Retrograde Signaling Via Dendritic Activation of Glial-Neuronal Circuits

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Abstract

Neuroendocrine cells in the hypothalamus secrete neuropeptides from both their axon terminals and their dendrites to regulate homeostatic function. Vasopressin is expressed in both vasopressin and corticotropin-releasing-hormone neurons and is released dendritically following exposure to homeostatic neuromodulators such as ghrelin and norepinephrine. Dendritically released vasopressin stimulates ATP release from astrocytes, which activates local upstream glutamate and GABA circuits. This *trans*-neuronal-glial retrograde signaling exploits the spatial domain of astrocytes to expand the reach of dendritic volume transmission from neuroendocrine cells to distal presynaptic partners to regulate local synaptic circuit inputs, thus providing a powerful means of neuroendocrine cell autoregulation of hormonal output.

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Keywords

Astrocytes · Tripartite synapse · Gliotransmitter · Vasopressin · Corticotropin-releasing hormone · Ghrelin · Norepinephrine

8.1 Introduction: Gliotransmission

8.1.1 The Tripartite Synapse

First proposed in the 1990s, the model of the **tripartite synapse** (Fig. 8.1) recognizes the essential role of glia in synaptic function by means of interleaving glial processes with presynaptic and postsynaptic neuronal structures (Araque et al. 1999). Since glial cells lack the ability to generate action potentials, they previously have been considered "quiet" cells, whose main role is to provide mechanical and metabolic support for neuronal cells in the brain. An abundance of data collected over the past two decades, however, highlights the many unique roles of glial cells in sensing changes in neuronal activity and influencing neuronal signals by releasing transmitters (termed **gliotransmitters**) such as taurine, ATP, D-serine, and glutamate.

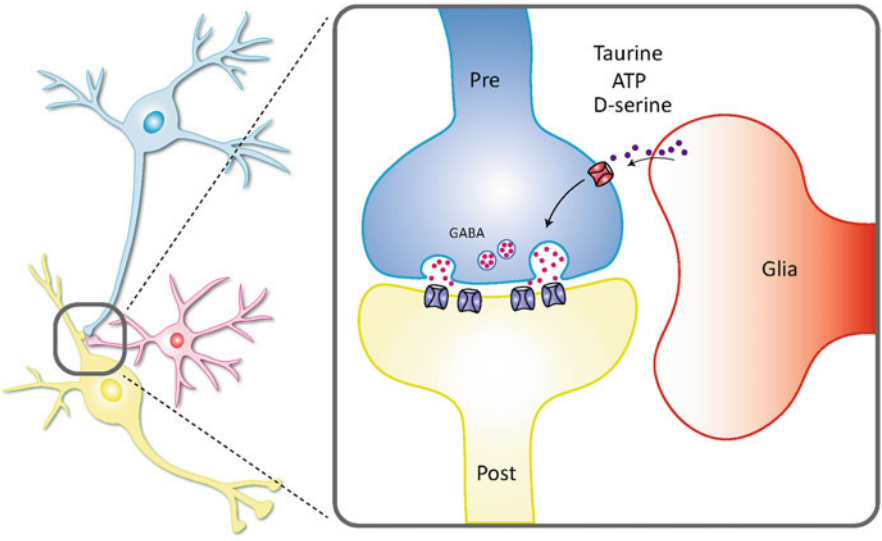


Fig. 8.1 The tripartite synapse and gliotransmission. Astrocyte processes are frequently in close proximity to presynaptic axon terminals and postsynaptic dendrites and dendritic spines, where they can respond to orthograde and retrograde chemical signals from neurons (i.e., neurotransmitters) and release chemical signals (i.e., gliotransmitters) onto neurons. Thus, they can modulate synaptic transmission both pre- and postsynaptically. In the example shown here, gliotransmitters such as taurine, ATP, and D-serine regulate synaptic release at GABAergic synapses

Astrocytes are the most abundant type of glial cell. During the past thirty years, advances in anatomical studies have provided support for the role of astrocytes in synaptic function by demonstrating a close juxtaposition of astrocytic processes to neuronal synaptic structures. Astrocytes extensively cover various parts of neurons, such as dendrites, axon terminals, and somata, with irregular shaped processes. By virtue of their proximity to neurons and neuronal processes, astrocytes can act as a critical modulator of neuronal function. Their ability to maintain homeostasis of the extracellular milieu and to modulate neuronal activity by releasing gliotransmitters has been well documented over the past 20 years (Araque et al. 2014; Sahlender et al. 2014).

The unique anatomical characteristics of astrocytes provide clues as to how they act as the third participant at the synapse, along with the presynaptic axon terminal and the postsynaptic neuronal dendrite, and are indispensable in neuronal circuit signaling. Recent advances in histology and imaging techniques have revealed that a single astrocyte, with its extremely elaborate processes, can contact more than 10,000 synapses in rodents and two million synapses in humans (Bushong et al. 2002; Fields et al. 2015). Astrocytic domains are characterized by unique territories that form a tile-like structure with minimal overlap with their neighboring astrocytes (Bushong et al. 2002), allowing each astrocyte to exercise quasi-exclusive control over neuronal synapses in its spatial domain. By providing extensive coverage of its territory, an astrocyte can effectively control a set of functionally defined synapses. In addition, the dynamic nature of the astrocytic processes offers a powerful means of regulation of synaptic activity relevant to synaptic plasticity. The astrocyte-specific glial fibrillary acidic protein (GFAP) is expressed primarily in the large stem processes of astrocytes, which are altered in response to physiological stimuli, suggesting that the astrocytic stem processes are dynamic. Furthermore, the GFAP-negative fine processes that extend from the stem processes are also highly flexible, showing significant morphological changes on a time scale of seconds to minutes that result from their lack of intermediate filaments and microtubules and low expression of actin filaments (Derouiche and Frotscher 2001).

The morphological flexibility of astrocytes has been particularly well demonstrated in the hypothalamus. In the suprachiasmatic nucleus, which is the circadian clock of the brain, morphological changes occur according to the light/dark cycle within 24 h. In the rostral preoptic area, astrocytes undergo steroid hormone-mediated changes in morphology during the estrous cycle that alter the astrocytic coverage of the surface of gonadotropin-releasing hormone (GnRH) neurons, resulting in corresponding changes in synaptic inputs to the GnRH neurons. In the supraoptic nucleus (SON) and paraventricular nucleus (PVN) of the hypothalamus, where magnocellular neurons that release the neurohypophysial hormones oxytocin and vasopressin are located, strong physiological stimuli such as dehydration and lactation induce dramatic changes in the astrocytic coverage, which also has a potent impact on neuronal signaling at excitatory and inhibitory synapses on the magnocellular neurons (described in detail in Chap. 2).

8.1.2 Neuronal Communication with Astrocytes

Unlike neurons, which generate electrical signals, glial cells signal via fluctuations in their intracellular calcium concentration. The seminal work by Kuffler and colleagues in the 1960s demonstrated that glial cells present an increase in intracellular calcium when neighboring neuronal cells are electrically stimulated, providing the first direct evidence that glial cells actively respond to neuronal signals (Orkand et al. 1966).

Astrocytes express a variety of ionotropic and metabotropic receptors that are activated by neurotransmitters. They express high levels of metabotropic receptors, which are G protein-coupled receptors. The activation of metabotropic receptors triggers intracellular G-protein and second-messenger signaling cascades that lead to an increase in the intracellular calcium concentration in astrocytes. Changes in intracellular calcium levels are caused primarily by release from intracellular calcium stores, the endoplasmic reticulum and mitochondria, but calcium can also enter the cell from the extracellular space through store-operated channels or transporters, such as the sodium-calcium exchanger (Verkhratsky et al. 2012). In addition, ionotropic receptors, which are transmitter-activated ion channels, have been shown to play a critical role in mediating fast-acting astrocytic responses to a variety of neurotransmitters. Glutamate receptors were among the first neurotransmitter receptors to be identified in astrocytes. They comprise both ionotropic and metabotropic receptors activated by the release of the excitatory neurotransmitter glutamate from neighboring neurons. Astrocytes also express both metabotropic and ionotropic receptors for the inhibitory neurotransmitter GABA, though GABA's actions in astrocytes are usually not inhibitory. Activation of the metabotropic $GABA_B$ receptors causes an increase in intracellular calcium levels via release from intracellular calcium stores. Activation of the ionotropic $GABA_A$ receptors on astrocytes causes a membrane depolarization due to the high intracellular chloride concentration and reversed chloride concentration gradient, which can subsequently activate voltage-gated calcium channels. In addition to glutamate and GABA receptors, astrocytes express several other types of receptor that can be activated by neurotransmitters such as norepinephrine (NE) and acetylcholine.

There have been significant improvements recently in identifying the source of neurotransmitter-induced calcium responses in astrocytes. Recent advances in calcium indicators have improved labeling of astrocytic processes. While many of the bulk-loaded dye and cytosolic calcium indicators do not effectively label the astrocytic processes with which most synaptic structures are intimately associated, the newer membrane-targeted genetically encoded calcium indicators effectively label fine astrocytic processes (Box 8.1). Furthermore, advances in *in vivo* imaging have enabled us to circumvent issues with altered calcium responses under *in vitro* conditions. With these advances, we now have gained a better understanding of the cellular mechanisms underlying the neuronal activation of astrocytes.

In the hypothalamus, astrocytes respond not only to classical neurotransmitters, but also to various neurohormones. Vasopressin and cholecystokinin, which are neurohormones secreted by neuroendocrine cells, have been shown to activate

hypothalamic astrocytes (Chen et al. 2019; Crosby et al. 2018; Haam et al. 2014). Astrocytes in the hypothalamus also respond to NE (Gordon et al. 2005). When activated, the neurohormone/neurotransmitter receptors on astrocytes trigger a signaling cascade that subsequently induces an increase in intracellular calcium and often the release of gliotransmitters. The gliotransmitters released into the extracellular space can reach neuronal receptors and modulate neuronal circuits relevant to the physiological function of the brain region, which will be discussed in more detail in the next few sections.

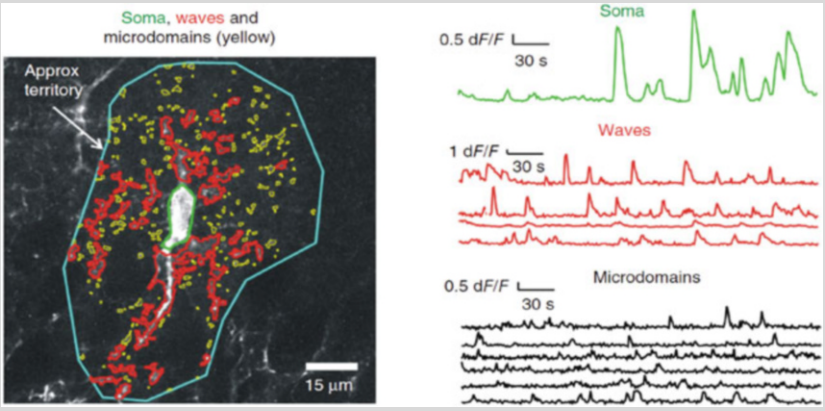
In addition to classical neurotransmitter release from axon terminals, somatodendritic release of neurotransmitters has also been well documented in neuroendocrine cells. Astrocytes that cover dendritic regions can receive dendritically released neurotransmitters and mediate a retrograde feedback signal to a group of neuroendocrine cells that are functionally related (Chen et al. 2019; Crosby et al. 2018; Haam et al. 2014). The hypothalamic astrocyte's capacity to respond to various transmitters, including neuroendocrine hormones, suggests that astrocytes can effectively sense local neuronal activity and participate in the modulation of the relevant neural circuits.

Box 8.1 Measuring glial activity using calcium imaging

Astrocytes use calcium signaling to code information, communicate with other cells, and release gliotransmitter. The activity of glial cells can be measured by using calcium indicators that change their fluorescence intensity with changes in the calcium concentration. Since the early days of chemical calcium indicators, which are loaded into cells using patch-clamp techniques or membrane-permeant acetoxymethyl (AM) esters, there has been great progress in calcium imaging in glial cells. Recent developments in genetically encoded calcium indicators (GECIs), such as GCaMP, have allowed not only the cell type-specific expression of calcium indicators in glial cells, but also the labeling of fine distal processes of glia, which chemical calcium dyes cannot easily reach. With the use of GECIs, calcium fluctuations can be observed not only in the somatic regions but also in fine processes (microdomains, figure below) (Srinivasan et al. 2015). Furthermore, advances in *in vivo* fluorescence measurement techniques such as multi-photon imaging and fiber photometry have allowed calcium measurement in freely behaving animals.

(continued)

Box 8.1 (continued)



Calcium fluctuations can manifest as three predominant subtypes depending on the location within a cell: somatic Ca^{2+} fluctuations (green), Ca^{2+} waves that spread between adjacent areas in processes (red), and microdomains, which are restricted to local spots in processes (yellow territories on the left, black traces on the right) (Adapted from (Srinivasan et al. 2015) with permission).

8.1.3 Astrocyte Communication with Neurons

It is well known that neuronal cells communicate with each other by releasing neurotransmitters at chemical synapses. Astrocytes not only release classical transmitters, such as glutamate and GABA, but also release various types of other transmitters, such as ATP, D-serine, and taurine, which exert diverse effects on other astrocytes and on neighboring neurons (Araque et al. 2014; Sahlender et al. 2014) (see also Chap. 2).

Neurotransmitters released from neighboring neurons bind to their cognate receptors and activate a variety of signal transduction pathways in astrocytes. Among those, an increase in intracellular calcium has been well characterized as the hallmark of astrocytic activation, which triggers the release of gliotransmitters (Araque et al. 2014; Sahlender et al. 2014; Verkhratsky et al. 2012). Extracellular gliotransmitters then activate receptors on neighboring neuronal cells, which triggers the opening of ion channels or a second messenger-mediated response (Araque et al. 2014).

Astrocytes have been shown to communicate with each other to expand their spatial signaling to enable regulation of a large population of neurons when necessary. Astrocytes communicate directly with other astrocytes via gap junctions,

through which second messengers as well as intracellular calcium ions can quickly spread. Glia-to-glia communications also can be achieved through gliotransmitters. Extracellularly released gliotransmitters can activate receptors on neighboring astrocytes in a paracrine fashion to propagate intercellular calcium waves and amplify astrocytic signals.

In the hypothalamic PVN and SON, changes in gliotransmission can happen slowly due to morphological changes in glia or rapidly in response to local signals such as neurotransmitters. Astrocytes present dramatic morphological changes during strong physiological stimuli such as lactation and dehydration, which result in an altered astrocytic coverage of magnocellular neuroendocrine cells. The retraction of astrocytic processes surrounding magnocellular neuroendocrine cells during lactation leads to a reduced capacity for synaptic plasticity at glutamate synapses due to a decrease in the astrocytic release of D-serine, which acts at excitatory synapses as a co-agonist of the NMDA receptor required for synaptic potentiation (Panatier et al. 2006) (see also Chap. 2). In addition, glial retraction after a chronic dehydration regime eliminates taurinergic gliotransmission, which normally activates glycine receptors expressed on magnocellular neurons (Hussy et al. 2001). Finally, as mentioned above, removal of the glial coverage of magnocellular neuronal membranes results in new glutamate and GABA synapse formation (see Chap. 2 for a detailed description).

8.1.4 ATP as a Gliotransmitter Regulating Neuroendocrine Cells

Although used also as a neurotransmitter, ATP has been described as a gliotransmitter in structures throughout the brain. In the PVN and SON, astrocytes release ATP to regulate neuroendocrine cell activity in response to multiple signals from both extrinsic and intrinsic sources. Briefly (see below for a detailed description), the dendritic release of vasopressin from vasopressinergic neuron dendrites activates ATP release from PVN and SON astrocytes, which stimulates recurrent GABAergic circuits to the vasopressin neurons (Haam et al. 2014). Norepinephrine activates alpha1 receptors directly on astrocytes in the SON/PVN to stimulate the astrocytic release of ATP, which controls the membrane trafficking of glutamate receptors in magnocellular neurons (Gordon et al. 2005). Recent evidence suggests that norepinephrine also activates alpha1 receptors on corticotropin-releasing hormone (CRH) neurons in the PVN to cause the dendritic release, interestingly, of vasopressin, which stimulates astrocytes in the PVN to release ATP and recruit recurrent GABA and glutamate circuit inputs to the CRH neurons (Chen et al. 2019).

8.1.5 Trans-astrocyte Inter-neuronal Communication

Bidirectional signaling between neurons and glia has been described in several brain areas to be critical for the proper function of neural circuits. The trans-neuronal endocannabinoid signaling in the hippocampus is an excellent example that shows

neuronal-glial bidirectional communication that is fundamental to synaptic function (Navarrete and Araque 2008). The type 1 cannabinoid receptor is expressed in hippocampal astrocytes and binds endocannabinoid released from neighboring neurons. The astrocytes respond by increasing intracellular calcium levels, which triggers the release of glutamate onto neighboring neurons and the subsequent activation of neuronal NMDA receptors (Navarrete and Araque 2008).

The "sandwich synapse" described by Stanley and colleagues also clearly demonstrates trans-glial neuronal signaling (Rozanski et al. 2013. At this synapse, two dorsal root ganglion neurons communicate with each other through an intercalated satellite glial cell. The stimulation of dorsal root ganglion cell somata induces the neuronal release of ATP to activate neighboring glial cells, which subsequently release a gliotransmitter to activate nearby neuronal cells.

8.2 Neuroendocrine Systems

8.2.1 Dendritic Neuropeptide Release in the Hypothalamic-Neurohypophysial System

The hypothalamic-neurohypophysial system (HNS), first described by German biologists Wolfgang Bargmann and Ernst Scharrer in the 1950s, is an important integrative structure that coordinates cardiovascular and reproductive functions. The HNS consists of magnocellular neuroendocrine cells in the SON and PVN of the hypothalamus that secrete the neuropeptides vasopressin and oxytocin from their axons, which run through the internal zone of the median eminence (ME) and terminate on the capillaries of the posterior lobe of the pituitary gland (Fig. 8.2). In response to hypovolemic and/or hyperosmotic stimuli, vasopressin (a.k.a. antidiuretic hormone) is released into the circulation, where it acts on the vascular smooth muscle to cause vasoconstriction and on the kidneys to regulate water conservation and restore fluid homeostasis. In females, oxytocin is involved in parturition and milk ejection by acting at the uterus and mammary glands, respectively.

In response to stimulation, magnocellular neurons in the HNS release vasopressin and oxytocin from their axonal terminals in the posterior lobe of the pituitary, as well as from their somata and dendrites in the SON and PVN. Large dense core vesicles (LDCVs), the organelles that store neuropeptides, are located throughout the neuroendocrine cell. The HNS is the best-studied model system for the detailed mechanisms of somato-dendritic release due to the relatively homogeneous neuroendocrine cell population in the SON and PVN, the relative lack of interneurons, and the unique anatomy that makes the neuroendocrine cells easily identifiable by antidromic stimulation of the pituitary.

Dendritic release of oxytocin occurs during parturition and lactation and contributes to the bursting activity of the oxytocin neurons and the pulsatile secretion of oxytocin into the blood. Synchronous firing among the oxytocin neurons is facilitated by positive feedback of dendritically released oxytocin and activation of

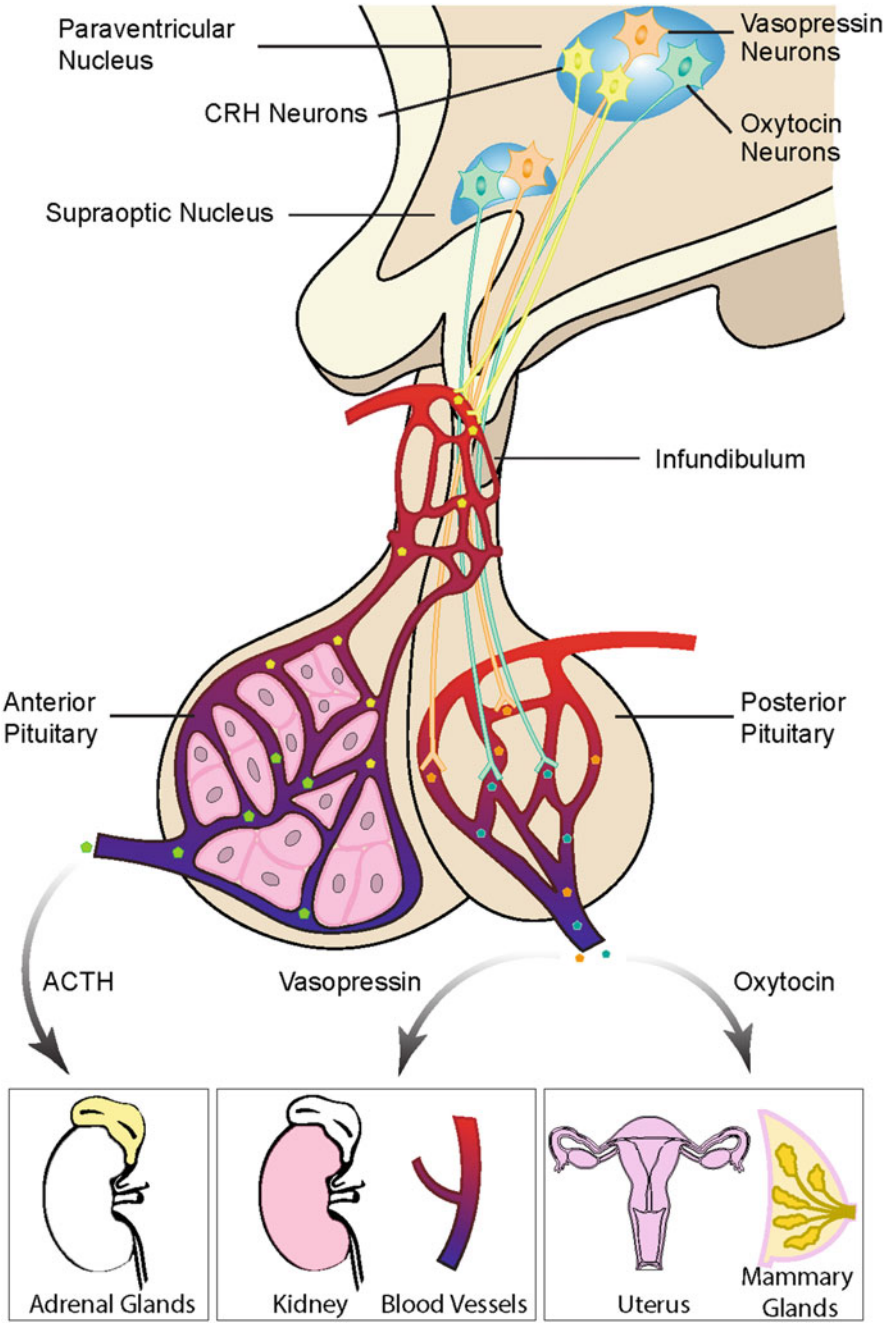


Fig. 8.2 The hypothalamic-neurohypophysial system and hypothalamic-pituitary-adrenal axis. Magnocellular neuroendocrine cells of the hypothalamic-neurohypophysial system reside in the hypothalamic supraoptic nucleus and paraventricular nucleus and secrete vasopressin and oxytocin from their axon terminals into the capillaries of the posterior lobe of the pituitary gland when stimulated. Circulating vasopressin binds to receptors in the kidney to cause water reabsorption and

oxytocin autoreceptors in the SON and PVN (Neumann et al. 1994). Oxytocin secretion into the blood promotes uterine contraction to facilitate the delivery process, and promotes milk let down by stimulating the milk ejection reflex during lactation. Dendritic release of oxytocin also contributes to the induction and maintenance of morphological changes in oxytocin neurons and astrocytes and to neuronal-glial interactions during reproduction (Theodosis et al. 1986). Dendritic release of oxytocin also occurs during exposure to stressors, especially social stressors such as social defeat and resident-intruder stress, and is believed to play a role in the maternal aggression during lactation that is critical for the defense of the offspring (Bosch et al. 2005).

Vasopressin neurons are osmosensitive, and dendritic release of vasopressin occurs in response to hypertonic stimuli. In contrast to the excitatory effect of oxytocin, the activation of autoreceptors on vasopressin neurons leads to a decrease in their firing and modulates their firing mode (Ludwig and Leng 1997). In addition to its autocrine effects, vasopressin can also function as a paracrine signal. Thus, dendritically released vasopressin can also diffuse to neighboring presympathetic neurons in the PVN to coordinate sympathetic outflow with homeostatic hormonal output (Son et al. 2013), or to nearby astrocytes that control local circuit activity in the SON and PVN (Haam et al. 2014).

Somato-dendritic release and axonal release of oxytocin and vasopressin can occur simultaneously or independently of one another. Parturition and suckling, as well as forced swim and shaker stress, increase oxytocin release simultaneously both centrally and peripherally, while social defeat only increases the dendritic release of oxytocin in the SON. Similarly, hyperosmotic stimulation increases both somato-dendritic and axonal release of vasopressin, while social defeat and forced swim selectively promote somato-dendritic vasopressin release (Engelmann et al. 2001; Neumann et al. 1993; Wotjak et al. 2001). The differential axonal and somato-dendritic release of neuropeptides in response to different stressors confers context-specific regulation of homeostasis.

The release of neuropeptide from dendrites and axons has distinct spatio-temporal profiles due to a different release machinery involved. First, axonal release depends on action potential generation, while somato-dendritic release depends on the mobilization of intracellular calcium stores (Ludwig et al. 2002). Thus, differential densities of intracellular organelles that store intracellular calcium, such as the endoplasmic reticulum and mitochondria, and differential expression of ion

Fig. 8.2 (continued) in blood vessels to cause vasoconstriction. Circulating oxytocin binds to oxytocin receptors in the uterus to stimulate uterine contractions and parturition and in the mammillary gland to stimulate milk ejection. CRH neurons of the hypothalamic-pituitary-adrenal axis reside in the hypothalamic paraventricular nucleus and secrete CRH and vasopressin from their axon terminals in the median eminence into the pituitary portal circulation. Portal CRH and vasopressin stimulate ACTH secretion into the general circulation from corticotropes in the anterior lobe of the pituitary gland. Circulating ACTH then stimulates corticosteroid synthesis and secretion from the adrenal cortex into the general circulation, from where it accesses target tissues, including the brain

channels, plasma membrane calcium pumps, and sodium/calcium exchangers all may contribute to the differential release from dendrites and axons. Second, release of neuropeptide depends on the exocytosis of LDCVs, and the proteins involved in exocytosis may be expressed differentially in somato-dendritic and axonal compartments.

Differential dendritic and axonal release is exemplified by the regulation of central oxytocin release by α-melanocyte stimulating hormone (α-MSH) (Sabatier et al. 2003). SON and PVN neurons express high levels of melanocortin-4 receptors and receive inputs from α-MSH neurons in the arcuate nucleus. Central administration of α-MSH increases c-fos expression in oxytocin neurons, but decreases the oxytocin neuron firing rate, selectively promoting the dendritic release of oxytocin. This is caused by the mobilization of intracellular calcium and the release of retrograde-signaling endocannabinoids, which reduce the excitatory synaptic inputs and drive to the oxytocin neurons.

8.2.2 Dendritic Neuropeptide Release in the Hypothalamic-Pituitary-Adrenal System

The hypothalamic-pituitary-adrenal (HPA) axis is responsible for the neuroendocrine component of the stress response. The stress response is a highly conserved, multi-faceted physiological reaction that is activated when an organism is challenged by internal and external disturbances. During the generalized stress response, corticotropin-releasing hormone (CRH) neurons (also referred to as corticotropin-releasing factor (CRF) neurons) located in the PVN, a key nucleus that integrates physiological and psychological afferent information, are activated. The activated CRH neurons release CRH from their axon terminals in the median eminence, which is located at the base of the brain. The secreted CRH transits through the portal circulatory system of the pituitary to the anterior lobe of the pituitary, where it activates the corticotrope cells to secrete adrenocorticotropic hormone (ACTH) into the general bloodstream. The secreted ACTH then acts on cells of the adrenal cortex to cause the synthesis and release of corticosteroids, the primary hormonal product of the HPA (Fig. 8.2). Corticosteroids impact nearly every cell in the body by activating glucocorticoid and/or mineralocorticoid receptors. Corticosteroids also feed back to the brain and pituitary to negatively regulate CRH and ACTH expression and secretion, thereby limiting the duration and impact of the neuroendocrine stress response and returning circulating corticosteroid concentrations to normal, unstressed levels.

In addition to its antidiuretic function, vasopressin also is co-expressed in PVN CRH neurons and potentiates the CRH stimulation of the release of ACTH from corticotropes in the anterior pituitary. Interestingly, the co-expression of vasopressin in CRH neurons is state-dependent and can be enhanced by specific stressors. Systemic stressors such as interleukin-1β (IL-1), lipopolysaccharide (LPS), brain surgery, electric foot shock, and immobilization (both acute and repeated) increase vasopressin expression in the CRH neurons (Bartanusz et al. 1993). Removing

circulating corticosteroid by adrenalectomy in rats induces vasopressin peptide expression in over 70% of CRH neurons in the PVN (Sawchenko et al. 1984). Indeed, transient suppression of corticosteroid release by central CRH immunoneutralization or by peripheral inhibition of corticosteroid synthesis increases vasopressin mRNA expression in CRF fibers in the median eminence (Schmidt et al. 1997). The corticosteroid-sensitive expression of vasopressin in the CRH neurons reveals a plasticity of the HPA in response to different stressors.

The somato-dendritic release of neuropeptide from the CRH neurons in the PVN is less well studied than the dendritic release from the MNCs in the SON and PVN, partly due to the heterogeneous populations of neurons in the PVN. With advances in optogenetics tools, recent studies have demonstrated that selective activation of CRH neurons excites neighboring neurons within the PVN via local CRH release and activation of type 1 CRH receptors (CRHR1), which suggests the possible dendritic release of CRH in the PVN (Jiang et al. 2018). In addition, studies of the mechanisms of NE excitation of CRH neurons suggest that CRH neurons can also release vasopressin from their dendrites to recruit astrocytes to amplify dendritic volume transmission (Chen et al. 2019).

8.3 Ghrelin and Norepinephrine Activate Neuronal-Glial Signaling

8.3.1 Ghrelin Activation of Vasopressin Neurons in the PVN Via a Neuronal-Glial Circuit

Ghrelin is a well-known hunger signal that is released from the gastrointestinal tract in response to a negative energy balance to mediate feeding-related behavior. In addition to a variety of functions in energy homeostasis, ghrelin also regulates fluid balance by modulating signaling involved in osmoregulation, cardiovascular function, and drinking behavior. The ghrelin signaling in the PVN that leads to the activation of vasopressin neurons demonstrates neuronal-glial signaling essential for neuroendocrine function; the signaling is mediated via neuronal activation of astrocytes (vasopressin neurons → astrocytes) and subsequent astrocytic activation of neuronal cells (astrocytes → GABAergic neurons) (Haam et al. 2014). In this section, we will review the vasopressin-expressing MNCs, the synaptic inputs that regulate vasopressin neurons, and the ghrelin signaling that stimulates vasopressin neurons via a neuronal-glial signaling mechanism.

Vasopressin neurons and oxytocin neurons are the two types of MNCs found in the PVN and SON (Fig. 8.2). Although co-expression of oxytocin and vasopressin is observed under certain conditions, such as in response to osmotic challenge, the majority of MNCs typically express only one of the two neuropeptides under baseline conditions, and the neuropeptide expression defines each of the two types of MNCs. In addition to playing roles in social behaviors, oxytocin and vasopressin mediate distinct physiological functions: oxytocin facilitates parturition and triggers milk ejection, while vasopressin regulates osmotic homeostasis by eliciting water

reabsorption in the kidney and vasoconstriction. Oxytocin also plays a role in osmoregulation by regulating sodium excretion in the kidney.

The excitability of MNCs is regulated by glutamatergic and GABAergic synaptic inputs. It has been shown that MNCs in the PVN and SON receive synaptic inputs from local glutamatergic and GABAergic neurons located in or near the nuclei (Boudaba et al. 1996, 1997). Both ionotropic and metabotropic receptors are expressed in MNCs to mediate the glutamatergic and GABAergic synaptic regulation of the MNCs. Unlike glutamate, GABA actions are significantly affected by intracellular ionic concentrations. At excitatory synapses, ionotropic AMPA and NMDA glutamate receptors are permeable to the cations Na^+, K^+, and Ca^{++}, and the opening of the receptor channels by glutamate causes membrane depolarization due to the significantly more positive reversal potential of the cationic synaptic currents (~0 mV) relative to the resting membrane potential. On the other hand, at inhibitory synapses, the ionotropic $GABA_A$ receptors are permeable to Cl^-, and because the equilibrium potential for Cl^- is normally negative to the MNC resting membrane potential, the opening of the $GABA_A$ receptor channels causes influx of Cl^- and hyperpolarization. The intracellular Cl^- concentration is maintained in MNCs by the ionic co-transporters K^+-Cl^- co-transporter 2 (KCC2), Na^+-K^+-Cl^- co-transporter 1 (NKCC1), and Na^+-K^+-Cl^- co-transporter 2 (NKCC2). While KCC2 decreases the intracellular Cl^- concentration by exporting Cl^- ions from cells using the outward K^+ concentration gradient, NKCC1 and NKCC2 increase the intracellular Cl^- concentration by importing Cl^- ions using the inward Na^+ concentration gradient. As the $GABA_A$ receptor-mediated ionic mechanism relies on the intracellular Cl^- concentration, any alteration in the intracellular Cl^- concentration, such as via a change in the expression or function of the Cl^- transporters, can change GABA's actions by altering the magnitude and direction of the Cl^- flow across the membrane. During development, a low level of KCC2 expression contributes to a high intracellular Cl^- concentration and shifts the Cl^- reversal potential positive, which results in the activation of $GABA_A$ receptors causing a membrane depolarization (Ben-Ari et al. 2012). In addition, potent physiological stimuli can alter Cl^- transporter function and increase the intracellular Cl^- concentration, which shifts GABA's actions to less inhibitory or excitatory. The intracellular Cl^- concentration is regulated in a cell type-specific manner and is modulated by signaling molecules, such as brain-derived neurotrophic factor, vasopressin, and oxytocin. Evidence suggests that GABA can have excitatory actions in vasopressin-expressing MNCs due to a low level of KCC2 expression in these cells (Haam et al. 2012; Kanaka et al. 2001). The nature of $GABA_A$ receptor signaling is a function of environmental conditions impacting Cl^- transporter expression and/or function because GABA actions can be either inhibitory or excitatory in vasopressin neurons at baseline under different conditions, and can shift to less inhibitory or excitatory with chronic stimulation of the vasopressin system (Choe et al. 2015; Haam et al. 2012; Kim et al. 2011).

Vasopressin neurons have the capacity to release vasopressin from their dendrites to exert neuromodulatory effects that are distinct from the classical axonal release of the peptide from the posterior pituitary that mediates vasoconstriction and water

reabsorption (Ludwig et al. 2002). Dendritically released vasopressin plays a critical role not only in providing autocrine and retrograde regulation to vasopressin neurons (Ludwig and Leng 1997), but also in recruiting neighboring astrocytes to amplify signals (Haam et al. 2014). When ghrelin activates the type 1a growth hormone secretagogue (ghrelin) receptor (GHS-R1a) expressed in vasopressin neurons, it induces G protein-coupled receptor signaling to trigger dendritic release of vasopressin (Haam et al. 2014) (Fig. 8.3). The dendritically released vasopressin then triggers calcium signaling in neighboring astrocytes by activating astrocytic vasopressin 1a (V1a) receptors (Haam et al. 2014). The V1a receptors are coupled to $G_{\alpha q/11}$ and activate phospholipase C, which hydrolyzes phosphatidylinositol 4,5-bisphosphate to form the second messengers inositol (1,4,5) trisphosphate (IP3) and diacylglycerol (DAG), which subsequently induce Ca^{2+} release from intracellular calcium stores.

The release of gliotransmitter is an important physiological phenomenon that results from an astrocytic calcium response and is responsible for the modulation of multiple neuronal and non-neuronal cells within the spatial sphere of influence of the astrocyte. When ghrelin triggers a calcium response in astrocytes via the dendritic release of vasopressin, the activated astrocytes release ATP, a well-known gliotransmitter (Fig. 8.3). Although ATP was originally known as a source of energy transfer in living cells, more recent studies have identified an exciting role of ATP as a gliotransmitter. ATP has a variety of neuromodulatory functions by acting at purinergic P2X and P2Y receptors, which are ionotropic and metabotropic receptors, respectively. The activation of P2X ionotropic receptors has been shown to stimulate GABAergic synaptic transmission in the hypothalamus (Crosby et al. 2018; Haam et al. 2014; Vavra et al. 2011). In the downstream response of vasopressin neurons to ghrelin, ATP elicits an increase in GABAergic synaptic inputs to the vasopressin neurons by activating P2X receptors to stimulate presynaptic GABAergic neurons (Haam et al. 2014). Since GABA is excitatory in vasopressin neurons under baseline conditions in our hands (Haam et al. 2012; Morton et al. 2014), this increase in GABAergic inputs can activates the vasopressin neurons (Haam et al. 2014).

8.3.2 Norepinephrine Activation of CRH Neurons in the PVN Via a Neuronal-Glial Circuit

Stress-relevant information converges in the PVN, and CRH neurons are positioned to generate rapid and accurate changes in hormonal, autonomic, and behavioral outputs in response to dynamic stress contexts and reward conditions. Abundant evidence indicates the role of brainstem catecholaminergic signaling in the activation of the HPA. CRH neurons in the PVN receive noradrenergic inputs from the dorsal and ventral medulla and express adrenergic receptors. Functionally, selective immunotoxin ablation of noradrenergic inputs from the A1 and A2 catecholaminergic cell groups of the medulla or selective blockade of adrenergic receptors within the PVN diminishes stress-induced activation of ACTH and corticosterone secretion (Ritter et al. 2003), suggesting that NE excites CRH neurons. *In vitro*

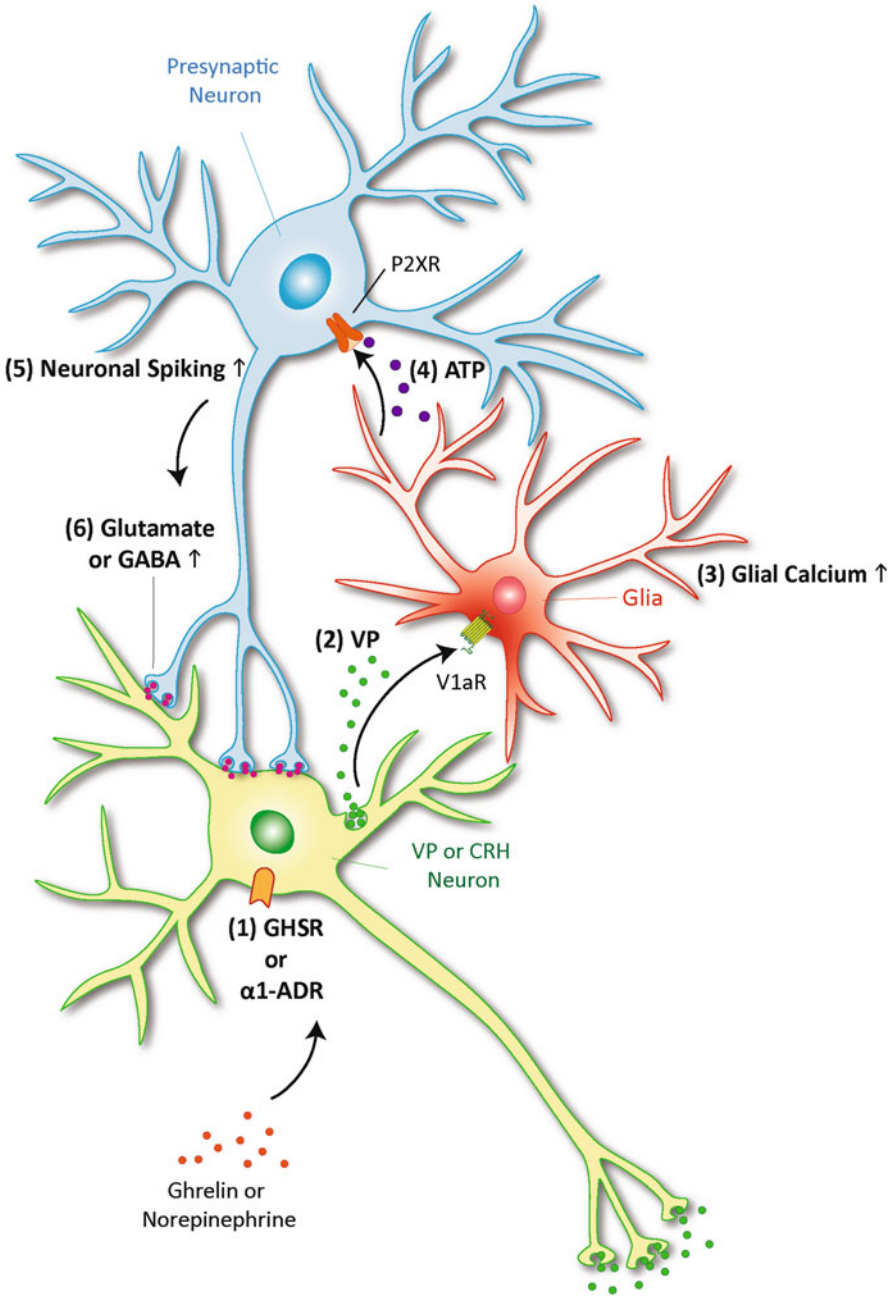


Fig. 8.3 Vasopressin as a retrograde messenger that activates astrocytes in ghrelin and NE signaling. Ghrelin stimulates vasopressin MNCs and norepinephrine stimulates CRH neurons in the PVN (1) to release vasopressin from their dendrites (2). The dendritically released vasopressin activates V1a receptors in astrocytes to trigger an increase in intracellular Ca^{2+} signaling (3). Activated astrocytes release the gliotransmitter ATP onto neighboring glutamatergic and/or GABAergic neurons. (4) ATP activates P2X receptors in the glutamatergic and GABAergic

electrophysiology showed that NE increases action potential firing in CRH neurons (Chen et al. 2019), consistent with studies showing that activation of medullary catecholaminergic inputs to the PVN or microinjection of NE directly into the PVN increases CRH release and elevates circulating ACTH and corticosterone (Itoi et al. 1994; Plotsky 1987).

Despite direct noradrenergic innervation of the CRH neurons in the PVN, early electrophysiological studies indicated that NE activates PVN neurons indirectly, through activation of local synaptic circuits (Daftary et al. 1998; Han et al. 2002). This discrepancy was reconciled with the finding that NE stimulates presynaptic circuits by activating postsynaptic alpha1-adrenoceptors on the CRH neurons and triggering the dendritic release of a retrograde messenger to activate local upstream glutamate and GABA circuits (Chen et al. 2019). In addition to activating both presynaptic glutamate and GABA circuits via an alpha1 receptor-dependent retrograde signaling mechanism in CRH neurons, NE also activates presynaptic alpha2 adrenergic receptors located directly on GABAergic axon terminals to cause a suppression of GABA release, thus causing a complex modulation of GABAergic synaptic inputs to CRH neurons.

Why would NE have opposing effects on synaptic excitation and inhibition in the same population of CRH neurons? It turns out that the differential effects of NE on glutamate and GABA neurotransmission have different concentration sensitivities and may therefore be recruited under different physiological conditions. The NE retrograde facilitation of glutamatergic synaptic inputs and presynaptic suppression of GABAergic synaptic inputs occur at lower concentrations of NE than the retrograde facilitation of GABAergic synaptic inputs. Therefore, at the initiation of the stress response when the NE concentration is low, the NE facilitation of glutamate transmission and suppression of GABA transmission may work together, if GABA is inhibitory (see Chap. 4), to excite the CRH neurons and activate the HPA. At a later phase of the stress response, when the NE concentration reaches its peak, the high-threshold NE facilitation of GABAergic inhibitory transmission may be recruited into the response to counter the excitatory actions of NE and dampen the activation of the CRH neurons and the HPA, which could contribute to the termination of the stress response and the return to homeostasis. The NE activation of GABAergic inputs to the CRH neurons at high concentration could also provide an inhibitory break on HPA activation in the case of extreme or prolonged activation of the ascending noradrenergic afferents. The valence of GABA signaling in CRH neurons can shift from inhibitory to excitatory following acute stress exposure (Hewitt et al. 2009), such that, paradoxically, increased GABA inputs at higher NE concentration could actually contribute to the excitation of the CRH neurons and amplify the activation of the HPA.

Fig. 8.3 (continued) neurons to stimulate spiking (5), which triggers an increase in the GABAergic/glutamatergic synaptic inputs to the postsynaptic vasopressin and CRH neurons, completing the retrograde volume transmission-activated recurrent signaling circuit

Endogenous NE release induced by optogenetic activation of the noradrenergic inputs greatly enhances excitatory synaptic transmission in CRH neurons, similar to the response to exogenous NE application. Interestingly, the effect is only partially suppressed by blocking alpha1-adrenergic receptors or by blocking G-protein signaling in the CRH neurons. Thus, it seems that the noradrenergic afferents that project to PVN CRH neurons co-release glutamate at their synapses (Chen et al. 2019), consistent with the co-expression of the vesicular glutamate transporter 2 with tyrosine hydroxylase in brainstem catecholaminergic neurons (Johnson et al. 2018).

The facilitatory effect of NE on glutamatergic and GABAergic transmission requires the activation of postsynaptic alpha1 adrenoreceptors on the CRH neurons, whereas the effect is generated by presynaptic spike-mediated glutamate and GABA release. This discordance in the pre- vs. postsynaptic sites of action indicates the involvement of a retrograde messenger, which, surprisingly, was found likely to be vasopressin (Chen et al. 2019) (Fig. 8.3). Antagonists of the vasopressin V1a receptor blocked the NE effect and exogenous vasopressin mimicked the NE effect on synaptic glutamate and GABA release. Ghrelin, which activates dendritic vasopressin release from neighboring vasopressin neurons in the PVN (see above), had no effect on the excitatory synaptic inputs to CRH neurons, suggesting that vasopressin release from vasopressin neurons does not spill over onto the CRH neurons. Also, blocking type 1 CRH receptors had no effect on the NE modulation of synaptic inputs, indicating that CRH is not the dendritic messenger. While somewhat surprising, the release of vasopressin from CRH neuron dendrites is not unfounded, since PVN CRH neurons express vasopressin in their somata and axon terminals, and vasopressin and CRH are independently regulated in CRH neurons by chronic stress (Bartanusz et al. 1993; Schmidt et al. 1997).

Chemical inhibition of astrocyte metabolism and genetic impairment of exocytosis specifically in astrocytes blocked the NE modulation of synaptic inputs to the CRH neurons, revealing the involvement of astrocytes in NE signaling. Astrocytes express vasopressin receptors, and both NE and vasopressin evoke V1a receptor-mediated calcium responses in astrocytes, likely via calcium release from intracellular stores. These observations together suggested that NE elicits the dendritic release of vasopressin from CRH neurons, and that this causes astrocyte activation (Chen et al. 2019).

Several gliotransmitters have been identified in the hypothalamus, such as glutamate, D-serine, taurine, ATP, and TNP-α (Theodosis et al. 2008) (see Chap. 2). As described above, the dendritic release of vasopressin from vasopressin neurons stimulates astrocytes to release ATP (Haam et al. 2014). The NE-induced vasopressin release from CRH neuron dendrites also stimulates astrocytes in the PVN to release ATP, which causes action potential generation in upstream glutamate and GABA neurons via ionotropic P2X purinergic receptor activation. Astrocytes can transmit signals to remote sites by propagating calcium waves among multiple astrocytes coupled electrotonically via gap junctions and/or chemically via ATP release. GABAergic neurons presynaptic to the CRH neurons are likely located outside of the PVN (Boudaba et al. 1996), which would necessitate the propagation of the NE-induced signal through a chain of astrocytes. Therefore, the recruitment of

astrocytes by dendritically released vasopressin can greatly expand the spatial domain of the NE-induced retrograde signaling.

8.4 Perspectives

During the past three decades, advances in neuroscience have revealed a significant role for glia in synaptic function. By virtue of their close spatial relationship with neuronal synaptic structures and their expression of a variety of neurotransmitter receptors, as well as their release of gliotransmitters, astrocytes are capable of reading neuronal signals and reciprocally influencing neuronal activity. The hypothalamic PVN and SON are brain regions in which physiological stimuli induce significant changes in astrocyte morphology, which cause important effects on neuroendocrine signaling, as described in Chaps. 2 and 3. Astrocytes in the PVN and SON actively modulate neuronal populations by releasing gliotransmitters in response to external stimuli. Here, we have described a novel form of neuronal-glial signaling in the hypothalamus in which the dendritic release of vasopressin from vasopressin and CRH neurons stimulates astrocytes. The activated astrocytes then transmit signals to local presynaptic GABAergic and/or glutamatergic neurons. In this way, postsynaptic neuroendocrine cells exert a robust control of the activity of their presynaptic partners via retrograde volume transmission, extending their influence on upstream synaptic circuits potentially by the reach of several astrocytic spatial domains.

8.5 Key Literature

Araque et al. (1999) The first paper that proposed the tripartite synapse model.

Bushong et al. (2002) Seminal paper demonstrating the non-overlapping spatial domains of astrocytes to create a tiling effect of astrocyte distribution.

Chen et al. (2019) This study demonstrated stress-induced NE activation of retrograde trans-neuronal-glial signaling that stimulates local glutamate and GABA circuits and elicits an increase in excitatory and inhibitory synaptic inputs to stress-related CRH neurons in the PVN.

Haam et al. (2014) This was the first study to describe retrograde trans-neuronal-glial signaling that activates upstream local circuits via dendritic volume transmission and astrocyte activation to modulate synaptic inputs to the postsynaptic neurons.

Orkand et al. (1966) A seminal study providing the first evidence of a glial calcium response to neuronal signals.

Parpura et al. (1994) One of the pioneering works that demonstrated gliotransmission by showing that calcium elevation in astrocytes triggers astrocytic glutamate release, which subsequently activates NMDA receptors on neurons.

Srinivasan et al. (2015) This study identified three different compartments of astrocytes that present distinct types of calcium fluctuations, including IP3 receptor-independent signaling.

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