Activity-Dependent Structural and Functional Plasticity of Astrocyte-Neuron Interactions

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I. INTRODUCTION

Neurons and glia are not independent cellular elements of the nervous system but two strongly interconnected components. Intensive research in the past decade has revealed new, important roles for the latter. Probably the least expected finding has been that information processing is not an exclusive property of neurons but that it
is shared by astrocytes, the most abundant glial cells in the central nervous system (CNS). Astrocytes thus participate in a number of interactions that are central to the development, function, and repair of the CNS. The wealth of new observations has resulted in a decided movement away from the prevalent neuron-centric vision of nervous system function. Without assuming an entirely gliocentric vision, it is obvious that astrocytes, in addition to their more ancillary functions of structural and nutritional support, intervene in the regulation of synaptic function and are active partners in information processing, a topic much reviewed recently (see, for example, Refs. 76, 125, 200).

Work over the past 25 years has rendered yet another dogma about the adult CNS obsolete. Ramon y Cajal (154) had concluded that its structure remains essentially stable once it reaches full development, changing only in response to increasing age and degeneration. Numerous observations have shown that this is not the case. Thus careful electron microscopic analyses of different neuronal systems described a surprising capacity of neurons and their synaptic connections to undergo significant morphological alterations. Today, direct fluorescent imaging approaches, especially on live tissues, confirm and extend many of these earlier findings. We can no longer ignore, therefore, that neurons can change their form throughout life, during a variety of physiological conditions that modify neuronal activity. Much attention has been given to the incredible turnover of dendritic spines, and presumably of excitatory synapses, under different conditions (reviewed in Ref. 2). However, it is not necessary to limit oneself to dendritic spine remodeling to illustrate the brain’s remarkable capacity for structural change. Examples of axonal and synaptic transformations under different physiological conditions are abundant (8, 32, 57, 184, 201). The shape and size of somata can change as well (8, 184), while large-scale structural changes in major dendritic branches have been documented in many systems (153, 168, 199).

The same methodologies have revealed that it is not only neurons that can undergo remodeling but glial cells as well. In particular, it is clear that astrocytes, a major class of glia in the vertebrate CNS, are remarkably dynamic. Structural changes can be detected not only at the level of their somata and large processes but most importantly for neuronal function, at the level of their fine, lamellate distal processes that ensheathe neuronal elements, including synapses. This review focuses on this latter form of astrocytic-neuronal plasticity. We shall show that it is experience-dependent, occurring during conditions as different as reproductive status, enriched sensory input, learning, and memory. We then describe how such morphological changes modify neuronal function. Finally, we discuss molecular and cellular mechanisms that may help us to understand how this kind of plasticity occurs in an organ that we thought overly endowed with enough cells and circuits to ensure its complex activities.

II. ASTROCYTE-NEURON INTERACTIONS DURING BASAL NEURONAL ACTIVITY

A. Distal Astrocytic Processes Ensheath Neurons and Their Synapses

Astrocytes are numerous in the vertebrate CNS, and their number is enhanced considerably with phylogeny and increasing brain complexity. For example, in the nervous system of lower species like Caenorhabditis elegans, neurons outnumber glia by 6:1, while in the mouse or rat cortex, the ratio of astrocytes to neurons is 1:3. In the human cortex, there are 1.4 astrocytes per neuron (125). They were the original neuroglia visualized by Ramon y Cajal, after examination of neocortical sections treated with a gold sublimate stain (154) (Fig. 1A). The stain targeted intermediate filaments, the most prominent cytoplastic feature of these cells, that we know now consist mainly of glial fibrillary acidic protein (GFAP). Recent work from various sources has confirmed and extended Ramon y Cajal’s observations.

The most common astrocytes in the gray matter, called stellate or protoplasmic (in contrast to fibrous astrocytes in white matter), have an intricate, complex morphology, consisting of a relatively small cell body with several thick processes extending into the neuronal network from which arise numerous fine processes (Fig. 1, A–D). The latter are generally 30–40 μm in length and appear as thin, veil-like lamellae within the neuropile (Figs. 1 and 2). Ramon y Cajal (154) had noted the complexity of these processes and appreciated their close relationship to neuronal and vascular elements (Fig. 1A). As seen with electron microscopy, these leafletlike processes ensheathe all neuronal somata, dendrites, and synapses (Fig. 2, A and C). However, the extent of this ensheathment varies in different systems, indicating that there are specializations of astrocytic-neuronal interactions at the local level, even under normal conditions. For example, in the neocortex, astrocytic processes only partially cover pre- and postsynaptic elements of axodendritic glutamatergic synapses (195), while in the cerebellar cortex, Bergmann glia processes almost totally encapsulate synapses between parallel fibers and Purkinje cell spines (65). In the rat visual cortex, layer I synapses have the least and layers II/III the most extensive astrocytic coverage (90).

The structural complexity of astrocytes also increases with phylogeny so that the human cortex contains the largest and most elaborate astrocytes (134) (Fig. 1, C and D). In addition, a given population of astrocytes in
any one brain region can be quite heterogeneous (48). In the supraoptic nucleus (SON) of the rodent hypothalamus in which they have been extensively analyzed, there are at least two types, with distinct morphologies and electrophysiological properties (17, 87). In this nucleus, in addition to classic protoplasmic astrocytes (Figs. 1B and 2A), there is a radial type that resembles interlaminal astrocytes in the adult primate cortex and polarized human cortical astrocytes (134).

While GFAP immunolabeling is still generally considered a reliable means to identify astrocytes, it should be interpreted with caution since it does not do full justice to the complex morphology of the cells. Indeed, under normal conditions, astrocytes may express no GFAP or so little that it remains undetectable with immunocytochemistry. When GFAP immunoreactivity is rendered visible, it is found in cell bodies and thick processes. This is illustrated clearly by reactive astrocytes that appear in many neuropathologies and for reasons that we still ignore, express high levels of GFAP (Fig. 1E), together with vimentin, another intermediate filament protein (157). In contrast, distal astrocytic processes contain little cytoplasm and lack most intracellular organelles, including intermediate filaments and therefore GFAP. Their simple morphology is illustrated well with electron microscopy (Fig. 2, A and C) (65, 103, 185), immunocytochemical labeling for intracellular proteins (Fig. 2, B–E) (39, 135), or live imaging of cells filled completely with different fluorescent markers (Fig. 1, B–D) (13, 24, 125). Because of the profusion of these distal processes, we should consider the overall morphology of astrocytes as polyhedral, rather than starlike.

Ramon y Cajal (154) had noted that there was very little overlap between the distal processes of two adjacent cells and suggested that individual glial cells delineate distinct domains within the neuropile. Indeed, intracellular filling of cells with different fluorescent markers, in the hippocampus (134) and cortex (135) for example, revealed that astrocytes occupy highly segregated domains, an anatomical configuration that is not evident when the cells are labeled for GFAP (Fig. 1E). Moreover, this kind of cell labeling established that astrocytic processes rarely extend beyond a 50-µm radius and that there is only limited overlap in the peripheral portions of neighboring cells (Fig. 1D) (69, 134, 135). A recent study (69) estimated that a single astrocyte enwraps 4–8 neuronal somata and 300–600 dendrites and creates a kind of synaptic island defined by its ensheathing processes. It is obvious, then, that the classical image of an astrocytic syncytium in a large area of nervous tissue has to be replaced by one of more restrained, independent glial microdomains. Functionally, this is of primary impor-
distance, since it means that astrocytes are capable of interacting with neurons in a restricted microenvironment.

**B. Astrocytes and the Extracellular Space**

The fine distal processes of astrocytes are interposed between all neuronal elements (Figs. 1A and 2; see also Fig. 8). By their mere presence, then, they can be considered a physical barrier to restrict spillover and diffusion of locally released, potentially active molecules into the extracellular space (ECS). Moreover, by their position, they contribute to the regulation of the microenvironment in which neurons develop and function, maintaining a tight control on local ion and pH homeostasis, delivering glucose, providing metabolic substrates, and clearing away metabolic waste. For example, the concentration of extracellular K⁺ needs to be tightly regulated since its accumulation in the ECS can alter neuronal excitability dramatically. Astrocytes take up this ion via the glial isoform of the Na⁺/H⁺/K⁺/H⁺ pump or through specific ion channels, like inward-rectifying K⁺ channels (79, 129). They then transfer it from sites of accumulation to areas with lower concentrations, to finally extrude it in the ECS and/or into the circulation, a kind of K⁺ spatial buffering that may depend on gap junction coupling (197). Another example is the regulation of extracellular levels of Na⁺, particularly important in sensory circumventricular organs like the subfornical organ and the organum vasculosum of the lamina terminalis, brain loci involved in the control of salt/water intake (164, 204). These structures contain brain Na⁺ sensors, the Na⁺/x sodium channels that accumulate on perineuronal astrocytic processes. In these areas, then, astrocytes play an important role in sensing the level of Na⁺ (164). As will be discussed in detail in the next section, astrocytes, by expressing different transporters (Fig. 2, B and C), are also involved in the clearance of synthetically released neurotransmitters, like glutamate and GABA. Taken together, such passive and active properties of astrocytes serve to limit interneuronal communication mediated by volume transmission and prevent spillover of transmitters originating in synapses, thus preserving point-to-point neuronal communication (171).

In neurohemal structures like the neurohypophysis and external layer of the median eminence, processes of astrocytic-like cells (pituicytes and tanycytes, respectively) enclose neurosecretory axons (193). They thus limit potential paracrine and/or autocrine actions of secreted peptides. In addition, these glial processes abut on perivascular spaces, thereby physically restricting access of neurosecretory terminals to the perivascular basal lamina (73, 176) (Fig. 3). Acting as a kind of physical barrier, then, astrocytic processes affect neurosecretion, since their presence limits diffusion of neurohormones released.
confined to the immature CNS, where they contribute to the dynamic cell interactions characterizing its development, that of molecules like PSA-NCAM and the tenascins persists in adult neuronal systems endowed with the capacity for plasticity (15, 89, 98, 180). Notable examples are the hypothalamoneurohypophysial system (HNS) (Fig. 4), the hippocampus, and the olfactory bulb.

C. Neuron to Astrocyte Signaling

Astrocytes can sense neuronal activity through a rich panoply of neurotransmitter receptors and ion channels that accumulate in their fine processes (see Refs. 7, 196). This is of fundamental importance and has been conserved evolutionarily in both invertebrate and vertebrate glia. Astrocytes respond to synaptically released neurotransmitters and other signaling substances with transient elevations of cytosolic Ca^{2+} (196). These Ca^{2+} transients, resulting from release of Ca^{2+} from internal stores, are induced by activation of metabotropic receptors. Initially described in astrocytes in culture, they have now been detected in vivo. For example, robust increases in cytosolic Ca^{2+} were recorded in the barrel cortex in response to repetitive whisker stimulation (203) and in the somatosensory cortex after sensory stimulation from the hindlimb (207). The responses appeared restricted to individual astrocytes. Increased extracellular levels of K^+ can also induce Ca^{2+} transients in astrocytes (71). Such signaling may alter gene expression and initiate morphological changes. It is at the basis of the astrocytic contribution to the maintenance of local microvascular tone as well (76). On the other hand, there has been a developing consensus that these Ca^{2+} elevations induce astrocytes themselves to release glutamate, which then activates adjacent neurons via pre- and postsynaptic receptor activation (reviewed in Refs. 76, 200). Nevertheless, the validity of the latter mechanism to explain rapid astrocyte-neuron interactions has been questioned, since Ca^{2+} elevations in hippocampal astrocytes did not induce any significant rises in cytosolic Ca^{2+} in adjacent pyramidal neurons nor did they affect their electrical activity (50).

Cytosolic Ca^{2+} elevations can develop into Ca^{2+} oscillations, or repetitive increases of Ca^{2+} within single cells (see Ref. 134). Modulation of the Ca^{2+} signal occurs at discrete regions of distal processes and controls intracellular propagation of the signal (143). Since one astrocyte covers many synapses (69), this would be a means to ensure that not all are affected in the same way by Ca^{2+} propagation. Cytosolic Ca^{2+} elevations may also be transformed into Ca^{2+} waves that propagate radially from one single cell to neighboring cells, thus transferring the glial signal at a distance from its site of origin. The principal mechanism of wave propagation appears to be release of ATP from one cell which then acts as a diffusible extra-

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**Fig. 3.** Astrocytic processes in neurohemal structures. As seen with electron microscopy, in the neurohypophysis under basal conditions of neurosecretion, fine processes of astrocytic-like cells, the pituicytes (pit.) ensheath neurosecretory axons and terminals (ter.) and contact —60% of the perivascular area (arrows); —40% is covered by neurosecretory terminals. This proportion is reversed after stimulation of neurosecretion which results in a retraction of pituicyte processes from the basal lamina. [Adapted from Theodosis and MacVicar (176) and Theodosios and Poullain (184).]
cellular messenger to activate neighboring cells (reviewed in Refs. 76, 125). Another mechanism is direct communication through gap junctions, clearly seen in cultured astrocytes from the striatum (61, 82) and cortex (196). While intercellular propagation of Ca\(^{2+}\) waves may explain communication between distant cells (31), the validity of this mechanism as a normal means of intercellular signaling is uncertain. It has been observed only after intense electrical stimulation, and the phenomenon shares many properties with spreading depression. In any case, both modalities of astrocytic signaling can affect surrounding neurons, which respond by prolonged increases in cytosolic Ca\(^{2+}\) (see Ref. 125).

Distal astrocytic processes are also enriched in transporters that assure rapid and efficient removal of synaptic neurotransmitters, in particular glutamate (Fig. 2, B and C). At very high concentrations, this excitatory amino acid can be highly toxic to neurons. Under normal circumstances, astrocytes quickly remove glutamate at synaptic sites and thus contribute actively to the regulation of synaptic transmission, a phenomenon that has led us to change dramatically our thinking about neurotransmission. Today we speak of the “tripartite” synapse in which information flows not only between the traditional pre- and postsynaptic neuronal partners but, in addition, between these elements and adjacent astrocytic processes (Fig. 5). This topic has gained ever-increasing attention and is the subject of several reviews (for example, Ref. 7). For our purposes, we need to keep in mind that if glutamate is not removed by astrocytic processes,
it will diffuse in the ECS. It can then interact with presynaptic metabotropic glutamate receptors on those very excitatory synapses from which it was released, thus inducing a feedback inhibition of its own release (138). In addition, the amino acid can diffuse to neighboring synapses and, via similar presynaptic mechanisms, influence the activity of other synapses, thereby contributing to intersynaptic cross-talk (139, 148) (see Fig. 8).

D. Gliotransmission

Astrocytes themselves release several neuroactive molecules, including glutamate, d-serine, ATP, taurine, and cytokines like tumor necrosis factor (TNF)-α.

1. Glutamate

As noted above, a rise in cytosolic Ca\(^{2+}\) is necessary and sufficient to induce glutamate release from astrocytes (7, 94), which then activates glutamate receptors located pre- or postsynaptically (Fig. 5). Thus activation of postsynaptic NMDA receptors produces a slow transient current that may contribute to network synchronization (5, 49) while activation of presynaptic NMDA receptors facilitates subsequent glutamate release, thereby favoring neurotransmission (92). On the other hand, interaction with metabotropic presynaptic receptors will inhibit neurotransmitter release (138).

There is increasing evidence that glutamate release from astrocytes occurs via regulated exocytosis (6, 92, 142). The cells express protein components of the vesicular secretory apparatus, and gliotransmitter-mediated actions are compromised when the exocytotic machinery is impaired with specific neurotoxins. Vesicles that could contain the amino acid prior to regulated exocytosis have been visualized in these cells, including within distal processes next to synapses (119). Nevertheless, other mechanisms of release are possible, such as diffusion through ionotropic purinergic receptors or unpaired connexons (hemichannels) on the cell surface, through volume-activated chloride channels opened in response to swelling, reversal of uptake by glutamate transporters, or via exchange of the amino acid by the cystine-glutamate antiporter (reviewed in Ref. 123). In any case, while a vesicular release mechanism may be of primary importance, one or other of these latter mechanisms can exist in parallel, especially under pathological conditions (see Ref. 125).

2. d-Serine

In some brain areas, astrocytes synthesize and release the amino acid d-serine (121, 141), considered an important intermediary in glutamate neurotransmission since it binds to the strychnine-insensitive glycine site and serves as an endogenous coagonist of NMDA receptors (see Fig. 9). Thus, in the hypothalamus (Fig. 2, D and E) (141), hippocampus (165, 211), and retina (169), NMDA receptor activity requires d-serine rather than glycine, usually considered the coagonist of these receptors. Through their release of d-serine, therefore, astrocytes have become key elements in all physiological and pathological processes implicating NMDA receptors, from synaptic plasticity (211) to excitotoxicity (165). Like glutamate, there is growing evidence that this amino acid accumulates in vesicles (M. Martineau, J. Puyal, R. Jahn, D. Theodosis, and J. Mothet, unpublished observations), presumably to be released by regulated exocytosis (121).

3. ATP and adenosine

Neurons have been considered the sole source of the neuromodulators ATP and adenosine in the CNS, but increasing evidence demonstrates that they derive from astrocytes as well. Astrocytic ATP is presently considered...
III. ASTROCYTE-NEURON INTERACTIONS ARE MODIFIED BY NEURONAL ACTIVITY

A. Mobility of Astrocytic Processes

The relationship of astrocytes to their neighboring neurons is not a stable one, and there is presently ample evidence demonstrating that astrocytes constantly change their morphology. First, GFAP immunolabeling shows gross modifications in certain systems, even under normal conditions, changes that are similar to those characterizing activated astrocytes under neuropathological conditions (157). They result in significantly altered expression of GFAP together with remodeling of somata and thick processes. They have been noted in hypothalamic areas like the SON (14), suprachiasmatic nucleus (SCN) (12, 60, 108, 109), arcuate (55), and preoptic (59, 88) nuclei in response to dehydration, circadian rhythmicity, and fluctuating sexual steroid levels, respectively. Such transformations also occur in the CA1 region of the hippocampus (101) and visual cortex (91), during conditions of enriched sensory input. In the visual cortex, for example, within 4 days exposure to an enriched environment, concomitant to dendritic growth, there is a significant increase in the surface density of GFAP immunopositive processes (91).

Whether gross changes in astrocytic morphology like those detected with GFAP immunolabeling are of consequence to neuronal activity is presently unknown. What is more certain is that the distal, lamellate processes of astrocytes, devoid of GFAP, are remarkably mobile, and this glial restructuring does have important direct effects on neuronal function. As will be discussed in detail below, accumulating evidence makes it clear that remodeling of distal astrocytic processes is closely linked to neuronal activity and often occurs in concert with morphological changes in neighboring neurons and synaptic inputs, a kind of astrocytic-neuronal plasticity that highlights the brain’s remarkable capacity for activity-dependent restructuring. In the hypothalamus, such remodeling results in reduced astrocytic coverage of neuronal elements, including synapses; in the hippocampus and cerebral cortex, it gives rise to a greater astrocytic volume associated with an enhanced number of synapses.

1. Hypothalamoneurohypophysial system

It is now over two decades that electron microscopy coupled to morphometric analyses of this neuroendocrine system (74, 181, 185, 192) provided the first clear evidence that astrocytes are surprisingly dynamic, continually changing their morphology in relation to the activity of neighboring neurons.

The hypothalamoneurohypophysial system (HNS) is composed of magnocellular neurons that secrete either...
OT or VP and that accumulate in well-defined regions of the hypothalamus, the paired SON and paraventricular (PVN) nuclei. Their axons project, via the internal layer of the median eminence, to the neurohypophysis where the neuropeptides are released directly into the bloodstream. As a neurohormone, OT intervenes in vital functions like parturition and lactation while VP is essential to osmotic and cardiovascular regulation. Both peptides are released centrally as well, in different regions, where they participate in various neurovegetative and limbic functions (reviewed in Ref. 105). Within the magnocellular nuclei, they are released mainly by a somatodendritic exocytotic mechanism (120) and ultimately facilitate the activity of their own neurons (156). OT and VP neurons respond to afferent stimulation by increased electrophysiological and secretory activities, with patterns specific to each type of neuron (150). At the same time, as shown by ultrastructural analyses of profiles immunoidentified as oxytocinergic or vasopressinergic, OT neurons undergo striking morphological changes. Their somata progressively hypertrophy (reviewed in Ref. 172), their dendrites change in size and branching (168), and their axons enlarge and ramify (73, 115).

In the hypothalamus, OT neurons usually occur in tightly packed clusters, but under basal conditions of neurosecretion, they remain separated by fine astrocytic processes (Figs. 2A and 6). In contrast, during parturition, lactation, chronic dehydration, or in response to elevated ambient levels of OT, there is a significant reduction in the astrocytic coverage of all portions of OT neurons (reviewed in Ref. 172). In the rat SON, for example, astrocytic processes cover ~90% of any OT soma under normal conditions, a proportion that is reduced to 70% during lactation or chronic dehydration (26, 174). At the same time, their surfaces, as that of their dendrites, become directly and extensively juxtaposed (Fig. 6). Where this occurs, neuronal membranes do not contact each other, and the extent of the ECS remains unaltered (Fig. 6, bottom image). Gap junctions are not visible between juxtaposed neuronal membranes, but they are detected between astrocytic profiles (106, 185). Astrocytic coverage of synapses contacting OT neurons is also significantly reduced (138). This plasticity, which has been analyzed in the rat (for a review, see Ref. 172), mouse (187), and several desert rodents (Ouali and Theodosis, unpublished observations), takes place throughout the hypothalamus, in all the nuclei containing OT neurons. In contrast, in response to stimulation for VP secretion, including severe chronic salt loading (26), astrocytic coverage of VP neurons is not altered and remains at ~90%. While VP neurons display some juxtapositions, their incidence is low, they are small, and more importantly, they show no variation with changing conditions of VP secretion (26, 174).
Concomitant glial transformations in the neurohypophysis result in a reduced glial ensheathment of the axons and terminals of these neurons (reviewed in Refs. 72, 176). At perivascular areas, this leads to a significantly enlarged neurovascular contact zone (Fig. 3). Thus, under basal conditions, ~40% of the perivascular basal lamina is contacted by neurosecretory terminals and ~60% by processes of astrocytic-like pituicytes. During all conditions that stimulate neurosecretion, there is retraction of pituicyte processes from the perivascular zone as well as proliferation of neurosecretory terminals, and these proportions are reversed (Fig. 3) (73, 115).

In vivo analyses had strongly suggested that this form of glial-neuronal transformation is rapid, since changes were detected within a few hours of the onset of parturition (117) or osmotic stimulation (192). In vitro analyses in acute slices of adult hypothalamus that include the SON (106, 175), or in hemineurohypophyses (115, 145), show clearly that such glial transformations occur very rapidly indeed, within 1 h of stimulated neurosecretion. When OT secretion returns to baseline levels (for example, after weaning the young or reduction of ambient OT), astrocytic processes reappear between neuronal profiles, and the structure of the magnocellular nuclei and neurohypophysis assume their unstimulated morphologies. This phenomenon can take a few hours as well (106, 115).

Whether juxtaposed neuronal surfaces are due only to retraction of astrocytic processes is not established. What is highly unlikely is that the processes are squeezed away passively by hypertrophied neuronal profiles. For example, this mechanism does not explain the significant reduction in astrocytic ensheathing of dendrites and the appearance of numerous dendritic bundles (see Ref. 184). The rapidity of the glial changes also precludes such a possibility, since they can be detected in tissues where hypertrophy of neuronal elements has not yet occurred (106). Moreover, VP neurons greatly hypertrophy during conditions like chronic dehydration, yet their surfaces do not become increasingly juxtaposed (26). A more likely explanation is that neuronal juxtapositions are the result of active retraction and elongation of astrocytic processes over surfaces of neuronal elements whose morphology is constantly changing. The altered expression of several cytoskeletal proteins in HNS neurons and glia (75, 114, 133) presumably reflects this remodeling. Compared with other systems (8), one expects that these cell transformations are accompanied by differential gene expression and de novo protein synthesis. In the SON, new macromolecular synthesis is essential, since there is no glial or neuronal remodeling when protein synthesis is blocked by agents like anisomycin (106).

2. **Basal hypothalamus**

Rapid, activity-dependent astrocytic remodeling is detectable in other areas of the basal hypothalamus. In the rat arcuate nucleus, composed of neurons that secrete reproductive hormones, the proportion of neuronal somatic surface covered by astrocytic processes fluctuates in relation with sexual steroid levels, being high when plasma estrogen is elevated (afternoon of proestrus and morning of estrus) and low 24 h later at metestrus when estrogen levels have diminished (reviewed in Ref. 54). Administration of estradiol to ovariectomized animals mimics the effects of the estrous cycle and shows that, like in the SON (106), the astrocytic-neuronal changes are rapid, occurring within 2 h of steroid injection. Similar phenomena have been recorded in the infundibulum and preoptic area of primates, areas analogous to the rodent arcuate nucleus. Thus, in Rhesus (209) and African green (54) monkeys, glial ensheathment of gonadotropin releasing hormone (GnRH) neurons varies significantly in relation to steroid levels, a remodeling that is reproducible in ovariectomized animals given estradiol replacement.

The axons of GnRH neurons project to the external layer of the median eminence where fluctuating steroid levels lead to axonal-glial changes similar to those characterizing the neurohypophysis. They result in retraction of end feet of tanycytes (modified ependymoglial cells) from the perivascular zone and exposure of GnRH terminals to fenestrated portal capillaries (36, 97). In neurohypophyseal structures, therefore, glial rearrangements ultimately permit direct access of neurosecretory axons to perivascular zones, an anatomical configuration that will favor release of neurosecretory products into the circulation.

3. **Suprachiasmatic nucleus**

Another hypothalamic nucleus that displays astrocytic-neuronal remodeling is the SCN, the master clock driving circadian rhythm. Earlier work had described pronounced circadian fluctuations in GFAP labeling in the hamster SCN (108, 109). This was confirmed recently in the rat where GFAP rhythm appeared closely dependent on light entrainment (12). By using ultrastructural analysis, the latter study clearly showed that fine astrocytic processes covering vasoactive intestinal polypeptide (VIP) and VP neurons continually undergo remodeling. The changes were closely linked to the light-dark cycle and were accompanied by altered proportions of directly juxtaposed somatic and dendritic profiles (12). Taken together, the observations provide convincing evidence that in the SCN, modulation of the morphology of astrocytic processes is closely related to the oscillatory activity of the biological clock synchronizing circadian rhythms to the 24-h light-dark cycle.
4. Brain stem

Two-photon microscopy of the brain stem in acute slices from postnatal transgenic mice have offered direct proof that astroglial processes display a high degree of motility (Fig. 7), closely linked to the activity of the synapses they ensheath (77). The astrocytes were rendered visible by expression of green fluorescent protein (GFP) and the synapses by uptake of the endocytic marker FM4-43. Two distinct modes of process motility were visualized, gliding of thin lamellopodia along neuronal surfaces and transient extensions and retractions of filopodial processes into the neuronal environment. These dynamic changes occurred within minutes while the morphology of astrocytic somata remained stable.

5. Hippocampus

Early electron microscopic analyses indicated that synaptic plasticity or long-term potentiation (LTP) is accompanied by astrocytic remodeling, since an increased density and closer apposition of astrocytic processes to the synaptic cleft of potentiated synapses was noted in the dentate gyrus 8 h after LTP induction in vivo (206). More recent studies using time-lapse imaging of fluorescently labeled live cells in acute slices have confirmed this earlier work and revealed that the fine processes of adult hippocampal astrocytes display motility in situ (13, 68, 126). As the astrocytic processes underwent different types of motility, similar to those visualized in the brain stem (77), filopodia emerged from astrocytic tips and there was a constant extension and retraction of lamellodia-like structures, over a time scale of minutes. Even splitting of individual processes was noted (68).

Not unexpectedly, actin-based cytoskeletal rearrangements are involved, and the astrocytic transformations were closely linked to transformations in neighboring neuronal elements. Thus, in rat hippocampal slice cultures (124, 131), as well as in acute slices (68), repetitive two-photon imaging revealed that astrocytic contacts enhanced both the lifetime and morphological maturation of pyramidal neuron dendritic spines. The motility of astrocytic processes exceeded that of dendritic filopodia, and the former showed directional protrusive activity towards dendrites. As will be discussed in more detail later, such observations strongly suggest that the direction and extent of astrocytic process motility is regulated by environmental cues, including diffusible factors from adjacent neuronal elements.

6. Cerebellum

Electrophysiological and morphological observations revealed that rather than engaging in static encapsulation, Bergmann glia require constant input from their associated synapses to maintain their relationship (83). It is AMPA receptors on these astrocytes that play the important role of maintaining ensheathment. The Ca\(^{2+}\)/H11001 permeability of AMPA receptors is determined by the presence of the GluR2 subunit, which markedly reduces Ca\(^{2+}\) entry into the cells. Removal of GluR2 subunits from these receptors reduced Ca\(^{2+}\) permeability in the cells and was accompanied by process retraction, leaving synapses uncovered by glia. The phenomenon was accompanied by delayed glutamate uptake (83). In vitro studies (84) supported these findings and showed that conversion of AMPA receptors to the Ca\(^{2+}\)-impermeable type resulted in glial process retraction while overexpression of Ca\(^{2+}\)-permeable receptors produced process extension. On a more general level, it is noteworthy that synaptogenesis induced by motor learning in the cerebellar cortex was associated with an increased volume of astrocytic processes, a parameter that remained unaffected by increased neuronal activity due to exercise (90).

7. Barrel cortex

In mice, sensory stimulation elicited by 24-h whisker stimulation caused surprising changes in astrocytic ensheathment of glutamate synapses. In contrast to events in the rat SON (172), stimulation here resulted in a significantly enhanced astrocytic coverage of axo-spinous glutamatergic synapses (58). This appeared to be due to remodeling of individual processes over particular spines,
since there were no changes in the total volume or surface area occupied by the processes.

8. Neocortex

In acute rat cortical slices, application of brain-derived neurotrophic factor (BDNF) rapidly modified the morphology of astrocytic processes via T1 activation and signaling (136). While in other areas astrocytic processes were seen to extend over very few micrometers (77), in this study they were noted to extend as far as 15 \( \mu \text{m} \).

9. Visual cortex

Quantification of fine astrocytic processes in layers I-IV of the rat visual cortex indicated changes in their volume per neuron roughly comparable to the increase in synapse number recorded after 30 days exposure to an enriched environment (90, 91). Moreover, the enriched environment enhanced the degree of surface contact made by fine astrocytic processes with axodendritic synapses, an increase which tended to be greater in layer IV but which was also apparent in layers I and II/III. On the other hand, in the cat visual cortex, a reduced neuronal activity induced by dark rearing during the critical period retarded astrocytic maturation and resulted in a reduced number of astrocytic processes in the neuropil (122).

B. Consequences on Extracellular Homeostasis

Since astrocytic processes are an important structural element of the space surrounding neurons and since they serve to maintain the homeostasis of the ECS, any structural modification of the astrocytic environment should affect the volume and geometry of the ECS as well as its molecular composition. The rat SON with its clear lactation-related astrocytic changes has served to test this possibility directly (148), using the real-time tetramethylammonium (TMA\(^{+}\)) iontophoretic method (130). The approach permits measurement of diffusion parameters like tortuosity (a measure of restriction on diffusion in the tissue compared with an obstacle-free medium), volume fraction (the volume of tissue occupied by the ECS), and nonspecific uptake. In virgin rats, diffusion in the SON was not equivalent in all directions (anisotropy), and tortuosity was hindered less along the ventrodorsal axis, probably reflecting the ventrodorsal orientation of most large astrocytic processes in this structure (17, 87). On the other hand, in lactating animals, in which there is remodeling of astrocytic processes (184), there was a significant reduction of volume fraction and tortuosity. As a consequence, diffusion became equivalent in all planes (isotropy), indicating that not only was the geometry of the ECS affected, but its diffusion properties as well (Fig. 8). This would then enhance the range of action of circulating molecules and their local concentrations, a phenomenon with important repercussions, especially on neurotransmission.

C. Consequences on Excitatory and Inhibitory Neurotransmission

Because astrocytic processes carry transporters that rapidly clear away neurotransmitters like glutamate (Fig. 2, B and C), they control the accumulation and diffusion of these transmitters in the ECS near the source of their release. It follows that the positioning of glutamate transporters close to the site of glutamate release is paramount if they are to remove it efficiently. In the barrel cortex, two-thirds of spines have astrocytic processes at the bouton-spine interphase. As noted in section IIIA, sensory stimulation induces these processes to adopt a more intimate contact with the region through which glutamate transmission occurs, a remodeling that is accompanied by a significantly enhanced expression of the astrocytic glutamate transporters GLAST and GLT1 (58). These changes ensure that the cortex guards against possible deleterious effects of high concentrations of glutamate in the ECS.

On the other hand, withdrawal of astrocytic processes from the synaptic cleft area has several consequences on synaptic transmission, including modulation of postsynaptic responses, modulation of feedback mechanisms on afferents, and the appearance of heterosynaptic cross-talk between neighboring synapses (Fig. 8). Electrophysiological observations obtained in acute slices of the rat SON have provided direct evidence for this. Like most CNS structures, the SON contains glutamate afferents that provide excitatory input to both its neurosecretory populations (47). Compared with virgin rats, in lactating (138, 147) and chronically dehydrated (18) animals, in which there are fewer astrocytic processes around OT neurons and their glutamatergic synapses (172) (Figs. 6 and 8), tonic activation of presynaptic metabotropic glutamate receptors on glutamate synapses was enhanced, which reduced synaptic efficacy (18, 138). This effect was similar to that induced by pharmacological blockade of glutamate transporters in virgin rats (147). Another system in which similar phenomena have been noted is the cerebellum (83), where retraction of Bergmann glia processes resulted in delayed glutamate uptake and modified glutamate neurotransmission.

Under certain conditions, accumulation of glutamate in the ECS may lead to heterosynaptic modulation of neighboring afferents. At basal levels of glutamatergic activity, glutamate is not sufficient to activate glutamate receptors located at a distance from glutamatergic syn-
apses, an observation consistent with earlier studies (reviewed in Ref. 139). However, heterosynaptic modulation of GABAergic transmission was noted when glutamatergic input activity was high enough to saturate glutamate transporters and enable diffusion of glutamate to remote receptors. In the SON of virgin rats, this heterosynaptic depression of GABAergic transmission occurred only when short conditioning trains of stimulation were applied to glutamatergic fibers (147). In lactating rats, because of anatomical remodeling (172) (Fig. 8), a similarly induced intersynaptic cross-talk was facilitated (147).

Taken together, these observations show that the presence of astrocytic processes close to the synaptic cleft enhances point-to-point synaptic communication and limits presynaptic inhibitory feedback and extrasynaptic communication. Astrocytic process remodeling, therefore, should be considered an important mechanism when examining factors that potentially can modify synaptic strength.

D. Effects on Gliotransmission

Repositioning of astrocytic processes will have further consequences on neuronal function, since it affects the concentration of neuroactive substances secreted by astrocytes themselves into the ECS. Again, observations from the hypothalamic magnocellular nuclei provide clear-cut evidence for this. One such gliotransmitter is D-serine, present in distal astrocytic processes (Fig. 2, D and E) and released by activation of glutamatergic receptors to act as a coagonist of glutamate at NMDA receptors (Fig. 9) (121, 141). In the SON of virgin female rats, under basal conditions of neurosecretion, there is extensive astrocytic coverage of neuronal elements and the amino acid almost completely saturates the glycine sites on NMDA receptors (141). In contrast, in lactating rats, consequent to the considerable withdrawal of astrocytic processes (172), there is a significant reduction in D-serine levels around synapses (Fig. 9). The occupancy of glycine sites is then low, and the activity of NMDA receptors is greatly reduced (141). As a consequence, NMDA receptor-dependent manifestations of synaptic plasticity, like LTP and long-term depression (LTD), are profoundly affected. In particular, the activity dependence of these processes is shifted towards higher values so that it takes a much stronger synaptic activity to induce LTP in lactating rats compared with virgin animals. Since D-serine levels are reduced in the ECS around synapses, fewer NMDA recep-

![Figure 8](http://www.prv.org/physrev/physiology.org/Downloaded-from/)
tors are activated and, therefore, less Ca\textsuperscript{2+} enters the postsynaptic neurons. NMDA receptor activity and the ability to induce LTP and/or LTD in lactating rats are completely rescued when saturating concentrations of D-serine are applied to the tissue (141).

A similar scenario occurs in the PVN of dehydrated rats where the withdrawal of astrocytic processes from the vicinity of magnocellular neurons (183) affects the accumulation of yet another gliotransmitter, ATP (64). The result is insufficient ATP to activate P2X receptors on the neurons, and synapse strengthening through AMPA receptor insertion is compromised. This form of synaptic plasticity can be restored by providing exogenous ATP to the tissue.

Another consequence of reduced astrocytic coverage of excitatory synapses may occur via release of cytokines, like TNF-\alpha. TNF-\alpha released from astrocytic processes enhances synaptic efficacy by promoting the insertion of AMPA receptors at neighboring glutamatergic synapses (11). Its continual presence, therefore, is a prerequisite for the maintenance of synaptic strength (167). Any change in TNF-\alpha concentration in the vicinity of glutamatergic synapses will modify AMPA receptor number and, therefore, synaptic strength.

It is obvious, therefore, that by affecting gliotransmission, remodeling of astrocytic processes has far-reaching consequences on glutamatergic transmission and long-term plasticity (see also Ref. 9).

E. Astrocytic Changes and Synaptic Remodeling

Astrocytes participate actively in synaptogenesis and synaptic remodeling in the developing and adult brain, and their contribution appears complex. On the one hand, their mere presence along the neuronal surface will hamper the formation of synapses; their absence will allow it. On the other hand, at least during development, astrocytes favor synaptogenesis by secreting factors that support synapse formation. These include cholesterol and thrombospondins that promote the formation and maturation of excitatory synapses (29, 112, 194). Moreover, through their release of substances like TNF-\alpha, they contribute to subsequent activity-dependent homeostatic establishment of synaptic connectivity (167). Nevertheless, it remains to be seen whether these secreted factors intervene in synapse remodeling during adulthood. Thrombospondin expression, for example, is low or nonexistent in the adult CNS (29).

We have known for decades that synapse formation with accompanying rearrangements of connectivity occurs in many adult neuronal systems, in response to a variety of physiological stimuli. There are many excellent reviews on this topic (see, for example, Refs. 8, 28, 32, 57), and we shall limit our discussion to those cases where there appears to be a participation of astrocytes, direct or indirect.

Several neuroendocrine systems provide clear evidence that certain hypothalamic circuits remain relatively
plastic throughout life, a plasticity coincident with re-structuring of adjacent astrocytes and dependent on the activity of neighboring neurons. In the magnocellular nuclei of rodents, changes in astrocytic coverage of OT somata and dendrites invariably occur together with synapse turnover. The most obvious manifestation is the appearance of boutons making synaptic contact onto more than one postsynaptic elements simultaneously ("multiple" synapses) (reviewed in Refs. 172, 188). Similar synaptic changes are visible in the cerebellum where retraction of Bergmann glial processes is accompanied by an increased number of multiple synapses formed by climbing fibers on Purkinje cells (83). In the OT system, synaptic density analyses revealed that synapse formation affects boutons making single synaptic contacts as well. Moreover, analyses of ultrathin sections in which synapses were immunoidentified as GABAergic, glutamatergic, or noradrenergic allowed to identify the circuits undergoing plasticity as inhibitory (GABAergic) and excitatory (glutamatergic and noradrenergic) (reviewed in Ref. 188). The most important increases affect GABAergic inputs so that in the SON of lactating rats (47, 62), ~50% of all axosomatic and axodendritic synapses are GABAergic, compared with ~35% in the SON of virgin rats under basal conditions (47). Once stimulation is over, synaptic densities revert to control values. Ultrastructural analyses in which both pre- and postsynaptic partners were identified confirmed that the inputs undergoing plasticity are those impinging on OT neurons (62, 178). This synaptic remodeling can be reproduced in vitro in acute hypothalamic slices that include the SON. Electron microscopy and patch-clamp electrophysiology showed that synapse formation is very rapid, occurring within an hour, is reversible within hours as well, and results in the formation of inhibitory, putative GABAergic synapses that are functional (175).

Another hypothalamic center in which there are synaptic changes clearly linked to astrocytic remodeling is the arcuate nucleus. In adult females, there is a natural phasic synaptic turnover that is linked to variations of the ovarian cycle (54). As clearly demonstrated by ultrastructural analyses, the number of axosomatic GABAAergic synapses on arcuate neurons falls significantly between the morning and afternoon of proestrus, remains low throughout estrus, and rises to baseline levels on metestrus, to fall again at proestrus. Concurrently, there is increased and decreased astrocytic ensheathment of neuronal profiles (55). This negative correlation between the level of glial ensheathment and number of synaptic inputs in relation to fluctuations in steroid levels has been detected in primates as well (54, 209). In sheep, on the other hand, seasonal variations in glial ensheathment and synaptic inputs occur in the preoptic area. Thus the number of synapses decreases while astrocytic coverage of neurons increases between the breeding and nonbreeding seasons in conjunction with the diminished pulsatile GnRH secretion that leads to anestrus (198). In the rodent arcuate, synaptic rewiring of inhibitory inputs on POMC neurons has been noted as well, in response to elevated circulating levels of the hormone leptin, that participates in the regulation of energy balance and growth hormone release (149). It is not unlikely that these latter synaptic changes are accompanied by astrocytic changes similar to those seen in response to estrogen.

An increasing number of reports describe synaptic transformations linked to changes in glial ensheathment in brain areas other than the hypothalamus. In the adult hippocampus, new synapse formation by subsets of mossy fiber terminals occurs in response to experience and age (53), while increases in dendritic spine synapses were noted in response to the hormone ghrelin (41). How astrocytic processes intervene under such conditions has yet to be examined, but it is noteworthy that astrocytic changes were detected in acute hippocampal slices during recuperative synapse formation after synapse loss due to cutting of the tissue (208). As noted in section IIIA, astrocytic processes in the hippocampus are capable of a motility rapid enough to account for these effects (68).

Recent work in different cortical areas offers further examples of glial participation in adult synaptic plasticity. This is particularly striking in the barrel cortex, where whisker stimulation modified astrocytic coverage of glutamatergic synapses and increased the density of putative excitatory and inhibitory synapses on spines (58). In animals exposed to an enriched environment, the occipital cortex displays gross changes in thickness, reflecting a 25–40% increase in dendritic branching, with a parallel increase in the number of synapses per neuron. Concomitantly, there are changes in the size and surface density of astrocytes (90).

Most observations of structural synaptic plasticity in the adult brain concern the appearance of new synapses. Yet it is clear from studies in neuroendocrine systems that synapse formation is followed by synapse elimination, coincident with arrest of stimulation (54, 184). Astrocytes may promote this synapse elimination as well. Early ultrastructural studies established that astrocytes phagocytose degenerating axonal boutons (1). Recently, a type of trans-endocytosis was observed between neurons and astrocytes in culture, which did not resemble this traditional astrocytic phagocytosis of degenerating axons but rather more fine structural remodeling between neuronal protrusions and astrocytes (107). Nevertheless, the professional phagocytes of the CNS are microglia (reviewed in Ref. 70), and it is highly likely that they participate in remodeling of synaptic connections by engulfing degenerated terminals. This phenomenon has been described in the rat neurohypophysis (151). It is noteworthy that degenerated synaptic profiles are not detected in the magnocellular or arcuate nuclei of the hypothalamus when...
synaptic densities return to unstimulated levels. In these structures, then, a reduced number of synapses may reflect a retraction of terminals with concomitant loss of presynaptic specializations or displacement of terminals with their presynaptic specializations to neighboring postsynaptic elements. One expects that intervening astrocytic processes participate in these processes. They cannot be interposed between pre- and postsynaptic partners if synapse formation is to occur, whereas their reinsertion could contribute to the undoing of synaptic contacts (see also Refs. 1, 54). After synapse elimination, pre- and postsynaptic protein components may simply be redistributed in their respective compartments, without involving degeneration of boutons.

These possibilities await experimental evidence. What is certain is that synapse formation must be accompanied by a coordinated development of pre- and postsynaptic molecular and structural specializations, requiring exchange of information between pre- and postsynaptic partners, as well as with adjacent astrocytes. As will be discussed in section VI, astrocytic membranes are equipped with numerous signaling and cell recognition molecules (transmitter receptors, transporters, adhesion molecules) that make the cells well suited for these purposes.

IV. PHYSIOLOGICAL CONSEQUENCES OF ASTROCYTE-NEURON PLASTICITY

A. Neuroendocrine Regulations

1. Parturition and lactation

The magnocellular system of the hypothalamus constitutes a physiologically controlled system that intervenes in the regulation of several vital neuroendocrine processes. Its morphological reorganization, described in earlier sections, involves both its neurons and glia. Since it is easily accessible in vivo and in vitro, it serves as an excellent model to analyze the importance of astrocytic process remodeling, not only at the level of synaptic function but in the more general context of system physiology as well. This is particularly clear in the case of the OT system that displays highly characteristic electrical, biosynthetic, and secretory activities at lactation (reviewed in Refs. 22, 150).

Peripheral information during suckling is transmitted to OT neurons through nearby glutamatergic neurons. During the milk ejection reflex, for example, OT neurons display high-frequency bursts of action potentials (150) that are triggered by bursts of glutamatergic postsynaptic potentials derived from a local glutamatergic pulse generator situated in the adjacent hypothalamus (85, 93). As discussed in section IV, patch-clamp recordings provided convincing evidence that excitatory information transmitted by glutamatergic inputs is affected by glial remodeling (139). Thus the reduced astrocytic coverage of OT neurons and their synapses during lactation leads to accumulation of glutamate in the ECS. The amino acid, via its action on metabotropic receptors on those very synapses from which it is released, will in turn inhibit glutamate release (138). On first sight, it seems paradoxical that these inputs exhibit a lower probability of release under a physiological condition that requires OT neurons to be strongly activated. However, bursts of action potentials occur every 5–15 min, separated by periods of low-frequency activity (see Ref. 150). Presynaptic inhibition depends on the level of presynaptic activity (140). The tonic presynaptic inhibition during the low-frequency period is likely to be overcome when the probability of release associated with Ca\(^{2+}\) accumulation in the terminals strongly increases during the brief, high-frequency train of action potentials occurring at milk ejection. Therefore, information transmitted via high-frequency excitatory synaptic activity would be less affected by negative glutamate feedback than information transmitted by low or moderate frequencies. Such a high-pass filter may serve to increase signal-to-noise ratio for information carried by high-frequency activities. It may also favor input from suckling over other inputs driving OT neurons, such as those involved in regulating osmotic stimulation and triggering rather low-frequency activity (see below). Moreover, accumulating glutamate in the ECS may exert additional heterosynaptic actions, especially during high-frequency firing. For example, it can inhibit GABAergic synapses in the vicinity of glutamatergic inputs (148), and this can lead to a local disinhibition of the somatodendritic compartment (113). The same considerations may apply to parturition as well, a condition in which OT neurons display bursting high-frequency discharges (170).

As discussed in detail in section IV, the presence or absence of astrocytic processes has important consequences on the ECS concentration of gliotransmitters like D-serine, which will affect NMDA receptor activation, intracellular Ca\(^{2+}\) concentrations, and ultimately, functional synaptic plasticity such as LTP and LTD (141). During lactation, fewer NMDA receptors are occupied by D-serine (141), and the threshold for synapse potentiation is elevated, making it more difficult for synapses to gain synaptic strength. Making potentiation harder to achieve may favor very active synapses, like those communicating the suckling stimulus to OT neurons, while other, less active, but potentially confounding inputs, will be depressed. Overall, these mechanisms will ensure that the pool of OT available for secretion will not be used in response to stimuli not pertinent for parturition or lactation when all available OT will be used for delivery of the young and milk ejection.
2. Osmotic regulation

One of the main functions of VP is to contribute to hydromineral homeostasis by the release of VP acting on the kidney. OT neurons are also involved in this regulation through the release of OT, inducing sodium excretion at the level of the kidney (reviewed in Refs. 22, 150). A puzzling observation is that reduction in astrocytic coverage in the magnocellular nuclei is enhanced during chronic osmotic stimulation, but specifically in the OT population (26). However, under such stimulation, OT neurons display an increased tonic activation, a pattern different from the periodic bursting activity seen during suckling (150). As speculated above, in lactating animals, which are in a state of steady osmotic imbalance (see Ref. 102), it is possible that the astrocytic changes filter out part of the incoming osmotic stimulus, by a mechanism of high pass filter. Indeed, OT release in response to osmotic stimulation is reduced in lactating rats, compared with virgin rats (102).

Another regulation that could be affected by the astrocytic changes is that related to the release of the gliotransmitter taurine, an amino acid that contributes to the control of osmotic balance (81). An altered astrocytic compartment should modify ECS concentrations of this amino acid as well, ultimately affecting the activity of magnocellular neurons via activation of strychnine-sensitive glycine receptors (37) (see also sect. III). On the other hand, astrocytic modifications have been linked to fluctuations of serotonergic activity, and it has been suggested that they could be regulated by the daily rhythm of serotonin release (63). On the other hand, astrocytic modifications have been linked to glutamatergic afferent activity originating in the retina. It has thus been proposed that SCN astrocytes regulate and mediate glutamate release by retinal terminals, thereby facilitating light signal transmission and contributing to the coordination of firing of SCN neurons (108). Seasonal variations have been noted as well (60) and were linked to alternating photic stimulation conveyed by retinofugal fibers to the nucleus. It is also noteworthy that there is a reduced astrocytic coverage of VP neurons in this nucleus at night, a remodeling which results in increased numbers of directly juxtaposed dendrites and soma and which could represent a substrate for nocturnal facilitation of their synchronized activity (12).

3. Sex-specific behaviors

Another hypothalamic neuroendocrine system where concurrent astrocytic and synaptic plasticity is of potential import to system activation is that secreting GnRH, the neurohormone that controls reproductive behaviors like ovarian cyclicity and puberty. Thus it is highly likely that the transformations in astrocytic ensheathment of GnRH somata and dendrites, their synaptic inputs, as well as GnRH terminals in the median eminence contribute to the generation of the preovulatory GnRH surge (54, 78, 137). For example, in the rodent arcuate nucleus, most neurons are hyperpolarized by GABA, and this inhibition is an important component of the regulation of GnRH and prolactin release (reviewed in Ref. 54). At proestrus, estrogen enhances astrocytic coverage of GnRH neurons, and this coincides with a marked reduction in their inhibitory GABAAergic synapses (54). A reduced inhibitory input will enhance their firing and therefore increase release of hormone from their terminals into the circulation just before ovulation. On the other hand, in monkeys, gonadal steroid-dependent astrocyte-neuron interactions contribute to the regulation of GnRH secretion at the onset of puberty (137, 209). Finally, in the rodent ventromedial hypothalamus, it is highly likely that astrocyte-neuron interactions participate in the mechanisms controlling sexual behavior, if only to allow synaptic rewiring taking place under conditions leading to lordosis (52).

4. Circadian rhythm

In the SCN, the biological pacemaker clock driving and controlling behavioral and physiological circadian rhythms, the gross changes in the morphology of astrocytes, detected with GFAP labeling (12, 60, 109), have been associated with fluctuations of serotonergic activation, and it has been suggested that they could be regulated by the daily rhythm of serotonin release (63). On the other hand, astrocytic modifications have been linked to glutamatergic afferent activity originating in the retina. It has thus been proposed that SCN astrocytes regulate and mediate glutamate release by retinal terminals, thereby facilitating light signal transmission and contributing to the coordination of firing of SCN neurons (108). Seasonal variations have been noted as well (60) and were linked to alternating photic stimulation conveyed by retinofugal fibers to the nucleus. It is also noteworthy that there is a reduced astrocytic coverage of VP neurons in this nucleus at night, a remodeling which results in increased numbers of directly juxtaposed dendrites and soma and which could represent a substrate for nocturnal facilitation of their synchronized activity (12).

B. Learning and Memory

How plasticity of astrocyte-neuron interactions participates in mechanisms of learning and remembering is only beginning to be appreciated. Long-term memory does involve structural rearrangements, especially of synaptic inputs (see, for example, Refs. 8, 90). Synaptic rewiring has been detected in the ocular dominance columns, occipital cortex, cerebellum, and hippocampus and has been implicated in the control of vision, memory, motor learning, LTP, and kindling, respectively. One expects that the synaptic changes are accompanied by remodeling of the astrocytic processes with which they are associated. As illustrated by the hypothalamic magnocellular nuclei (see sect. III), even if the effects of this kind of remodeling are subtle, they can have far-reaching effects.
on neuronal function and ultimately on the regulation of phenomena as complex as learning.

V. ASTROCYTE-NEURON INTERACTIONS AND NEUROPATHOLOGICAL CONDITIONS

While the main emphasis in this review has been on physiological astrocytic-neuronal interactions, one cannot ignore that astrocytes, by their position and because of their release of neuroactive substances, are potential sources of pathological conditions as well. Until recently, for example, they were considered passive bystanders or victims in conditions like epilepsy, in spite of observations showing that focal epilepsy is accompanied by pathological changes in astrocytes, including hypertrophy and hyperplasia (189). In an experimental model of epilepsy, electrophysiological recordings demonstrated that glutamate release was sufficient to elicit paroxysmal-type depolarizations in neurons while adjacent astrocytes displayed coincident slow depolarizations of longer duration. These observations thus raise the interesting possibility that epileptiform activity in neurons may initially activate neighboring astrocytes which then maintain and propagate neuronal activity independent of synaptic interactions, a mechanism that may explain synchronization of firing during focal seizures.

Another serious neuropathological condition in which astrocytes appear to be involved is schizophrenia (160). Astrocytes produce factors like Bornea disphosphates which astrocytes appear to be involved in schizophrenia, including hypertrophy and hyperplasia (189). In an experimental model of epilepsy, electrophysiological recordings demonstrated that glutamate release was sufficient to elicit paroxysmal-type depolarizations in neurons while adjacent astrocytes displayed coincident slow depolarizations of longer duration. These observations thus raise the interesting possibility that epileptiform activity in neurons may initially activate neighboring astrocytes which then maintain and propagate neuronal activity independent of synaptic interactions, a mechanism that may explain synchronization of firing during focal seizures.

VI. CELLULAR MECHANISMS UNDERLYING ASTROCYTE-NEURON PLASTICITY

A. Permissive Molecular Factors

The structural plasticity described in this review connotes dynamic cell-cell and cell-matrix interactions that bring into play cell surface and ECS molecules, as well as soluble factors, many of which have been identified by molecular neurobiology in developing systems undergoing similar glial and neuronal transformations. In the developing brain, the effects of these molecules ultimately determine the architecture of nervous tissue and are a result of mutual interactions on the cell surface and activation of intracellular signal transduction mechanisms through diverse cell surface receptors. It is not too surprising then that adult neural systems capable of remodeling continue to express many of these “juvenile” molecules. The list includes cell adhesion molecules like the cadherins, ephrins, neurexins, and members of the immunoglobulin superfamily, like NCAM. Complex ECS glycoproteins, like the chondroitin sulfate proteoglycans and tenascins, as well as their receptors, also contribute (34, 100, 180, 201). Many reviews describe these molecules and their interactions, especially in the context of axon outgrowth and synaptic plasticity (see, for example, Refs. 43, 46, 110). Here, we focus on the highly sialylated isoform of NCAM, PSA-NCAM. Our choice is based essentially on the fact that we have direct evidence that this particular cell surface molecule does intervene in the dynamic cell interactions responsible for astro-neuronal remodeling (reviewed in Ref. 180). It thus serves as a good model to describe the experimental approaches used to analyze the localization and expression of such molecules and, in particular, their contribution to cell processes responsible for morphological plasticity.

While PSA-NCAM is abundant in developing tissues, most adult tissues contain NCAM with little PSA. However, as we noted in section uB, it continues to be strongly expressed in adult systems endowed with the capacity for morphological plasticity (reviewed in Refs. 15, 16). In the HNS, for example, PSA-NCAM is made by astrocytes and neurons throughout life (16, 99, 173, 186). In the hippocampus, PSA-NCAM expression is linked to neuronal activity (98), but in the HNS (146), as in other neurosecretory systems (80), its expression is constitutive and not markedly affected by changing conditions of neurosecretion. This does not mean that it is of no consequence to activity-dependent plasticity. On the contrary, it is a prerequisite for all its dynamic phases. Thus specific enzymatic removal of PSA from NCAM in the SON in situ inhibited glial and neuronal remodeling associated with lactation and chronic dehydration; there was no effect if PSA was removed once morphological changes had al-
ready taken place (173). Likewise, enzymatic removal of PSA from cell surfaces in the neurohypophysis prevented stimulation-related induction and reversal of axon and glial changes but had no effect once remodeling had occurred (115). There is strong, constant expression of PSA-NCAM in the GnRH system of rodents (16, 80, 162), sheep (198), and primates (144) where it also appears essential for astrocytic-neuronal plasticity, since removal of PSA inhibited the onset of astrocytic and synaptic changes associated with reproductive status (80, 162).

How PSA intervenes to permit morphological changes remains undetermined, but a possible mechanism is that its presence on cell surfaces attenuates adhesion via physical impedance or charge repulsion (159). Cells can then detach from their neighbors or from the extracellular matrix and undergo changes in their conformation. In addition, PSA-NCAM may intervene by transducing intracellular signals resulting in astrocytic shape changes and/or synapse formation (42).

We consider a molecule like PSA-NCAM permissive for morphological plasticity in the neuroendocrine centers, since its expression is not tightly linked to neuronal activity yet it appears obligatory for their morphological plasticity. This was clearly evident from enzyme perturbation experiments (80, 115, 173). Nonetheless, there can be compensatory replacement by other molecules, since remodeling does occur in lactating and salt-loaded transgenic mice genetically deficient in NCAM (187), which reflects a redundancy in the molecular systems permitting such plasticity. Similar phenomena have been described in other neuronal systems in which NCAM is highly expressed and which continue to undergo plasticity in NCAM −/− mutants (45). In a sense, the specific identity of molecules permissive for remodeling may not be important provided that they activate the same intracellular mechanism. On the other hand, the response of neurons and astrocytes implicated in remodeling would be invariant and independent of the identity of the factor, provided the proper stimulus intervenes.

B. Signaling Molecules

If molecules like PSA-NCAM act as primers, providing a permissive environment for remodeling, then there must be molecules whose expression is particular to each potentially plastic system that act as specific stimuli, capable of triggering cascades of intracellular events that result in glial and neuronal transformations. As will be discussed in detail below, such molecules include those expressed at the onset of enhanced activity to bring on remodeling as well as molecules, not necessarily identical to the former, which signal the system to revert to its original condition. Taking together available observations, there appear to be many candidates for such functions, including neuropeptides, neurotransmitters, steroids, and trophic factors that are released by neighboring elements and diffuse to astrocytic processes to direct the extent and direction of the latter’s motility. Nevertheless, we still have little knowledge of how these signaling pathways consequently operate, through genomic and nongenomic pathways, to influence the morphology of the brain’s cells and its synaptic wiring.

The hypothalamic magnocellular nuclei again offer many observations that shed some light on the action of such molecules. In the OT system, the physiological conditions during which astrocytic remodeling is most striking are parturition and lactation (172), conditions characterized by enhanced release of the peptide in the periphery as well as in the hypothalamic nuclei. Intraneuronal OT has a positive-feedback action, since it facilitates the activity of the OT system (35, 85, 93, 156). It can also induce its morphological plasticity, since intracerebroventricular infusion of the peptide, presumably mimicking this central release, induced neuronal-glial and synaptic changes in the SON similar to those detected under physiological stimulation (177). These effects can be reproduced in vitro, in acute slices of the adult hypothalamus that include the SON. It was thus possible to show clearly that the neuropeptide acts specifically via its receptors since its action was mimicked by close analogs and inhibited with specific OT receptor antagonists (106). Addition of structurally similar peptides like VP had no effect. The receptor-mediated action of the neuropeptide may explain why morphological changes in the HNS are specific to the OT system. In the hypothalamic magnocellular nuclei, OT receptors occur on OT but not on VP neurons (10) and on astrocytes (66).

OT, however, does not act alone but in synergy with sexual steroids, especially estrogen (106, 116). In view of the rapidity of remodeling (106), it is probable that the peptide and steroid act via a plasmalemmal mechanism (155), mobilizing cytosolic Ca2+ and inducing signaling cascades that result in activation of gene expression and ultimately remodeling. In the magnocellular nuclei, the steroid does have rapid effects on the electrical activity of OT neurons (86), and it enhances OT gene expression (33) and central (202) and peripheral (210) peptide release. The oxytocin gene promoter, but not the VP promoter, possesses estrogen-responsive elements (see Ref. 22). Nevertheless, although OT, with or without steroids, appears essential for remodeling in the magnocellular nuclei, there are other candidates for such signaling, and OT may act in a secondary fashion, by facilitating their expression.

Of these, glutamate is an excellent candidate, since it can serve as a bidirectional signal to transfer information between activated neurons and adjacent astrocytes (see sect. uC). In vitro, astrocytes display a rapid structural response to the amino acid by increasing the number of
surface filopodia within minutes of its application (31). It is to be expected then that changes in glutamate transmission may lead to rapid and concerted modifications in the morphology of neurons and astrocytes. This does appear to be the case in several brain areas. Thus, in the cerebellum, activation of AMPA receptors on Bergmann glia is required for the maintenance of neuron-glia interrelationships (83). In the hippocampus, spine and astrocytic growth and retraction are coordinated in ~50% of cases (68). In the SON, exposure to a combination of metabotropic and ionotropic glutamate receptor antagonists prevented OT/estrogen-mediated remodeling (106). Likewise, such a treatment inhibits the appearance of new GABA synapses (A. Trailin and D. Theodosis, unpublished observations). In the magnocellular nuclei, then, glutamate itself may be a more immediate signaling agent than OT. Interestingly, in the hypothalamic preoptic area, a mediator of astroneuronal communication via glutamate appears to be prostaglandin E_2 (PGE_2). PGE_2 induces astrocytic release of glutamate which in turn activates glutamate receptors on adjacent neurons, an effect which can have important consequences on neuronal morphology and dendritic spine formation (3).

Another neurotransmitter that may signal glial-neuronal changes is nitric oxide (NO). Electron microscopic observations of the external layer of the median eminence indicate that vascular endothelial cells use a signaling pathway mediated by NO to regulate neural plasticity (36). Thus activation of endogenous NO release induced a rapid structural remodeling, resulting in freeing of GnRH terminals from ensheathing astrocytic processes.

Other excellent signaling candidates are the purines, especially in the neurohypophysis. Neurohypophysyal pituicytes possess purinergic receptors (111, 158), ATP can be released from neurosecretory granules in the terminals (166), and adenosine, secondary to the metabolism of ATP, induces pituicyte stellation (114, 158). Activated RhoA GTPase activation is a key event in coupling purine receptor activation and stellation (158). In line with these results are preliminary observations of the SON in the acute slice preparation where adenosine can substitute for OT in inducing astrocytic and synaptic changes similar to those visible under physiological conditions (A. Trailin, S. Oliet, D. Poulain, and D. Theodosis, unpublished observations).

Recent studies have revealed that ephrins and their tyrosine kinase receptors are important intercellular signaling molecules, affecting astrocytic and postsynaptic structure, at least in the hippocampus. In this brain area, astrocytes express ephrin A3 and ephrin A receptors which accumulate on their peripheral processes (124, 126, 191). Activation of neuronal ephrin A receptors induced a rapid coordinated retraction of dendritic spines and extension of astrocytic filopodia (124), while exposure to exogenous or endogenous ephrin A gave rise to the outgrowth of GFP-labeled astrocytic processes around glutamatergic synapses within minutes (126). Concomitant with the morphological changes, ephrin A reduced the frequency of NMDA-mediated currents in CA1 pyramidal cells, an effect elicited by a diminished glutamate release from astrocytes (126).

Finally, we have abundant evidence that gonadal steroids themselves signal the onset of morphological changes, both in the hypothalamus and elsewhere. In the arcuate nucleus, administration of one single dose of 17β-estradiol resulting in plasma levels similar to those at proestrus led to synaptic and astrocytic transformations similar to those detected at proestrus (54). The effects of estradiol on arcuate astrocytes were clearly dose-dependent and were blocked by the simultaneous administration of progesterone (54). GnRH neurons themselves do not express estrogen receptors so that the effects of the steroid must be mediated through effects on presynaptic axons and/or astrocytes (209). Indeed, in this area of the hypothalamus, not only are astrocytes targets for estrogen action, they themselves release factors under the influence of gonadal steroids that then intervene in morphological rearrangements. Thus estrogens facilitate astrocytic expression of growth factors of the epidermal growth factor family and their tyrosine kinase receptors, thereby contributing to neuron-glia signaling implicated in the initiation of puberty (137). Moreover, astrocytes and tanyocytes in this area release factors like PGE_2, insulin growth factor I (IGF-I) and transforming growth factor (TGF)-α that contribute to estrogen-induced synaptic remodeling in the arcuate nucleus and GnRH terminal remodeling in the external layer of the median eminence (56, 137).

In other central areas, growth factors like BDNF appear to set off remodeling. In the adult CNS, BDNF is synthesized and secreted from pre- and postsynaptic neurons in a manner dependent on neuronal activity (see Ref. 104). In slices of adult neocortex (136), activation of T1, a major BDNF-specific receptor, appears to intervene in the regulation of astrocyte process morphology via Rho GTPases. Within 1 h, BDNF induced elongation and branching of astrocytic processes, while it had no effect when T1 receptors were inactivated. Whether this occurs in vivo remains to be seen.

C. Cytoskeletal Changes

The intracellular signaling pathway involved in directional motility of fine astrocytic processes has not been identified. To approach understanding of such mechanisms, many investigators use primary cultures of astrocytes of various sources. Astrocytic processes display extraordinary capacity for growth and motility in culture, a phenomenon called stellation (see, for example, Ref.
stimulation of adenylyl cyclase-coupled catecholamine via Rho GTPase, which inhibit Rho activity (19) and promote extension of cytoskeletal elements and inactivating Rho A-dependent pathways. For example, adenosine-induced stellatization in the neurohypophysis depends on downregulation of Rho GTPase via F-actin depolymerization and microtubule reorganization (114, 158). Another proposed mechanism involves adenyyl cyclase-coupled catecholamine stimulation of β-adrenergic receptors and subsequent enhancement of cytosolic cAMP levels (72).

VII. CONCLUSIONS AND PERSPECTIVES

From the numerous examples offered in this and other recent reviews (76, 125, 200), it is obvious that the traditional notion that neurons control all CNS activity must be revised and that we have to take into account the active participation of astrocytes in functional neuronal networks. Moreover, this review has assembled convincing evidence that astrocytes are highly plastic elements in the adult CNS. This means that dynamic astrocyte-neuron interactions are part of the physiology of several adult neuronal systems. A number of hypothalamic neuroendocrine systems, in particular that secreting the neurohormone/neuromodulator OT, illustrate remarkably well the close association of neuronal and glial activities regulating several homeostatic systems in the body. Similar astrocyte-neuron interactions have now been described elsewhere in the adult brain, including within the brain stem, cerebellum, hippocampus, and cortex. Taken together, the observations offer strong evidence that because of their capacity for remodeling, distal astrocytic processes provide a kind of environmental sensitivity to synaptic activity that ultimately will influence the overall activity of the neuronal network.

Given that astrocytic processes oppose most synapses in the brain, it is important presently to appreciate further the extent of this kind of remodeling and to see whether it is a general phenomenon, coming into play whenever the proper stimulus intervenes. While a number of observations provide clues about molecular and cellular mechanisms responsible for such plasticity, we are still far from a thorough identification and analysis of the molecular forces driving astrocytic and neuronal transformations, especially those involving lamellate astrocytic processes that appear devoid of most cytoskeletal elements. Evidence presented in this review indicates that there are molecules whose expression is permissive for this kind of plasticity (cell surface adhesion molecules, ECS components) and that they are common to neuronal systems that display it. On the other hand, there are molecules that signal or reverse the morphological changes (neurotransmitters like glutamate and purines, neuropeptides like OT, intramembranous proteins like ephrins and their receptors), and they appear to act specifically in each system that can undergo plasticity. It is now essential to dissect subsequent intracellular signaling pathways and to identify genomic and translational mechanisms ultimately resulting in the complex morphological transformations responsible for coordinated, activity-dependent astrocytic-neuronal remodeling.

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REFERENCES

95. Kapoor JR, Sladek CD. Substance P and NPY differentially potentiate ATP and adrenergically stimulated vasopressin and oxytocin...


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178. Theodosis DT, Trailin A, Poulain DA. Remodeling of astrocytes, a prerequisite for synapse turnover in th adult brain? Insights from...


