretical results regarding the dependence of synaptic transmitter release on perisynaptic astrocyclic Ca fluxes are presented in Fig. 11.4. These results, derived from the model outlined in Fig. 10, indicate that astrocyclic Ca fluxes could modulate synaptic transmission very strongly.

(4) Many neuroactive substances, including peptide neuromodulators, neurotransmitters, nitrogen oxides and lipid metabolites, have been shown to be synthesized or secreted by astrocytes (see Barres, 1991). Most of these agents are of types where synthesis or secretion is usually regulated by cytosolic Ca ions. Astrocytic Ca waves might therefore

RESULTS OF SIMULATED CHANGES IN SYNAPTIC CLEFT

A. One Glial Calcium Channel Opens 3 msec Before AP:

![Diagram]

Presynaptic Ca influx decreases by 3%

Fourth power of Ca influx decreases by 11%

B. Cleft in Annulus and Cuff Widenad from 15 nm to 25 nm:

![Diagram]

Presynaptic Ca influx increases by 24%

Fourth power of Ca influx increases by 236%

Fig. 11. Predictions of the magnitudes of simulated changes in astrocytic status on pre-synaptic Ca influx and neurotransmitter release. The model outlined in Fig. 10 is used and it is assumed that neurotransmitter release varies as the fourth power of Ca influx (see Augustine et al., 1987). A. Effect of opening one astrocyclic Ca channel prior to the pre-synaptic action potential. The open channel draws Ca ions from the synaptic cleft: within a few milliseconds, a new – lower – equilibrium cleft [Ca] value is established. This lowering of [Ca] reduces the influx of Ca into the nerve terminal if a pre-synaptic action potential should fire. This, in turn, will reduce the probability or quantity of neurotransmitter release – here by 11%. Such effects would summate for multiple astrocyclic Ca channel openings – and there may be as many as 100 astrocyclic Ca channels within the wrapping of a single synapse. Ca pumps or exchangers located in the astrocytic cleft membrane might also have large, but oppositely, effects on pre-synaptic terminal function. B. The effect of changing perisynaptic cleft width, as might result from a change in astrocyte volume. Widening the perisynaptic clefts increases the extracellular volume and therefore the size of the extracellular Ca reservoir in utilizable proximity to the active zone. The widened cleft also allows for easier diffusion of more distant Ca ions into the Ca sink created by the opening of pre-synaptic Ca channels. Both of these factors will promote larger pre-synaptic Ca influx per action potential and more neurotransmitter release. The figure shows that modest and plausible changes in cleft width can have quite enormous effects.

1.8 mM. Movements of Ca ions through the perisynaptic cleft geometry indicated in B were calculated from the diffusion equation with $d = 6 \times 10^{-6}$ cm$^2$/sec and the boundary condition that [Ca] was constant at 1.8 mM outside the specified astrocyclic cuff. The three traces in the lower panel show [Ca] at the three different locations specified by the inset drawing. Note that [Ca] within the active zone (and therefore Ca influx per open channel) declines by from 60% to nearly 90% within 100 $\mu$s during the action potential downstroke. As the channels close, [Ca] is promptly restored by diffusion from outside the astrocytic cuff. (In actual synaptic neuropil, the reservoir of fixed Ca concentration (our boundary condition) placed outside the astrocytic cuff is probably absent, so recovery may be a bit slower than in this simulation.) D. Spatial profiles of [Ca] at two different timepoints, 110 and 505 $\mu$s after action potential downstroke, from the same simulation as C. The minimum values of active zone [Ca] are reached at approximately the 110 $\mu$s timepoint. The ordinate axis used to generate this plot is shown in relation to the model geometry by the inset drawing.
govern neuronal function by regulating the release of neuroactive agents from astrocytes.

(5) Based on the general cell biology of Ca and ion transport and the cytoskeleton, cytosolic Ca ions are likely to be major players in shape change phenomena like those that have been demonstrated in cultured astrocytes (e.g., Olson et al., 1990; Cornell-Bell et al., 1990b; Kimelberg, 1991; O'Connor and Kimelberg, 1991). Since the extracellular volumes in brain are very, very small in comparison to cell volumes, it is to be expected that proportionally small changes in astrocytic shape could have proportionally very large effects on dimensions of the extracellular spaces. Changes in extracellular space, in turn, would then modulate any effects on neurons of extracellular current flow or extracellular ion accumulation. Ca-dependent changes in astrocyte structure could therefore allow the astrocytic Ca wave to modulate neuronal function by modulating the dimensions of narrow extracellular spaces. Some preliminary theoretical work suggesting that such effects could actually be quite substantial are presented in Fig. 11B.

(6) Astrocytes play a major role in the metabolism of neurotransmitter at CNS synapses (e.g., Hertz and Schousboe, 1986). There are substantial reasons to believe that Ca may regulate some of the astrocytic enzymes involved (Benjamin, 1987; Nicholls and Attwell, 1980). Moreover, the electrogenic transporters responsible for astrocytic uptake of the neurotransmitters GABA and glutamate are known to be sensitive to transmembrane potential, and thus subject to indirect Ca regulation via the Ca-dependent potassium channels. The astrocytic Ca wave therefore might regulate synaptic transmission through modulation of astrocytic neurotransmitter uptake or metabolism.

(7) Very exciting PET and MRI studies of human subjects have shown that cerebrovascular function responds rapidly and in intricate pattern to neural activity events of almost incredible specificity, down to the level of specific cognitions. The mechanisms of such vascular regulation are not well-characterized at present, but many investigators suspect that astrocytes play a major role in both vasomotor and capillary permeability components of cerebrovascular regulation (Bradbury, 1985; Janzer and Raff, 1987; Barres, 1991). One possible mode of astrocytic vasomotor regulation involves the production of nitrogen oxides (NO compounds). At least part of the astrocytic NO synthesis capacity is known to be Ca-dependent (Murphy et al., this volume). Vascular regulation may also be effected somehow at the specialized array of membrane proteins that has been described at the sites of contact between astrocytes and endothelial cells (Landis and Reese, 1981); there has been speculation that one component of these arrays is an astrocytic potassium channel. Potassium fluxes through such channels might be regulated directly by cytosolic Ca (if they are Ca-dependent potassium channels), or indirectly via Ca actions on other potassium channels which alter astrocytic membrane potential and therefore electrochemical gradient for potassium ion movement. Ca-dependent potassium fluxes across the astrocyte membrane could also govern blood vessels by influencing the potassium gradient across endothelial or smooth muscle membrane. Finally, it may be significant that astrocytic Ca is regulated by receptors for the vasoregulatory peptide endothelin. This signaling linkage may reflect operation of a feedback loop. It is certain that cerebrovascular regulation must ultimately feed back to influence neuronal activity, although the detailed mechanisms and physiological properties of such effects are not presently well known. It is not unlikely, however, that cerebrovascular effects on neuronal activity could be comparable in speed and potency to actions of the neuromodulatory transmitters. Cerebrovascular regulation is thus another possible link between the astrocytic Ca wave and neuronal activities.

(8) Astrocytes possess a major fraction of CNS carbohydrate energy stores, in the form of conspicuous glycogen granules distributed throughout their cytoplasm (Hertz and Schousboe, 1986). There are indications that this energy store is mobilized in response to neuronal activity, and that an astrocytic Ca signal might serve as the immediate trigger to such mobilization (Pearce et al., 1988). An astro-
cytic Ca wave initiated by neuronal activity is an excellent candidate to explain many of the known connections between neuronal activity and brain carbohydrate metabolism. *Neuronal function depends very directly on the availability of sugar substrates, so astrocytic carbohydrate metabolism provides yet another possible link from the astrocytic Ca wave to neuronal activity.*

It should be emphasized that the above is only a list of hypotheses. None of the hypothesized links between the astrocyte Ca wave and neuronal function have yet been documented by unequivocal physiological experimentation. On the other hand, all of these hypotheses seem both plausible and testable by practical means.

**Summary and discussion**

This chapter has attempted to build the case for a shift in our view of the astrocyte from that of a purely supportive cell toward that of a cell which participates actively and fully in the functions of neural information processing. This attempt was inspired by the recent experimental discovery that astrocytes may, after all, possess a form of excitability. The Ca excitability of astrocytes differs in many ways from the electrical excitability of neurons, but there are close formal analogies. I have argued that astrocytic Ca excitability may also be functionally analogous to neuronal electrical excitability—that is, it may enable networks of cells to encode and process information.

It is interesting to reflect on how the argument put forth here has depended just as much on new information about neuronal function as it has on new information about astrocytes. Not too long ago, it would have been assumed that all neuronal information processing would be explained by fast synaptic interactions occurring on the millisecond time scale. Then it was discovered that many signals between neurons were mediated by the class of second messenger mechanisms originally defined in studies of metabolic regulation. These signals express their effects over very slow time scales of seconds or even minutes—yet this is the class of signals that is now the basis for most of our known psychopharmacology, and it is the class of signals that presently absorbs much of our thinking about the mechanisms of learning and memory. It is the discovery of very slow signaling by neurons that has enabled us to think about possible information processing by the relatively slow astrocytic signaling system.

To summarize, we might characterize the past, the present and the (possible) future of thought about astrocytes in terms of a progression through three views as summarized in Table III. The “past” ideas about supporting roles are surely true, but definitely incomplete. The “present” idea acknowledges that evidence for astrocytic receptors and responses to neural activity strongly suggest that astrocytic support is more or less subtly regulated by neurons and their secretions. This idea now has very broad support among critical neurobiologists. The third idea is the possible future view for which this chapter has argued: that astrocytic responses to neuronal activity amount to a re-encoding of information, and that subsequent astrocytic signaling followed by feedback onto neuronal networks amounts to a processing and retransmission of that information. The transition from the second to the third of these ideas seems an awesome one, as it would undermine the so-called “Neuron Doctrine”. Most neurobiologists have long thought of this doctrine—stating that neurons are the brain’s sole information-processing elements—as the central dogma of their discipline. On the other hand, now that studies of neuromodulatory signaling have blurred the distinctions between information-bearing neuronal signals and metabolic regulation, it

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### Table III

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<th>Conceptions of the astrocyte role in brain function</th>
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begins to seem like a very slippery slope from "present" to "future".

It remains to be seen whether future investigation and our growing understanding of neural information processing will uphold the third, seemingly radical idea about the astrocyte. What is quite certain are the facts that a great deal remains to be learned about astrocytic function, and that our view of brain function will be greatly enriched as this information becomes available.

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335 – 340.


