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## Chapter 4

### Somato-dendritic secretion of neuropeptides

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## **Abstract**

In addition to classical neurosecretion at the synapse, many neurons also secrete neurotransmitters from their cell bodies and dendrites. By contrast to synaptic transmission, somato-dendritic secretion is best characterized as modulating the overall excitability of the target cells over a longer time-course via actions at presynaptic and extrasynaptic receptors. Magnocellular neurons of the hypothalamic supraoptic and paraventricular nuclei synthesize the neuropeptides, vasopressin and oxytocin, and were among the first neurons shown to secrete neurotransmitters from their cell bodies and dendrites by exocytosis. These neuropeptides modulate the activity of the neurons from which they are secreted, as well as the activity of neighboring neurons, to provide intra- and inter-population signals that coordinate the endocrine and autonomic responses for control of cardiovascular and reproductive physiology, as well as behavior.

## **Keywords**

oxytocin, vasopressin, magnocellular neurons, supraoptic nucleus, paraventricular nucleus, dendrite

## **Glossary**

**Antidiuretic hormone:** see *vasopressin*.

**Magnocellular neurons:** large (~20  $\mu\text{m}$  diameter cell body) neurons (also called magnocellular neurosecretory cells) that are principally located in the supraoptic and paraventricular nuclei of the hypothalamus that each project a single axon to the posterior pituitary gland via the internal zone of the median eminence. The axons branch extensively within the posterior pituitary gland and each branch possesses swellings and terminals that exocytose either vasopressin or oxytocin.

**Magnocellular neurosecretory cells:** see *magnocellular neurons*.

**Oxytocin:** a nonapeptide secreted into the bloodstream from the posterior pituitary gland as a hormonal regulator of birth and lactation, and into the brain as a neurotransmitter/neuromodulator to influence behavior and autonomic function

**Neurohypophysis:** see *posterior pituitary gland*.

**Parvocellular neurons:** a collective term (also called parvicellular neurons) used to describe non-magnocellular neurons in the hypothalamic paraventricular nucleus. Some parvocellular neurons project to the median eminence of the hypothalamus where they secrete corticotrophin releasing hormone or thyrotropin releasing hormone to stimulate secretion of adrenocorticotrophic hormone or thyroid stimulating hormone from the anterior pituitary gland, respectively. Other parvocellular neurons (that principally secrete oxytocin, vasopressin or endogenous opioid peptides) project to other brain areas to modulate behavior or autonomic function. The neurons that control autonomic function are called preautonomic neurons.

**Posterior pituitary gland:** an endocrine gland and circumventricular organ at the base of the brain from which vasopressin and oxytocin are released into the circulation. After exocytosis, the hormones diffuse through the extracellular space into the general circulation through fenestrated capillaries. Specialized glial cells (called pituicytes) physically modulate the access of vasopressin and oxytocin to the general circulation, depending on the prevailing physiological conditions.

**Preautonomic neurons:** a subset of paraventricular nucleus parvocellular neurons that project to the brainstem and spinal cord to modulate sympathetic and parasympathetic nerve activity of input to a variety of organs.

**Vasopressin:** a nonapeptide (also known as antidiuretic hormone) secreted into the bloodstream from the posterior pituitary gland as a hormonal regulator of body fluid balance and blood pressure, and into the brain as a neurotransmitter/neuromodulator to influence behavior and autonomic function.

## 4.1 Introduction

The prevailing view of neuronal communication in the brain is of point-to-point transmission at synapses within neural networks. However, knowledge of other modes of communication has rapidly expanded in recent years, providing insight into the complexity of information transfer in the nervous system. These other modes of communication are all paracrine communication that are broadly categorized in neuroscience as volume transmission because they involve neurotransmitter diffusion through the extracellular space to activate extrasynaptic receptors, or intracellular effectors (Sykova, 2004). While synaptic transmission allows for specific one-on-one interactions between individual neurons, volume transmission lends itself to communication at the population level. Many neurons have been shown to release neurotransmitters from their cell bodies and **dendrites** and **magnocellular neurons** (also known as magnocellular neurosecretory cells) of the hypothalamic **supraoptic and paraventricular nuclei** are among those for which the mechanisms and consequences of somato-dendritic exocytosis are best characterized.

## 4.2 Magnocellular neurons

Magnocellular neurons secrete the hormones, **vasopressin** (aka, antidiuretic hormone) or **oxytocin** into the general circulation. Vasopressin maintains body fluid balance and blood pressure by activation of renal  $V_2$ -receptors to increase water reabsorption from the urine and, when blood pressure/volume is decreased, by activation of vascular  $V_1$ -receptors to cause vasoconstriction (Brown, 2016). Oxytocin triggers uterine contractions during birth and milk ejection during lactation (Brown, 2016).

Each magnocellular neuron extends a single axon (that typically arises from the primary dendrite) to the posterior pituitary gland (neurohypophysis), where each axon branches extensively into several thousand neurosecretory axon swellings and terminals that are tightly packed with dense core vesicles containing either vasopressin or oxytocin, as well as lesser amounts of other neurotransmitters/neuromodulators (Brown et al., 2008). Some magnocellular neurons extend axon collaterals to other brain areas to modulate behavior (Knobloch et al., 2012).

Most magnocellular neurons extend between one and three simple dendrites that are smooth (*i.e.* do not have dendritic spines), thick, varicose and a few hundred micrometers in length. Magnocellular neuron dendrites are also tightly packed with dense core vesicles that undergo **exocytosis** (Pow and Morris, 1989) to release vasopressin or oxytocin, along with other neurotransmitters/neuromodulators (Figure 1). The dendrites of supraoptic nucleus magnocellular neurons largely extend to the ventral surface of the nucleus where they bundle together within the ventral glial lamina (a layer of astrocytes within the supraoptic nucleus) and the dendrites of paraventricular nucleus magnocellular neurons extend towards the subependymal region of the third ventricle (Brown, 2016).

### **4.3 Regulation of somato-dendritic secretion in magnocellular neurons**

While axons of magnocellular neurons have morphological structures (swellings and terminals) from which release occurs, somato-dendritic secretion appears to occur from any part of the cell body or dendrites (Morris and Pow, 1991), more reminiscent of endocrine cells than synaptic transmission. Similarly to magnocellular neuron terminals in the posterior pituitary gland, the cell bodies and dendrites contain a network of sub-plasmalemmal

filamentous actin (cortical F-actin) that might limit access of dense core vesicles to the plasma membrane for secretion (Figure 2). Again similarly to secretion from the terminals, F-actin depolymerization increases somato-dendritic vasopressin and oxytocin secretion and F-actin polymerization inhibits stimulated somato-dendritic secretion (Tobin and Ludwig, 2007) , although it remains to be determined whether specific sites in the cortical F-actin network provide dense core vesicles privileged access to the surface membrane.

Somato-dendritic exocytosis from magnocellular neurons appears to be mediated by the soluble N-ethylmaleimide sensitive factor attachment receptor (SNARE) complex, which allows vesicle fusion with the plasma membrane. Tetanus toxin cleaves vesicle-associated membrane protein-2 (VAMP-2, a core SNARE complex protein) and inhibits secretion from isolated magnocellular neurons (bereft of axons and dendrites, so secretion presumably is only from cell bodies) (de Kock et al., 2003). While many SNARE proteins have been identified in magnocellular neuron axon terminals, labelling for VAMP-2 and synaptosomal-associated protein 25 (SNAP-25, another core SNARE complex protein) is not evident in the cell bodies and dendrites of magnocellular neurons (Tobin et al., 2012). Hence, somato-dendritic exocytosis from magnocellular neurons might involve other isoforms of the core SNARE complex proteins than those involved in axon terminal release.

While neuropeptides can be released by partial (kiss-and-run) fusion with the plasma membrane, this is unlikely to occur in magnocellular neurons because their dense core vesicles are tightly packed with ~10,000 neuropeptide molecules, which is near the maximum possible due to physico-chemical constraints. Indeed, tannic acid perfusion fixation shows that the entire vesicle core is exocytosed (Pow and Morris, 1989). Hence, it is not surprising that magnocellular neurons might express a non-canonical suite of exocytotic machinery.



The dendrites of magnocellular neurons support back propagation of action potentials (Bains and Ferguson, 1999) and it has been proposed that single action potentials trigger somato-dendritic exocytosis from magnocellular neurons from studies of membrane capacitance (de Kock et al., 2003). However, a one-to-one relationship between action-potential firing and somato-dendritic dense core vesicle exocytosis is unlikely because exocytosis of ~6,000 vesicles per second would be sufficient to maintain effective concentrations of vasopressin and oxytocin within the hypothalamus (Brown et al., 2007). There are ~10,000 magnocellular neurons in the rat hypothalamus (and over 100,000 in the human) and so each magnocellular neuron would need to release (on average) only one vesicle every couple of seconds to maintain basal hypothalamic vasopressin and oxytocin concentrations. However, magnocellular neurons have a mean firing rate of ~5 action potentials per second. Hence, a one-to-one relationship between action potential firing and dense core vesicle exocytosis would flood the extracellular space with vasopressin and oxytocin. Indeed, somato-dendritic oxytocin secretion can occur in some cases when action potential firing is reduced (Sabatier et al., 2003).

Increased intracellular calcium is obligatory for exocytosis. By contrast to classical synaptic transmission, exocytosis from somato-dendritic dense core vesicles requires a sustained increase in intracellular calcium and the associated calcium sensor has a high calcium affinity. Therefore, specialized release sites are likely not essential for somato-dendritic exocytosis to occur because the dense core vesicles do not need to be close to membrane calcium channels, but this remains to be confirmed.

Magnocellular neurons express a suite of voltage-operated calcium channels that can be activated by action potential firing or by smaller sustained membrane depolarization.  $Ca_{v2.2}$  (N-type) channels carry a comparatively small current in magnocellular neurons compared to

other voltage-operated calcium channels but nevertheless are most important for somato-dendritic oxytocin release (Tobin et al., 2011). While action potential activity can activate voltage-operated calcium channels, vasopressin and oxytocin neurons express receptors for their cognate neuropeptides and the neuropeptides activate these receptors to produce a cell-type specific rise in intracellular calcium concentration (Dayanithi et al., 2000) that could drive somato-dendritic exocytosis. Calcium permeation through extrasynaptic N-methyl-D-aspartate (NMDA) receptors provides another means to trigger somato-dendritic exocytosis of vasopressin and oxytocin from magnocellular neurons (Son et al., 2013, de Kock et al., 2004). While the initial signal is a calcium influx from the extracellular space, calcium release from intracellular stores also evokes somato-dendritic secretion of neuropeptides from magnocellular neurons (Ludwig et al., 2005, Ludwig et al., 2002) and calcium buffering mechanisms regulates somato-dendritic exocytosis from magnocellular neurons through a variety of mechanisms that limit increases in intracellular calcium (Dayanithi et al., 2012).

In addition to triggering somato-dendritic secretion, increased intracellular calcium also ‘primes’ somato-dendritic secretion by promoting movement of dense core vesicles toward the cell surface (Tobin et al., 2004) and by recruitment of  $Ca_v2.2$  channels. Priming increases the somato-dendritic secretory response to other signals that mobilize intracellular calcium, such as vasopressin or oxytocin themselves. Without a priming stimulus, vasopressin and oxytocin elicit little somato-dendritic secretion but after priming each neuropeptide elicits robust somato-dendritic secretion from their respective neuronal phenotypes (Ludwig et al., 2002, Ludwig et al., 2005).

## **4.4 Physiological functions of somato-dendritic neuropeptide secretion**

The physiological functions of somato-dendritic secretion include autocrine effects on the activity of the neuron from which neurotransmitters are released, as well as paracrine effects on neighboring neurons and glia. While it has been proposed that somato-dendritic secretion of vasopressin and oxytocin might act as hormone-like signals in the brain (Ludwig and Leng, 2006), it has yet to be established whether neuropeptides released from the cell body and dendrites can impact neurons in distant brain areas, although there is some evidence that magnocellular neuron dendrites might mediate vasopressin and oxytocin activation of nearby neurons.

### ***4.4.1 Autocrine regulation of vasopressin neuron activity***

Vasopressin-containing dense core vesicles express  $V_{1a}$  and  $V_{1b}$  receptors (Hurbin et al., 2002). These receptors are presumably inserted into the cell membrane during somato-dendritic exocytosis of vasopressin and so will be exposed to high vasopressin concentrations, providing an ideal system for autocrine feedback regulation of vasopressin neuron activity.

While vasopressin neurons express a range of activity patterns, many display rhythmic ‘phasic’ activity, in which they alternate between periods of activity (bursts) and silence that each last tens of seconds and that increase the efficiency of vasopressin secretion from the posterior pituitary gland. Autocrine feedback regulation of phasic activity has been largely characterized by microdialysis drug application directly into the supraoptic nucleus

while recording spontaneous action potential discharge in anesthetized rats (Box 1 , Movie 1).

**Box 1. In vivo extracellular single-unit recording from the supraoptic nucleus with microdialysis drug application in anesthetized rats.** *In vivo* recording of the activity of magnocellular neurons in anesthetized rats allows the impact of (patho)physiological and pharmacological manipulations on the neuronal activity to be measured, without the confounding influence of uncontrolled variables evident in conscious animals. The anatomical separation of magnocellular cell bodies from their secretory terminals in the posterior pituitary gland allow for electrophysiological identification of the neurons (by antidromic stimulation from the posterior pituitary and recoding from the cell body (Movie 1); and the localization of magnocellular neurons to the hypothalamic supraoptic nucleus makes these neurons amenable to microdialysis drug administration during *in vivo* electrophysiological experiments (Movie 1) without disruption of their afferent inputs or cellular integrity. We have used this technique to apply various drugs into the supraoptic nucleus while recording phasic activity, which has identified several dendritically-released factors as modulators of this activity pattern (Brown et al., 2008).

While application of exogenous vasopressin has been reported to drive all vasopressin neurons towards phasic activity (Gouzenes et al., 1998), this is unlikely to occur naturally because it would increase circulating vasopressin concentrations to levels that would cause robust vasoconstriction that is not evident under basal conditions. Indeed, antagonism of V<sub>1a</sub>-receptors within the supraoptic nucleus consistently increases the activity of phasic neurons throughout the active period (Ludwig and Leng, 1997), indicating that endogenous vasopressin is inhibitory, probably via V<sub>1a</sub>-receptor-mediated inhibition of excitatory postsynaptic potential (EPSP) amplitude (Kombian et al., 2000) and increased inhibitory

postsynaptic potential frequency (Hermes et al., 2000). While vasopressin neurons express vasopressin receptors, the simplest explanation for the effects of vasopressin on synaptic potentials is that vasopressin also acts as a retrograde modulator of synaptic transmission as well as an autocrine feedback inhibitor of activity. Overall, somato-dendritic vasopressin secretion contributes to the generation of phasic activity patterning in vasopressin neurons necessary for efficient release of vasopressin into the bloodstream under basal conditions (Figure 3).

In addition, somato-dendritic vasopressin secretion functions as a negative feedback mechanism to restrain excitation of vasopressin neurons at a population level. This hypothesis is supported by the observation that osmotic stimulation of vasopressin neurons increases vasopressin release into the blood and brain. However, central release is delayed (perhaps while priming is established) and prolonged compared to peripheral release (Ludwig et al., 1994), such that central release is increasing while peripheral release is decreasing as would be expected by negative feedback regulation of vasopressin neuron activity by somato-dendritic vasopressin secretion.

#### **4.4.1.1 Autocrine regulation of vasopressin neuron activity by co-released neurotransmitters**

Magnocellular neurons synthesize and secrete number of neuropeptides in addition to vasopressin and oxytocin, although in much lower quantities than the principal neuropeptides. For vasopressin neurons, these neuropeptides include apelin, dynorphin, galanin, neuroendocrine regulatory peptides (NERPs), pituitary adenylate cyclase activating polypeptide (PACAP) and secretin (Brown et al., 2008).

While most neuropeptides in magnocellular neurons are found within the same dense core vesicles as vasopressin or oxytocin, at least apelin and galanin appear to be segregated from vasopressin. Apelin is packaged in a distinct group of dense core vesicles from vasopressin while some dense core vesicles contain only galanin or vasopressin (although others contain both galanin and vasopressin). Clearly, segregation might allow for differential release of vasopressin or oxytocin and their co-expressed neuropeptides. Indeed, this does appear to be the case because the co-expressed neuropeptides and galanin-containing vesicles are preferentially trafficked to the dendrites rather than the axons (Landry et al., 2003).

It is not known whether co-expressed neuropeptides other than apelin and galanin are also differentially segregated in dense core vesicles of vasopressin neurons but, if so, differential release of co-expressed neuropeptides might reinforce or restrain peripheral secretion of vasopressin because apelin, PACAP and secretin excite vasopressin neurons while dynorphin, NERPs and galanin inhibit vasopressin neurons (Brown, 2016).

While it possible that there is some segregation of dynorphin and vasopressin, it is clear that dynorphin and vasopressin are present in many of the same dense core vesicles and that these vesicles also express  $\kappa$ -opioid receptors (Shuster et al., 2000), for which dynorphin is the canonical agonist, and  $\kappa$ -opioid receptors are inserted into the plasma membrane along with vasopressin receptors during somato-dendritic exocytosis. Similarly to vasopressin, with which it will be co-secreted, dynorphin inhibits phasic activity in vasopressin neurons but unlike vasopressin, dynorphin inhibition is activity-dependent; *i.e.* it is absent at the onset of phasic bursts and increases over the course of bursts, even during osmotic stimulation (Figure 3) when prodynorphin mRNA expression is up-regulated (Scott et al., 2009). Hence, somato-dendritic secretion of dynorphin appears to be involved

in generation of phasic activity in vasopressin neurons. While the membrane current that sustains activity during phasic bursts in vasopressin neurons is inhibited by endogenous dynorphin (Brown and Bourque, 2004), similarly to vasopressin, dynorphin also acts as a retrograde modulator of synaptic transmission (Iremonger and Bains, 2009) and this too might contribute to activity patterning.

Remarkably, differential somato-dendritic secretion of dynorphin might also contribute to the adoption of different activity patterns across the population of vasopressin neurons. Similarly to phasic neurons, action potential firing in vasopressin neurons displaying irregular activity is robustly excited by administration of a  $\kappa$ -opioid receptor antagonist, indicating that they are also under  $\kappa$ -opioid inhibition (Scott et al., 2009). By contrast, action potential firing in continuously-active vasopressin neurons is unchanged by  $\kappa$ -opioid receptor antagonist administration despite being strongly inhibited by  $\kappa$ -opioid agonist administration (Scott et al., 2009), indicating that continuously-active vasopressin neurons express functional  $\kappa$ -opioid receptors but do not release dynorphin. Hence, it appears that vasopressin neurons that escape autocrine dynorphin inhibition enter continuous activity.

Overall, it appears that autocrine regulation of activity patterning in vasopressin neurons regulates phasic activity under basal conditions. Intuitively, it could be hypothesized that a relaxation of autocrine inhibition is a mechanism by which vasopressin neurons increase their activity under stimulated conditions. However, this does not appear to be the case, at least for dynorphin feedback, which counter-intuitively increases during chronic osmotic stimulation by dehydration (Scott and Brown, 2010). Hence, autocrine inhibition of vasopressin neurons by somato-dendritic secretion probably functions as a restraining mechanism to prevent increased activation of afferent inputs precipitating over-secretion of

vasopressin into the bloodstream, providing for a graded hormonal response to graded (patho)physiological stimuli.

#### ***4.4.2 Paracrine regulation of preautonomic neuron activity by somato-dendritic vasopressin secretion***

Unlike the supraoptic nucleus, which essentially comprises only magnocellular neurons (and glia), the paraventricular nucleus also contains parvocellular neurons that are involved in the regulation of hormone secretion from the anterior pituitary gland, autonomic function through projections that control activity of autonomic regulatory (preautonomic neurons) and behavior.

Preautonomic neurons modulate sympathetic and parasympathetic nerve activity to a variety of organs, including the heart, blood vessels and kidneys to ultimately influence cardiovascular function and body fluid homeostasis, among other physiological systems. As their name implies, preautonomic neurons do not project directly to the target organs in the periphery. Rather, they project to sympathetic and parasympathetic centers in the brainstem and spinal cord, including the nucleus tractus solitarius, rostral ventrolateral medulla and dorsal motor nucleus of the vagus in the brainstem and preganglionic sympathetic neurons in the intermediolateral cell column of the spinal cord that project outside the central nervous system to regulate autonomic function.

While there is some anatomical compartmentalization of magnocellular and parvocellular neuron cell bodies within the paraventricular nucleus, some vasopressin neuron dendrites approach the cell bodies of preautonomic neurons in the parvocellular division of the paraventricular nucleus and preautonomic neurons express vasopressin receptors (Son et al., 2013), providing a framework for somato-dendritic vasopressin secretion from magnocellular



neurons to modulate autonomic function by paracrine actions within the paraventricular nucleus. Indeed, activation of individual vasopressin neurons by uncaging N-methyl-D-aspartate (NMDA) to activate individual vasopressin neurons increased action potential firing in neighboring preautonomic neurons that retrogradely-labeled from the rostral ventrolateral medulla (Son et al., 2013). The responses of preautonomic neurons to activation of magnocellular vasopressin neurons occur with a delay of several seconds, depend on intracellular calcium and are blocked by superfusion of a vasopressin receptor antagonist. Taken together, these observations strongly suggest that the excitation is mediated by diffusion of somato-dendritic vasopressin through the extracellular space. Most remarkably, excitation of a single vasopressin neuron excites several neighboring preautonomic neurons, suggesting that this represents a population-to-population communication rather than point-to-point communication between individual neurons. Indeed, administration of a vasopressin receptor antagonist alone reduces the activity of preautonomic neurons and the effectiveness of the antagonist depends on the activity level of the vasopressin neurons population; when the vasopressin neurons are more active the antagonist is more effective at reducing the firing rate of preautonomic neurons. Hence, it appears that preautonomic neurons are under tonic modulation by neighboring magnocellular vasopressin neurons that likely coordinates the humoral (vasopressin) neuronal (sympathetic) responses to (patho)physiological perturbations (Figure 4).

**Box 2. Retrograde labelling of axon projections.** This technique allows the identification of the cell bodies of the neurons with axons that terminate in the brain area of interest by injecting retrograde label (e.g. fluorescent microspheres) into the region of interest. It is important that a retrograde label is used that is only taken up at axon terminals to prevent confounding results caused by label that could be taken up by projections passing through the

area of interest. After several days, the retrograde label can be visualized in the cell bodies of projecting neurons, which can be double-labelled using immunohistochemistry to identify the phenotype of the projecting neurons. Or, as we have done (Son et al., 2013), retrograde labelling can be completed in animals that have the neuronal phenotype of interest labeled by genetic modification (e.g. enhanced green fluorescent protein, eGFP) to allow identification of retrogradely-labeled neurons of a particular phenotype to be studied (e.g. by patch-clamp electrophysiology in brain slices).

#### ***4.4.3 Autocrine regulation of oxytocin neuron activity***

Similarly to somato-dendritic vasopressin secretion, somato-dendritic oxytocin secretion also has autocrine paracrine effects. However, by contrast to vasopressin, the paracrine effects of somato-dendritic oxytocin secretion on oxytocin neurons are mediated by endocannabinoids that are liberated after autocrine receptor activation to cause retrograde inhibition of synaptic transmission (Hirasawa et al., 2004). While endocannabinoid retrograde inhibition of synaptic transmission is triggered by somato-dendritic oxytocin secretion, the overall effect of somato-dendritic oxytocin secretion is excitation, but only under specific (patho)physiological conditions.

##### **4.4.3.1 Burst firing in oxytocin neurons**

Under basal conditions, magnocellular oxytocin neurons typically fire slowly and continuously at 1 – 5 action potentials per second to sustain circulating concentrations of 1 – 3 pg per ml, with higher concentrations during sleep. However, as a hormone, oxytocin is best known for its stimulation of uterine contraction during birth and of milk ejection

during suckling. Indeed, oxytocin comes from the Greek, *oxutokia*, meaning ‘sudden delivery’. Both uterine contraction and contraction of the mammary ducts for milk ejection are episodic, with each contraction occurring several minutes apart. It is the coordinated secretion of pulses of oxytocin by the population of oxytocin neurons that generates the pattern of contractions required for normal birth and for milk delivery to the offspring and somato-dendritic secretion plays a fundamental part in generating the pulsatile secretion pattern through its influence on oxytocin neuron activity.

Pulsatile oxytocin secretion during birth and suckling results from bursts of action potentials in oxytocin neurons (Brown, 2016). Unlike phasic bursts in vasopressin neurons, bursts fired by oxytocin neurons are generally much faster (up to 100 action potentials per second), relatively short (1 – 3 seconds) and, most remarkably, are coordinated across the population of oxytocin neurons in the bilateral supraoptic and paraventricular nuclei (Belin et al., 1984).

While oxytocin neurons do not normally fire bursts under basal conditions, they can be induced to do so in brain slices by treatment with an  $\alpha_1$ -adrenoceptor agonist in a low calcium medium (Wang and Hatton, 2005), suggesting that noradrenergic inputs might trigger bursts. Indeed, the number of noradrenergic inputs to oxytocin neurons is increased in late pregnancy and lactation and norepinephrine levels are increased in the supraoptic nucleus during birth. However, noradrenergic excitation of bursts in oxytocin neurons is likely mediated via stimulation of somato-dendritic oxytocin secretion because noradrenergic receptor stimulation is required for suckling-induced somato-dendritic oxytocin secretion, which might be part of a positive feedback loop that builds towards bursts because oxytocin increases norepinephrine levels within the supraoptic nucleus. Indeed, oxytocin levels rise within the supraoptic nucleus immediately preceding bursts in lactating rats (Moos et al.,

1989) and bursts are blocked by oxytocin receptor antagonist administration, suggesting that (regardless of what the initiating event might be) somato-dendritic secretion of oxytocin is required for bursts to occur.

Bursts can also be evoked in oxytocin neurons of anaesthetized virgin rats by coordinated activation of neighboring oxytocin neurons and this is enhanced by agents that induce priming by mobilization of intracellular calcium, including oxytocin itself (Ludwig et al., 2002). Hence, while plasticity in oxytocin neurons, their surrounding astrocytes and their afferent inputs over the course of pregnancy favors the emergence of bursting (Augustine et al., 2018), these changes are not necessary for bursting to occur. Rather, it appears that the core component of the system necessary for bursts is somato-dendritic oxytocin secretion (Figure 5). Indeed, in anaesthetized lactating rats, central oxytocin receptor antagonist administration inhibits burst firing during suckling. Nevertheless, somato-dendritic oxytocin secretion is likely not sufficient to induce bursts in oxytocin neurons because simple administration of oxytocin does not induce bursting in virgin rats.

The role of endocannabinoids in burst firing of oxytocin neurons is not known but, if bursts liberate large amounts of endocannabinoids these might contribute to burst termination and perhaps also the short silent period that often follows oxytocin neuron bursts.

#### **4.4.3.3 Morphine dependence in oxytocin neurons**

In addition to its endocrine regulation of reproductive function, oxytocin modulates a wide range of behaviors, including addictive behaviors and is emerging as a potential treatment for drug addiction, including opiate addiction (McGregor and Bowen, 2012). Remarkably, oxytocin neurons develop dependence during chronic administration of the  $\mu$ -opioid

receptor agonist, morphine (Brown and Russell, 2004), which is revealed by a long-lasting hyper-excitation upon removal of chronic morphine treatment.

Both norepinephrine and oxytocin levels are increased in the supraoptic nucleus during morphine withdrawal and acute antagonism of supraoptic nucleus  $\alpha_1$ -adrenoceptors or oxytocin receptors reverses morphine-withdrawal excitation of oxytocin neurons (Brown et al., 1997), consistent with noradrenergic stimulation of somato-dendritic oxytocin secretion driving increased activity during withdrawal through autocrine (and perhaps paracrine) communication (Figure 6). Oxytocin neurons are under tonic inhibition by endogenous opioid peptides during pregnancy and this inhibition is relaxed at the end of pregnancy. However, this is likely not the trigger for birth because oxytocin neurons do not develop dependence on endogenous  $\mu$ -opioid peptides (Doi et al., 2001). Furthermore, while morphine-withdrawal excitation evokes somato-dendritic oxytocin secretion as occurs during birth and suckling, it does not result in bursts of action potentials in oxytocin neurons. Rather, morphine-withdrawal excitation is a pharmacological phenomenon that precipitates a sustained increase in oxytocin neuron firing rate and of oxytocin secretion into the circulation. Hence, while somato-dendritic oxytocin secretion might be the core component of the system necessary for bursts to occur, there must be modifying elements, probably in the timing of interactions between each element in the system, as revealed by the emergence of burst firing during coordinated activation of neighboring oxytocin neurons (Ludwig et al., 2002); *i.e.* morphine withdrawal leads to sustained increases in somato-dendritic oxytocin secretion to underpin a sustained increase in firing rate across the population (Brown et al., 1997), whereas uterine contraction and suckling lead to fluctuations in somato-dendritic oxytocin secretion to underpin coordinated burst firing across the population.

#### **4.4.3.3 Co-expressed neuropeptides**

Similarly to vasopressin neurons, oxytocin neurons also synthesize other neuropeptides, principally the endogenous opioid peptides, the enkephalins and dynorphin. However, it appears unlikely that these have a substantive role in orchestrating oxytocin neuron activity because administration of the broad-spectrum opioid receptor antagonist, naloxone, does not affect oxytocin neuron activity under basal conditions (Brown and Russell, 2004). Rather, it appears that these co-released opioid peptides might restrain oxytocin secretion at the level of the posterior pituitary gland because opioid receptor antagonist administration increases oxytocin secretion under basal conditions without changing oxytocin neuron firing rate (Brown et al., 1998).

#### ***4.4.4 Paracrine regulation of behavior by somato-dendritic oxytocin secretion***

In addition to its endocrine regulation of reproductive function, central oxytocin modulates a wide variety of behaviors (Ross and Young, 2009). Some of this behavioral modulation by oxytocin is likely through axonal projections to the nucleus accumbens and central amygdala, which include axon collaterals from magnocellular oxytocin neurons (Knobloch et al., 2012). However, central oxytocin also appears to modulate some behaviors via actions in brain areas that receive little or no axonal projections from oxytocin neurons, suggesting that these areas might respond to somato-dendritic secretion of oxytocin.

#### **4.4.4.1 Regulation of energy balance by somato-dendritic oxytocin**

Centrally-released oxytocin inhibits food intake and there is emerging evidence that oxytocin mediates the central effects of the primary anorexigenic hormone, leptin, which is secreted by adipocytes (Leng and Sabatier, 2017). Leptin is sensed by pro-opiomelanocortin neurons in the arcuate nucleus of the hypothalamus that project to the supraoptic and paraventricular

nuclei where they secrete  **$\alpha$ -melanocyte stimulating hormone** ( $\alpha$ -MSH) to activate melanocortin-3 and -4 receptors.

The effects of  $\alpha$ -MSH on oxytocin neurons are complex; while  $\alpha$ -MSH inhibits the activity of oxytocin neurons to reduce secretion into the circulation, it mobilizes intracellular calcium to increase somato-dendritic oxytocin secretion. The dissociation of somato-dendritic and peripheral secretion of oxytocin results from the actions of endocannabinoids, which mediate the inhibition of activity without impacting somato-dendritic oxytocin secretion (Sabatier and Leng, 2006).

The effects of somato-dendritic oxytocin secretion on food intake are likely mediated by actions within the ventromedial nucleus of the hypothalamus, in addition to other brain areas where there is high expression of oxytocin receptors but essentially no oxytocin neuron axons (Leng and Sabatier, 2017). Given the close proximity of the ventromedial nucleus to the supraoptic and paraventricular nuclei, it appears likely that somato-dendritic oxytocin from magnocellular neurons could diffuse through the extracellular space in sufficient quantities to activate oxytocin receptors, which have nanomolar affinity for oxytocin.

#### **4.4.4.2 Regulation of social behavior by somato-dendritic oxytocin**

Central oxytocin impacts many behaviors, but there is little clear evidence in most cases as to whether this results from oxytocin secretion from axon terminals, or somato-dendritic secretion. There is strong evidence that the effects of oxytocin on fear conditioning are mediated via synaptic activation of central amygdala neurons by magnocellular neuron axon collaterals (Knobloch et al., 2012). While the central amygdala contains oxytocin neuron axons, the neighboring medial amygdala (which lies immediately lateral to the supraoptic nucleus) has few, if any, oxytocin neuron axons but does contain some oxytocin neuron

dendrites. Oxytocin receptor antagonist injection into the medial amygdala attenuates preference for a stranger induced by activation of the supraoptic nucleus (Takayanagi et al., 2017). Hence, it appears likely that it is somato-dendritic oxytocin secretion from the supraoptic neurons enhances social recognition.

## 4.5 Perspectives

The autocrine effects of somato-dendritic secretion from magnocellular neurons are well-established, as are the local paracrine effects on their afferent inputs. Similarly, there is strong evidence to suggest that nearby neuronal populations, specifically those in the paraventricular nucleus, ventromedial nucleus and medial amygdala are regulated by somato-dendritic neuropeptide secretion from magnocellular neurons. However, there is still debate as to whether vasopressin and oxytocin act as hormone-like signals on distant targets in the brain after diffusion through the extracellular space. Certainly, the neuropeptides are present in the cerebrospinal fluid (CSF) but the levels measured in various brain areas do not always parallel CSF levels (Landgraf et al., 1988). Furthermore, while vasopressin and oxytocin have half-lives of ~20 min in the CSF, extracellular peptidases likely breakdown the neuropeptides more rapidly in the parenchyma, and the tortuosity of the parenchyma likely limits diffusion, either from the site of release or from the ventricles (*e.g.* the paracrine effects of somato-dendritic vasopressin on preautonomic neurons that are only ~100 – 200  $\mu\text{m}$  away is delayed by ~2 – 5 seconds). A further confounding factor is that many of the distant brain areas on which the neuropeptides act (such as the nucleus accumbens and nucleus tractus solitarius) receive afferent projections from magnocellular neuron axon collaterals or paraventricular nucleus parvocellular neurons. Nevertheless, the possibility remains that there is long-distance hormone-like signaling by vasopressin and oxytocin in the brain over a much longer timescale, particularly in brain regions in which there are neuropeptide receptors but no



neuropeptide axons, such as the olfactory bulb. In addition, somato-dendritic secretion of vasopressin and oxytocin could modulate excitability of the same target neurons to which they (or parvocellular neurons) send axonal projections to modulate excitability on a population level on which the short-term point-to-point modulation achieved by synaptic transmission is superimposed. Resolving this long-standing debate remains an ongoing challenge for the field.

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## **4.7 Further Recommended Reading**

### ***Somato-dendritic secretion***

Dendritic Neurotransmitter Release. (2004) ed. LUDWIG, M. pp xvi + 334, Springer, New York. ISBN 10: 0387229337 ISBN 13: 9780387229331

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### ***Phasic activity patterning in vasopressin neurons***

BROWN, C. H. & BOURQUE, C. W. 2006. Mechanisms of rhythmogenesis: insights from hypothalamic vasopressin neurons. *Trends Neurosci.*, 29, 108-115.

### ***Morphine dependence in oxytocin neurons***

BROWN, C. H. & RUSSELL, J. A. 2004. Cellular mechanisms underlying neuronal excitability during morphine withdrawal in physical dependence: lessons from the magnocellular oxytocin system. *Stress*, 7, 97-107.



### ***Inter-population signaling***

STERN, J. E. 2015. Neuroendocrine-autonomic integration in the paraventricular nucleus: novel roles for dendritically released neuropeptides. *J Neuroendocrinol*, 27, 487-497.

### ***Reproductive regulation of oxytocin neuron activity***

AUGUSTINE, R. A., SEYMOUR, A. J., CAMPBELL, R. E., GRATTAN, D. R. & BROWN, C. H. 2018. Integrative neuro-humoral regulation of oxytocin neuron activity in pregnancy and lactation. *J. Neuroendocrinol.* 30, e12569. <https://doi.org/10.1111/jne.12569>.

### ***Regulation of energy balance by oxytocin***

LENG, G. & SABATIER, N. 2017. Oxytocin - The sweet hormone? *Trends Endocrinol. Metab.*, 28, 365-376.

### ***Regulation of social behavior by oxytocin***

ROSS, H. E. & YOUNG, L. J. 2009. Oxytocin and the neural mechanisms regulating social cognition and affiliative behavior. *Front. Neuroendocrinol.*, 30, 534-547.

## **Index**

$\alpha$ -melanocyte stimulating hormone

Antidiuretic hormone

Dynorphin

Endocannabinoid

Exocytosis

Magnocellular neurons

Neurohypophysis

Oxytocin

Lactation

Morphine dependence

Parturition

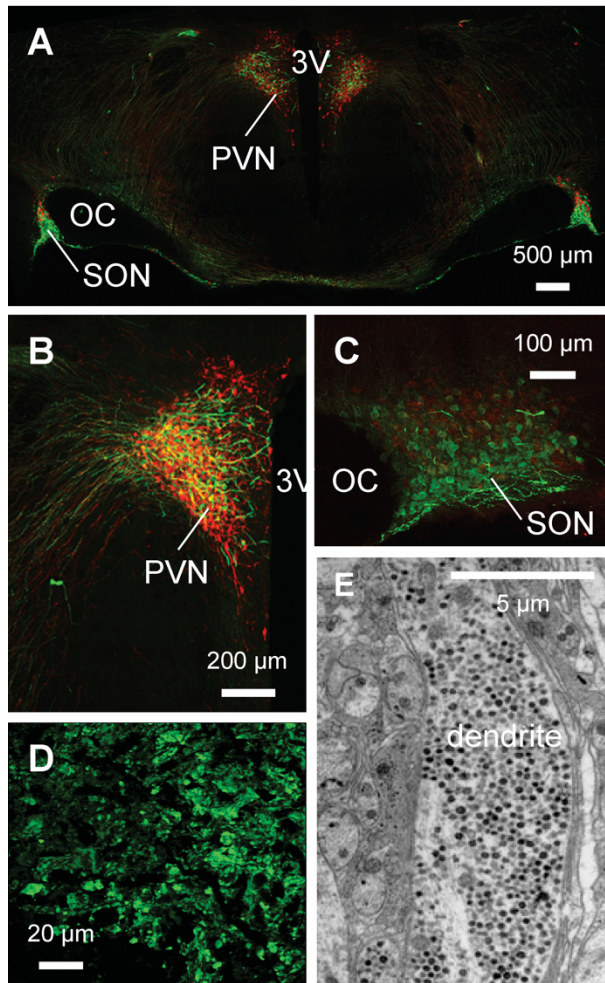
Posterior pituitary gland

Preautonomic neuron

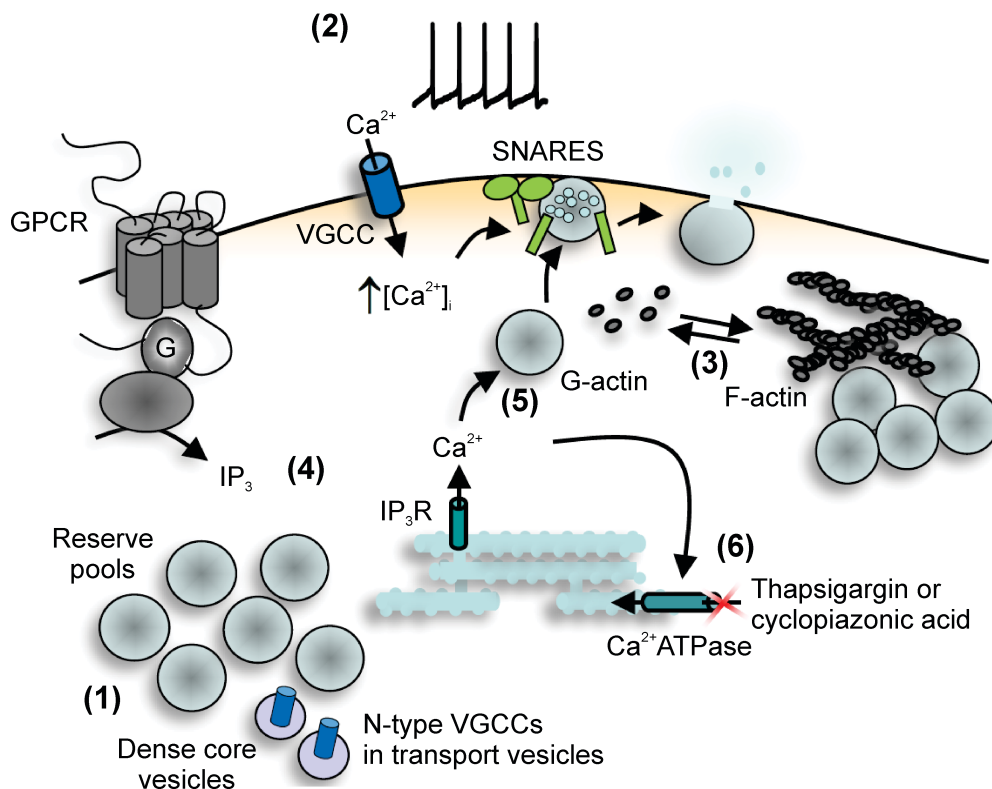
Somato-dendritic secretion

Vasopressin

Brown et al Figure 1

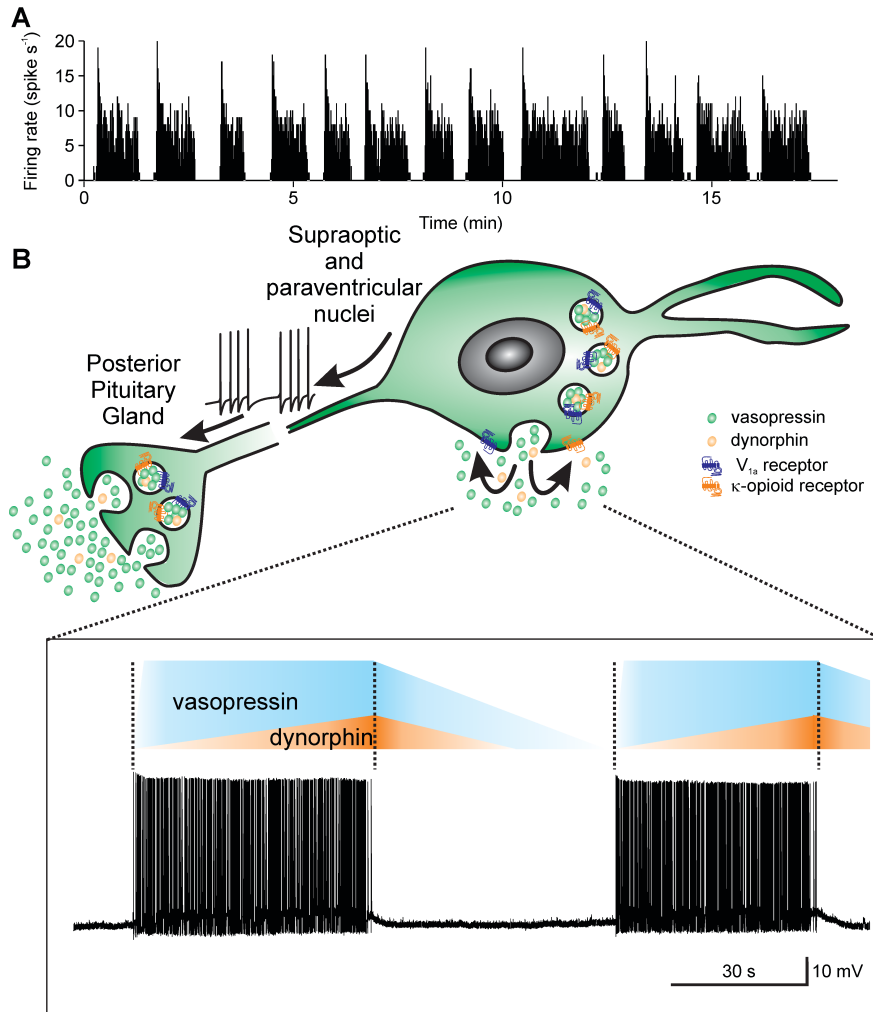


**Figure 1. The magnocellular neurosecretory system.** **A – C**, Photomicrographs of coronal sections of rat hypothalamus (**A**), in which vasopressin neurons are immunostained with fluorescent green and oxytocin neurons with fluorescent red. Magnocellular cell bodies are principally found in the hypothalamic supraoptic nucleus (**SON**) (**B**), lateral to the optic chiasm (**OC**), and paraventricular nucleus (**PVN**) (**C**), lateral to the third cerebral ventricle (**3V**). The **SON** contains only magnocellular neurons that project predominantly to the posterior pituitary gland, whereas the **PVN** also contains parvocellular oxytocin and vasopressin neurons (as well as other parvocellular neurons) that project elsewhere in the brain. **D**, Photomicrograph of vasopressin axon terminals in the posterior pituitary gland. **E**, Electron micrograph showing magnocellular dendrites densely packed with dense core neurosecretory vesicles (small black dots). Reproduced from (Brown et al., 2013), with permission.

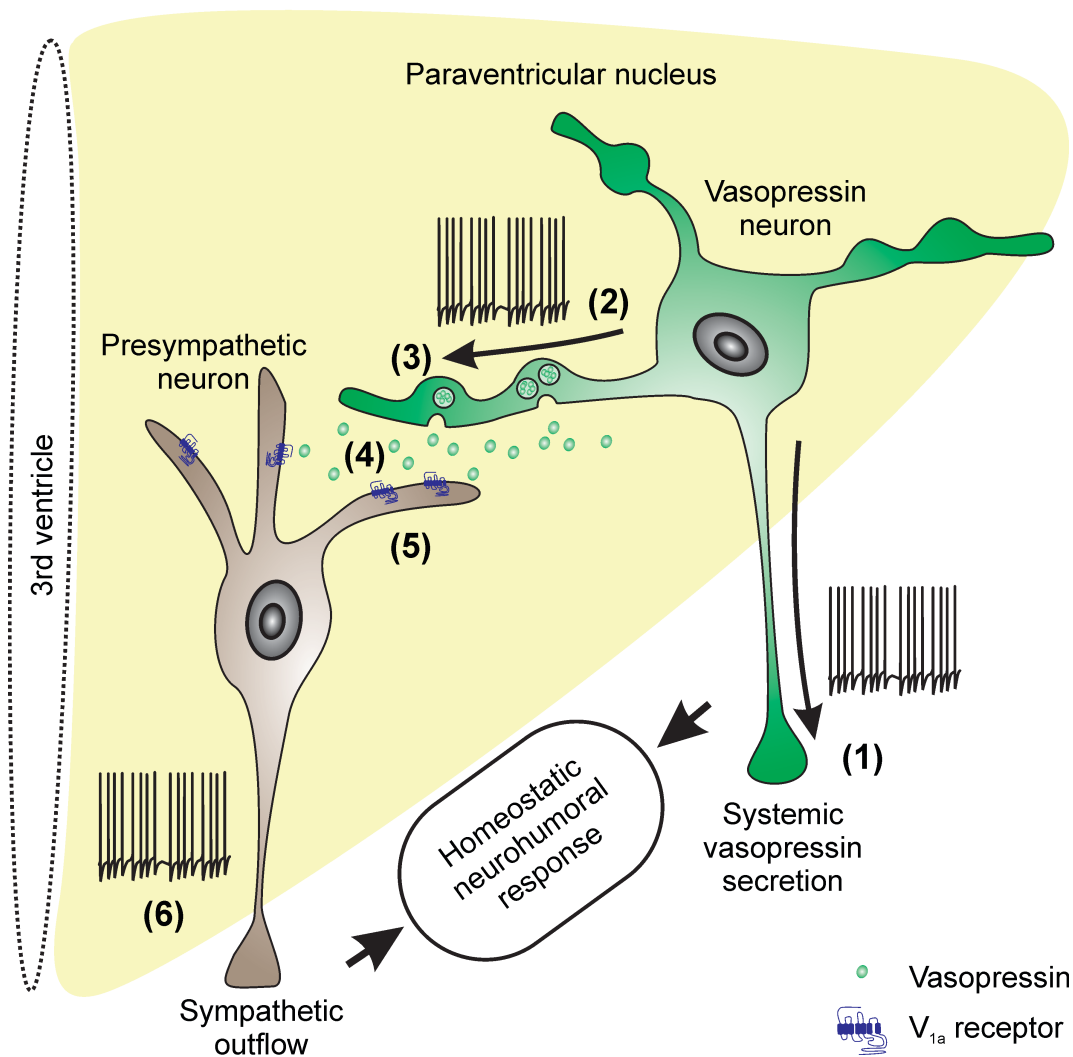


### Figure 2. Somato-dendritic neuropeptide secretion from magnocellular neurons.

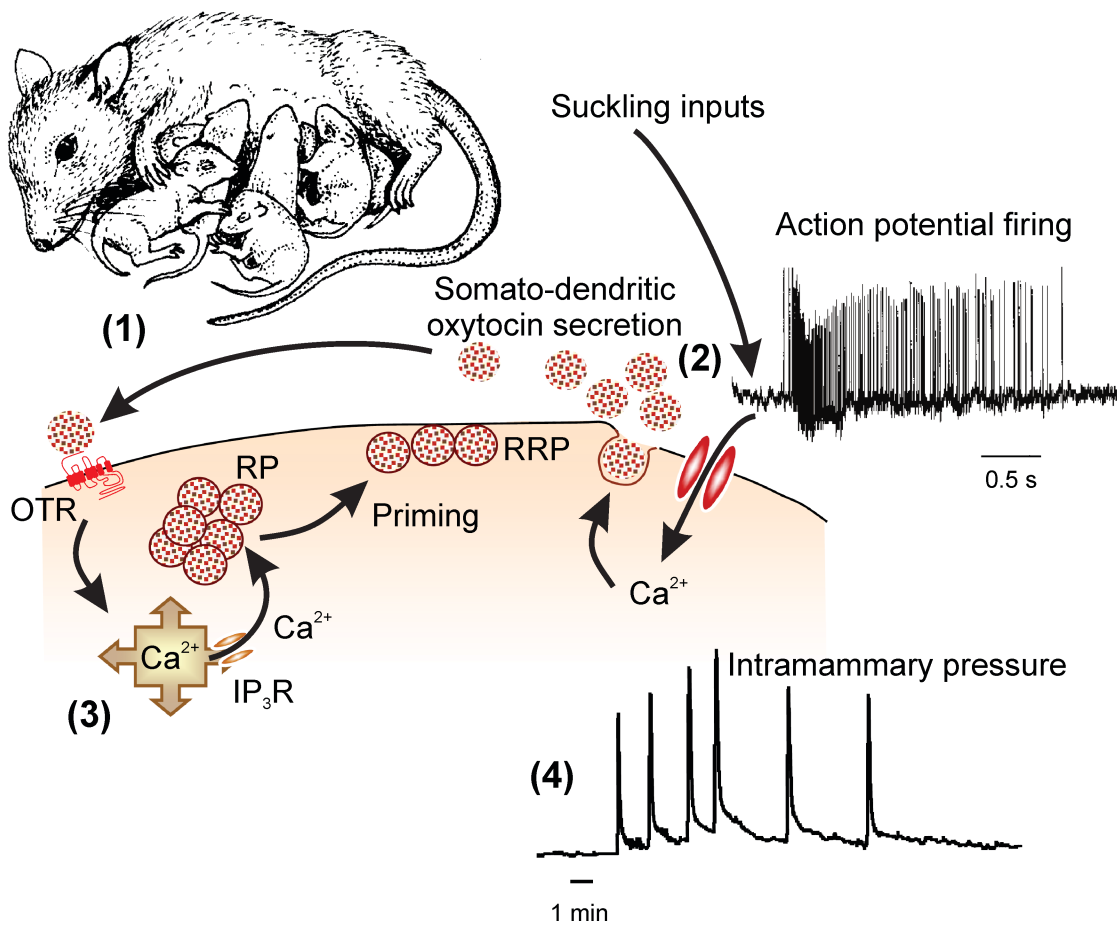
Neuropeptides are synthesized and packaged in the cell body and stored in a reserve pool (RP) containing large numbers of dense core vesicles in the cell body and dendrites (1). Depolarization-induced calcium influx through voltage gated calcium channels (VGCCs) stimulates exocytosis to secrete neuropeptide into the extracellular space (2). Access of dense core vesicles to the plasma membrane requires the depolymerization of F-actin to G-actin (3). In addition, activation of G<sub>q</sub>-protein coupled receptors (GPCRs), including the oxytocin receptor, mobilizes calcium from inositol triphosphate (IP<sub>3</sub>)-dependent intracellular stores (4) and increases the number of dense core vesicles and N-type VGCCs at the plasma membrane (5), priming the exocytotic machinery for subsequent activity-dependent release. Direct stimulation of calcium release from intracellular stores with thapsigargin or cyclopiazonic acid also causes priming (6). Although some members of the SNARE family are detectable by immunocytochemistry, there appears to be little or no VAMP, SNAP-25 and synaptotagmin-1 in the cell bodies and dendrites of magnocellular neurons, with their function presumably being replaced by other SNARE proteins. Modified from (Ludwig et al., 2017), with permission.



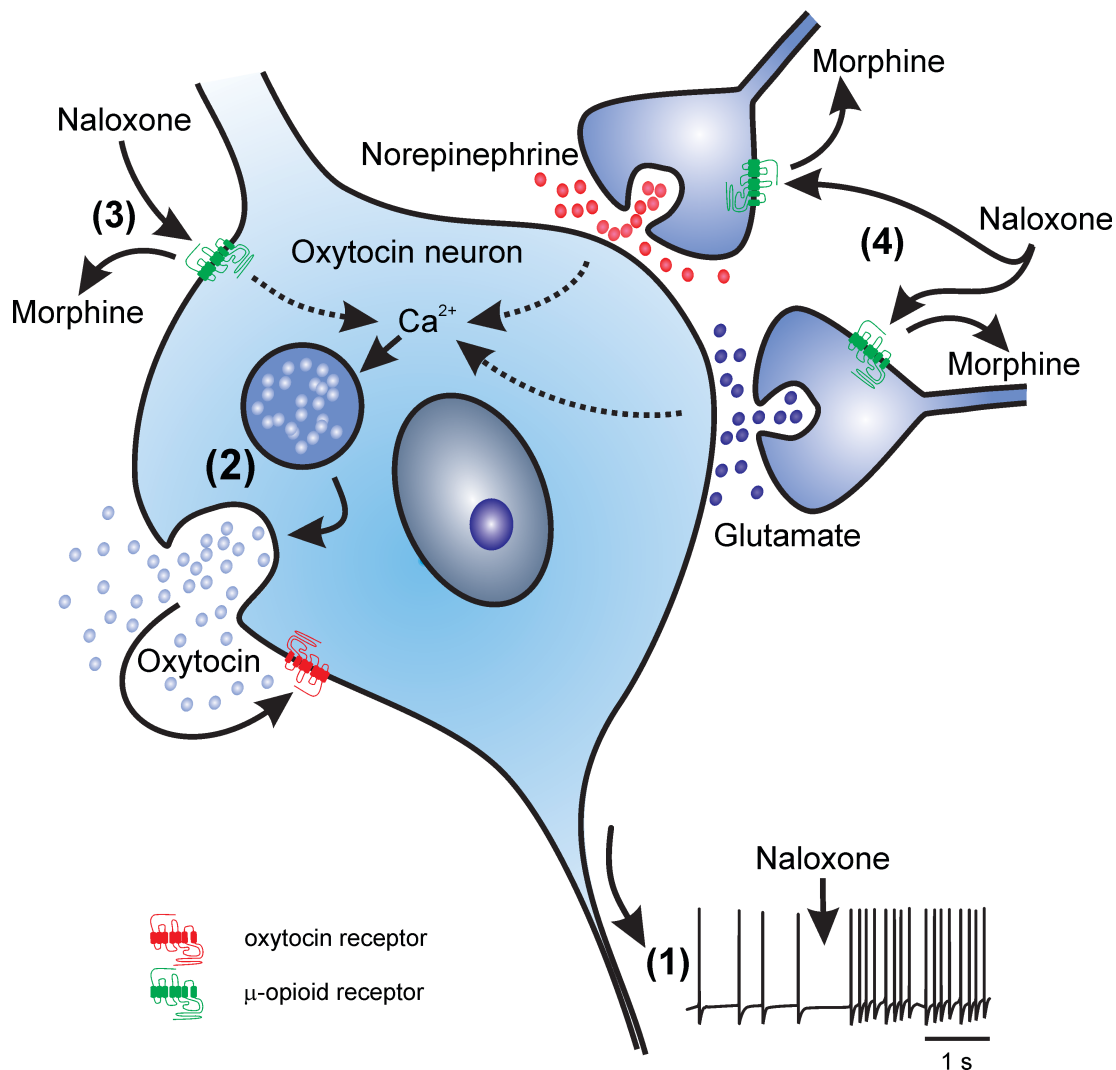
**Figure 3. Autocrine inhibition of phasic activity in vasopressin neurons by somato-dendritic neuropeptide secretion.** **A.** *In vivo* extracellular recording of the firing rate (averaged in 1 s bins) of a vasopressin neuron. The neuron displays the highly organized phasic activity pattern characteristic of vasopressin neurons, with periods of activity (bursts) and silence that each last tens of seconds. **B.** Schematic representation of autocrine feedback inhibition of phasic activity in vasopressin neurons. Once a burst starts, somato-dendritic vasopressin and dynorphin secretion occurs with concurrent insertion of  $V_{1a}$  and  $\kappa$ -opioid receptors into the cell membrane. The inset illustrates the temporal profile of vasopressin and dynorphin feedback. Vasopressin inhibition reaches maximum in within the first few seconds of burst initiation and is maintained through the burst. Dynorphin inhibition progressively increases during the burst, eventually helping to terminate firing. After burst termination, vasopressin and dynorphin are cleared by diffusion and extracellular peptidases and the cycle restarts with the beginning of the next burst.



**Figure 4. Autonomic activation by somato-dendritic vasopressin secretion.** (1) Activation of magnocellular vasopressin neurons leads to a burst of action potentials that increase vasopressin secretion into the circulation from the posterior pituitary gland. (2) Concurrently, the action potentials also back-propagate into the dendrites to (3) trigger somato-dendritic vasopressin release into the paraventricular nucleus. (4) Vasopressin passively diffuses through the extracellular space and (5) binds to V<sub>1a</sub> receptors on presympathetic paraventricular nucleus neurons to cause membrane depolarization and thus increased firing, leading in turn to increased sympathetic outflow to peripheral organs. Somato-dendritic vasopressin secretion contributes to the coordination of the neurohumoral response to physiological challenges that is required to maintain homeostasis. Reproduced from (Stern, 2015) with permission.



**Figure 5. Autocrine excitation of oxytocin neurons during suckling.** Suckling during lactation (1) activates a relay of afferent inputs from the mammary glands that ultimately stimulates bursts of action potentials in oxytocin neurons as well as calcium influx (2) that triggers somato-dendritic oxytocin secretion that primes further oxytocin release by activating oxytocin receptors (OTR) to increase intracellular calcium by activating inositol triphosphate receptors (IP<sub>3</sub>R), which mobilizes somato-dendritic dense core vesicles from the reserve pool (RP) to the readily-releasable pool (RRP) near the cell surface (3). The released oxytocin might act as a paracrine signal that coordinates bursts across the population of oxytocin neurons to releases large pulses that causes episodic milk let-down (4) in response to a continuous stimulus. Modified from (Leng et al., 2012), with permission.



**Figure 6. Autocrine excitation of oxytocin neurons during morphine withdrawal.**

Administration of the broad-spectrum opioid receptor antagonist, naloxone, displaces morphine from chronic occupancy of  $\mu$ -opioid receptors, which results in an immediate and sustained increase in oxytocin neuron firing rate (1) due, in part, to increased somato-dendritic secretion of oxytocin (2). Increased somato-dendritic oxytocin secretion during morphine withdrawal presumably results from increased intracellular calcium because excitation is prevented by administration of a calcium channel blocker (Blackburn-Munro et al., 2000). However, it is currently unknown whether calcium influx is directly triggered by naloxone antagonism of  $\mu$ -opioid receptors on oxytocin neurons (3) or their principal excitatory afferent inputs, norepinephrine and glutamate, which also express  $\mu$ -opioid receptors (4).



