Glial cells in the mammalian central nervous system come in a variety of types, shapes, and sizes. There are astroglia, ependymoglia, microglia, and oligodendroglia and at least some variation within each of these types. Oligodendroglia associate chiefly with the axonal portions of neurons as myelinating cells, although in the olfactory bulb there are myelinated dendrites. Microglia wander around the neurons, performing their phagocytic tasks and secreting bioactive agents as needed, not being particularly selective with which portions of neurons they are found. Ependymoglia line the walls of the ventricles and the spinal canal and contribute to the production of cerebrospinal fluid. Except for select groups of neurons that inhabit areas in and around the subependymal zones, the ependymoglia have little in the way of direct association with mature neurons. Most variable in type, most intimately associated with all parts of neurons, and thus most interesting functionally in their relationships with neurons are the astroglia, or astrocytes. For these reasons, this brief review will focus mainly on astrocyte-neuron interactions.

Textbooks that pay any attention at all to glial cells usually present them, at the end of a chapter on nerve cell types, as interesting structures that inhabit the spaces left unoccupied by the neurons. Further treatment of astrocytes in these sources usually includes discussions of glial morphology as viewed in preparations immunostained for the intermediate filament

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Astrocytes: A Closer Look

Conceptualization of astrocyte morphology from immunostains for cytoskeletal proteins such as GFAP can be grossly misleading, because such stains reveal so little of the cell’s structure. An elegant demonstration of just how little is revealed of the total volume of astrocytes by GFAP stains was recently published (4). Hippocampal astrocyte volumes were estimated from injection of a fluorescent dye that filled the entire cell. Immunostaining of the same cells for GFAP delineated only ~15% of the cells’ total volumes. Driven home by this finding is the idea that astrocytes occupy a far greater amount of the space between the neurons than would be projected by the commonly published light microscopic images of these glia. Knowing this, one can at least attempt to mentally fill in the other large percentage (perhaps as much as ~85%) when viewing such micrographs (Fig. 1). Bushong et al. (4) went on to show that the astrocytes in the CA1 region of the hippocampal formation have nonoverlapping domains, helping to correct yet another misconception, as it is easy to assume from GFAP images that there is considerable overlap in their occupation of interneuronal spaces.

In addition to intermediate filaments, astrocytes also express the microfilament actin, along with many actin-binding proteins (15). Actin bundles are found associated with the cytoskeleton and the plasma membrane. This most likely accounts, in large part, for the ability of astrocytes to extend and retract their processes in response to activation of receptors, such as β-adrenergic receptors, that influence the activity of adenyl cyclase and the accumulation of cAMP (see next section).

Astrocyte-Neuron Juxtapositions

An obvious reason why one finds astrocytes associated with all parts of neurons is that astrocytes are consistent inhabitants of synaptic regions, and virtually all parts of neurons are involved in synapses. Shown in Fig. 2 is an example of this relationship between synaptic boutons, dendritic spines, and astrocytes. Although this micrograph is from cerebellar cortex, it could as well have come from many other brain regions. It is clear that the astrocyte (blue) in close apposition with several of the neural structures...
would be exposed to many neurotransmitters/modulators that are released and escape from the synaptic cleft. Thus this glial cell stands to receive perhaps several different signals, depending on the nature of the synapses and the types of receptors expressed by the astrocyte. Abundant evidence exists that transmitters do indeed escape from the cleft in quantities sufficient to trigger responses in neighboring cells (6). Not only do astrocytes express transporters for uptake of these molecules, they also have receptors for many, if not all, of these transmitters, e.g., glutamate, GABA, acetylcholine, etc. Furthermore, many modulators, such as neuropeptides, are not even released into the synaptic cleft, but rather into the perisynaptic spaces (for review see Ref. 1). Dense-core vesicles, representing these modulators, are rarely seen around the active zones of synapses or synaptoid contacts (see Fig. 12). Because astrocytes can respond to neural signals with shape changes, it may well be that the relationship among the cells shown in Fig. 2 is only a snapshot in time and that a different picture might be seen after activation of certain of the synapses shown.

Illustrative of the shape changes produced in astrocytes by classical neurotransmitters are the micrographs in Fig. 3. Primary cultures of astrocytes maintained in standard culture medium display a flattened morphology, as seen in Fig. 3A. Treatment of such cultures for 30 min with 100 nM epinephrine produces the changes seen in Fig. 3B, in which the astrocytes retract from their former spread-out positions and become process bearing or stellate in shape. Epinephrine, by definition a β-adrenergic agonist, was most potent in inducing the response shown in Fig. 3. Norepinephrine, a mixed-adrenergic agonist, was only slightly less potent, and such responses are se-
lectively blocked by β-antagonists (11). β-Adrenergic receptors are linked to the activation of adenylyl cyclase and the accumulation of cAMP. It is this second-messenger pathway that is involved in astrocyte shape changes (3). Therefore, the astrocyte highlighted in Fig. 2 may change its relationship with any or all of the synapses upon release of a sufficient amount of a particular transmitter from one or more of the terminals. Data from both in vivo and in vitro studies support the idea that these morphological changes in astrocytes are all completely reversible.

MODEL SYSTEM ILLUSTRATES MORPHOLOGICAL PLASTICITY

To illustrate the myriad ways in which such astrocyte shape changes play a role in brain function, a brief review is in order of the model system in which astrocyte morphological plasticity was first described as part of a physiological process. More extensive recent reviews of this phenomenon are available for the interested reader (9, 17, 26). Figure 4 presents an artist’s three-dimensional rendition of a rat brain, cut away at the level of the hypothalamic supraoptic (SON) and paraventricular (PVN) nuclei, revealing the parvocellular PVN projections to the median eminence and the projections of the magnocellular neurons, via the pituitary stalk, to the neurohypophysis. It is the magnocellular hypothalamo-neurohypophysial system, in particular the supraoptic-neurohypophysial portion, in which the glial-neuronal plasticity and functional interactions were first discovered and have been extensively studied. A brief overview of this system follows.

Production of the hormones oxytocin (OT) and vasopressin (VP) is the responsibility of the two SON neuron populations. Released in response to physiological challenges such as dehydration, parturition, and suckling of the young, OT and VP have well known actions on peripheral tissues of the kidney, uterus, myoepithelial cells of the mammary gland, and certain vascular beds. Under basal conditions (i.e., in the absence of such physiological challenges), SON neurons are spontaneously active, although relatively quiescent. Physiological activation of the system leads
to the development of two distinct, largely intrinsically generated firing patterns that characterize the two cell types (for review see Ref. 2). As discussed below, astrocyte signaling appears to have roles in both the maintenance of quiescence under basal conditions and the production and maintenance of the activated states.

Neurons in the rat SON are large (15–25 μm in diameter) and densely packed compared with hypothalamic neurons in general. Also compared with that reference group, the SON displays a higher degree of organization, a factor contributing to its model system status. Depicted diagrammatically in Fig. 5 are some of the essential features of the SON involved in its plastic capabilities. Positioned immediately lateral to the optic chiasm/tract, the SON consists of somatic and dendritic zones and a row of astrocytes that makes up the ventral glial lamina (VGL). SON somata generally give rise to a dorsally projecting dendrite from which the parent axon usually arises (not shown) and one to three ventrally projecting dendrites. Because these latter dendrites turn to run rostrocaudally in parallel with one another, they are seen in cross section in this coronal plane (Fig. 5). Filled ovoid shapes representing the astrocyte nuclei are seen at the dorsal aspect of the VGL. As is the case in virtually all areas where brain parenchyma meets extraparenchymal tissue, there is a basal lamina (small arrows) separating the astrocytes of the VGL from the pia mater (open arrows). Under basal conditions, the VGL astrocytes project long processes dorsally throughout the dendritic zone (DZ) and the somatic zone (SZ), wrapping the dendrites and insinuating themselves between adjacent somata (see Figs. 7 and 9). Astrocytic membrane that is not involved in the dorsal projections fills the space between the DZ and the pial surface (Fig. 6A). Visualized with GFAP immunostaining, dorsally projecting processes from the VGL astrocytes are shown wending their way through the OT neuronal dendrites and somata (Fig. 6B). Unstained are the VP neurons that comprise the other 50% of SON nerve cells. Also unseen in this GFAP immunostain is the remaining 15% of each astrocyte (see Fig. 1), which fills the spaces that appear to be open between the neighboring dendrites or cell bodies.

Electron micrographs reveal the close relationships of the SON neurons to their associated astrocytes. Readily seen in Fig. 7A are the fine astrocytic processes that are interposed between these magnocellular neurons of a prepartum pregnant animal. Strikingly different is the picture one sees in the animal during lactation (Fig. 7B), in which large areas of neural membrane are closely apposed to (but not in contact with) other neural, rather than glial, membrane. Correlated with this glial withdrawal is an increase in the number of multiple synapses (i.e., one terminal forming synapses with two or more postsynaptic elements). An example of such a synapse from a lactating rat SON is shown in Fig. 8A, in which the terminal is making both an axodendritic and an axosomatic synapse. Terminals may also make multiple synapses exclusively with somata or with dendrites (Fig. 9B), and as many as seven postsynaptic elements have been observed being synaptically contacted by one terminal. That multiple synapses increase in number whereas single conventional synapses decrease, that there is no ingrowth of new terminals, and that the time course of multiple synapse formation has...

FIG. 5. Diagram of the SON and the pial-glial limitans in coronal plane. Profiles representing somata are lateral to the myelinated fibers of the optic tract (OT) in the somatic zone (SZ). Ventral to the SZ are the parallel-projecting dendrites (unfilled small circles), depicted in cross section, and constituting the dendritic zone (DZ). Mingling with only the most ventral dendrites are the astroglial cell nuclei (larger filled circles), whose ventrally projecting processes (shown in Fig. 6) fill the clear space between the basal lamina (small arrows) and the most ventral dendrites. These glial cell bodies and processes constitute the ventral glial lamina (VGL). Dorsally projecting processes from these glia fill most of the space not occupied by the somata and dendrites. Ventrolaterally projecting dendrites are not included here. Pia mater is indicated by open arrows.
been observed to be too rapid for axonal sprouting to occur have forced the interpretation that multiple synapses in this system arise from single ones. How this might happen is easily imagined from the situation shown in Fig. 8B, where a conventional synapse is evident with the soma on the left (arrowhead). Retraction of the fine astrocytic process (arrow) would allow a second synapse to be made with the soma on the right.

Another plastic change that accompanies activation of this system is an increase in gap junctional intercellular communication among neurons, as inferred from dye and tracer coupling studies as well as from in situ hybridization for connexin protein mRNA (12, 16). Such coupling among SON neurons is dendrodendritic and is observed exclusively between cells expressing the same peptide (i.e., either OT or VP). Because dendrites that are wrapped by glia and isolated from one another the way they are under basal conditions (e.g., Fig. 9A) could not readily form gap junctions, the glial retraction must play at least a permissive role.

FUNCTIONAL SIGNIFICANCE OF MORPHOLOGICAL PLASTICITY

Among the functional implications of these altered glial-neuronal relationships are the following possibilities. 1) There is reduced uptake and spatial buffering
of $K^+$, allowing higher concentrations of $K^+$ to be achieved in the extracellular space. This would lead to enhanced neuronal activity. 2) Glial uptake of glutamate from perisynaptic zones would be reduced, with the consequence that this excitatory transmitter would have prolonged action and might reach different sites than when the glia are interposed (22). 3) Enhanced multiple synapse and gap-junctional communication establishes some basis for the synchro-

FIG. 7.
Electron micrographs of SON neurons illustrating the changes in somasomatic direct apposition that occur with activation of this system. A: day 1 postpartum; small direct apposition (open arrow). B: lactating animal; large areas of apposition (filled arrows). Scale bar, 2 $\mu$m.

FIG. 8.
Electron micrographs of SON neurons showing types of synapses common in lactating but rare in virgin rats. A: axodendritic and axosomatic multiple synapse. B: axon terminal making synaptic contact with one soma and separated from another only by a thin astrocytic process. Scale bar, 1 $\mu$m.
nous firing of thousands of OT neurons that precedes
the milk ejection reflex.

Weaning of pups terminates lactation and returns the
SON to its prelactation morphology. Similar changes
are induced by dehydration produced by either water
deprivation or saline drinking, and these, too, are
reversible upon rehydration. Dehydration-induced
morphological plasticity in the DZ is illustrated in Fig.
9. In the well hydrated rat SON, the dendrites are
mostly separated from one another and are even
wrapped by processes of nearby astrocytes (Fig. 9A).
In contrast, the dendrites of a dehydrated animal form
bundles (i.e., direct appositions with no intervening
glial processes) upon the retraction of the astrocytic
processes from their previous interdendrite positions
(Fig. 9B). As in the case of the morphological plastic-
ity in vitro (shown in Fig. 3), changes in the dendritic
zone in situ have been observed after 20–30 min of
osmotic stimulation (27). Plastic changes of the type
shown here, occurring in response to physiological
stimulation, also have implications for the fate of the
OT and VP that are released from the dendrites (25,
21). Very likely, dendritic release of peptide would be
tonically inhibited under basal conditions (see be-
low), but any peptide that was released would imme-
diately be in contact with astrocyte membrane (Fig.
9A). In contrast, peptide released from dendrites un-
der the stimulated conditions (Fig. 9B) would have
immediate access to the membrane of the neighbor-
ing dendrites. Because OT and VP have been found to
have receptor-mediated actions on these neurons (5,
13, 20), the glial presence or absence is likely to be of
some functional significance.

SON astrocytes contain the amino acid taurine (7). In
the example shown in Fig. 10, the taurine-containing

![Fig. 9](image9.jpg)

**Fig. 9.** Electron micrographs of SON dendritic zones of animals under basal (A) and activated (B) conditions. In A, dendrites tend to be separated from one another and wrapped by glial processes (arrows). In B, dendritic membrane tends to be in apposition with that of other dendrites, forming dendritic bundles. Those dendrites with similar numbers are in bundles. A multiple synapse is indicated (arrows). As, astrocyte nucleus. Scale bar, 2 μm.

![Fig. 10](image10.jpg)

**Fig. 10.** Electron micrograph of a taurine-immunoreactive astrocyte completely surrounding an axon (ax) and its bouton (b) making contact with a supraoptic dendrite (D). Visible in this astrocyte are glial filaments (gf). GSSP, gold-substituted silver periodate. Scale bar, 290 nm.
astrocyte is engulfing a portion of a SON dendrite and a presynaptic bouton. Release of taurine from SON astrocytes (8) occurs in the well hydrated animal and has an inhibitory effect on the firing of VP neurons, in particular via glycine receptor activation (14).strychnine blockade of these glycine receptors in a water-loaded rat results in increased firing of VP neurons. Acute dehydration reduces taurine release, and retraction of the glial processes accompanies chronic dehydration, thereby further removing possible inhibitory influences on the activation of the system in response to the physiological challenge.

PLASTICITY IN THE NEUROHYPOPHYSIS

Axons from the magnocellular neurons terminate in the neurohypophysis (Fig. 4), where there is a population of pituitary astrocytes that display the same type and degree of plasticity as those in the SON. These astrocytes also contain large amounts of taurine, which they release under normal to low osmotic conditions (18). Plasticity and signaling phenomena in the neurohypophysis parallel those in the SON. Under basal conditions, the flattened, spread-out astrocytes (comparable to those in Fig. 3A) engulf the neurosecretory terminals and tend to occupy a large proportion of the basal lamina, through which secreted peptides must pass to enter the fenestrated capillaries leading to the general circulation (Fig. 11A). With activation of the system, in this case parturition, the pituitary astrocytes quickly become process bearing (comparable to those in Fig. 3B), release the engulfed terminals, and retract from their positions along the basal lamina (Fig. 11B). Neurosecretory terminals are then found to abut most of the basal lamina along the perivascular space. Note the terminals devoid of dense-core vesicles (arrowheads) in Fig. 11B. Neurohypophysial astrocytes remain retracted throughout lactation. Studies of dehydration-induced plastic responses in these astrocytes have revealed that the terminals abutting the basal lamina enlarge, whereas those associated with the glia become smaller. Accompanying these plastic changes are decreases in expression of certain extracellular matrix proteins, presumably playing a permissive role in allowing this reorganization of cellular processes (19).

Of special interest in this bidirectional signaling story are the synaptoid contacts that occur in the neurohy-
pophysis between neurosecretory terminals, containing both clear microvesicles and dense core vesicles, and the astrocytes (Fig. 12). Synaptoid contacts were reported long ago to increase in frequency with dehydration (28), suggesting that they were involved in the activation of this system. Such contacts are called synaptoid rather than synaptic, because there is only a presynaptic specialization and not a postsynaptic one as there is in a true synapse. Nonetheless, the juxtaposition is obviously favorable for two-way signaling between the cells. Release of taurine from the pituitary astrocyte can provide appropriate signals to the nerve terminal, and release of peptide from the terminal can influence the glial cell. For instance, VP released from the neurosecretory granules is likely to trigger a sizeable intracellular calcium rise, as has been shown in cultured pituitary astrocytes, mediated by a V₁ receptor (10). As discussed below, rises in intracellular free calcium in astrocytes can lead to release of neuroactive substances, such as glutamate, which in this case could enhance secretion of peptide. Interruption of this apparently positive feedback relationship would come while the intracellular calcium stores in the astrocyte reloaded.

DIRECT ASTROGLIAL-NEURONAL SIGNALING

Because astrocytes have receptors for many neurotransmitters and neuromodulators, neuronal-glial signaling is well established. New and accumulating evidence that astrocytes engage in receptor-mediated release of neuroeffectormolecules establishes the glial-neuronal signaling pathway. In addition to the astrocytic release of the inhibitory amino acid taurine mentioned earlier, astrocytes have now been shown to release glutamate in response to a variety of signaling molecules that induce significant rises in astrocyte intracellular calcium (e.g., VP, bradykinin, ATP). HPLC was used to show that the bradykinin-induced calcium rise in astrocytes results in glutamate release (Fig. 13A). Data from a variety of studies designed to

**FIG. 12.** High-power electron micrograph of a neurosecretory axon containing both dense-core vesicles and clear microvesicles, making a synaptoid contact with a pituitary astrocyte (P). Scale bar, 224 nm.

**FIG. 13.**

A: bradykinin (BK; 10 nM) releases measurable quantities of glutamate from astrocytes, as measured by HPLC. B: cultures of forebrain astrocytes loaded with the fluorescent indicator fura 2 and ratio imaged for calcium response to bradykinin (10 nM) stimulation. Ordinate, ratio responses to two excitation wavelengths, 340 and 380 nm. Yellow-red pseudocolor =1 μM [Ca²⁺].

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eliminate other possibilities (e.g., release via reversed glutamate transporter) suggest that this release is exocytotic and calcium dependent. In earlier studies, calcium rises in astrocytes were evoked using methods that left open the question of what mechanism actually led to release. For example, either mechanical stimulation or application of certain neuropeptides, such as bradykinin, could evoke large calcium responses in cultured astrocytes (Fig. 13B). More recent experiments have established that a rise in internal free calcium from a resting level of \( \sim 85 \) to \( \geq 140 \) nm is sufficient to release glutamate. Data from one illustrative series of experiments (23) are presented below and were kindly provided by Dr. V. Parpura, University of California, Riverside.

To establish the intracellular calcium rise itself as sufficient to release glutamate, the elegant experiment depicted in Fig. 14 was performed. Single neurons were grown in culture on beds of several astrocytes (as in Fig. 13B). Both the neuron and the astrocytes were loaded with the fluorescent free-calcium indicator fluo-3 and the caged calcium compound NP-EGTA (NPE). A patch electrode was then used in whole-cell configuration to dialyze out the loaded compound from the neuron, after which a pulse of UV light photolyzes some of the caged calcium in the astrocytes. Simultaneous monitoring of the fluorescence generated by the calcium rise in the glia and the inward current flow across the membrane of the neuron is shown in Fig. 15. Two successive pulses of UV light produce increments in the levels of intracellular calcium and in the amplitude of inward current. Demonstration that this inward current was generated by activation of glutamate receptors on the neuron was shown in a parallel experiment (Fig. 16). Astrocyte fluorescence (Fig. 16A, top trace) and inward current in the associated neuron (Fig. 16A, bottom trace) followed a pulse of UV light, as before. In the presence of the caged calcium (NP-EGTA) in the glia and the absence of the \( N \)-methyl-D-aspartate (NMDA) and non-NMDA antagonists \( \omega \)-2-amino-5-phosphonopentanoic acid (\( \omega \)-AP5) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), these responses were robust (Fig. 16B, left bars). When these antagonists were added to the medium, little or no inward current was observed despite a still substantial calcium rise in the glia (middle bars). In the absence of both the antagonists and the caged compound, neither response was appreciable (right bars).

**CONCLUSIONS**

Taken together, these data strongly indicate that receptor-mediated intracellular calcium increases are capable of inducing glutamate release from astrocytes in sufficient quantities to generate sizeable currents in
neighboring neurons. There are, of course, clear implications in this glial-neuronal signaling for synaptic transmission and neuromodulation, even away from the synapse. That the astrocytic processes are mobile and able to be retracted, also in response to receptor activation, adds yet another probable level of complexity to this phenomenon. Although there is much work yet to be done in this exciting area, it seems that the two-way communication between astroglia and neurons is firmly established.

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