



Functional Consequences of Morphological Plasticity in the Adult Hypothalamo-Neurohypophysial System

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Abstract

The adult hypothalamo-neurohypophysial system (HNS) comprises the cell bodies of the magnocellular neurons of the hypothalamus, located in the supraoptic and paraventricular nuclei, and their axons that project onto the neurohypophysis, where they release oxytocin and vasopressin directly in the bloodstream. Oxytocin governs parturition and lactation, while vasopressin is the antidiuretic hormone and a vasopressor. The HNS undergoes a remarkable reversible, activity-dependent morphological neuroglial plasticity during lactation and dehydration. Here we summarize how this made it a seminal model to study the physiological contribution of the astrocytic environment to synaptic signaling. We show first that reduction in glial processes modifies local glutamate level at the synapse by reducing its uptake, and thus affects homosynaptic strength. Second, it also changes neurotransmitter diffusion, and as a result contributes to inter-synaptic crosstalk between glutamate and GABAergic inputs through metabotropic and kainate receptors. Finally it hampers the contribution of astrocytes to glutamatergic synaptic communication through D-serine gliotransmission. Astrocytes thus dynamically dictate the tenor of brain signaling.

Keywords

Hypothalamo-neurohypophysial system · Oxytocin · Vasopressin · Astrocyte · Plasticity

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2.1 Introduction: Structural Glial Plasticity in the Adult HNS

2.1.1 Anatomy of the HNS System

The hypothalamo-neurohypophysial system (HNS) is the first identified brain peptidergic system and oxytocin and vasopressin the first peptides to be chemically synthesized in the laboratory. The anatomical organization of the HNS, with neuronal cell bodies clearly identifiable and clustered in small, specific nuclei, together with highly accessible secretory terminals in the neurohypophysis, made it a seminal model to study the synthesis and maturation of peptides, co-synthesis and co-secretion, stimulus-secretion coupling, pulsatility of hormonal release, rhythmogenesis of electrical activity and dendritic release of neurotransmitters. It also proved to be a critical model to study the functional impact of structural glial plasticity (reviewed in Theodosis et al. 2008).

The HNS is made up of the cell bodies of magnocellular neurons located in the hypothalamic supraoptic, paraventricular and accessory nuclei and their axons that project through the internal layer of the median eminence into the posterior pituitary lobe, as illustrated in Fig. 2.1 (for review see Tasker et al. 2017). The supraoptic nuclei are located in the anterior and lateral hypothalamus, bilaterally on either side of the optic chiasm/tract. The paraventricular nuclei are located bilaterally on either side of the third ventricle. Two distinct populations of these neurons synthesize the precursors of the nonapeptidic hormones oxytocin and vasopressin, as shown in Fig. 2.2. The newly synthesized peptides are included in vesicles, where they mature and are transported toward the distal axon terminals that release them directly into the general circulation through exocytosis. Magnocellular neurons possess axons that ramify in numerous terminals in the posterior lobe of the hypophysis and each terminal contains many secretion granules. Magnocellular neurons also send axons to the amygdala and other brain regions involved in emotional, social, and affiliative behaviors. Action potentials from the soma invade the axon, depolarize the terminals, opening voltage gated calcium channels, which triggers exocytosis of neurosecretory granules. Each peptide can also be secreted from the dendrites in the central nervous system, independently of action potential activity, and act in an autocrine or paracrine manner, or through diffusion into the cerebrospinal fluid, contributing to volume transmission.

Magnocellular neurons have a relatively large soma (20–35 μm in diameter) compared to other hypothalamic neurons. They synthesize either oxytocin or vasopressin and also, to a lesser extent, a variety of other peptides and neurotransmitters, such as dynorphin, galanin, ATP (Brown et al. 2013). Most of them possess 2–3 primary dendrites that, in the supraoptic nucleus, ramify in the ventral glial lamina, a region that is rich in dendro-dendritic appositions and makes an important site of reception of synaptic inputs. There are similar numbers of oxytocin and vasopressin neurons in the supraoptic nuclei. The oxytocin neurons tend to cluster rostrally and dorsally in the nucleus, while the vasopressin neurons are preferentially distributed ventrally and caudally, as shown in Fig. 2.2. The paraventricular nuclei contain three times fewer magnocellular neurons than the supraoptic nuclei, but also contain

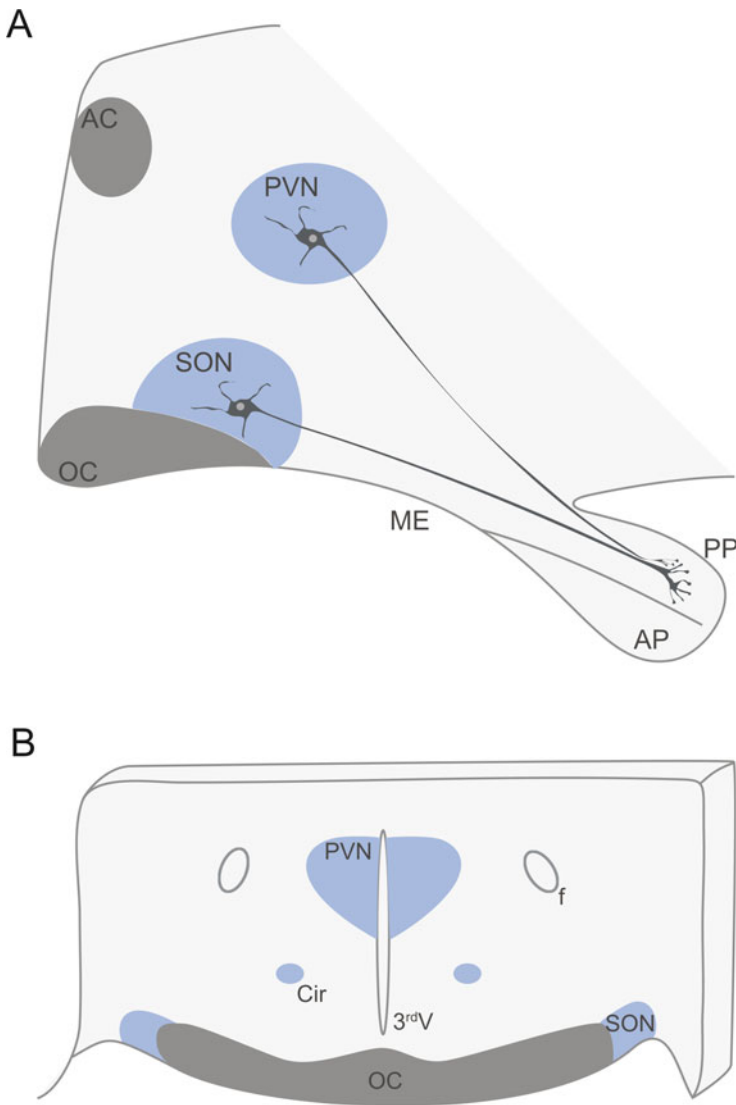


Fig. 2.1 Anatomical organization of the hypothalamo-neurohypophysial system. (a) Schematic drawing showing a sagittal view of the location of magnocellular neurons in the rat supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus and their axonal projections into the posterior pituitary (PP). (b) Schematic tracing of a coronal section through the hypothalamus showing the location of the SON, PVN, and circularis nucleus (Cir). AC, anterior commissure; AP, anterior pituitary; f, fornix; ME, median eminence; OC, optic chiasma; 3rd V, third ventricle

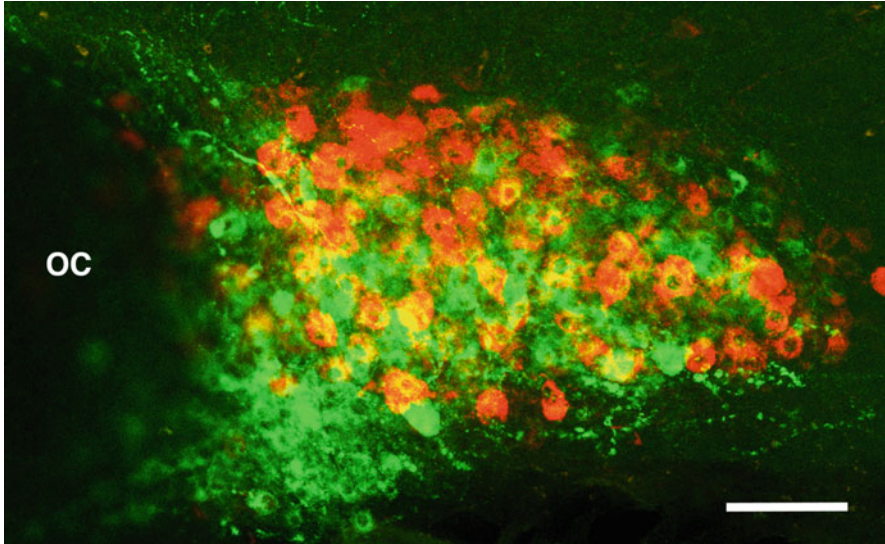


Fig. 2.2 Oxytocin and vasopressin neurons in the supraoptic nucleus. Confocal microscopy image showing the distribution of oxytocin (in red) and vasopressin (in green) neurons in an unfixed 250 μ m-thick coronal section of the supraoptic nucleus. The labeling results from the expression of a vasopressin-enhanced green fluorescent protein (eGFP) fusion protein and an oxytocin-monomeric red fluorescent protein 1 (mRFP1) fusion protein in a 3.5-month old double-transgenic virgin female Wistar rat. OC, optic chiasma. Calibration bar, 100 μ m. Courtesy of Drs ZS Thirouin and CW Bourque, McGill University

parvocellular neurons, some of which express oxytocin or vasopressin and project to the external layer of the median eminence and centrally (Armstrong 2015).

The main afferent inputs of the magnocellular neurons are glutamatergic and GABAergic projections that, respectively, provide the fast excitatory and inhibitory synaptic control over oxytocin and vasopressin release, while noradrenergic projections act primarily to modulate these inputs. In normal conditions of secretion, around 45% of HNS synapses are GABAergic, 25 % glutamatergic, and both types are often in close proximity. Around 10% of HNS synapses are noradrenergic.

2.1.2 Functions of Oxytocin and Vasopressin

Oxytocin and vasopressin released in the general circulation are vital for the species and the individual, while their central release governs crucial social behaviors and visceral functions (for review see Tasker et al. 2017).

Oxytocin is present in all mammals. Oxytocin produced by magnocellular neurons and released from the posterior pituitary acts on the uterus and contributes to its contraction during parturition, although it is not absolutely required for successful delivery. Oxytocin released in the general circulation is also the efferent

pathway of the milk ejection reflex, and this function is vital to allow pups to receive milk in response to suckling. Indeed, oxytocin knockout mice are fertile, and females are able to deliver their litters, however, the pups do not successfully suckle and die within 24 h without milk in their stomachs. In the rat, oxytocin is also a natriuretic hormone, regulating sodium excretion from the kidneys. Centrally released oxytocin is involved in the control of visceral functions, including cardiovascular regulation, food intake, sexual functions, inhibition of nociception. It also modulates anxiety and fear and plays a major role in social cognition and affiliative behaviors.

Vasopressin produced by magnocellular neurons and released from the posterior pituitary promotes water reabsorption from the kidney, and this function is vital to maintain body fluid balance. Vasopressin also increases blood pressure through vasoconstriction. Centrally released vasopressin is also an important modulator of diverse social behaviors.

2.1.3 Electrophysiology of Oxytocin and Vasopressin Neurons

Peripheral release of oxytocin and vasopressin is driven by the highly specific electrical activity of each cell population, under the main control of glutamatergic, GABAergic, and noradrenergic afferents (for review see Tasker et al. 2017). Central release of the neuropeptides does not always parallel peripheral release.

Since oxytocin and vasopressin neuron terminals do not sustain intrinsic repetitive action potential discharge, the release of both peptides in the neurohypophysis is mainly determined by the frequency and pattern of action potential discharge initiated at the cell bodies. Different mechanisms contribute to the generation of that electrical activity, including the intrinsic membrane properties of the magnocellular neurons, their synaptic inputs, autoregulation by local release of each peptide and other co-transmitters, factors released by neighboring glial cells, and influence of circulating hormones.

Under normal conditions, oxytocin and vasopressin neurons show a low level of irregular electrical activity (1–3 action potentials/s) that allows a constant and low release of hormone into the general circulation. In response to physiological stimuli, they progressively increase their firing rate and also produce cell-type specific and stereotyped patterns of bursting activity (Poulain and Wakerley 1982). These patterns are important for generating the appropriate secretion of each peptide to meet the current physiological demands. They are related to the frequency facilitation and fatigue properties of oxytocin and vasopressin neuron terminals. In contrast to sustained electrical activity, relatively brief bursts of action potentials facilitate release, whereas pauses between bursts allow for recovery of the mechanisms of exocytosis. Schematically, in response to the asynchronous, phasic bursting activity of vasopressin neurons, vasopressin is released in a tonic fashion into the blood. This allows to precisely adjust water reabsorption from the kidney and corrects changes in osmolality (Bourque 2008). By contrast, during parturition and suckling, oxytocin neurons discharge intermittent high-frequency bursts of action potentials that are synchronized across their entire population, leading to the pulsatile release of

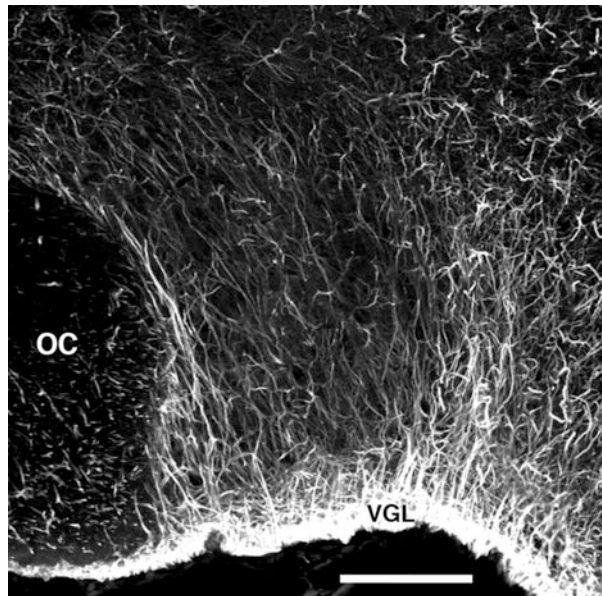
oxytocin that acts on the uterus and the myoepithelial cells of the mammary glands, which facilitates the delivery of pups and promotes ejection of milk, respectively (Poulain and Wakerley 1982).

Somatodendritic release of oxytocin and vasopressin can be regulated independently of that from the axon terminals in the neurohypophysis, which requires refined regulatory mechanisms. Somatodendritic, but not axon terminal release, can be modulated by changes in intracellular calcium concentration by release of calcium from intracellular stores, resulting in priming of dendritic pools of secretory granules for activity-dependent release (Ludwig and Stern 2015). Locally released peptides may provide a local feedback regulation of the electrical activity of magnocellular neurons and synaptic inputs.

2.1.4 Glial Cells of the HNS

Three types of astrocytes are present in the supraoptic nucleus (Theodosis et al. 2008). As illustrated in Fig. 2.3, a large population of astrocytes have a radial glia-like morphology, with cell bodies located along the ventral glia lamina (VGL), long processes traversing the nucleus in the coronal plane, horizontally-oriented processes that form a dense network in the VGL, and a short process oriented toward the pia. They are immunoreactive for glial fibrillary acidic protein (GFAP) and vimentin, an intermediate filament protein of immature glial cells and a marker for radial glia, and they are not dye-coupled. A second population of astrocytes, less numerous than the radial type, is located close to the subarachnoid space. They are

Fig. 2.3 Radial glia-like astrocytes of the supraoptic nucleus. Confocal microscopy image showing immunostaining for glial fibrillary acidic protein (GFAP) in the supraoptic nucleus of a male Wistar rat. Long, thick GFAP-positive fibers coming from the ventral glia lamina (VGL) extend dorsally through the nucleus. OC, optic chiasma. Calibration bar, 100 μ m. Courtesy of Dr M Prager-Khoutorsky, McGill University



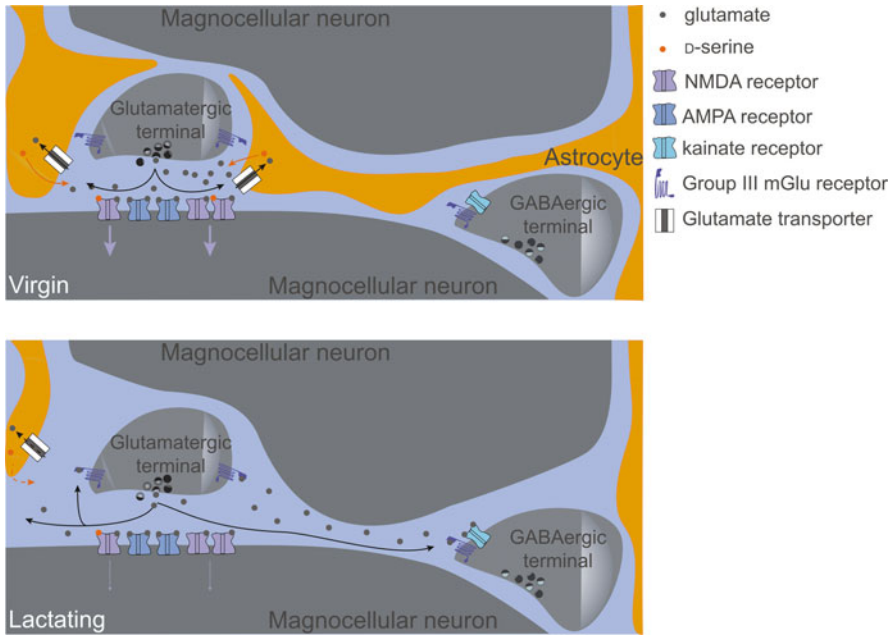


Fig. 2.4 Structural glial plasticity in the adult HNS. During lactation, when the electrical and secretory activities of oxytocin neurons are greatly enhanced, the astrocytic coverage of magnocellular neurons is reduced, compared to virgin animals. Astrocytic processes no longer wrap around synapses and neuron somata, and dendrite surfaces become directly juxtaposed. Consequently, glutamate uptake by astrocytic transporters is reduced, which favors homosynaptic regulation of glutamate release through presynaptic metabotropic glutamate receptors (mGluRs). Diffusion is facilitated, which allows inter-synaptic crosstalk through glutamate actions at mGluRs and kainate receptors expressed by GABAergic terminals. D-serine availability at the synapse is greatly reduced, which impairs NMDA receptor-mediated synaptic transmission and long-term synaptic plasticity. In this oversimplified representation, the extracellular space appears very large, whereas in fixed tissues, it is about 10 nm between contiguous cellular elements. Adapted with permission from Theodosis et al. (2004)

characterized by small and round cell bodies with few processes, show little immunoreactivity for GFAP and are dye-coupled. The third population is made of typical stellate astrocytes, similar to that of most astrocytes in the adult central nervous system, and found scattered in the nucleus. As shown in Fig. 2.4, under basal conditions of secretion, the thin distal astrocytic processes separate the magnocellular neurons, although these are often tightly packed in clusters of cells. There are also astrocyte-like cells (pituicytes) in the neurohypophysis.

The functional significance of each type of supraoptic astrocyte is still unknown. Astrocytes generally contribute to the regulation of the microenvironment in which neurons function. The HNS astrocyte plasmalemma is enriched with glycoproteins that contribute to the molecular composition and complexity of the extracellular space, such as the highly sialylated isoform of the neural cell adhesion molecule PSA-NCAM. By expressing different transporters, HNS astrocytes may be involved

in the clearance of synaptically released neurotransmitters, like glutamate and GABA (Fig. 2.4). Through the interposition of their fine distal processes between all neuronal elements, they make a physical barrier to restrict spillover (i.e. the escape of neurotransmitters from the synaptic cleft) and diffusion of locally released neuroactive molecules into the extracellular space (Fig. 2.4). Finally, HNS astrocytes can detect synaptic activity through the expression of specific receptors at their surface, which may lead to the release of signaling molecules that are collectively named gliotransmitters (Fig. 2.4). These include glutamate, D-serine, ATP, taurine, and cytokines. In the neurohypophysis, processes of pituicytes ensheath neurosecretory axons and may affect neurosecretion by limiting the diffusion of secreted peptides into perivascular spaces and thus, into the general circulation.

2.1.5 Structural Glial Plasticity in the Adult HNS

Magnocellular nuclei undergo extensive and reversible morphological transformations under conditions of intense activity such as lactation, parturition, and chronic dehydration (for review see Miyata and Hatton 2002; Salm 2000; Theodosis et al. 2008). In these conditions, increased numbers of synapses are observed and magnocellular neuronal somatic and dendritic surfaces are no longer separated by astroglial processes, but become directly and extensively juxtaposed as a result of active retraction of astrocytic processes covering their surfaces (Fig. 2.4). The reduction of astrocytic coverage impacts essentially the oxytocin neurons and their synapses. It is rapid, since changes can be detected within a few hours of the onset of stimulation. However, juxtaposed neuronal membranes do not contact each other and the intervening extracellular space remains constant (≈ 10 nm). Such structural modifications of the astrocytic environment affect the volume and geometry of the extracellular space, as well as its molecular composition. Changes are fully reversible upon cessation of stimulation. Rapid glial plasticity also occurs at the level of the neurohypophysis, including a dynamic change in the interaction between pituicytes and nerve terminals.

The structural glial plasticity implies dynamic cell–cell and cell–matrix interactions, requiring cell surface, extracellular fluid molecules, and soluble factors to come into play. For instance, PSA-NCAM, the expression of which is not related to neuronal activity, still is a required permissive factor for HNS glial plasticity, since specific enzymatic removal of PSA from NCAM in the supraoptic nucleus can inhibit the glial remodeling associated with lactation and chronic dehydration. Other molecules such as glutamate and oxytocin, acting in synergy with sex steroids, provide the signaling message that triggers the mechanisms leading to HNS glial plasticity.

2.1.6 Investigating the Functional Consequences of Structural Plasticity

The adult HNS thus undergoes a remarkable reversible, activity-dependent morphological neuroglial plasticity. In this chapter, we summarize how this made it a seminal model to study the physiological contribution of the astrocytic environment to synaptic strength, inter-synaptic crosstalk, and neuronal signaling through the release of the gliotransmitter D-serine. We also open perspectives related to these discoveries and to future research on neuroglial plasticity in the HNS.

As illustrated in Fig. 2.4, investigating the functional consequences of structural plasticity took advantage of the reversible reduction in the astrocytic coverage of oxytocin neurons that is induced during lactation, when the surfaces of oxytocin neurons become extensively juxtaposed and synapses are no longer tightly ensheathed by astrocytic processes. In normal conditions, the fine distal processes of astrocytes that ensheath synapses contribute to point-to-point synaptic transmission by expressing different transporters that are involved in the clearance of synaptically released neurotransmitters, such as glutamate and GABA. Moreover, by their position, astrocytic processes provide a barrier to prevent neurotransmitter spillover and limit volume transmission. Finally, they may themselves contribute to synaptic transmission by releasing neuroactive molecules, such as glutamate, D-serine, ATP, taurine. The consequences of the absence of glial coverage were investigated in the supraoptic nucleus of lactating animals considering three possibilities: transporters may no longer be available at the synapse; astrocyte processes may no longer hinder diffusion in the extracellular space; the contribution of astrocytes to synaptic communication through gliotransmission may be hampered. Identification of these consequences fueled the now fundamental concept that astrocytes are dynamic partners of brain signaling.

2.2 Contribution of the Astrocytic Environment to Homosynaptic Strength

Glutamate is the main excitatory neurotransmitter in the HNS. Its clearance depends on uptake and diffusion. Uptake of synaptically released glutamate relies essentially on the astrocytic high-affinity glutamate transporter GLT-1. Changes in glutamate uptake may produce local variations of glutamate concentrations close to the release sites, and thus affect synaptic transmission at both post- and presynaptic levels. Additionally, a prolonged alteration in glutamate uptake may lead to excitotoxicity through the chronic activation of glutamate receptors.

Box 2.1: Investigating the Presynaptic Origin of Drug Action Using Paired-Pulse Facilitation

To determine whether presynaptic mechanisms are involved in the action of a drug on evoked postsynaptic currents/potentials, one may use paired-pulse facilitation (PPF). PPF is a form of short-term synaptic plasticity. Two presynaptic spikes are evoked in close succession and the responses of the postsynaptic cell are measured in terms of postsynaptic potentials or currents. PPF is expressed as the amplitude ratio (S_2/S_1) of the second synaptic response (S_2) over the first synaptic response (S_1). In PPF, the second postsynaptic response, S_2 , is larger than the first (S_1). In most cases, PPF reflects a presynaptic phenomenon, due to an increase in the number of vesicles released by the presynaptic element, even if postsynaptic contributions must also be considered. Different mechanisms have been proposed to explain the presynaptic facilitation. They converge on a build-up of calcium in the presynaptic terminal that leads to an increased probability of neurotransmitter release upon the second spike.

In presynaptic elements with a low initial probability of release, the first spike causes a small postsynaptic response, but the build-up of calcium in the presynaptic terminal increases the probability of neurotransmitter vesicle release upon the second spike. The second postsynaptic response is thus greater than the first one, showing facilitation. In presynaptic elements with a high initial probability of release, the first spike depletes part of the available readily releasable pool of vesicles and even if calcium concentration is higher after the second pulse than after the first one, less transmitter is available to be released due to the reduced number of readily releasable vesicles. The second postsynaptic response is thus smaller than the first one, showing a depression.

Although not conclusive, a change in PPF ratio after a drug treatment suggests that the drug may be acting at the presynaptic level. If the drug is acting at the postsynaptic level, it may change the amplitude of individual evoked currents, but the release of neurotransmitter should not be affected and neurotransmitter interaction with receptors should be equally affected by the drug during the two pulses, leaving PPF unchanged. If the drug is acting at the presynaptic level, the probability of neurotransmitter release is affected, which alters the facilitation process and thus PPF.

In addition to PPF, one may assess whether presynaptic mechanisms are involved in the action of a drug by analyzing miniature postsynaptic currents or potentials and trial-to-trial fluctuation of the evoked responses using the inverse of the squared coefficient of variation ($1/CV^2$) ($CV=SD/mean$, coefficient of variation) of the postsynaptic response amplitude.

To test the possibility that a deficiency in the availability of glutamate transporters at the synapse may result from the absence of glial coverage that normally wraps glutamatergic synapses and secondarily affects synaptic transmission, the following

questions were addressed (Oliet et al. 2001): (1) What are the effects of a deficiency in glutamate uptake upon excitatory synaptic transmission? (2) What are the mechanisms of these effects? (3) Do changes in glial coverage affect excitatory synaptic transmission in the same way? (4) Do changes in glial coverage affect glutamate concentration and/or time course in the synaptic cleft?

2.2.1 Role of Glutamate Uptake on Homosynaptic Efficacy

To answer the first question, the effects of inhibiting glutamate transporters in the supraoptic nucleus were measured on glutamate-mediated, evoked excitatory postsynaptic currents (EPSCs) using whole-cell patch clamp recordings. Supraoptic nuclei were taken for virgin rats, in which the glial coverage of synapses is normal. Both a specific inhibitor of GLT-1 or PDC, a broad-spectrum glutamate transporter blocker, inhibited reversibly EPSCs by about 50%, which suggests that the most part of their effect was mediated by inhibition of GLT-1. As illustrated in Fig. 2.5a, analysis of miniature EPSCs (mEPSCs) showed that PDC decreased the frequency, but not the size of these events, suggesting a presynaptic origin of the modulation of synaptic currents by excess ambient glutamate when glutamate transporters are inhibited. This was confirmed by analysis of the paired-pulse facilitation (PPF) ratio of evoked currents that showed an increase when glutamate transporter antagonists were applied (see Box 2.1). It appears therefore that the increase in local glutamate concentration after inhibition of glutamate transport is sufficient to activate presynaptic glutamate receptors controlling neurotransmitter release.

2.2.2 Control of Homosynaptic Efficacy Through Metabotropic Glutamate Receptors

One likely mechanism underlying these effects could be the activation of group III metabotropic glutamate receptors (mGluRs), as they were known to induce presynaptic inhibition of glutamate release from excitatory synapses in the supraoptic nucleus. To test this hypothesis, the effects of blocking glutamate transport on excitatory synaptic inputs were tested in the presence of agonists and antagonists of group III mGluRs. As shown in Fig. 2.5b, the mGluR agonist reduced evoked EPSCs and subsequent addition of the transporter blocker had no further effect on EPSC amplitude, which suggested that the blockade of glutamate transport was occluded by agonist activation of mGluRs. Reciprocally, the mGluR antagonist increased EPSC amplitude, which indicated that mGluRs are tonically activated in normal conditions. Blocking group III mGluRs also prevented the effects of increasing extracellular glutamate with blockade of glutamate uptake. Together, these data show that presynaptic mGluR activation underlies the inhibitory effect on synaptic transmission of increased glutamate concentration resulting from glutamate uptake reduction.

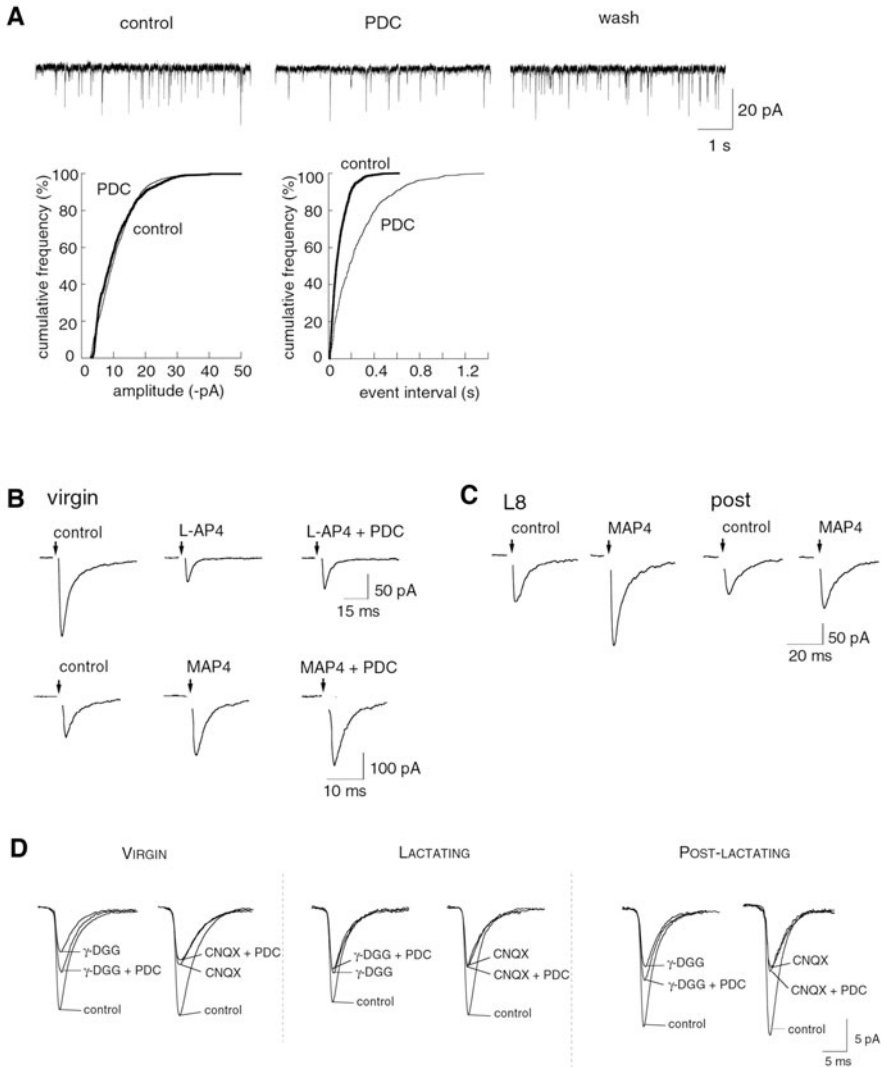


Fig. 2.5 Control of glutamate uptake and synaptic efficacy by glial coverage of neurons. **(a)** Glutamate transporter blockade induces presynaptic inhibition of EPSCs. Top, traces from a recording of a magnocellular neuron showing a reversible reduction in mEPSC activity with application of a glutamate transporter blocker, PDC. Bottom, the corresponding cumulative amplitude distributions obtained in the presence and absence of PDC were not statistically different ($P > 0.05$), whereas the cumulative event interval distribution was significantly shifted to the right with PDC ($P < 0.05$), which corresponds to a reduction in mEPSC frequency. **(b)** Glutamate transporter blockade causes glutamate activation of presynaptic group III mGluRs. Sample traces of evoked EPSCs obtained in virgin rats in the absence and presence of a group III mGluR agonist (L-AP4, upper panel) and antagonist (MAP4, lower panel). Subsequent blockade of glutamate transport with PDC had no effect on the EPSC amplitude under both conditions. **(c)** Reduction in astrocytic coverage affects presynaptic group III mGluR activation. Sample recordings of evoked EPSCs obtained in magnocellular neurons in the presence and absence of the group III mGluR antagonist MAP4 in slices from lactating (L8) and postlactating (post) rats. **(d)** Glial coverage of

2.2.3 Glial Coverage Controls Homosynaptic Efficacy

If the absence of glial coverage in lactating conditions results in a deficiency in the availability of glutamate transporters at the synapse, it should lead to increased glutamate concentration. Therefore, presynaptic mGluRs should be more activated, leading to a decrease of release probability and reduced evoked EPSCs. The effect should be the same as the one induced in virgin rats by the application of a specific antagonist of glutamate transporters. In addition, this transporter antagonist should have either a small or no effect on excitatory synaptic transmission in lactating rats, in which glutamate concentration is already increased. Accordingly, glutamate transporter blockade was less effective in reducing evoked EPSCs in lactating rats, suggesting just such a partial occlusion effect. In postlactating rats, the effects of glutamate transporter blockade reversed to what was found in virgin rats, as expected in a condition when astrocytic coverage reverts back to basal conditions. In addition, antagonizing presynaptic mGluRs induced a larger increase of evoked EPSC amplitude in lactating rats than observed in virgin or postlactating rats (Fig. 2.5c), suggesting that the tonic activation of mGluRs by ambient glutamate is increased in lactating rats. Altogether these data show that presynaptic mGluR activation underlies the inhibitory effect on synaptic transmission resulting from a reduction in glial coverage of synapses in lactating rats.

2.2.4 Glial Coverage Controls Concentration and/or Time Course in the Synaptic Cleft

To test whether changes in glial coverage affect glutamate concentration and/or time course in the synaptic cleft, γ -D-glutamylglycine (γ -DGG), a low-affinity, competitive AMPA receptor antagonist, whose effect is sensitive to the concentration and/or time course of glutamate in the synaptic cleft, was used. γ -DGG reduced the amplitude of miniature EPSCs (mEPSCs) in virgin and postlactating rats by about 50% (Fig. 2.5d). When used in combination with glutamate uptake blockade, the reduction was only by about 35%, as expected from the presence of an increased concentration of glutamate in the synaptic cleft. Accordingly, in lactating rats, γ -DGG only reduced the amplitude of mEPSCs by about 35%, and additional blockade of glutamate reuptake did not change the amount of reduction. The reduction of mEPSCs by a high-affinity, slowly dissociating, competitive AMPA



Fig. 2.5 (continued) supraoptic neurons affects the relative glutamate concentration and/or time course in the synaptic cleft. Examples illustrating the inhibition of mEPSCs observed under different conditions (control, γ -DGG alone, γ -DGG + PDC, CNQX alone, CNQX + PDC) in virgin, lactating, and postlactating animals. γ -DGG is a low-affinity, competitive AMPA receptor antagonist, whose effect is sensitive to the concentration and/or time course of glutamate in the synaptic cleft, which is not the case for CNQX, another AMPA receptor antagonist. Adapted with permission from Oliet et al. (2001)

receptor antagonist was similar in all three conditions, around 55%, and remained unaffected by addition of reuptake blocker, which is consistent with the fact that the effect of the high-affinity AMPA antagonist is not sensitive to the concentration and/or time course of glutamate in the synaptic cleft. These data strongly suggest that changes in glial coverage affected glutamate concentration and/or time course in the synaptic cleft.

2.2.5 Physiological Consequences

Together, these data show that the absence of glial coverage of glutamatergic synapses in lactating rats results in a reduction in the availability of glutamate transporters, leading to a deficiency in glutamate clearance, leading to an increase in local glutamate concentration, which further activates presynaptic mGluRs and causes a reduction in homosynaptic strength. Thus, astrocytes control the level of the negative feedback exerted by ambient glutamate on its own release at HNS excitatory synapses. Similar physiological findings were found in the supraoptic nucleus of chronically dehydrated rats, in which it is known that structural glial plasticity also takes place (Boudaba et al. 2003).

As a consequence, low to moderately active synapses should undergo a clear presynaptic inhibition. At very active synapses, however, the higher firing rate of presynaptic inputs should potentiate the increase in Ca^{2+} in presynaptic terminals, which would overcome presynaptic inhibition and facilitate glutamate release. In other words, increased presynaptic inhibition due to a reduction in glial coverage in lactating animals sets a functional high-pass filter. In the context of parturition and the milk ejection reflex, that could serve to prevent activation of oxytocin secreting neurons in response to low-frequency stimuli that are not directly related to uterine or mammary gland inputs, thus saving oxytocin for its two most important functions in such a context. The high-pass filter function would also result in a decreased synaptic noise, thus increasing postsynaptic membrane resistance and consequently raising the gain of the input–output neuronal response. Whether this would tune the responsiveness of oxytocin neurons to pertinent synaptic inputs remains to be explored (Jourdain et al. 1998).

2.3 Contribution of the Astrocytic Environment to Inter-synaptic Crosstalk

The demonstration that the reduction in glial processes modifies the local glutamate concentration at the synapse by reducing its uptake, and thus affects homosynaptic strength, led to the question whether such remodeling might also change neurotransmitter diffusion, and as a result contribute to inter-synaptic crosstalk. Indeed, astrocyte processes make a physical barrier to diffusion in the extracellular space, hindering the movement of molecules through this space, and changes in their coverage may thus influence heterosynaptic modulation of adjacent synapses.

GABA is the main inhibitory neurotransmitter in the HNS, and GABAergic synapses are often located close to glutamatergic synapses. In addition, it has been shown that presynaptic mGluRs located on inhibitory terminals in the supraoptic nucleus can be activated by changes in ambient glutamate, provided these changes reach a certain magnitude, which is not the case in basal conditions (Piet et al. 2003). However, when activated, presynaptic mGluRs may reduce GABA release. Inter-synaptic crosstalk between glutamatergic and GABAergic synapses can thus be explored in the supraoptic nucleus, provided the extracellular concentration of glutamate reaches sufficient levels to saturate the transporter system and diffuse away to affect adjacent synapses.

To test the possibility that astrocyte processes may no longer hinder diffusion in the extracellular space in lactating rats, the following questions were thus addressed (Piet et al. 2004): (1) Do changes in glial coverage during lactation affect diffusion in the supraoptic nucleus? (2) Do such changes in diffusion facilitate heterosynaptic activity through metabotropic glutamate receptors? (3) Is inter-synaptic crosstalk prevented when diffusion is limited?

Kainate receptors (KARs) are ubiquitous ionotropic glutamate receptors in the central nervous system. Activation of presynaptic KARs may regulate GABA release and this regulation has been shown to be bidirectional in several brain areas, with experimental increase in the concentration of KAR agonist resulting in a switch from facilitation to inhibition of GABA release. The HNS offered thus an excellent model to study the impact of changes in glutamate levels resulting from a reduction in glial coverage on the mechanisms and function of KAR switch upon GABA synaptic release. To address this issue, three steps were followed (Bonfardin et al. 2010): (1) Establishing the presence of functional KARs on GABAergic terminals in the supraoptic nucleus; (2) Showing that physiological enhancement of extracellular levels of glutamate resulting from glial withdrawal can lead to a switch in KAR activity; (3) Identifying the underlying mechanisms for KAR facilitation and inhibition.

2.3.1 Glial Coverage Controls Diffusion in the Extracellular Space

To answer the question whether changes in glial coverage during lactation affect diffusion in the HNS, diffusion parameters in the extracellular space were measured in the supraoptic nucleus. The diffusion parameters in the extracellular space were measured using the real-time TMA⁺ (tetramethylammonium) iontophoretic method (Fig. 2.6a). In brief, the extracellular marker TMA⁺, to which cell membranes are relatively impermeable, is administered in the tissue by iontophoresis and its concentration is measured at distance (about 100 μ m) by a selective microelectrode filled with an ion exchanger. The concentration profile depends on the diffusion properties of the extracellular space. The diffusion curves that are obtained can be compared with that obtained in obstacle-free conditions and fitted to extract the following parameters: volume fraction (α), tortuosity (λ), and nonspecific uptake (k'). Volume fraction is the proportion of tissue volume occupied by the extracellular space.

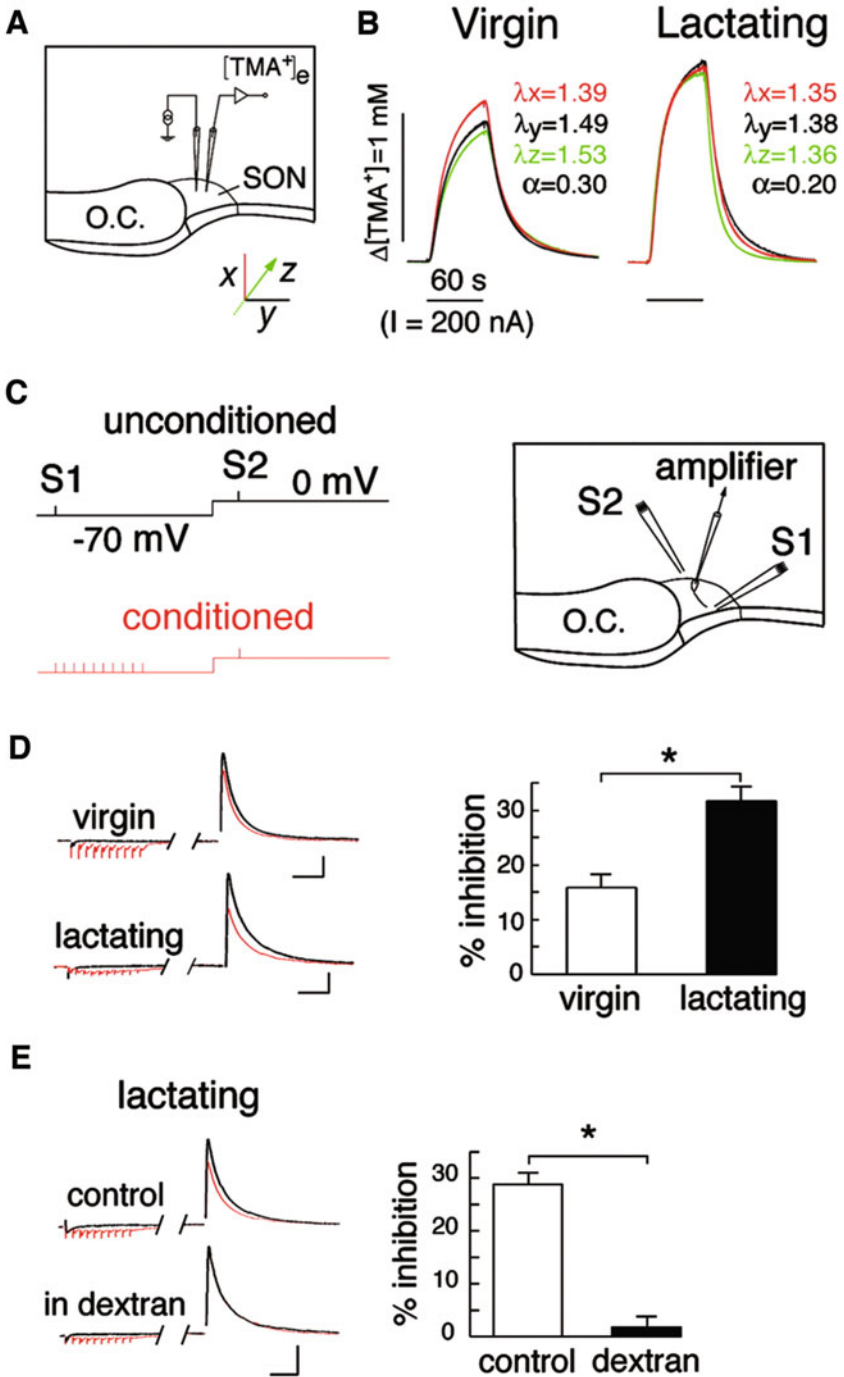


Fig. 2.6 Physiological contribution of the astrocytic environment of neurons to inter-synaptic crosstalk. (a) Experimental set-up illustrating the real-time TMA⁺ iontophoretic method applied to the supraoptic nucleus (SON) in acute slices. Measurements were made along three perpendicular

Tortuosity is a measure of restriction of diffusion in the extracellular space in comparison with an obstacle-free medium.

Lactation induced changes in diffusion parameters in the supraoptic nucleus, but not in the cortex, used as a control (Fig. 2.6b). In virgin animals, tortuosity was lower in the ventrodorsal axis than in the rostrocaudal and mediolateral axes, showing that the tissue is anisotropic, i.e. with properties that change according to the direction. This likely reflects the ventrodorsal orientation of radial-like glia in the supraoptic nucleus. In contrast, in lactating animals, tortuosity was reduced and similar in the three axes, indicating that the tissue becomes isotropic during lactation. Diffusion is thus enhanced in lactating animals and becomes equivalent in all directions as a result of glial remodeling. Volume fraction was also decreased in the SON from lactating animals, as a probable consequence of the reduction in interneuronal space resulting from the withdrawal of astrocytic processes.

2.3.2 Changes in Diffusion Facilitate Heterosynaptic Activity Through Metabotropic Glutamate Receptors

To ask whether such changes in diffusion facilitate heterosynaptic activity, the presynaptic mGluRs located on inhibitory terminals in the supraoptic nucleus were used as sensors of the spillover of glutamate (Fig. 2.6c). GABA-mediated, evoked inhibitory postsynaptic currents (IPSCs) were measured using whole-cell patch clamp recordings from supraoptic neurons, in response to stimulating an afferent pathway. This was preceded by the stimulation of a second, independent pathway with brief high-frequency trains of stimuli, in order to produce a sustained release of glutamate. Recordings were performed in the presence of a GABA_B receptor antagonist to avoid presynaptic changes that could result from the activation of these receptors by GABA spillover associated with the conditioning train. The conditioning train caused a significant reduction in IPSC amplitude in both virgin and lactating rats, but the reduction was twice as big in lactating ($\approx 30\%$) as in virgin ($\approx 15\%$) animals (Fig. 2.6d). Analysis of the paired-pulse facilitation ratio and trial-to-trial fluctuations of the evoked-GABAergic responses (see Box 2.1) showed that



Fig. 2.6 (continued) axes (x , y , and z), as illustrated. O.C., optic chiasm. **(b)** Example of diffusion curves obtained in virgin and lactating rats. Values for tortuosity and volume fraction extracted from three curves recorded along the x , y , and z axes are indicated. **(c)** Experimental set-up illustrating the stimulation paradigm (left) and the position of the stimulating electrodes (S1, S2, right) to measure heterosynaptic depression of evoked IPSCs. **(d)** Sample traces (Left) and summary histogram (right) showing the heterosynaptic depression of evoked IPSCs obtained in virgin ($n = 18$) and lactating ($n = 18$) rats. (scale bars = 200 pA, 40 ms). **(e)** Diffusion in the extracellular space affects glutamate spillover. Left, sample traces obtained from a magnocellular neuron from a lactating rat in the absence and presence of dextran in the extracellular solution. Dextran increases extracellular viscosity and decreases passive diffusion. (Bars = 200 pA, 40 ms). On the right, histogram summarizing the heterosynaptic depression in the absence and presence of dextran. Adapted with permission from Piet et al. (2004), copyright (2004) National Academy of Sciences, U.S.A

the origin of this depression was presynaptic. It was blocked by the mGluR antagonist in virgin as well as in lactating rats, indicating that it also involved mGluRs. Additional experiments involving variations in the delay between the beginning of the conditioning train and the IPSC stimulation, and variations in the stimulation frequency of the conditioning train, showed that reduction in IPSC amplitude was directly related to the amount of glutamate that was released, but this was always larger in lactating animals. These data therefore imply that inter-synaptic crosstalk between glutamatergic and GABAergic afferent inputs depends on the concentration of glutamate reaching the GABAergic terminals. They also show that the action of glutamate at a distance from its release sites is facilitated in the supraoptic nucleus of lactating animals. In other words, the enhanced diffusion in the extracellular space that results from a reduction in glial coverage is paralleled by an increased heterosynaptic activity.

2.3.3 Limiting Changes in Diffusion Affect Glutamate Spillover

If changes in diffusion interfere with inter-synaptic crosstalk, reducing the coefficient of diffusion of glutamate should limit the concentration of the excitatory amino reaching neighboring synapses and, as a consequence, alter heterosynaptic modulation. As illustrated in Fig. 2.6e, adding 5% dextran (40 kDa) in the perfusion solution, which is known to increase viscosity and reduce the coefficient of diffusion of glutamate in the extracellular space, led to an abolition of the heterosynaptic depression of evoked IPSCs measured in the supraoptic nucleus of lactating rats. Together, these data show that the glial environment of HNS neurons is an important regulator of glutamatergic communication between independent synapses by setting the parameters of diffusion in the extracellular space (Piet et al. 2004).

2.3.4 Functional Presynaptic Kainate Receptors on GABAergic Terminals in the HNS

The possibility that glutamate might modulate GABAergic synapses through KARs was also investigated. Kainate was found to increase the mIPSC frequency in supraoptic neurons, without changing their amplitude, which indicates a presynaptic action on GABAergic terminals. The GluK1-specific KAR agonist ATPA had the same effect, and it was blocked by UBP302, a selective antagonist of GluK1-containing KARs. Functional presynaptic GluK1-containing KARs are thus present on GABAergic terminals in the supraoptic nucleus (Fig. 2.7a).

Interestingly, ATPA was less efficient than KA in increasing mIPSC frequency, and the increase in mIPSC frequency triggered by kainate was only partially abolished by UBP302, which suggests that an additional KAR subtype is present on these inhibitory terminals. This was confirmed, since the remaining kainate-induced response was completely blocked by a broad-spectrum AMPA/KA receptor

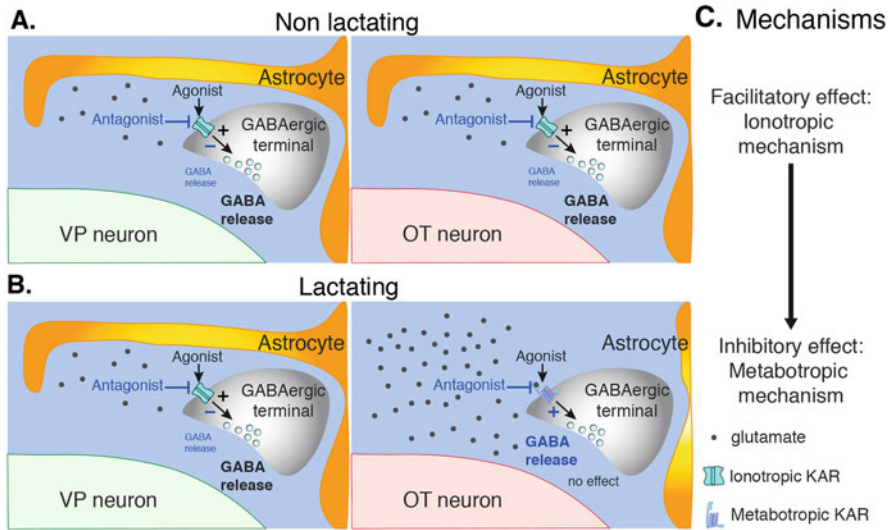


Fig. 2.7 Astrocyte-dependent switch of presynaptic kainate receptor action. (a) Presynaptic kainate receptors (KARs), including GluK1-containing KARs, are present on GABAergic terminals synapsing on vasopressin (VP) and oxytocin (OT) neurons in the supraoptic nucleus. In non-lactating rats, they are positively coupled to GABA release onto OT and VP neurons, so that application of KAR agonists facilitates GABA release and application of KAR antagonists inhibits GABA release. Ambient glutamate facilitates GABA release onto OT and VP neurons through GluK1-containing KARs. (b) In lactating rats, blocking KARs still has an inhibitory effect on GABA release onto VP neurons, but the effect switches to increased GABA release onto OT neurons. In lactating rats, increased levels of ambient glutamate in the vicinity of GABAergic synapses impinging on OT neurons change the coupling of KARs to GABA release, leading to an inhibition of GABA release. The increased levels of ambient glutamate also occlude the effect of KAR agonists. (c) The mechanisms for facilitation and inhibition of GABA released by KARs are mediated through an ionotropic and a metabotropic mode of action of KARs, respectively. It is not known whether the ionotropic and metabotropic actions of KARs are mediated by the same or by two distinct types of GluK1-containing receptors

inhibitor. Two different types of KARs are thus present on GABAergic terminals impinging on magnocellular neurons in the supraoptic nucleus.

In both identified oxytocin and vasopressin neurons, application of the antagonist UBP302 alone induced a reversible reduction in mIPSC frequency, whereas mIPSC amplitude remained unchanged (Fig. 2.7a). Blocking the remaining AMPA/KA receptors did not induce any further reduction in mIPSC activity. Together, these data show that only GluK1-containing KARs are activated by ambient glutamate and they regulate GABA release in a similar manner in both oxytocin and vasopressin neurons.

2.3.5 Changes in Glial Coverage Switches Kainate Receptor Presynaptic Action

Experiments designed to test whether physiological enhancement of extracellular levels of glutamate resulting from glial withdrawal can lead to a switch in KAR activity were performed in the presence of an mGluR antagonist, MAP4, as presynaptic mGluRs are activated by increased levels of ambient glutamate and can control GABA release. In lactating rats, blocking KARs with UBP302 still had an inhibitory effect on mIPSC frequency in vasopressin neurons, but the effect switched to excitation in oxytocin neurons (Fig. 2.7b). This suggests that in lactating rats, increased levels of ambient glutamate in the vicinity of GABAergic synapses impinging on oxytocin neurons change the coupling of KARs to GABA release. The KAR agonist ATPA still facilitated GABA transmission in vasopressin neurons, but was without effect in oxytocin neurons. The hypothesis that this lack of effect of ATPA results from an occlusion by increased ambient glutamate levels was confirmed when slices from lactating rats were incubated with an enzymatic glutamate scavenger to reduce ambient levels of the excitatory amino acid: when scavenging was efficient enough to desaturate the GluK1 receptors, but without affecting their coupling to GABA release, ATPA was found to decrease mIPSC frequency in oxytocin neurons.

To confirm that increasing glutamate concentration switches GluK1-containing KAR activity, extracellular glutamate was experimentally increased in the supraoptic nucleus of non-lactating rats using the broad-spectrum inhibitor of glutamate transporters D,L -TBOA. Under these conditions, the KAR antagonist UBP302 induced a reversible increase in mIPSC activity in both oxytocin and vasopressin neurons. The opposite KAR-mediated regulation of GABA transmission as a function of extracellular glutamate levels was also observed in supraoptic neurons when monitoring IPSCs evoked by afferent input stimulation.

Together, these data establish that lactation induces a selective switch in GluK1 activation in oxytocin neurons, in relation to increased extracellular glutamate levels resulting from a reduction in glial coverage (Bonfardin et al. 2010).

2.3.6 Mechanisms for Facilitation and Inhibition by KARs

On one hand, the facilitation of inhibitory transmission by presynaptic KARs in the supraoptic nucleus was found to be mediated through the ionotropic mode of action of KARs, as it was prevented by phallotoxin-433, a blocker of Ca^{2+} -permeable AMPA / KARs (Fig. 2.7c). Such an effect is likely to be caused by the KAR-induced membrane depolarization and / or the direct permeation of Ca^{2+} ions through KARs. On the other, the inhibitory effect of KAR activation on GABA release was not due to an indirect mechanism involving the activation of other presynaptic receptors, since adding a mixture of antagonists to block GABA_B receptors, adenosine A1 receptors, mGluRs, NMDA receptors, CB1 receptors, nicotinic receptors, dopamine D4 receptors, and adrenergic receptors did not affect the inhibitory action of GluK1-

containing KARs on GABA release. The inhibitory effect appeared to be mediated by a metabotropic mode of action of KARs, as it was prevented in the presence of a phospholipase C inhibitor and unaffected by philantotoxin-433 (Fig. 2.7c).

2.3.7 Physiological Consequences

The reduction in glial processes, by changing neurotransmitter diffusion, contributes to inter-synaptic crosstalk. This has also proven to be the case for endocannabinoids (Di et al. 2013) and ATP (Gordon et al. 2005) in the HNS. Other neuroactive substances that are released in the HNS nuclei such as neurosteroids, oxytocin, and vasopressin would also see their concentration and range of action increase, except, of course those released from the glial processes that have retracted. For somatodendritically released oxytocin that provides a positive feedback regulation of the electrical activity of oxytocin neurons, a facilitated diffusion would have important physiological consequences. After local release, it could reach distant targets and contribute further to the recruitment and synchronization of additional bursting of oxytocin neurons.

Enhanced heterosynaptic inhibition of GABA transmission by glutamate acting on mGluRs or KARs could boost transmission of high-frequency excitatory information, by inhibiting nearby GABAergic synapses and thus creating a local disinhibition. This could favor the excitation of oxytocin neurons during parturition and suckling (Jourdain et al. 1998). In other brain areas where similar glial changes occur, disinhibition could also play a physiological role or be involved in the pathophysiology of neurodegenerative diseases, epilepsy, and ischemia, which are associated with greatly enhanced levels of extracellular glutamate. Under such conditions, the disinhibition may amplify the excitotoxic action of glutamate and further reduce neuronal survival.

2.4 Gliotransmission in the HNS: A Case for D-Serine

Since astrocytic processes are closely juxtaposed to synapses, they are ideally located to detect synaptic activity through the expression of specific receptors at their surface, and in response, to release signaling molecules, named gliotransmitters by analogy to neurotransmitters (see Box 2.2), such as glutamate, D-serine, ATP, and taurine (Savtchouk and Volterra 2018). Among them, the amino acid D-serine (see Box 2.3) is considered as an important co-agonist of the synaptic NMDA receptors that are known to play a key role in synaptic plasticity in the HNS, as well as in the brain and spinal cord. Considering the hypothesis that the retraction of astrocytic processes could hamper the contribution of astrocytes to glutamatergic synaptic communication through D-serine gliotransmission, the consequences of the absence of glial coverage on D-serine availability, NMDA receptor activity and plasticity were investigated in the supraoptic nucleus (Panatier et al. 2006). The step-by-step

approach involved identifying D-serine production in supraoptic astrocytes, demonstrating that D-serine, and not glycine, is the endogenous co-agonist of NMDA receptors in the supraoptic nucleus, and showing that the D-serine level in the synaptic cleft depends on the coverage of synapses, with consequences for long-term synaptic plasticity.

Box 2.2: Is Gliotransmission a Physiological Phenomenon?

Gliotransmission can be defined as the active transfer of information from glia to neurons through neuroactive molecules that activate neuronal receptors located at synaptic and/or extrasynaptic sites. According to the concept of the tripartite synapse, peri-synaptic astrocytic processes are not only structural, but also functional partners of the pre- and postsynaptic neuronal elements, through the release of gliotransmitters.

Astrocytes express a large number of channels, transporters, and receptors, many of which are the same as the ones expressed by neurons. It has been shown that astrocytes may respond to synaptically released neurotransmitters, in acute brain slices as well as in vivo. Although astrocytes are not electrically excitable, they may sense synaptic activity by responding with different intracellular signaling cascades, including cyclic adenosine monophosphate elevations and calcium mobilization from internal stores via G protein-coupled receptor stimulation or calcium influx through transient receptor potential channels. Upon activation, astrocytes may release gliotransmitters (such as glutamate, GABA or ATP) using various mechanisms, including exocytic release from vesicles or release from the cytosol via plasma membrane ion channels and pumps. The molecular mechanisms leading to gliotransmitter release are still intensely disputed. Once released, gliotransmitters produce a variety of effects on synaptic activity, according to the specific receptors involved and their pre- or postsynaptic location.

The concept of gliotransmission has been harshly criticized in recent years based on the general argument that gliotransmission should be defined as the Ca^{2+} -dependent release of neurotransmitters by astrocytes and that no changes in physiologically relevant astrocyte Ca^{2+} have ever resulted in any detectable gliotransmission by astrocytes (see Fiacco and McCarthy 2018). However, gliotransmission appears to be a much more complex phenomenon than originally thought, since it is not triggered by a single neurotransmitter receptor, does not necessarily involve a single Ca^{2+} source or code, and does not necessarily imply neuron-like vesicular transmitter release (Savtchouk and Volterra 2018). Astrocytes do not behave like neurons, and they process information over a much slower time scale. The main function of gliotransmission seems to be to fine-tune neuronal processing according to more general brain states.

(continued)

Box 2.2 (continued)

In the HNS, the amino acid taurine was identified as a putative gliotransmitter in the late 90s (for review see Hussy et al. 2000). Taurine is one of the main osmolytes used by cells to compensate for changes in extracellular osmolarity. It is highly concentrated in supraoptic astrocytes, and released by them in response to hypo-osmotic stimuli through volume-sensitive anion channels. Taurine inhibits magnocellular neuron activity by binding to neuronal glycine receptors. Definite proof that taurine is a gliotransmitter in the HNS came from the work of Choe et al. (2012) showing that supraoptic neurons are unable to release taurine themselves and selective depletion of taurine eliminates the inhibitory tone on glycine receptors, ruling out the possibility that glycine receptors could be activated by other agonists. These data show that, in the HNS, gliotransmission plays an active part in a physiological regulatory loop.

Box 2.3: Is D-Serine a Gliotransmitter?

D-serine is a potent co-agonist at the glycine site (GluN1) of the NMDA receptor. For the receptor to open, either glycine or D-serine must bind to it and glutamate must simultaneously bind to its recognition site on the GluN2 subunits. The synthesis of D-serine requires serine racemase to convert L-serine into D-serine. Early reports identified D-serine as a potential gliotransmitter based on the localization of D-serine and serine racemase in astrocytes. Further and more recent results support the concept that D-serine originates from astrocytes, acts at synaptic NMDA receptors and is taken up by neurons, where it is degraded (reviewed in Papouin et al. 2017).

The concept has been harshly criticized recently by different groups arguing that D-serine is of neuronal and not glial origin, based on the development of serine racemase knockout and conditional knockout mice (Wolosker et al. 2016). According to the alternative hypothesis, astrocytes may still affect D-serine levels by synthesizing L-serine, which shuttles to neurons to fuel the neuronal synthesis of D-serine. According to this view, immunostaining for D-serine in astrocytes would be an artifact resulting from the high concentrations of L-serine in these cells. However, in our hands, not only D-serine, but also serine racemase was found to be exclusively localized in supraoptic astrocytes, and not in magnocellular neurons. This does not preclude, however, the possible presence of D-serine and serine racemase in neurons elsewhere in the brain.

2.4.1 D-serine Is Synthesized and Expressed by Astrocytes in the Supraoptic Nucleus

Several lines of evidence suggested D-serine expression in astrocytes of the supraoptic nucleus. First, immunoblot analysis revealed the high expression of the synthesizing enzyme of D-serine, serine racemase, in the supraoptic nucleus. Second, immunocytochemistry showed that serine racemase was localized throughout the supraoptic nucleus in astrocytic cell bodies and in the neuropil surrounding magnocellular somata (Fig. 2.8a). Third, high levels of D-serine were detected in the supraoptic nucleus by HPLC analysis. Finally, immunofluorescence for D-serine showed a similar distribution to that of serine racemase and double immunofluorescence for D-serine and the astrocytic marker GFAP confirmed the presence of the amino acid in cell bodies and processes of all supraoptic astrocytes. Together, these data identified D-serine production in supraoptic astrocytes.

2.4.2 D-Serine Is the Endogenous Co-agonist of NMDA Receptors in the Supraoptic Nucleus

To demonstrate that D-serine is an endogenous ligand of NMDA receptors in the supraoptic nucleus, NMDA receptor-mediated synaptic responses were recorded from magnocellular neurons in hypothalamic slices treated with D-amino acid oxidase (DAAO), an enzyme specifically degrading D-serine. Due to the high variability in NMDA evoked responses from cell to cell, and since supraoptic neurons are driven by excitatory glutamatergic afferents acting via postsynaptic AMPA receptors and NMDA receptors, the AMPA/NMDA ratio was used to evaluate the contribution of NMDA receptors to EPSCs. The AMPA/NMDA ratio corresponds to the ratio of the peak amplitude of the EPSCs recorded at -60 mV, representing the AMPA receptor-mediated response, over the peak amplitude of the EPSCs recorded at $+40$ mV in the presence of an AMPA receptor antagonist, revealing the NMDA receptor-mediated response. As illustrated in Fig. 2.8b, the NMDA component of EPSCs was reduced significantly in DAAO-treated slices from virgin animals. Such a reduction was not due to a deleterious action of D-serine oxidation by-products on NMDA receptors since acute application of D-serine restored the AMPA/NMDA ratio to values measured in the absence of the enzyme.

In addition, glycine oxidase (GO), which selectively degrades glycine, was ineffective in altering NMDA receptor-mediated currents, showing that glycine is not an endogenous ligand of NMDA receptors in the supraoptic nucleus. Care was taken to check, using HPLC measurements, that DAAO and GO specifically and efficiently degraded D-serine and glycine, respectively. Together, these data showed that D-serine, and not glycine, is the endogenous co-agonist of NMDA receptors in the HNS, which is consistent with the paucity of glycinergic fibers in this region.

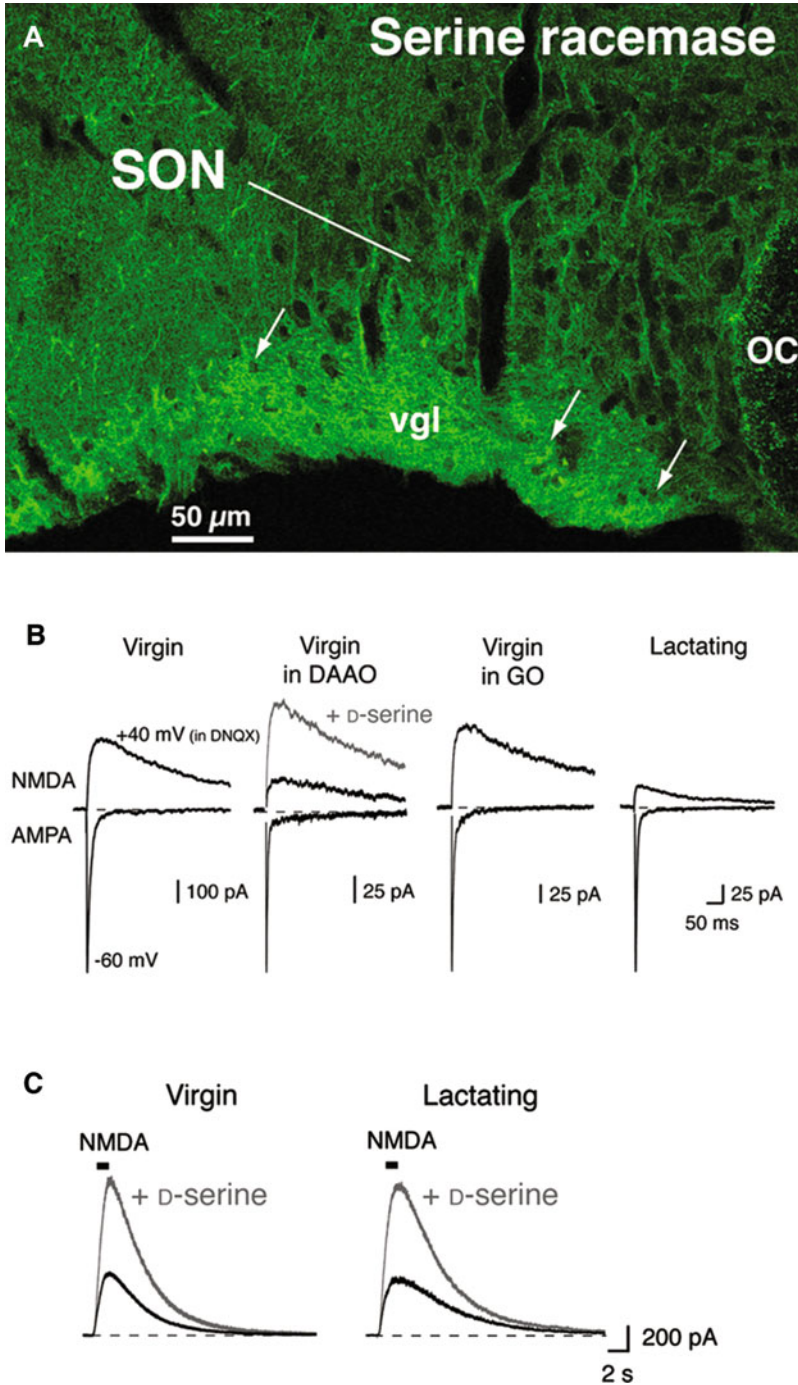


Fig. 2.8 Astrocyte-derived D-serine controls NMDA receptor activity. (a) Localization of serine racemase in the rat supraoptic nucleus (SON). Immunofluorescence revealed a strong reaction for serine racemase throughout the nucleus, localized in large processes along blood vessels, in the

2.4.3 Astrocytes Regulate D-Serine Concentration in the Synaptic Cleft

To demonstrate that the D-serine level in the synaptic cleft depends on the coverage of synapses, the AMPA/NMDA ratio was measured in the supraoptic nucleus of lactating rats and found to be larger than that measured in virgin animals and similar to that observed in DAAO-treated virgin slices (Fig. 2.8b). Since AMPA receptor-mediated responses in magnocellular neurons are not altered by lactation (Oliet et al. 2001), such a change in the ratio implied a decrease in NMDA receptor-mediated EPSCs. Pharmacological data obtained using the selective NR2B antagonist ifenprodil showed that this was not due to a switch in NMDA receptor NR2 subunits that are known to confer distinct biophysical properties to NMDA receptors. In addition, NMDA receptor-mediated synaptic responses recorded in slices from lactating rats recovered when the perfusion medium was supplemented with saturating concentrations of D-serine. Together, these data showed that the level of occupancy of the glycine site of synaptic NMDA receptors is critically controlled by the extent of astrocytic coverage of the neuron.

Finally, as shown in Fig. 2.8c, responses elicited by local applications of NMDA, reflecting activation of mainly extrasynaptic NMDA receptors, were enhanced considerably by D-serine and to a similar extent in slices from virgin and lactating rats. These data indicate that the level of occupancy of the glycine site of extrasynaptic NMDA receptors is unsaturated and unaffected by the relative changes in glial coverage. In other words, astrocytes regulate D-serine concentration preferentially in the synaptic cleft.

2.4.4 Astrocytes Control Long-Term Synaptic Plasticity

Glutamatergic synapses in the supraoptic nucleus exhibit activity-dependent long-term synaptic plasticity similar to that prevailing in other brain areas, which could play an important role in the context of physiological responses, like dehydration or lactation, where the activity of presynaptic glutamatergic neurons is strongly

Fig. 2.8 (continued) neuropil surrounding immunonegative magnocellular somata and in the ventral glial lamina (vgl), where the cell bodies of astrocytes (arrows) accumulate. OC, optic chiasma. **(b)** D-serine is the endogenous co-agonist of synaptic NMDA receptors in the supraoptic nucleus. Evoked EPSCs recorded at -60 mV to isolate synaptic AMPA currents and at $+40$ mV in AMPA receptor antagonist (DNQX) to isolate synaptic NMDA currents in supraoptic neurons. An increase in the AMPA/NMDA ratio was seen in supraoptic neurons from lactating compared to virgin rats and after D-serine degradation with D-amino acid oxidase (DAAO), but not after glycine degradation with glycine oxidase (GO). All recordings were carried out in the presence of bicuculline. **(c)** Astrocyte regulation of D-serine concentration does not occur outside the synaptic cleft. The D-serine-induced enhancement of the amplitude of extrasynaptic responses elicited by local puffs of NMDA ($50 \mu\text{M}$) was comparable in virgin and lactating rats. Adapted from Panatier et al. (2006)

increased. The induction of long-term potentiation (LTP) and long-term depression (LTD) relies on Ca^{2+} entry through NMDA receptors. Therefore, changes in D-serine concentration in the synaptic cleft resulting from changes in glial coverage should alter the induction of LTP and LTD.

This hypothesis was confirmed, since an experimental protocol that induced NMDA receptor-dependent LTP in control conditions elicited NMDA receptor-dependent LTD in lactating rats (Fig. 2.9). In addition, a stimulation protocol that induced NMDA receptor-dependent LTD in the supraoptic nucleus of virgin animals was ineffective in lactating rats. Finally, when synapses were stimulated strongly enough using high-frequency stimulation of afferent inputs, NMDA receptor-dependent LTP was produced in the supraoptic nucleus of both virgin and lactating rats.

The most likely explanation for this modification of LTP threshold is that a reduction in the number of NMDA receptors activated during the induction protocol, due to a reduced D-serine availability following astrocyte process withdrawal, resulted in a smaller postsynaptic Ca^{2+} rise, which favored phosphatases over kinases, thus reducing the postsynaptic response to glutamate, which manifests as LTD. In hypothalamic slices from lactating rats, application of saturating concentrations of D-serine increased the number of NMDA receptors available for activation and entirely reversed this effect. In other words, astrocytes control synaptic plasticity and its direction in the HNS through the action of D-serine.

2.4.5 Physiological Consequences

These results confirm that glial cells participate in synaptic signaling and show that they can drive the direction of synaptic plasticity. Retraction of astrocytic processes hamper the contribution of astrocytes to glutamatergic synaptic communication through D-serine gliotransmission, thus reducing NMDA receptor activity at synapses in the HNS.

The shift in LTP threshold in HNS neurons of lactating rats should favor potentiation at glutamatergic inputs displaying high activity, and depress synapses exhibiting lower activity. This could favor the excitation of oxytocin neurons during parturition and suckling and reduce their activation in response to other stimuli, thus preserving oxytocin for its primary functions.

2.5 Perspectives

In this chapter, we have summarized how the adult HNS, through its remarkable reversible, activity-dependent morphological neuroglial plasticity, has proved to be a seminal model to study the physiological contribution of the astrocytic environment to synaptic strength, inter-synaptic crosstalk, neuronal signaling and synaptic plasticity. Such a contribution has fueled the now fundamental concept that astrocytes are dynamic partners of brain signaling. That distal processes, as proven by the

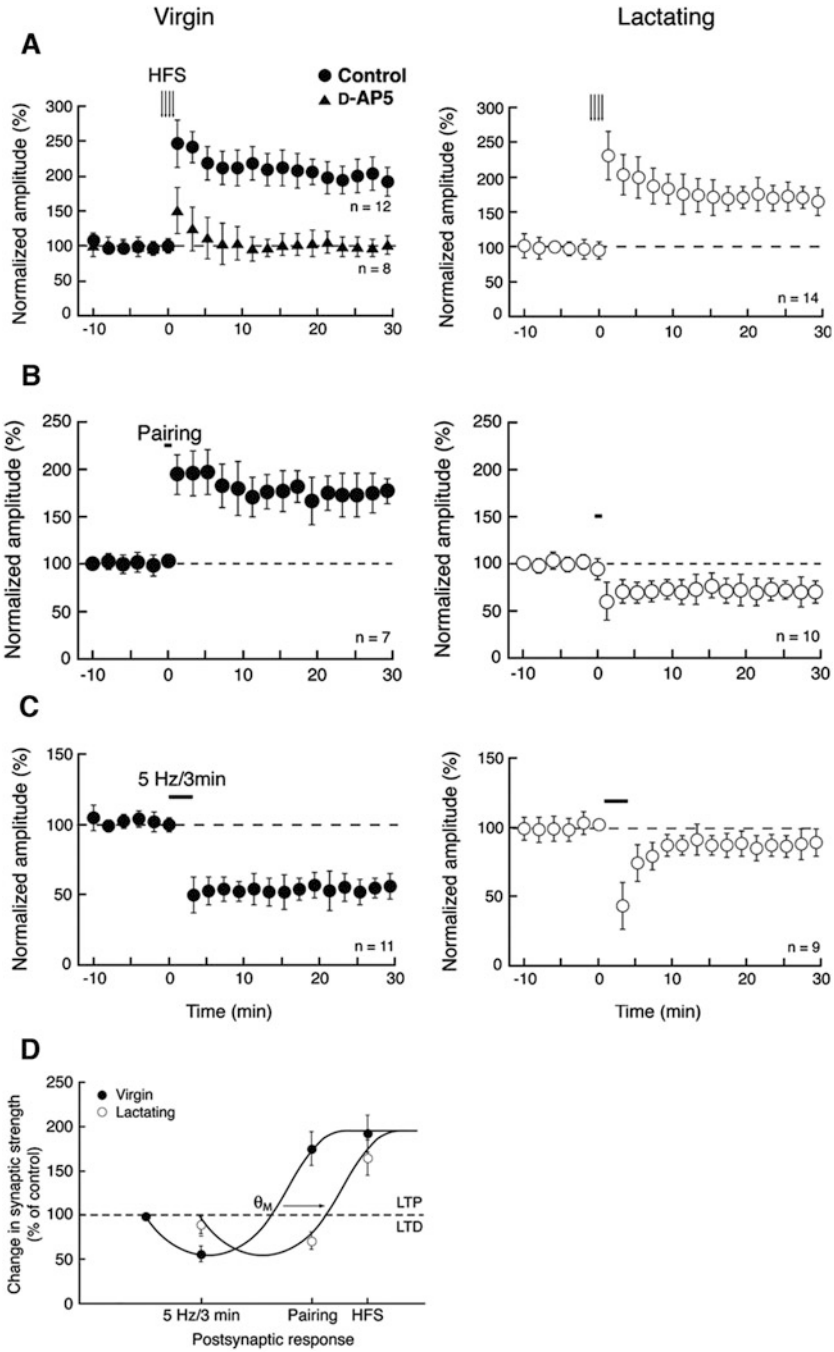


Fig. 2.9 Astrocyte-derived D-serine controls synaptic plasticity. (a) High-frequency stimulation (HFS) induced NMDA receptor-dependent long-term potentiation (LTP) of EPSCs in supraoptic neurons of virgin (left) and lactating (right) rats. The LTP was blocked by the NMDA receptor antagonist D-AP5. (b) A pairing stimulation paradigm, pairing postsynaptic membrane

effects of their retraction, can play such an important role in the dialog between astrocytes and neurons has been emphasized recently by the fact that small, rapid, and localized Ca^{2+} responses can be regulated in small compartments along the astrocytic processes by minimal synaptic activity (Panatier et al. 2011).

While HNS neuroglial plasticity was initially seen as a possible morphological basis for the synchronization of oxytocin neuron firing during lactation, this proved not to be the case. When glial withdrawal and synaptic remodeling were prevented by removing polysialic acid from the neural cell adhesion molecule using the enzyme endoneuraminidase, parturition and the milk ejection reflex remained normal (Catheline et al. 2006). Neuroglial remodeling thus is not essential to parturition and lactation. It rather seems that remodeling serves to isolate the oxytocin neurons from external excitatory influences, like those carrying stress or osmotic information, in order to preserve oxytocin for parturition and milk ejection.

Still, the high morphological plasticity of astrocytes in the HNS provides a flexible system to adapt and enrich the functioning of magnocellular neurons in response to physiological demand, and future research will need to further address this issue as well as others. For instance, the impact of glial changes on the finely tuned properties of the receptors mediating the astrocyte response to neurons will have to be studied, as well as on how astrocytes encode and integrate incoming inputs from different sources. What are the consequences of astrocytic process retraction on calcium signaling and travel in astrocytes? Are there different second messenger systems involved? Does this influence the mechanisms by which astrocytes release gliotransmitters? D-serine has a very precise site of action in the synaptic cleft, while taurine targets are more diffuse on the neuronal membrane (Deleuze et al. 2005), still the action of these two gliotransmitters is hampered in conditions of astrocyte retraction (Panatier et al. 2011; Choe et al. 2012): how does this work? Might the same astrocyte release these two gliotransmitters? What is the impact of glial morphological plasticity on the coordination of vasopressin neuron firing during osmotic challenges? Finally, we have little information about the plasticity of the different types of astrocytes in the HNS and we need to know the possible interactions they may develop with other non-neuronal cells, such as



Fig. 2.9 (continued) depolarization with stimulation of presynaptic glutamate axons at 2 Hz for 45 s (pairing, bar), potentiated the amplitude of evoked EPSCs in supraoptic neurons of virgin rats (left), but induced a persistent depression of the evoked EPSC amplitude in supraoptic neurons of lactating rats (right). (c) Stimulating afferent glutamatergic axons at 5 Hz for 3 min caused long-term depression (LTD) in supraoptic neurons from virgin rats (left), but not in supraoptic neurons from lactating rats (right). (d) Curve of astrocyte-dependent shift in plasticity threshold. Points represent percent changes in EPSC amplitude measured 30 min after the induction of LTD, pairing LTP, and HFS-LTP, respectively. These points were positioned arbitrarily on the x -axis. A curve was drawn to fit the data obtained in virgin rats (filled circles) and then shifted to fit the points obtained in lactating animals (empty circles). Note that the threshold for LTP induction (θ_M) is shifted toward higher activity values in lactating rats. Data are reported as means \pm SEM. Adapted from Panatier et al. (2006)

vascular endothelial cells and microglia, during the process of active morphological plasticity.

2.6 Key Literature

- Bonfardin et al. (2010) This study revealed that physiological astrocytic plasticity modifies the mode of action of presynaptic kainate receptors, thereby inverting their coupling with GABA release.
- Oliet et al. (2001) This seminal paper demonstrated that astroglial wrapping of neurons, by controlling glutamate clearance by way of the GLT-1 transporter, plays a significant role in regulating the efficacy of glutamatergic neurotransmission.
- Panatier et al. (2006) Key paper that revealed that the degree of astrocytic coverage of neurons governs the level of glycine site occupancy on the NMDA receptor, thereby affecting NMDA receptor availability for activation and thus the activity dependence of long-term synaptic changes.
- Piet et al. (2004) Established how astrocytes, by hindering diffusion in the extracellular space, regulate intersynaptic communication between neighboring synapses.

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