Published in final edited form as:
J Neuroendocrinol. 2013 August ; 25(8): . doi:10.1111/jne.12051.

Physiological regulation of magnocellular neurosecretory cell activity: Integration of intrinsic, local and afferent mechanisms

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Abstract

The hypothalamic supraoptic and paraventricular nucleus contain magnocellular neurosecretory cells (MNCs) that project to the posterior pituitary gland where they secrete either oxytocin or vasopressin (the anti-diuretic hormone) into the circulation. Oxytocin is important for delivery at birth and is essential for milk ejection during suckling. Vasopressin primarily promotes water reabsorption in the kidney to maintain body fluid balance, but also increases vasoconstriction. The profile of oxytocin and vasopressin secretion is principally determined by the pattern of action potentials initiated at the cell bodies. While it has long been known that the activity of MNCs depends upon afferent inputs that relay information on reproductive, osmotic and cardiovascular status, it has recently become clear that activity depends critically on local regulation by glial cells, as well as intrinsic regulation by the MNCs themselves. Here, we provide an overview of recent advances in our understanding of how intrinsic and local extrinsic mechanisms integrate with afferent inputs to generate appropriate physiological regulation of oxytocin and vasopressin MNC activity.

Keywords

Paraventricular nucleus; supraoptic nucleus; oxytocin; vasopressin; osmoregulation; reproduction

Introduction

Oxytocin and vasopressin (the anti-diuretic hormone) are secreted into the systemic circulation from the posterior pituitary gland. While oxytocin and vasopressin have many peripheral and central functions (1), circulating oxytocin is primarily known for its essential role in milk let-down during suckling and for its involvement in the normal progression of parturition, whereas circulating vasopressin principally acts in the kidney to promote water reabsorption, and on the vasculature to increase blood pressure via vasoconstriction. At least in the rat, oxytocin complements vasopressin actions in the kidney via promotion of natruiresis (2) to help maintain plasma osmolality.

There has been only one oxytocin receptor identified to date (3, 4). The many different cellular actions of oxytocin are mediated by activation of various G-protein (Gq/11, Gs and G_i) coupled intracellular signalling pathways (5, 6), the best characterised of which is

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activation of phospholipase C via $G_{q/11}$ (7). By contrast, the effects of vasopressin are mediated by V_{1a}, V_{1b} (V_3) and V_2 receptors, which are differentially distributed throughout the body. In the periphery, $G_{q/11}$-coupled V_{1a} receptors are highly expressed in vascular smooth muscle and induce vasoconstriction in response to strong hypotensive or hypovolaemic stimuli (8); $G_{q/11}$-coupled V_{1b} receptors are expressed in the anterior pituitary gland and mediate vasopressin-induced secretion of adrenocorticotrophic hormone (9); and G_s-coupled V_2 receptors are highly expressed in the collecting duct principal cells of the kidney and mediate water and sodium reabsorption by increasing insertion of aquaporin-2 (10) and epithelial sodium channels (11) into the luminal membrane.

While regulation of receptor numbers and signal transduction can modulate the efficacy of hormone signalling, the temporal organisation of the effects oxytocin and vasopressin are largely determined by the profile of hormone secretion. The secretion of both hormones is principally regulated by the action potential discharge of the oxytocin- and vasopressin-synthesising neurones (magnocellular neurosecretory cells; MNCs) within the hypothalamus.

Under basal conditions, vasopressin MNCs maintain plasma vasopressin concentrations at ~1 - 3 pg ml^{-1} (12) to cause the reabsorption of ~30 litres of water from the urine each day in humans. Despite being exposed to the same prevailing physiological conditions, vasopressin MNCs display a wide range of activity patterns (13); some fire no spontaneous spikes (silent), some fire spikes irregularly, some fire continuously and some fire in a rhythmic ‘phasic’ pattern of bursts (Figure 1), during which there is clear spike frequency adaptation (a reduction in firing rate with time), separated by silent periods that each last tens of seconds (14, 15). It is the integrated output of the population of vasopressin MNCs produces the circulating concentration of vasopressin (16).

Similarly to vasopressin MNCs, oxytocin MNCs display a range of activity patterns under basal conditions: silent, irregular or continuous firing that maintains circulating concentrations of oxytocin relatively constant at ~1 – 3 pg ml^{-1} (12). However, during parturition and lactation, oxytocin MNCs adopt a firing pattern comprised of high frequency co-ordinated bursts (17) that cause large pulses in oxytocin concentrations to induce rhythmic contraction of the uterus for birth and contraction of the milk ducts for milk ejection (Figure 1E).

The adoption and maintenance of the appropriate hormone secretion profile for the prevailing physiological conditions relies upon afferent inputs that relay information on reproductive and osmotic status. However, the regulation of activity is more complex than passive following of afferent input activity. It has recently become clear that the activity of MNCs is strongly influenced by glial cells and also that the patterning of activity is dependent upon intrinsic regulation by the MNCs themselves. A further layer of complexity is evident at a population level because hormone output is determined by the integrated activity of the population as a whole; for vasopressin MNCs this requires that some MNCs adopt different activity patterns in response to the same physiological conditions, whereas oxytocin MNCs must adopt the same activity pattern across the whole population under specific circumstances. Nevertheless, the biological message encoded by the neuronal population as a whole can be deduced from the physiological response to the secreted hormone, which allows interpretation of the function of activity patterning across the population of MNCs. In this review, we provide an overview of the means by which local and intrinsic mechanisms translate afferent information into appropriate activity patterns that generate the required physiological response.

The magnocellular neurosecretory system

Anatomy of the hypothalamo-neurohypohysial system

There are over 100,000 MNCs in the human (18) and ~10,000 in the rat (19). While MNCs are principally located in the hypothalamic supraoptic nucleus (SON) and paraventricular nucleus (PVN) (Figure 2A – C), there are substantial numbers in some smaller accessory cell groups, of which the largest is the nucleus circularis (accessory magnocellular nucleus), located approximately mid-way between the PVN and SON (20).

In terms of neuronal phenotypes, the SON contains essentially only MNCs, which synthesise either oxytocin or vasopressin. By contrast, the PVN also contains parvocellular neurones that project to the median eminence as well as to other brain regions (21). MNCs each project a single axon caudally and medially to collect in the hypothalamo-neurohypophysial tract, which courses through the internal zone of the median eminence to the posterior (neural lobe of the) pituitary gland (the neurohypophysis) where oxytocin and vasopressin are secreted into the general circulation (22). Within the posterior pituitary gland, each axon arborises extensively to give rise to several thousand neurosecretory axon swellings and terminals (Figure 2D). MNC axon terminals are tightly packed with dense-core (neurosecretory) vesicles that contain vasopressin or oxytocin, as well as a distinct population of microvesicles that contain glutamate (23). While has been widely accepted that MNCs project only to the posterior pituitary gland, it has recently been shown that some oxytocin MNCs (particularly those with cell bodies in the nucleus circularis) project axon collaterals to (at least) forebrain areas, including the central amygdala and nucleus accumbens (24).

Most MNCs have between one and three dendrites (25). In the SON, most dendrites course ventrally to the ventral glial lamina (a layer of glial cells on the ventral surface of the SON), where they form a plexus of dendritic bundles (26). In the PVN, the dendrites mainly track dorsally and medially towards the subependymal region of the third ventricle (27). MNC cell bodies and dendrites are also densely packed with dense-core vesicles (Figure 2E).

Secretion from magnocellular neurosecretory cells

Secretion of oxytocin and vasopressin from axon swellings and terminals in the posterior pituitary gland occurs by exocytosis of dense-core vesicles in response to action potential invasion of the neurosecretory membrane. Once released into the extracellular space, the hormones enter the general circulation by diffusion through fenestrated capillaries in the posterior pituitary gland (28). MNC terminals do not sustain intrinsic repetitive action potential discharge (29) and so hormone secretion is principally determined by the frequency and pattern of action potential discharge initiated at the cell bodies. Nevertheless, various factors can modulate stimulus-secretion coupling at MNC terminals in the posterior pituitary gland, including ionic conditions (30), purines (31) and neuropeptides (32).

The secretory response to increasing action potential firing rate is highly non-linear (Figure 3); in response to trains of electrical stimuli, oxytocin and vasopressin release from isolated posterior pituitary glands is facilitated as the frequency of stimulation increases (33). This ‘frequency facilitation’ of hormone release differs between oxytocin and vasopressin terminals. Frequency facilitation of oxytocin release is most marked between ~5 and 25 Hz, but release continues to increase (albeit with a slower rate of increase) beyond ~50 Hz. By contrast, frequency facilitation of vasopressin release is maximal at ~13 Hz and sustained higher frequency stimulation actually results in less vasopressin release (33, 34). Frequency facilitation of hormone release has been ascribed to increased calcium entry through voltage-gated channels to enhance exocytosis, as a consequence of action potential broadening during high frequency firing, depolarisation due to accumulation of potassium in

the extracellular space, and a progressively more extensive invasion of the neurosecretory terminal field in the posterior pituitary gland (35). Frequency facilitation of hormone release is not sustained upon continuous stimulation, with a more pronounced 'fatigue' in facilitation of vasopressin release than oxytocin release (36). This fatigue is rapidly reversed when stimulation is stopped for a few tens of seconds. These properties of MNC terminals are important for generating the appropriate secretion of oxytocin and vasopressin to meet the prevailing physiological demands. A corollary of frequency facilitation of release is that MNCs firing at less than ~4 spike s^{-1} likely releases little or no hormone from the posterior pituitary gland *in vivo* (34).

Hence, while the profile of posterior pituitary secretion is orchestrated by the pattern of action potential discharge at the cell bodies, the terminals actively modulate the spread of the signal within the neurosecretory terminal field, as well as the efficacy of stimulus-secretion coupling, to fine-tune the gain of the secretory output generated.

Remarkably, MNC cell bodies and dendrites also release oxytocin and vasopressin by exocytosis (37) in response to mobilisation of intracellular calcium stores (38). A number of other neuropeptides are also synthesised by MNCs. For vasopressin MNCs, these include the κ-opioid peptide, dynorphin (39), as well as apelin (40), galanin (41), neuroendocrine regulatory peptides (NERPs) (42, 43), pituitary adenylate cyclase-activating polypeptide (PACAP) (44) and secretin (45), each of which are co-localised with vasopressin in dense-core vesicles (46). Similarly, oxytocin MNCs also synthesise other neuropeptides, including proenkephalin A-derived μ-opioid peptides (47) and dynorphin (48). In addition to neuropeptides, MNCs express high levels of nitric oxide synthase (49) and their dense-core vesicles contain large amounts of ATP (50) that is presumably secreted along with the neuropeptides during periods of activity. The physiological function of these co-released substances will be described in more detail later this review.

The glutamate contained within MNC microvesicles is also released in response to action potential invasion of the posterior pituitary gland. However, high frequency action potential discharge is thought to trigger release of neuropeptides in preference to classical neurotransmitters (51) and so, if glutamate is released from the axon terminals and this release is regulated as it is in other neurons, the profile of glutamate secretion is likely to be distinct from that of hormone secretion, with glutamate more readily released at low firing rates.

Activity patterning in magnocellular neurosecretory cells

Continuous activity in oxytocin and vasopressin magnocellular neurosecretory cells

While oxytocin and vasopressin MNCs can display markedly different action potential firing patterns, particularly when facing specific physiological challenges such as dehydration or pregnancy and lactation, most MNCs fire continuously under basal conditions, regardless of phenotype (52). While water deprivation for as little as six hours was initially reported to induce phasic activity in the majority of vasopressin neurons (51), we have found that the majority of MNCs are still continuously active after 48 h of water deprivation (13).

Although both oxytocin and vasopressin MNCs display continuous activity, there are marked differences in the rate and organisation of firing in continuous oxytocin and vasopressin MNCs *in vivo*. Oxytocin MNCs generally fire action potentials at a slower rate (~2 – 4 spikes s^{-1}) than vasopressin MNCs (~4 – 6 spikes s^{-1}) under basal conditions. The difference in basal spontaneous firing rate might be caused by differences in the organisation of continuous firing between the two cell types; these differences can be revealed by

calculating the probability of the next action potential firing with time after the preceding action potential to generate a hazard function.

The hazard function reflects the post-spike excitability of MNCs (54), and shows that vasopressin MNCs are generally more excitable soon after each action potential than oxytocin MNCs (55) (Figure 4). For 10 – 20 ms after each action potential, both oxytocin and vasopressin MNCs are unexcitable and will not fire a further spontaneous action potential (the relative refractory period). Thereafter, excitability increases progressively in both oxytocin and vasopressin MNCs. However, while oxytocin MNC post-spike excitability increases from zero at ~20 – 30 ms after each spike to reach a steady-state hazard from ~70 ms after each spike, vasopressin MNC post-spike excitability overshoots (in both continuous and phasic vasopressin MNCs) before returning to a similar steady-state excitability as evident in oxytocin MNCs (55). These differences in post-spike excitability might contribute to the differences in spontaneous firing rate between oxytocin and vasopressin MNCs because chronic blockade of the overshoot of post-spike excitability in vasopressin MNCs (54) reduces vasopressin MNCs firing rate to ~1 spike s^{-1} and the firing rate of these MNCs is increased to ~4 spikes s^{-1} by acute reversal of the blockade (52).

Phasic activity in vasopressin magnocellular neurosecretory cells

While many vasopressin MNCs are silent, irregular or continuous under basal and stimulated conditions, it is generally accepted that phasic activity is the most efficient for secretion of vasopressin from the posterior pituitary gland (35).

Phasic activity is characterised by periods of activity that last at least 15 s (with the probability of burst termination being essentially stochastic thereafter) separated by silent periods that last for at least 10 s (with the probability of the next burst starting being essentially stochastic thereafter) (56). At the onset of bursts, vasopressin MNCs typically achieve firing rates of ~15 – 25 spikes s^{-1} for the first 5 – 10 s, before accommodating to a steady-state firing rate of ~ 6 spikes s^{-1} for the remainder of the burst (14, 15).

Given that vasopressin release is maximal at ~13 Hz (33, 34), the largest secretion of vasopressin is likely achieved during the first 5 – 10 s of each burst. As alluded to earlier, vasopressin release fatigues during continuous stimulation but this fatigue is reversed when stimulation is stopped for a few tens of seconds (36). Hence, the silent periods between bursts probably reset vasopressin MNCs to release further vasopressin at the onset of the next burst, when the typical firing rate is again in the range that is most efficient for vasopressin release.

The importance of the silent periods and burst duration for efficient vasopressin release is illustrated by the protection of these parameters from change during chronic osmotic stimulation (13, 53, 57, 58). By contrast, the firing rate within bursts has consistently been reported to be increased during chronic osmotic stimulation (13, 53, 57, 58). Hence, the increased intra-burst firing rate will keep phasic vasopressin MNC activity in the optimal range for efficient vasopressin release for longer during each burst but there will still be adequate time for recovery from fatigue of release between bursts.

The secretory profile of vasopressin depends on the integrated activity of the entire population of vasopressin MNCs, rather than the activity of any particular individual MNC. Phasic bursts are not synchronous across the population of vasopressin MNCs (14); this, coupled with the dampening effect of the (less efficient) continuous release from continuously-active vasopressin MNCs likely accounts for the smooth profile of vasopressin concentrations in the bloodstream in the face of highly variable vasopressin secretion from each phasic MNC. Hence, to maintain the appropriate level of vasopressin in the

bloodstream (under both basal and stimulated conditions), individual vasopressin MNCs must not only sense the prevailing physiological conditions but co-ordinate their response with their neighbours to prevent over- or under-recruitment of MNCs to the actively-secreting phasic MNC pool. The mechanisms by which this co-ordination is achieved are unknown but might involve somato-dendritic release from MNCs.

While recruitment of all vasopressin MNCs to phasic activity has been reported during prolonged water deprivation (53), we and others have found that chronic osmotic stimulation does not increase the proportion of MNCs displaying phasic activity compared to the proportion evident under basal conditions it is unlikely (13, 57, 58). Plasma vasopressin concentrations are ~1 pg ml^{-1} in conscious rats and this increases to ~10 pg ml^{-1} during chronic osmotic stimulation (59). However, plasma concentrations can approach 100 pg ml^{-1} in response to extreme acute osmotic stimuli (60). Presumably, these very high concentrations are achieved when all vasopressin MNCs adopt phasic activity. Hence, it is likely that only the most extreme stimulation would induce phasic activity in all vasopressin MNCs because this would be expected to increase plasma vasopressin to concentrations well above the reported range. Thus, even under chronic stimulation, individual vasopressin MNCs must not only sense the changing physiological conditions but co-ordinate their individual response with those of their neighbours to mount the appropriate physiological response across the population of MNCs (16).

Generation of phasic activity in vasopressin magnocellular neurosecretory cells—While phasic activity was first observed in anaesthetised rats in the early 1970s (61), the mechanisms that generate phasic activity patterning are still the subject of debate. Phasic bursts were originally demonstrated to result from activation of an intrinsic after-potential in MNCs recorded from brain slices, specifically an afterdepolarisation (ADP) that sustains regenerative activity during each burst (62). However, the most recent data from organotypic cultures prepared from juvenile rats suggest that phasic activity is driven by glutamatergic synaptic inputs because EPSP frequency and amplitude appear to be increased during phasic bursts (63). It is likely that both processes contribute to the generation of phasic activity *in vivo*, as comprehensively reviewed elsewhere (14, 15).

Excitatory synaptic input activity is clearly essential for action potential discharge in vasopressin MNCs *in vivo* because administration of glutamate receptor antagonists will silence phasic MNCs (64-66), even under stimulated conditions (66). Hence, it is probable that the final event that causes membrane potential to cross threshold and trigger each action potential *in vivo* is an EPSP. Nevertheless, the probability of action potential firing *in vivo* is influenced by the occurrence of action potentials, as revealed by the hazard function analyses of post-spike excitability described above. These changes in post-spike excitability modulate the probability that any EPSP will reach threshold to trigger the next action potential and are thought to result from changes in post-spike potentials that follow each action potential in MNCs (55) (Figure 4): a large-amplitude fast after-hyperpolarisation (fAHP) that lasts <10 ms (67), a small-amplitude medium AHP (mAHP) that lasts several hundred ms (68, 69) and a very small-amplitude slow AHP (sAHP) that lasts several seconds (68, 70) as well as a large-amplitude fast ADP (fADP) that lasts a few hundred ms (71) and low-amplitude slow ADP (sADP) that lasts several seconds (72, 73). The influence of these post-spike potentials on activity patterning in phasic MNCs waxes as wanes because each has a different amplitude and time-course that allow them to dominate membrane potential at different times following each action potential, and to summate over different timescales to impact activity at different times over the course of bursts.

Briefly, for ~10 s after termination of the previous burst, MNCs remain silent while the neurone recovers from a prolonged post-burst hyperpolarisation (56) that is probably

generated by sAHP summation. Thereafter, bursts are stochastically initiated (56), presumably when EPSPs cross threshold to generate an action potential. The large fADP that follows each action potential bootstraps further action potential firing to establish the burst (71) and probably, along with increased EPSP frequency and amplitude (63), supports the very fast firing evident in the first few seconds of each burst. The longer-lasting sADP summates to establish a plateau potential that maintains membrane potential relatively close to threshold to sustain activity during bursts (56). The influence of the sADP is moderated by the mAHP, which summates to induce spike frequency adaptation over the first ~5 – 20 s of bursts (67, 69), as well as by activity-dependent inhibition of the sADP (74). In addition to the sADP and mAHP, the fAHP and sAHP probably also modulate the amplitude of the plateau potential: the fAHP paradoxically increases firing rate during bursts via activation of a hyperpolarisation-activated inward current (I_H) (75) and sAHP summation decreases firing rate (70). As stated above, bursts typically last at least 15 – 20 s, with the probability of burst termination being essentially stochastic thereafter (56). Hence, it appears that the dynamic interaction of the various post-spike potentials establishes each burst but that the opposing influences of various AHPs and ADPs eventually reach a steady state to maintain a relatively stable plateau potential, during which time burst termination occurs stochastically as stochastic spontaneous EPSPs eventually fail to reach threshold (or when a burst of IPSCs fires (74, 75)), at which point the plateau potential decays decreasing the probability of on-going EPSPs reaching threshold and the burst terminates.

Counter-intuitively, GABA might also be involved in initiating, as well as terminating, phasic bursts; bursts of action potential-independent miniature IPSCs (mISPCs) that last a few seconds have been recorded from SON MNCs in hypothalamic slices, and current injections simulating IPSC bursts initiated bursts of action potential discharge in silent MNCs via a rebound depolarization, as well as terminating on-going action potential discharge by driving the plateau potential away from threshold (77). However, these observations are difficult to reconcile with the observation that local antagonism of SON $GABA_A$ receptors does not excite vasopressin MNCs *in vivo* (78) and the recent *in vitro* finding that GABA is always excitatory on vasopressin MNCs (but not oxytocin MNCs) from adult rats (79).

Burst firing of oxytocin magnocellular neurosecretory cells during birth and lactation

While oxytocin is essential for milk ejection when the young suckle, it does not appear to be essential for delivery of the young at parturition because transgenic mice lacking oxytocin deliver live young that die of starvation unless fostered by mice with oxytocin (80). Nevertheless, oxytocin is required for the normal progression of birth because administration of an oxytocin receptor antagonist to mice during parturition delays (but does not prevent) the delivery of subsequent pups (81, 82). Oxytocin is the most powerful uterotonic agent known, but also stimulates release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) from the endometrium (83). At the end of pregnancy, $PGF_{2\alpha}$ release triggers luteolysis in rats and thus causes withdrawal of progesterone to induce labour. There is a striking increase in the expression of uterine oxytocin receptors at the end of pregnancy (84), which is also triggered by $PGF_{2\alpha}$ release; deletion of the $PGF_{2\alpha}$ receptor prevents the rise in uterine oxytocin receptor expression, rendering the uterus insensitive to oxytocin and thus causing delivery to fail (85). Hence, the interplay of oxytocin and $PGF_{2\alpha}$ appears to be required for normal delivery.

As described earlier, the mean firing rate of oxytocin MNCs is generally ~2 – 4 spikes s^{-1}. However, during parturition and suckling, intermittent high-frequency bursts occur every 5 – 10 min that are responsible for rhythmic contraction of the uterus and of the milk ducts. These bursts are superimposed upon this slow/irregular firing (86-89); bursts each last only

1 – 2 s, but the action potential activity is intense, often with firing rates of 50 – 100 spikes s^{-1}. After each burst, oxytocin MNCs typically fall silent for several seconds, presumably to allow the neurones to recover from fatigue of hormone secretion. Because these bursts occur every few minutes, last for only a couple of seconds and are followed by a period of inactivity, the overall increase in firing rate is only about 1 – 2 spikes min^{-1} for each oxytocin MNC.

Oxytocin MNCs exhibit bursts only at parturition and during lactation; all other physiological stimuli increase the activity of oxytocin MNCs in a graded manner. This sustained release does not trigger uterine contraction or milk ejection; rather, sustained activation, such as elicited by hyperosmotic stimulation, actually inhibits bursting (90). Furthermore, pulses of oxytocin do not increase natriuresis in the kidney (91). Hence, these superimposed firing patterns allow oxytocin MNCs to simultaneously perform two distinct physiological functions.

Each burst triggers the transient release of a bolus of oxytocin from the posterior pituitary gland that causes the episodic contraction of the uterus, critical for delivery of the young, or of the milk ducts, essential for milk ejection (89). Administration of the μ-opioid receptor agonist, morphine, or the L-type calcium channel blocker, verapamil, silences oxytocin MNCs (92, 93) and prevents stimulation of oxytocin secretion (92, 94). Morphine administration interrupts parturition for up to an hour (95). Similarly, verapamil injected during lactation interrupts milk ejections for up to an hour (92). The morphine-induced interruption of parturition can be overcome by episodic administration of oxytocin to mimic the endogenous secretion profile, but not by continuous administration of the same amount of oxytocin (95). Similarly, episodic contraction of the milk ducts can be precipitated by repeated systemic injection of oxytocin, but not by strong stimulation of sustained oxytocin secretion (96). Hence the pattern of oxytocin release from the posterior pituitary gland generates the pattern of uterine and milk duct contraction.

Generation of burst firing in oxytocin magnocellular neurosecretory cells— The electrical activity of SON MNCs is correlated with intrauterine pressure during parturition (Figure 1E) and the phase relationship between MNC firing and uterine activity suggests that uterine contractile activity increases the acitivty of oxytocin MNCs, rather than simple uterine distension (97). These observations are consistent with the bursting activity of oxytocin MNCs being driven by afferent input activity in response to peripheral signals. However, the mechanisms that cause burst firing in oxytocin MNCs have been most extensively studied in lactation, when milk-ejection bursts occur every 5 – 10 min during suckling of the young, and have been extensively reviewed elsewhere (98). It is more difficult to explain how afferent inputs translate the continuous suckling of the young into episodic activation of oxytocin MNCs. Nevertheless, it has long been believed that the initial trigger for bursts is gated by afferent input because milk-ejection bursts only occur during active suckling *in vivo*. Furthermore, the number of action potentials in each burst increases as the number of pups suckled increases (99), consistent with afferent gating of bursts.

However, burst-like activity (although with lower intra-burst firing rates) has been induced *in vivo* in virgin female rats by mobilisation of calcium release from oxytocin MNC intracellular stores (38) and *in vitro* by α_1-adrenoceptor agonist administration in a low-calcium medium to MNCs from lactating rats (76) and male rats (100), suggesting that bursting might be an emergent property of the oxytocin MNCs themselves within their local environment. As with phasic activity in vasopressin MNCs, burst firing of oxytocin MNCs likely arises from a combination of intrinsic and extrinsic processes.

During basal firing, the intervals between consecutive action potentials in oxytocin MNCs are typically at least 20 ms (55, 101). By contrast, during suckling-induced milk-ejection bursts in anaesthetised rats, almost half of the intervals between consecutive action potentials are less than 20 ms (101). Moreover, analysis of action potential discharge patterns *in vivo* shows that short inter-spike intervals are usually followed by long inter-spike intervals but that during bursts, short intervals are more likely to be followed by further short intervals (38). Taken together, these observations suggest that post-spike potentials in oxytocin MNCs change to favour the emergence of bursting activity during parturition and lactation. Indeed, over the course of pregnancy, the proportion of oxytocin MNCs that express a sADP increases to be more than double that evident in virgin rats (102, 103); this might cause the depolarisation that occurs during pharmacologically-induced bursts (76) and hence the ability of oxytocin MNCs to fire action potentials with very short inter-spike intervals during endogenous bursts. Whether a similar plasticity occurs in the fADP of oxytocin MNCs has yet to be determined. Counter-intuitively, the mAHP and sAHP are also increased in oxytocin MNCs of late pregnant and lactating rats (102-104), a change that appears to be dependent on central oxytocin receptors (105) as well as on prolonged calcium transients in oxytocin MNCs (104). The increased mAHP and sAHP might limit the duration of oxytocin MNC bursts; in this scenario, burst initiation results in summation of sADPs (and perhaps fADPs) to cause a marked depolarisation that triggers rapid action potential discharge while progressive activation of the mAHP would quickly reverse this depolarisation to reduce firing rate and terminate the burst (analogous to, but more pronounced than, spike frequency adaptation in phasic vasopressin MNCs). The concurrent activation of the more prolonged sAHP might then cause the silent period evident for a few seconds after each burst *in vivo*.

While plasticity in post-spike potentials favours the emergence of bursting activity during parturition and lactation, such changes are unlikely to trigger each individual burst. Synaptic glutamate release on oxytocin MNCs is enhanced during lactation (106), suggesting that increased excitatory synaptic efficacy might contribute to burst firing. The ability of mISPC bursts to trigger action potential discharge described above is evident in oxytocin MNCs as well as vasopressin MNCs (77), suggesting that a rebound depolarization after a burst of IPSPs might cause bursting in oxytocin MNCs. Indeed bursts of IPSPs might be more effective in late-pregnant and lactating rats because a switch in $GABA_A$ receptor subunit expression increases the duration of IPSCs at these times (107). In addition, the sustained outward potassium current that facilitates a rebound depolarization following a burst of IPSCs is enhanced in oxytocin MNCs from lactating rats (103, 108), favouring enhanced summation of IPSPs during birth and lactation when bursts of action potentials are required for delivery of the offspring and for delivery of milk to the new-born.

Co-ordination of burst firing across oxytocin magnocellular neurosecretory cells—While the adoption of burst firing by oxytocin MNCs is remarkable, an even more striking feature of these bursts is that they are co-ordinated across the population of oxytocin MNCs located in two distinct bilateral nuclei (17, 109); while bursts of action potentials from each individual oxytocin MNC will release oxytocin from that MNC, co-ordination among MNCs is required for the increased release to be translated into pulsatile release of oxytocin from the population of MNCs; this pattern of secretion causes the rhythmic contraction of the uterus during normal parturition as well as the episodic contraction of the milk ducts for milk ejection (97).

Although bursts are certainly co-ordinated, they are not entirely synchronous; there are no leaders and followers, and there is no synchrony between individual action potentials across the population (17, 109). Hence, the recruitment of the entire population of oxytocin MNCs

to co-ordinated bursts of activity during parturition and lactation does not result from pacemaker activity within the population.

As explained earlier, bursts of mIPSCs have been recorded from SON MNCs that can initiate bursts of action potentials (77). Remarkably, some of these bursts were co-ordinated between recorded pairs of MNCs (77). Hence, co-ordinated bursts of mIPSPs might contribute to the co-ordination of bursts across the population of MNCs as well as the generation of bursts in individual MNCs.

Somato-dendritic secretion from magnocellular neurosecretory cells

MNC cell bodies and dendrites are densely packed with dense-core vesicles from which they release oxytocin and vasopressin by exocytosis (37). Somato-dendritic release is can be activity dependent (110, 111). However, it has been estimated that, on average, each MNC releases less than one vesicle every second from the somato-dendritic compartment (112). Hence, somato-dendritic exocytosis of individual dense-core vesicles from MNCs is unlikely to be triggered by individual action potentials. Indeed, sustained depolarisation, such as can be elicited *in vitro* by high extracellular potassium concentrations, markedly increases somato-dendritic release (38). Furthermore, somato-dendritic secretion can also be triggered by increasing intracellular calcium levels (38, 113, 114), which allows some stimuli, such as a α-melanocyte stimulating hormone, to increase somato-dendritic secretion while inhibiting electrical activity (115). Hence, the profile of somato-dendritic release does not necessarily follow the profile of secretion into the bloodstream.

Somato-dendritic vasopressin release

The membranes of vasopressin-containing neurosecretory vesicles contain V_{1a} and V_{1b} receptors (116) that are presumably inserted into the cell membrane and exposed to high local concentrations of vasopressin during exocytosis. Antagonism of V_{1a}-receptors within the SON increases the activity of phasic MNCs *in vivo* (117), an effect that is evident from the onset of bursts (110), implying that inhibition by endogenous vasopressin emerges very rapidly at the onset of bursts, or that phasic MNCs are tonically inhibited by endogenous vasopressin. Given that vasopressin can be recovered from the extracellular fluid of the SON under basal conditions (118) and that phasic activity can be triggered in some silent MNCs by local administration of a V_{1a}-receptor antagonist (117), it seems likely that at least some MNCs are tonically inhibited by endogenous vasopressin.

Exogenous vasopressin inhibits EPSC amplitude (119, 120), possibly via retrograde inhibition of excitatory synaptic transmission (121) as well as by autocrine inhibition. In addition, vasopressin increases IPSC frequency (122). Hence, somato-dendritic vasopressin release, which itself increases somato-dendritic release (123), provides a mechanism for autocrine/retrograde inhibition of vasopressin MNC activity (121, 124) to restrain the activity of the population of vasopressin MNCs.

While local administration of vasopressin inhibits vasopressin MNCs displaying robust phasic activity or continuous activity (117, 125), it has also been reported to excite vasopressin MNCs displaying irregular activity (125), suggesting that somato-dendritically released vasopressin should drive vasopressin MNCs towards phasic activity. Intracellular calcium levels are increased during phasic bursts (126, 127) and, consistent with the effects of vasopressin on phasic activity, application of vasopressin blocks spontaneous intracellular calcium oscillations in MNCs but triggers oscillations in non-oscillatory MNCs (128), providing a mechanism for the differential effects of vasopressin on MNC activity. The inhibitory actions of vasopressin are probably mediated by V_{1a}-receptors because V_{1a}-receptor antagonism increases the activity of phasic MNCs *in vivo* (110, 117), whereas the

excitatory actions might be mediated by V_{1b}-receptors. While there is no evidence to support the involvement of V_{1b}-receptors in the excitation, vasopressin MNCs express V_{1b}-receptors (116) and vasopressin activates phospholipase C (PLC) in vasopressin MNCs. Given that V_{1b}-receptor activation stimulates PLC to increase adrenocorticotropic hormone secretion from anterior pituitary corticotrophs (129), it is possible that V_{1b}-receptor mediated activation of PLC might trigger vasopressin-induced excitation of MNCs (130).

However, while exogenous vasopressin can drive MNCs towards phasic activity (125), most vasopressin MNCs do not display phasic activity under basal conditions (52, 53). Hence, while the activity of some MNCs are tonically regulated by endogenous vasopressin (117), it appears unlikely that there is sufficient vasopressin within the supraoptic nucleus to drive all vasopressin MNCs to adopt phasic activity. It has been estimated that each MNC only secretes one vesicle per second under basal conditions (112), and that this is sufficient to maintain extracellular concentrations of vasopressin in the PVN and SON that can be measured by microdialysis (131). Hence, vasopressin MNCs might be continuously exposed to vasopressin at levels that reflect the average activity of the vasopressin MNC population as whole. This has led to the proposal that somato-dendritic vasopressin might also act as a 'population feedback signal' that optimises activity across vasopressin MNCs via paracrine actions (112, 132). However, to date, there is no evidence that vasopressin released by one MNC can impact the activity of neighbouring MNCs, likely due to the mopping-up of vasopressin by extracellular peptidases (74). Indeed, when two vasopressin MNCs are recorded by a single extracellular electrode (and are thus presumably only tens of μm apart), there is no synchronisation of the onset or termination of phasic bursts between the two MNCs (14). Hence, it appears that somato-dendritic release of vasopressin from one MNC does not affect the activity of its neighbours, at least under basal conditions.

Neuropeptides co-released with vasopressin

Many other neuropeptides are also synthesised by MNCs (133) but the physiological significance of relatively few have been characterised to any great extent (124). For vasopressin MNCs, the co-localised neuropeptides with the best characterised effects on MNC activity are apelin (40), dynorphin (39), galanin (41), NERPs (42, 43), PACAP (44) and secretin (45). Similarly to vasopressin itself, vasopressin MNCs express receptors for most (and perhaps all) of these neuropeptides (44, 45, 134-138), providing a mechanism for autocrine feedback regulation of vasopressin MNCs by these co-released neuropeptides. Apelin was originally isolated from bovine stomach but is also expressed throughout the nervous system (139). Vasopressin MNCs express apelin (139, 140) as well as apelin receptors (APJ receptors) (138). While apelin is synthesised by vasopressin MNCs, there is a marked segregation of apelin and vasopressin labelling within MNCs (143), suggesting that the two peptides might be stored in distinct neurosecretory pools, and could therefore be differentially released in response to specific stimuli.

APJ receptor knockout mice have compromised responses to osmotic challenges (141). Furthermore, centrally-administered apelin inhibits vasopressin MNCs (40) to reduce basal vasopressin release (40, 142), suggesting that apelin might be an autocrine inhibitor of vasopressin MNCs. Consistent with this role, dehydration increases apelin and APJ receptor expression within the SON (40, 137), which might permit apelin to prevent over-excitation of vasopressin MNCs. However, these observations are confounded by the finding that intra-SON administration of apelin directly excites vasopressin MNCs (including phasic MNCs) by activation of non-specific cation channels (138). In addition, apelin reduces somato-dendritic vasopressin release (138), which would be expected to relieve the inhibitory vasopressin tone from at least some vasopressin MNCs.

It is possible that, similar to exogenous vasopressin (125), the effects of apelin depend on the activity of the vasopressin MNCs; the observations of apelin inhibition were made in lactating rats (40) in which vasopressin MNCs are hyperactive to preserve body water in the face of the increased demands of milk production (144), whereas the excitatory actions of apelin were evident under basal conditions (138).

While oxytocin MNCs also express APJ receptors, their activity is little affected by apelin administration under basal conditions (138), suggesting that apelin might only be effective against oxytocin MNCs under stimulated conditions.

Probably the best-characterised autocrine modulator of vasopressin MNC activity is the κ-opioid peptide, dynorphin (112). While κ-opioid binding sites have been demonstrated in the cortex of the kidney (145), as has mRNA for the κ-opioid receptor, the major diuretic effect of peripherally-applied κ-opioids appear to be mediated by inhibition of vasopressin MNCs (52, 54) to reduce vasopressin release because κ-opioids do not elicit diuresis in Brattleboro rats, which do not secrete vasopressin (146). Vasopressin MNCs express dynorphin (39) and κ-opioid receptors (135), but unlike apelin, these are located in the same neurosecretory vesicles as vasopressin (46, 147) but at much lower levels than vasopressin itself (148).

The κ-opioid inhibition of MNC activity is mediated by a reduction of evoked EPSP amplitude (149), mini EPSC frequency (150) and sADP amplitude (151). The inhibitory effects of exogenous κ-opioids reflect the actions of endogenous κ-opioid peptides because administration of a κ-opioid receptor antagonist prolongs phasic bursts *in vivo* (52, 56) and *in vitro* (56, 74) by increasing sADP amplitude (74) to increase plateau potential amplitude during phasic bursts (56), indicating that endogenous κ-opioid peptides reduce sADP amplitude to decrease plateau potential amplitude and thus decrease the probability of spike firing during bursts. Furthermore, κ-opioid receptor antagonism also prevents retrograde inhibition of EPSCs (150), indicating that endogenous κ-opioid peptides depress excitatory synaptic transmission, which would further reduce the probability of spike firing during bursts.

In contrast to the tonic vasopressin inhibition of phasic MNCs, endogenous κ-opioid inhibition of phasic MNCs is absent at the onset of each burst but emerges as bursts progress (110), suggesting that the progressive activity-dependent activation of κ-opioid receptors over the course of each burst helps shape phasic activity by contributing to the termination of phasic bursts (14, 15). Vasopressin MNCs displaying irregular activity appear to be more sensitive to endogenous κ-opioid inhibition than phasic MNCs (13, 152). Hence MNCs exhibiting increased irregular activity might be more sensitive than phasic MNCs to κ-opioids, which would cause more rapid termination of activity than evident in phasic MNCs, to generate the short periods of relatively high frequency firing often evident in irregular vasopressin MNCs. By contrast to endogenous κ-opioid inhibition of phasic and irregular vasopressin MNCs, vasopressin MNCs displaying continuous activity are not inhibited by endogenous κ-opioids (52), even under stimulated conditions (13). While continuously-active MNCs can be inhibited by exogenous κ-opioid peptides, they are not affected by a κ-opioid receptor antagonist delivered at the same doses that excite phasic MNCs (13, 52), suggesting that continuously-active vasopressin MNCs express functional κ-opioid receptors but are not exposed to endogenous dynorphin. Hence, changes in somato-dendritic dynorphin release might be involved in the transition between firing patterns in individual vasopressin MNCs (152).

Like dynorphin, galanin is co-expressed in vasopressin-containing neurosecretory vesicles. However, some neurosecretory vesicles contain galanin, but not vasopressin, and these

vesicles are differentially targeted to the dendrites of vasopressin MNCs (41). Thus, galanin might be specifically involved in autocrine/paracrine regulation of MNCs.

While central galanin administration increases peripheral vasopressin secretion under basal conditions, it inhibits secretion during acute osmotic stimulation (153, 154), perhaps by inhibition of SFO neurone activity (155), modulation of monoaminergic inputs (156), presynaptic inhibition of mEPSC frequency (157), inhibition of the sADP (158) and/or direct MNC hyperpolarisation (158). The inhibition of mEPSC frequency is more profound in MNCs from dehydrated rats (157), perhaps as a result of GAL1 receptor up-regulation in MNCs during dehydration (136). Hence, galanin might provide a restraining influence on MNC activity, particularly under stimulated conditions, to prevent over-excitation of vasopressin MNCs.

NERPs 1-3 are co-localised with vasopressin in MNCs (42, 43) and the MNC expression of all three NERPs is increased during dehydration (43, 159), suggesting a role for regulation of vasopressin MNC activity by somato-dendritic release of NERPs.

MNCs express PACAP and its cognate receptor, both of which are up-regulated in the SON during dehydration (44). PACAP depolarises MNCs to increase their firing rate (126) but also increases somato-dendritic vasopressin release, which will presumably limit the duration of the PACAP-induced excitation (114).

MNCs express secretin as well as secretin receptors and central secretin administration increases plasma vasopressin concentrations (45), suggesting that somato-dendritic secretin might stimulate systemic vasopressin release. Chronic osmotic stimulation also increases plasma secretin concentrations (45) and, similar to vasopressin, secretin increases insertion of aquaporin-2 into the luminal membrane of the kidney to increase water reabsorption (160). Hence, secretin might act at multiple levels to assist in maintaining body fluid balance (161). However, systemic secretin stimulates vasopressin (and oxytocin) MNC activity via activation of noradrenergic inputs (162) and so the increased secretin levels evident during dehydration (160) would be expected to have a positive feedback effect; presumably this positive feedback loop is overridden by other negative feedback effects to prevent runaway activation of vasopressin secretion.

Somato-dendritic oxytocin release

Similar to vasopressin receptor expression by vasopressin MNCs, oxytocin MNCs express oxytocin receptors (163) to provide a potential mechanism for autocrine feedback regulation of activity. By contrast to the inhibitory effects of vasopressin on vasopressin MNC activity, the feedback effects of oxytocin appear to be stimulatory and are important for the positive feedback regulation of oxytocin secretion evident during parturition and lactation (164).

Oxytocin release into the SON increases immediately prior to each milk-ejection burst in MNCs (165). Central oxytocin administration facilities milk-ejection bursts in suckling rats (166) as does oxytocin injection into the SON or PVN (167).

While the overall effect of somato-dendritic oxytocin is excitation, the effects are complex and might be involved in burst generation by combined excitatory and inhibitory processes. Oxytocin suppresses IPSC amplitude, but not frequency (168), which would be expected to excite oxytocin MNCs by shifting MNC membrane potential closer to the threshold for spike initiation. Counter-intuitively, oxytocin also induces retrograde inhibition of EPSCs (169) by triggering release of endocannabinoids from the postsynaptic MNC (170), which (in combination with mAHP and sAHP activation (102-104)) might contribute to termination of milk ejection bursts and to the post-burst silence in oxytocin MNCs. Hence,

somato-dendritically released oxytocin likely has a postsynaptic positive feedback effect to contribute to activity during bursts while activating endocannabinoid release for slower retrograde inhibition of excitatory synaptic drive to terminate bursts.

Remarkably, unilateral injection of oxytocin into one nucleus facilitates bursting of MNCs in contralateral nuclei (167), suggesting that somato-dendritic oxytocin release contributes to co-ordination of bursts across the population of oxytocin MNCs as well as to the generation of bursts in each MNC. Bursting has been modelled as an emergent behaviour of oxytocin MNCs sparsely connected by dendro-dendritic interactions (171). MNC dendrites tend to be separated from one another by glial processes under basal conditions but in late pregnancy and lactation, MNC dendrites are found in close apposition with other dendrites, forming dendritic bundles (172, 173). Each MNC has between one and three dendrites (25) and, in the model, each MNC has two dendrites that are each in different dendritic bundles, forming a sparse network of connections that allow somato-dendritic interactions between MNCs via oxytocin-induced release of endocannabinoids (171). This model replicates many of the features of co-ordinated milk ejection bursts in oxytocin MNCs, with bursting being initiated at any of the dendritic bundles (each of which contains only a few dendrites) and spreads rapidly through the remaining bundles (171).

Priming of somato-dendritic oxytocin release

The consequences of somato-dendritic oxytocin release are more complex than simple autoregulation of the electrical activity of the cells of origin. Somato-dendritic secretion does not always follow the action potential discharge that drives systemic secretion; e.g. systemic osmotic stimulation leads to prompt and long-lasting activation of oxytocin (and vasopressin) secretion into the systemic circulation but the associated increase somato-dendritic oxytocin (and vasopressin) release, is delayed by at least an hour compared to secretion into the circulation (174). Remarkably, after exposure of the SON to thapsigargin to mobilise calcium release from intracellular stores, somato-dendritic peptide release in response to osmotic stimulation (and other forms of stimulation) is dramatically potentiated *in vivo* and *in vitro* (38). This effect is long-lasting; priming oxytocin MNCs enhances somato-dendritic release in response to subsequent activity-dependent release for at least 90 min after exposure to thapsigargin. Priming in oxytocin (and vasopressin) MNCs is not a consequence of long-lasting elevation of intracellular calcium induced by thapsigargin. The thapsigargin-induced elevation of intracellular calcium lasts for ~5 min (175) but the potentiation of somato-dendritic release is only evident after 30min.

Thapsigargin induces an increase in intracellular calcium by inhibiting the endoplasmic reticulum calcium-ATPase to block the refilling of inositol triphosphate (IP3)-sensitive intracellular calcium stores. As a consequence, thapsigargin might also open store-operated cation channels to increase the effectiveness of subsequent stimuli such as depolarisation. Store-operated cation channels in MNCs are calcium permeable and are activated at membrane potentials more negative than –40mV (176). The irreversible depletion of intracellular calcium stores will permanently activate these currents. The resulting small but persistent calcium current might contribute to the activation of the calcium-sensitive exocytotic machinery, or increase voltage-dependent calcium influx by increasing the membrane potential range at which calcium influx occurs through voltage-gated channels.

Priming does not occur as a result of changes in translation/protein processing, but involves a translocation of dense-cored vesicles closer to the dendritic membrane. Thapsigargin treatment increases the number of dense-core vesicles located in the readily-releasable pool near the plasma membrane (177), indicating that the thapsigargin-induced priming involves a relocation of dense-core vesicles closer to sites of secretion, probably via actions on actin filaments to allow dense-core vesicles to leave the reserve pool (178).

Perhaps the most striking feature of priming is that oxytocin itself induces priming in oxytocin MNCs (38) (Figure 5) and oxytocin facilitates somato-dendritic oxytocin release (179). In contrast, vasopressin does not induce priming in vasopressin MNCs (113) and does not facilitate somato-dendritic vasopressin release (179). Somato-dendritic oxytocin release precedes milk-ejection bursts in oxytocin MNCs (165) and central oxytocin administration promotes bursting (180). Remarkably, induction of priming allows electrical stimulation to induce milk-ejection burst-like activity in oxytocin MNCs recorded from virgin female rats (38). Hence, oxytocin-induced priming might be required for bursting behaviour in oxytocin MNCs.

Neuropeptides co-released with oxytocin

By contrast to neuropeptides synthesised by vasopressin MNCs, the effects of somato-dendritic release of other neuropeptides synthesised by oxytocin MNCs are poorly characterised. Oxytocin MNCs also synthesise proenkephalin A-derived μ-opioid peptides (47) and dynorphin (48), as well as μ- (181) and κ-opioid receptors (182). While oxytocin MNCs are inhibited by administration of μ- and κ-opioid receptor agonists (96) and release from chronic opioid inhibition increases somato-dendritic oxytocin release (183, 184), it is unclear whether the endogenous opioid peptides are released from the soma and dendrites in sufficient quantities to alter oxytocin MNC activity under basal conditions; neither μ- (185) nor κ-opioid (52) receptor antagonism significantly affect the activity of oxytocin MNCs *in vivo*. It appears that the principal function of these endogenous opioid peptides might be as auto-inhibitors of secretion from the neurosecretory terminals in the posterior pituitary gland (32, 186).

Non-exocytotic somato-dendritic neurotransmitter/neuromodulator release

In addition to neuropeptides, MNC neurosecretory vesicles contain large amounts of ATP (50) and MNCs express various P2X receptors (187-189) as well as P2Y receptors (190), providing further potential mechanisms for somato-dendritic regulation of MNC activity (124). ATP injection into the SON induces anti-diuresis in anaesthetised rats (191) presumably via increased vasopressin secretion because ATP increases vasopressin (and oxytocin) secretion from hypothalamic explants (192). The ATP-induced increase in hormone secretion likely arises from a direct ATP-induced depolarization of MNCs (193) in combination with an ATP-induced increase in glutamate and GABA release from presynaptic neurones (194). While ATP is released by MNCs themselves, ATP can also be released from neighbouring glia (195) as well from noradrenergic afferents (196) to excite MNCs.

ATP is rapidly catabolised to curtail its actions (197), which generates extracellular adenosine. In anesthetised rats, adenosine A1 receptor antagonism increases the firing rate of phasic vasopressin MNCs (198). While the firing rate of continuously-active oxytocin and vasopressin MNCs are not affected by A1 receptor antagonism, the variability of firing is increased in both (198), suggesting that both are sensitive to endogenous adenosine feedback, although the physiological impact of this subtle action has yet to be determined. Endogenous adenosine causes activity-dependent enhancement of the mAHP in all MNCs (199) and in phasic MNCs this deepens spike frequency adaptation to shorten bursts (198) and might contribute to termination of milk-ejection bursts in oxytocin MNCs. Inhibition of the mAHP likely does not account for all of the effects of endogenous adenosine on MNCs because A1 receptor antagonism also inhibits activity-dependent depression of IPSCs and EPSCs via presynaptic receptor activation (200). MNCs also express A2A receptors and activation of these receptors causes a depolarisation-induced increase in firing rate (201). Nevertheless, the major functional role of endogenous adenosine appears to be inhibition because inhibition of adenosine uptake strongly inhibits spike discharge (202).

In addition to substances released by exocytosis, MNCs also release nitric oxide (NO). MNCs express high levels of nitric oxide synthase (NOS) (49) and neuronal NOS is upregulated in the SON during dehydration (203). Inhibition of NOS increases osmotically-stimulated secretion of oxytocin and vasopressin (204, 205), suggesting that MNC NO production restrains peripheral hormone secretion in response to osmotic stimulation. This restraint likely arises from NO inhibition of the firing rate of SON MNCs (206, 207) by increasing IPSC frequency and amplitude (207, 208). NO also restrains MNC responses to other stimuli (205, 209), suggesting that NO generation is a general inhibitory feedback regulator of MNC activity, regardless of the specific stimulus and of the phenotype of MNC.

MNCs also synthesize and release carbon monoxide (CO) in an activity-dependent manner; both oxytocin and vasopressin MNCs express the CO-synthesizing enzyme heme-oxygenase I, and this expression is enhanced during dehydration (210). In contrast to NO, CO appears to act as an excitatory gaseous transmitter, promoting firing activity in these neurons (210). Thus, there appears to be a balance between these two functionally opposing gaseous tranmsittser in the regulation of MNC activity, particularly during conditions of high hormonal demand.

Glial regulation of magnocellular neurosecretory cell activity

Glia have long been regarded as non-excitable cells that support and nourish neurones. However, it is now clear that glia are important participants in neuronal communication (Figure 6). In particular astrocytes actively modulate signalling at the tripartite synapse (211, 212). Activation of astrocytic α_1-adrenoceptors releases ATP as a gliotransmitter at the tripartite synapse; this ATP activates P2X7 receptors on postsynaptic MNCs to increase calcium influx as well as activation of phosphotidyl inositol 3-kinase, and thus increase AMPA receptor insertion into the postsynaptic membrane to increase MNC sensitivity to synaptic glutamate (195). Similarly, presynaptic glutamate release can also liberate glial ATP via activation of metabotropic glutamate receptors (213, 214). Hence, astrocytes are active components of excitatory synaptic signalling to MNCs.

Remarkably, astrocytes withdraw their processes from around MNCs during dehydration (215) and lactation (216) and this ‘glial retraction’ modulates the MNC responses to the prevailing physiological conditions (Figure 7). Indeed, glia retract only from oxytocin MNCs during lactation (217), suggesting that this might have a functional impact on bursting activity of oxytocin MNCs. However, preventing glial retraction by disruption of neural cell adhesion molecules does not alter milk-ejection bursts in oxytocin MNCs (218). Nevertheless, glial retraction has been shown to impact the excitability of MNCs in both dehydration and lactation, and might increase dendritic bundling to enhance intercellular communication between oxytocin MNCs and facilitate co-ordinated bursting during birth and lactation.

Glia act as a physical barrier to neurotransmitter/neuromodulator diffusion and also mop-up the major neurotransmitters via high affinity glutamate and GABA transporters (219, 220). When glia retract during lactation, extracellular neurotransmitter concentrations rise, increasing tonic activation of presynaptic metabotropic glutamate receptors (mGluRs), which inhibits further transmitter release (220, 221) to reduce synaptic efficacy at glutamatergic inputs on MNCs. Glutamatergic synaptic efficacy is also reduced by glial retraction during lactation due to a reduction in D-serine, which is released from glia to act as a co-agonist at the NMDA receptor (NMDAR) (222). Glial retraction during lactation decreases the physical barrier to diffusion away from the synaptic cleft, which also allows synaptically-released glutamate to more easily diffuse away from its site of release and activate presynaptic metabotropic glutamate receptors on GABAergic terminals to inhibit

GABA release (223) to disinhibit MNCs. A further consequence of glial retraction is a switch in presynaptic kainate receptor (KAR)-mediated facilitation of GABA transmission in virgin rats (mediated by presynaptic GluK1-containing KARs), to KAR-inhibition of GABA transmission in lactating rats when extracellular glutamate levels are increased to activate metabotropic KARs (224).

In addition to activation of conventional postsynaptic receptors within the synaptic cleft, glutamate can also activate extrasynaptic receptors to induce a persistent 'tonic' current that influences global neuronal excitability, shown by a sustained outward shift in holding current during bath application of NMDAR blockers (225). Glia surrounding MNCs express the glutamate transporter, GLT-1 (220, 226), and GLT-1 blockers enhance the tonic NMDA current in MNCs. The tonic NMDA current is also enhanced during dehydration, as well as in rats with heart failure (227), a pathological condition characterized by abnormally elevated levels of circulating vasopressin. In both cases, the enhanced tonic NMDA current is likely the result of a diminished efficacy of GLT-1 (225, 227). Thus, enhanced activation of extrasynaptic NMDA receptors, due to blunted glial GLT-1 clearance, contributes to increased MNC activity and hormone release under physiological (dehydration) and pathological (heart failure) conditions.

Recently, extrasynaptic NMDARs (eNMDARs) were shown to participate in functional crosstalk with $GABA_A$ receptors. Activation of eNMDARs resulted in a transient and reversible potentiation of postsynaptic $GABA_A$ receptors, via a calcium- and phosphorylation-dependent mechanism (228). This inter-receptor crosstalk likely acts as a compensatory, counterbalancing mechanism to dampen glutamate-mediated over-excitation (227). Hence, glia play a key role determining the status of the glutamate/GABA synaptic balance in MNCs, acting at pre-, post-, and extrasynaptic loci.

In addition to transporters for glutamate (221), glia express GABA transporters (219) that also modulate ambient GABA levels. Similarly to glutamate, GABA generates a persistent hyperpolarisation via the activation of extrasynaptic receptors (219, 229). Counter-intuitively, dehydration increases intra-SON GABA release (as well as glutamate release), evident as an increase in the frequency of spontaneous IPSCs (and EPSCs) in MNCs from dehydrated rats (230). This increased GABA might dampen the glutamate-driven hyperosmotic excitation of MNCs to prevent over-excitation, as it does during acute osmotic stimulation (231).

Glia surrounding MNCs also release taurine to inhibit MNCs via extrasynaptic glycine receptors (232). Glial taurine release is increased in hypo-osmotic conditions to increase MNC inhibition and is reduced in hyperosmotic conditions to disinhibit MNCs. However, mice that lack the taurine transporter concentrate urine normally during dehydration, but continue to generate hyperosmotic urine upon rehydration, when the urine of wild-type littermates rapidly returns to normal osmolality (233). Hence, decreased glial taurine release appears to have little impact on MNC activity during chronic dehydration whereas hypo-osmolality-induced increases in taurine release inhibit MNCs.

Afferent inputs to magnocellular neurosecretory cells

Afferent inputs are essential for action potential firing in MNCs *in vivo*; selective pharmacological antagonism of individual excitatory inputs (64, 65) silences these neurones, even during intense stimulation (66, 185). These synaptic inputs arise from various afferent projections from many other brain areas (Figure 8) that have been extensively described elsewhere (35, 234).

Perinuclear inputs

The region immediately dorsal to the SON, the perinuclear zone, is such a rich source of glutamatergic and GABAergic innervation of the SON that stimulation of this zone is frequently used in *in vitro* electrophysiology experiments to elicit evoked excitatory and inhibitory postsynaptic potentials/currents (EPSP/Cs and IPSP/Cs) in SON MNCs. While a large proportion of perinuclear zone axons enter the SON, many of these neurones have extensive axon collaterals that project elsewhere (235), including the parvocellular and magnocellular PVN (236), providing a potential mechanisms for co-ordination of activity between the different magnocellular nuclei. Indeed, stimulation of the perinuclear zone elicits glutamatergic EPSPs in MNCs of the ipsilateral and contralateral PVN (237). In addition to perinuclear zone inputs, stimulation of different sites shows that the PVN is essentially ringed in the horizontal plane by glutamatergic and GABAergic projections from an area that includes parts of the anterior hypothalamic area, the posterior bed nucleus of the stria terminalis (BNST) and the dorsomedial hypothalamus (238, 239).

Because the pattern of synaptic potentials recorded is essentially stochastic, it has been proposed that activity in these local inputs might generate background ‘noise’ in MNC membrane potential, against which the ability of brainstem and forebrain inputs to evoke specific responses is enhanced by a phenomenon referred to as ‘stochastic resonance’ (35). However, at least for the glutamatergic inputs, it appears that the perinuclear zone might play an active role in relaying information from distant inputs, such as the brainstem noradrenergic input (240, 241). At first glance, a local excitatory relay for a distant excitatory input might appear redundant but, in addition to monosynaptic excitatory postsynaptic potentials (EPSPs), single electrical stimuli applied to the perinuclear zone evoke multiple EPSPs in some SON MNCs (239), suggesting that there are local polysynaptic excitatory circuits within the perinuclear zone that modulate activity of SON MNCs. Hence, relaying the excitatory signals through local glutamatergic neurones might amplify the distant inputs to drive a stronger response in MNCs.

While perinuclear inputs are generally regarded as being principally glutamatergic or GABAergic, a large proportion of neurones in the perinuclear zone of the SON also synthesise acetylcholine (242). SON MNCs express nicotinic receptors (243) that mediate synaptic activation by perinuclear zone stimulation *in vitro* (244) and central administration of the muscarinic receptor agonist, carbachol, markedly increases the firing rate of both oxytocin and vasopressin MNCs in the SON (245). It is likely that PVN MNCs are similarly innervated because they are directly excited via nicotinic receptors *in vitro* (246). While the physiological significance of this prominent cholinergic innervation has not been well-characterised, it has been proposed to contribute to the osmotic stimulation of vasopressin secretion (247).

Forebrain inputs and osmoregulation

Among the major afferent inputs to MNCs are those that lie adjacent to the third ventricle: the median preoptic nucleus (MnPO), the organum vasculosum of the lamina terminalis (OVLT) and the subfornical organ (SFO). These regions convey information on the osmotic status of the animal to the MNCs (248, 249). Lesion of the region anterior and ventral to the third ventricle (AV3V, which encompasses the MnPO and OVLT) reduces oxytocin and vasopressin release in response to hyperosmotic stimulation (as does lesion of the SFO) by reducing the osmotically-stimulated increase in MNC action potential discharge (250). These neurones also promote thirst via projections to other brain regions, including the anterior cingulate cortex and insular cortex (251) and lesion of the AV3V has long been known to abolish osmotically-driven thirst (252). Thus, the AV3V appears to be a centre for

co-ordinating the appropriate physiological and behavioural responses to changes in plasma osmolality and so are often referred to as the 'osmoregulatory complex'.

While the principal action of vasopressin is antidiuresis in the kidney to conserve body water when plasma osmolality increases, increasing osmolality also activates oxytocin MNCs (231) and consequently the secretion of oxytocin (253), which (at least in the rat) is also involved in regulating the osmotic status of the animal via promotion of natruiresis in the kidney (2).

The OVLT and the SFO lack a blood-brain barrier and so neurones in these regions can be directly influenced by plasma osmolality, as well as factors circulating in the blood. OVLT (254) and SFO (255) neurones are directly osmosensitive; the former increase their action potential discharge via hyperosmotic depolarisation mediated by activation of transient receptor potential vanilloid 1 (TRPV1) channels (254).

In addition to intrinsic osmosensitivity, neurones of the osmoregulatory complex express receptors for many different circulating hormones (256), including AT1 receptors for angiotensin II (257). Angiotensin II production is increased via the renin-angiotensin system when blood pressure to the kidneys is reduced. Its principal actions are to increase arterial blood pressure by vasoconstriction of arterioles, and to increase renal retention of salt and water directly, as well as by stimulation of aldosterone secretion from the adrenal glands. Oxytocin and vasopressin release are also potently stimulated by circulating angiotensin II via the SFO (258) to provide a further level of co-ordination of the whole-body response to reduced kidney perfusion.

The OVLT and SFO project directly to the PVN and SON and indirectly via the MnPO, which also has reciprocal connections with both the OVLT and the SFO (232). While the overall effect of osmotic stimulation via the osmosensory complex is excitation of oxytocin and vasopressin MNCs and consequent secretion of hormones from the posterior pituitary gland, the projection neurones are both excitatory and inhibitory; while a direct osmosensitive glutamatergic input to the SON from the OVLT has been identified *in vitro* (259), both MnPO and OVLT stimulation can elicit GABA-mediated inhibition of SON MNCs *in vivo* (231, 260). In addition to the components mediated by glutamate and GABA, several peptides are also expressed in forebrain projections to the MNCs, principal among these is angiotensin II itself in the SFO projection to the MNCs, which mediates at least some of the SFO-induced excitation of MNCs (261).

It might appear counter-intuitive to have an excitatory stimulus relayed by a mixed population of excitatory and inhibitory afferent neurones. However, it is important that the osmotic response of MNCs is graded over a wide physiological range to allow the appropriate homeostatic response to variations in the osmotic status of the organism. To this end, it appears that the excitatory component of the osmosensory complex input provides the drive to increase MNC firing rate when plasma osmolality exceeds the osmotic threshold for vasopressin secretion of ~280 mOsmol kg^{-1}, while the critical function of inhibitory component is to reduce the gain of the excitatory response to allow a graded response to increasing plasma osmolality over the 'physiological' range of 280 – 340 mOsmol kg^{-1} (231). Indeed, mathematical modelling indicates that the graded increase in MNC firing rate in response to increasing osmolality *in vivo* probably involves an equal activation of excitatory and inhibitory osmosensory inputs (231).

While afferent inputs are required for osmotic activation of MNCs, MNCs are themselves osmosensitive via stretch-inactivated TRPV1 channels (262). The full osmotic response of

MNCs requires concurrent activation of afferent inputs and intrinsic TRPV1 channels (263, 264).

Noradrenergic inputs

Among the most prominent projections to MNCs in the PVN and SON are the ascending brainstem noradrenergic projections from the A1 cell group in the ventrolateral medulla (VLM) and the A2 cell group in the nucleus tractus solitarius (NTS). While these areas have been implicated in osmoregulation of MNC activity (264), they are widely accepted as primarily relaying visceral information from the periphery to the MNCs. Indeed, osmotic activation of these brain areas might be downstream of hypothalamic activation because water deprivation activates pre-autonomic PVN neurones that project to the brainstem (265, 266) and intra-carotid hypertonic saline infusion increases renal sympathetic nerve activity concomitant with an increase in Fos protein expression in OVLT neurones that project to the PVN (267, 268).

Noradrenergic inputs are well characterised as relaying cardiovascular information arising from baroreceptors and chemoreceptors whose afferents terminate in the NTS, as well as the area postrema and nucleus ambiguus (269). In addition to cardiovascular regulation, these inputs are also involved in mediating reproductive, gastrointestinal and somatosensory influences on MNC activity (35, 270). VLM neurones project preferentially to vasopressin MNCs (271), while NTS inputs project preferentially to oxytocin MNCs (272). Interruption of synaptic transmission through the VLM inhibits NTS excitation of vasopressin MNCs, but not oxytocin MNCs, suggesting that the NTS projection to oxytocin MNCs is predominantly direct while that to the vasopressin MNCs is mediated via the VLM (272).

Noradrenaline directly excites SON MNCs via a α_1-adrenergic receptor mediated depolarisation (273, 274). Intra-SON antagonism of α_1-adrenergic receptors profoundly inhibits oxytocin MNCs *in vivo* (185), indicating that oxytocin MNCs, at least, are under excitatory noradrenergic tone. For vasopressin MNCs, both excitation and inhibition by noradrenaline have been reported, with low doses of noradrenaline being excitatory via a α_1-adrenergic receptors, while high doses are inhibitory via β-adrenergic receptors (275). Furthermore, excitation of vasopressin MNCs by stimulation of the VLM projection is reduced by α_2-adrenergic receptor activation, indicating that excitatory transmitter release is likely inhibited by presynaptic α_2-adrenergic receptors (276). The NTS input to oxytocin MNCs is likely also under presynaptic α_2-adrenergic receptor modulation because stimulation of oxytocin release is reduced by α_2-adrenergic receptor activation (185).

While most neurones that project to MNCs from the VLM and NTS are characterised as noradrenergic, various neuropeptides are co-expressed with noradrenaline in different subsets of neurones: the NTS noradrenergic neurones co-express enkpehalin (277) and galanin (278) and the VLM noradrenergic neurones co-express enkpehalin (277), neuropeptide Y (279) and substance P (280). Furthermore, an appreciable number of NTS neurones that project to MNCs do not express noradrenaline (281), but rather express the neuropeptides, inhibin β, somatostatin and the μ-opioid, encephalin, in varying proportions (282).

Indeed excitation of vasopressin MNCs by A1 stimulation is not completely blocked by noradrenergic antagonists (283), indicating that it might result from the actions of co-transmitters (or of non-noradrenergic neurones), including ATP, which excites MNCs via P2X receptor-mediated depolarisation (284) and potentiates noradrenergic stimulation of oxytocin and vasopressin release from hypothalamic explants (192), possibly via a synergistic increase in intracellular calcium concentrations (285). Other possible candidates for mediating A1 stimulation of MNCs, at least in part, are neuropeptide Y, which also

potentiates noradrenergic stimulation of oxytocin and vasopressin release (286) and substance P, which synergises with ATP to cause the release of oxytocin and vasopressin (286).

Noradrenergic involvement in cardiovascular regulation of vasopressin magnocellular neurosecretory cells—Vasopressin secretion is tonically inhibited by afferent inputs from stretch receptors (baroreceptors) in the left atrium, aortic arch and carotid sinus. A reduction in baroreceptor activity, such as occurs during haemorrhage, increases vasopressin (and oxytocin) MNC activity as well as plasma vasopressin (and oxytocin) concentrations (287), while an increase in baroreceptor activity inhibits MNCs (288).

Haemorrhage increases noradrenaline levels in the SON (289) and lesion of the locus coeruleus, which contains the A6 cell group, reduces haemorrhage-induced oxytocin and vasopressin secretion (290) and inhibits baroreceptor activation of MNCs (291). However, there is little evidence of a direct projection from the locus coeruleus to MNCs and noradrenaline depletion within the SON or PVN does not prevent baroreceptor-induced inhibition of vasopressin MNCs (292). The locus coeruleus (and NTS) projects to the diagonal band of Broca (293) and it is likely that this relays baroreceptor-mediated effects on MNC activity via activation of inputs (294-298) that inhibit vasopressin MNCs via GABA interneurones in the perinuclear zone (299).

The defence of blood pressure by vasopressin does not operate in isolation from the homeostatic regulation of plasma osmolality; hypovolaemia or hypotension increases the gain of the secretory response to increasing osmolality and hypervolaemia or hypertension decreases the gain of the response (300). These changes in osmotic gain might be caused by plasticity in the vasopressin MNCs themselves because chronic osmotic stimulation prevents baroreflex inhibition of MNCs by changing the chloride gradient in MNCs to switch GABA from being inhibitory to being excitatory (301).

Noradrenergic involvement in reproductive regulation of oxytocin magnocellular neurosecretory cell activity—The noradrenergic innervation of oxytocin MNCs is increased in lactation (302), suggesting that remodelling of the noradrenergic input to MNCs over the course of pregnancy and lactation might be involved in signalling the reproductive status of the organism. Afferents from the uterus and vagina terminate in the NTS (303) and noradrenergic NTS neurones express Fos protein in response to activation of stretch receptors in the birth canal during fetal expulsion (304). Noradrenergic (and non-noradrenergic) neurones of the VLM and NTS retrogradely-labelled from the SON express Fos protein during parturition (305) and noradrenaline levels are increased within the SON at parturition (306). While NTS noradrenergic neurones are also activated during lactation, suckling (rather than the presence of pups) increases Fos protein expression in VLM noradrenergic neurones without further increasing expression in NTS neurones (307), suggesting that the specific noradrenergic pathways activated by uterine contraction and suckling of the young might be different.

While noradrenaline directly excites MNCs, its effects in pregnancy and lactation are likely more complex than simple excitation. Noradrenaline release within the SON is increased in late pregnancy (308). Noradrenaline facilitates oxytocin release within the SON and PVN during pregnancy and lactation (309, 310) and oxytocin increases noradrenaline release within the SON (311). Hence, the noradrenergic input to oxytocin MNCs might facilitate the emergence of oxytocin autoexcitation to promote bursting activity in oxytocin MNCs necessary for delivery of the young and of milk to the new-born. Indeed, stimulation of

central noradrenergic (and histaminergic) receptors is necessary for intranuclear release of oxytocin in response to suckling (312).

Noradrenergic inputs might also be important for the co-ordination of bursts across the population of oxytocin MNCs. Bilateral retrograde tracer injection into the right and left SONs results in co-localisation within individual neurones only in the VLM and anterograde tracer injection into the VLM labels axons that branch at the optic chiasm to innervate both SONs (180). Critically, lesion of the optic chiasm dramatically reduces the co-ordination of bursts between oxytocin MNCs recorded from the left and right SON (180). Hence, VLM neurones project to MNCs on both sides of the brain and are likely involved in co-ordinating bursts in oxytocin MNCs.

While direct noradrenergic projections to oxytocin MNCs contribute to the co-ordination of bursts, there might also be indirect actions of noradrenaline neurones on bursting in oxytocin MNCs. There is a dense noradrenergic projection to the bed nucleus of the stria terminalis (BNST) (313) where noradrenaline is released (314). Stimulation of the BNST (with oxytocin) facilitates bursting in oxytocin MNCs in the SON (315) and the cyclical activity of BNST neurones is correlated with milk-ejection bursts in oxytocin MNCs (316), suggesting that the BNST might also be involved in gating milk ejection bursts.

Noradrenergic involvement in gastrointestinal regulation of magnocellular neurosecretory cell activity—Peripheral administration of the gastrointestinal peptide, cholecystokinin (CCK), causes a dose-dependent increase in oxytocin, but not vasopressin, secretion into the bloodstream (317). The increased oxytocin secretion is caused by an increase in the firing rate of oxytocin MNCs (55, 318).

While CCK can directly activate MNCs via activation of stretch-inactivated cation channels (319), the excitation of oxytocin MNCs by peripheral CCK is blocked by gastric vagotomy (317), destruction of peripheral fibres (320, 321), or by peripheral administration of CCK-A (but not CCK-B) receptor antagonists (322). Thus peripheral CCK appears to excite oxytocin MNCs *in vivo* by activating CCK-A receptors located on gastric vagal afferent fibres. These vagal signals are likely mediated by noradrenergic neurones because peripheral CCK increases noradrenaline levels in the SON (323) and the increased firing rate seen in oxytocin MNCs after peripheral CCK is blocked by iontophoretic administration of an α_1-adrenoreceptor antagonist (324). Similarly, neurotoxic lesion of noradrenergic neurones abolishes CCK-induced oxytocin secretion and Fos protein expression in MNCs (185, 325). While both NTS and VLM neurones (but not other areas that project to MNCs) are activated by CCK, it has been demonstrated that only noradrenergic neurones of the A2 (NTS) cell group express Fos protein after peripheral CCK (325). Hence, peripheral CCK acts via CCK-A receptors on gastric vagal afferents to activate the A2 cell group projection to oxytocin MNCs to increase oxytocin secretion.

While peripheral CCK increases oxytocin secretion into the blood, the physiological significance of this response is unknown. CCK-induced oxytocin secretion can be mimicked by gastric distension (318) and gastric vagotomy reduces both CCK-induced inhibition of food intake and CCK-induced oxytocin secretion (317), suggesting that peripheral oxytocin might mediate CCK effects on food intake. However, the CCK-induced inhibition of food intake and gastric emptying is longer lasting than its stimulation of oxytocin secretion (326), and peripheral oxytocin administration does not induce food intake (317), indicating that peripheral oxytocin release does not mediate the CCK-induced inhibition of food intake. Nevertheless, oxytocin might play a pivotal role in food intake, and thus body weight regulation, via release into the brain because central (but not peripheral) oxytocin receptor antagonist administration blunts CCK-induced inhibition of food intake (327).

While these initial studies focussed on oxytocin as a satiety factor because this is the major role played by CCK in the regulation of food intake, there has been a recent resurgence in interest in central oxytocin as a long-term regulator of bodyweight because disruption of the *SIM1* gene causes profound monogenic obesity in humans via increased food intake (328) and *SIM1* knockout mice lack PVN and SON oxytocin MNCs (329, 330).

Serotoninergic inputs

Retrograde tracing studies suggest that MNCs receive a sparse serotoninergic projection from the dorsal and median raphe nuclei (331). However, these projections were not confirmed by anterograde labelling using *Phaseolus vulgaris* leucoagglutinin (PHA-L) injection into the dorsal or median raphe nucleus (332, 333). Nevertheless, intracerebroventricular (ICV) serotonin administration increases plasma oxytocin and vasopressin levels (334). PHA-L injections show a moderate innervation of the regions surrounding the PVN and SON (332, 333), which might mediate the serotonin excitation of MNCs.

Similar to noradrenaline, serotonin release in the SON is increased by intravenous injection of CCK (335) and so serotonin might play a similar role to noradrenaline in mediating the transfer of visceral information to MNCs. However, serotonin is not a mere adjunct to the noradrenergic inputs because, unlike noradrenaline, serotonin concentrations in the SON do not change over the course of parturition (306) and serotonin antagonists do not interfere with the progress of parturition in rats (336), suggesting that serotonin inputs might not be specifically involved in activation of oxytocin MNCs during parturition. However, intravenous CCK increases intra-SON serotonin concentrations more in late pregnant rats than in virgin rats (308), suggesting that serotonin might have a facilitatory role supporting oxytocin MNC activity during parturition.

Dopaminergic inputs

The main dopaminergic projections to MNCs arise from the A9 cell group of the pars compacta of the substantia nigra, the A10 cell group of the ventral tegmental area, as well as the A14 and A15 cell groups in the preoptic periventricular /anterior hypothalamic region (337, 338). Central dopamine administration increases oxytocin and vasopressin secretion under basal conditions (339) via an enhancement of MNC activity (340). While dopaminergic synapses have been visualised on MNCs in the PVN and SON using electron microscopy (341), and dopamine depolarises MNCs via D2 receptors (342), more recently it has become clear that dopamine also modulates MNC activity via activation of presynaptic D4 receptors, which inhibits GABA release onto MNCs (343, 344). Hence, dopaminergic stimulation of neurohypophysial hormone release appears to result from a combination of direct depolarisation and a reduction of inhibitory GABA influences.

The physiological role of dopaminergic inputs to MNCs have not been well-characterised but the facilitation of MNC activity is also evident under stimulated conditions, enhancing oxytocin MNC activity during lactation, as well as vasopressin MNC activity during water diuresis evoked by alcohol administration (340). Furthermore, dopamine release within the SON is increased during the second half of parturition (306), suggesting that increased activation of dopaminergic inputs might specifically contribute to excitation of oxytocin MNCs during parturition.

However, dopaminergic regulation of MNC activity is likely more complex than simple excitation because D4 receptor activation also inhibits glutamate release onto MNCs (345); this restraint of excitatory inputs might prevent over-excitation by dopaminergic inputs, or refine the temporal profile of the excitation.

Histaminergic inputs

Histaminergic projections to MNCs arise from the tuberomammillary nucleus (346) and appear to contribute to delivery of the young at parturition (347). The tuberomammillary nucleus receives afferent inputs from many brain areas (348), including synaptic inputs from noradrenergic and serotoninergic neurones (349).

Histamine has differential effects on oxytocin and vasopressin MNCs. Histamine excites vasopressin MNCs via an H_1 receptor-mediated reduction of the K^+ leak current (350), inhibition of EPSCs (351) and by enhancing the sADP (352) via H_1-receptor activation of a non-specific cation current (353). In contrast to its effects on vasopressin MNCs, histamine was initially reported to inhibit oxytocin MNCs via H_2 receptor activation of chloride-mediated hyperpolarisation (354). However, central histamine administration increases oxytocin secretion into the circulation (355). Histamine neurones express elevated levels of mRNA for the histamine synthetic enzyme, histidine decarboxylase, in late pregnant and lactating rats (347) when excitation would be expected and stimulation of central histaminergic (and noradrenergic) receptors is necessary for intranuclear release of oxytocin in response to suckling (312). Hence, it appears that the direct histaminergic inhibition of oxytocin MNCs is overcome by induction of somato-dendritic oxytocin release, possibly to facilitate bursting during parturition and lactation.

Histamine might also be involved in co-ordination of milk-ejection bursts across the population of oxytocin MNCs; hypothalamic lesions incorporating the tuberomammillary nucleus disrupts co-ordination of milk-ejection bursts between pairs of oxytocin MNCs recorded from the left and right SON of anaesthetised rats (356).

Endogenous opioid peptide inputs

Proopiomelanocortin (POMC) neurones in the brain are predominantly located in the hypothalamic arcuate nucleus and POMC fibres originating in the arcuate nucleus have been visualised in the PVN and SON, particularly in the oxytocin-rich parts of these nuclei (357). Oxytocin MNCs are under active opioid restraint over the course of pregnancy, presumably to protect the offspring from oxytocin secretion triggered by other stimuli such as inflammation (164, 358, 359). Administration of morphine inhibits oxytocin MNCs by inhibition of noradrenergic inputs (335), as well as by direct hyperpolarization of oxytocin MNCs (360). The endomorphins, which are highly potent and specific endogenous μ-opioid peptides, are present in the hypothalamus (361). While ICV endomorphin 1 inhibits both oxytocin and vasopressin MNCs, it potently inhibits oxytocin MNCs by a direct action (similar to exogenous agonists) whereas its weaker inhibition of vasopressin MNCs is mediated by indirect actions (362). While MNCs develop dependence on chronic morphine (363, 364), at least in part through changes in their intrinsic properties (360, 365), they do not develop dependence on chronic endomorphin 1 (362), similar to other neuronal responses to chronic activation by peptides (366), suggesting that release from on-going endogenous opioid inhibition is unlikely to trigger the increase in oxytocin MNC activity required for parturition.

Suprachiasmatic inputs

Oxytocin and vasopressin are secreted in a circadian pattern (12) that is mediated by a mixed excitatory (glutamatergic) and inhibitory (GABAergic) projection to the SON from the suprachiasmatic nucleus (367) and might be entrained to the light-dark cycle by a retinal projection via the perinuclear zone (368). These inputs are likely of particular importance in preventing night-time enuresis but might also impact the time of day at which delivery of the offspring occurs.

Olfactory inputs

MNCs receive direct afferent inputs from the main and accessory olfactory bulb (369-371) that excite MNCs via glutamatergic receptor activation (372). Remarkably, activation of olfactory inputs increase the incidence of dye coupling between MNCs but only in animals expressing maternal behaviour (373, 374), suggesting that these inputs might be important in the generation of the appropriate physiological responses of MNCs for maternal care of the offspring.

Hormonal regulation of magnocellular neurosecretory cell activity

Steroid regulation of oxytocin magnocellular neurosecretory cell activity

During pregnancy, the maternal brain, including oxytocin MNCs, adapts to maintain the pregnancy and to prepare for birth, lactation and for nurturing of the baby (164). In the rat, parturition uses about one third of the oxytocin stored in the pituitary gland and in preparation for the demands of parturition (and lactation), pituitary oxytocin stores increase during pregnancy.

Circulating oestrogen and progesterone levels increase markedly over the course of pregnancy (164) and oestrogen promotes oxytocin gene expression (375), suggesting that the high sex steroid levels of pregnancy might stimulate oxytocin synthesis in preparation for parturition. However, oxytocin MNCs do not express oestrogen receptor α (ERα), and express little ERβ (376). Nevertheless, oestrogen does appear to directly activate the oxytocin gene promoter, but not via the classical oestrogen response element; while oestrogen action is dependent on the presence of ER, it does not involve ER binding to DNA, rather oestrogen actions appear to be mediated by a nuclear orphan receptor binding site in the oxytocin gene promoter (377).

MNCs strongly express the non-classical ER, GPR30 (378), which might mediate rapid actions of oestrogen *in vivo* to enhance the excitation evident upon removal of opioid inhibitory tone (379) and *in vitro* to promote somato-dendritic oxytocin (and vasopressin) secretion (380) and to potentiate the kainate-induced depolarisation in MNCs recorded from slices from rats in late lactation (381).

While MNCs do not express the ER, many of their inputs express ER, particularly those from the osmoreceptor complex (382). Oestrogen potentiates systemic oxytocin and vasopressin secretion induced by hyperosmotic stimulation (383). Hence, oestrogen might promote fluid retention in the face of the decreased plasma osmolality that develops over the course of pregnancy (384).

Prolonged progesterone treatment inhibits oxytocin MNCs (385, 386) and progesterone administration at the end of pregnancy delays parturition by inhibiting oxytocin secretion (387). However, oxytocin MNCs do not express progesterone receptors (PRs), even at the end of pregnancy (388), and so these inhibitory effects are likely mediated by afferent inputs.

The primary metabolite of progesterone, allopreganolone, is an allosteric modulator of $GABA_A$ receptors and the expression of the α_1 subunit of the $GABA_A$ receptor by oxytocin MNCs increases through pregnancy (389), a process that is driven by the elevated progesterone levels of pregnancy (390). While increased α_1 subunit expression would normally decrease IPSC duration, α_1 subunit-containing $GABA_A$ receptors are more sensitive to allopregnanolone, which is abundant in late pregnancy and prolongs IPSC duration (107). As parturition approaches, despite the continued presence of progesterone, the subunit expression switches to favour α_2 subunits, which removes the allopregnanolone

sensitivity to decrease IPSC duration in time for parturition and this persists into lactation (107), removing the enhanced inhibitory influence on oxytocin MNCs in time for delivery and lactation.

By contrast to their inhibitory effects, allopregnanolone (and to a lesser extent, progesterone) increase somato-dendritic oxytocin release (as well as some vasopressin release) by a GABA-dependent mechanism (391), which would be expected to contribute to the bursting of oxytocin MNCs during partition and lactation.

Regulation of oxytocin secretion by non-steroidal pregnancy hormones

In addition to sex steroids, several hormones alter the activity of oxytocin MNCs; the best characterised of these is relaxin. Ovarian relaxin secretion increases over the course of pregnancy until term, becoming detectable in mid-pregnancy and continuing to increase until delivery (392). While relaxin ripens the cervix, softens the ligaments of the pubic symphysis and inhibits uterine contraction (384) in preparation for parturition, it also increases the firing rate of oxytocin MNCs to increase systemic secretion (393). However, this excitation is unlikely to be involved in stimulating oxytocin secretion during parturition because the excitation is lost in late pregnancy (and lactation) (394). Rather it appears that relaxin excitation of MNCs contributes to resetting the control of body fluid balance over the course of pregnancy, when there is a marked increase in blood volume and a decrease in plasma osmolality due to mild hyponatraemia in the face of sodium retention (384); late-pregnant relaxin knockout mice do not exhibit reduced plasma osmolality (395). Vasopressin MNCs are also excited by relaxin (393). While relaxin receptors are expressed in the PVN and SON (396), circulating relaxin is unlikely to enter the brain and so its effects on MNCs might be mediated via activation of receptors in the SFO (396).

Oxytocin MNCs express prolactin receptors (397) and are inhibited by central administration of prolactin in virgin female rats (398). Prolactin also inhibits oxytocin MNCs *in vitro*, with both excitatory and inhibitory effects reported (399), with the inhibition probably mediated by activation of potassium channels (400). The effects of prolactin on oxytocin MNCs appear to depend on the physiological status of the individual because prolactin increases oxytocin secretion in lactating rats, although this stimulatory effect might be mediated by actions on the neurosecretory terminals in the posterior pituitary gland, rather than by modulation of action potential firing rate (401).

Kisspeptins are a recently discovered group of peptides secreted by the placenta that are agonists at the GPR54 receptor (402). Peripheral kisspeptin administration excites oxytocin MNCs via activation of vagal afferents (321) to increase circulating oxytocin levels (402, 403). While placental secretion of kisspeptin increases dramatically over the course of pregnancy in humans (404), the impact of placental kisspeptin on oxytocin MNCs during pregnancy or lactation has yet to be determined.

Stress responses of oxytocin magnocellular neurosecretory cells in pregnancy

Oxytocin MNCs are activated by a wide range of stressors, including behavioural, immune and visceral stressors (359). Clearly, stress activation of oxytocin MNCs would increase the risk of pre-term delivery, particularly in late pregnancy when uterine oxytocin receptor expression is upregulated in preparation for delivery (84). To reduce the risk of inappropriate activation of uterine contraction, a wide range of stressors are less effective at causing oxytocin secretion in late pregnancy, including behavioural stressors (405), hyperosmotic stimulation (406) and immune challenge (358). The reduced stress responses of oxytocin MNCs in late pregnancy is due, at least in part, to active inhibition by endogenous opioid peptides (304), which might be induced by allopregnanolone (407).

Oxytocin, vasopressin and behaviour

The largest explosion of research on oxytocin and vasopressin in recent years has been the investigation of the impact of these neuropeptides on behaviour when released into the brain. The effects of oxytocin (and to a lesser extent, vasopressin) on social behaviour appear to be via actions on the brain reward pathway and have been extensively reviewed elsewhere (408, 409). These remarkable findings have led to a focus on dysfunction of the central oxytocin system as a key element of behavioural disorders, and for stimulation of central oxytocin receptors as a potential therapy for autism spectrum disorder (410), as well as for drug addiction (411). In addition to effects on social behaviour, central oxytocin has again come to prominence as a regulator of food intake (412).

Oxytocin and vasopressin do not cross the blood-brain-barrier and so centrally-acting oxytocin must be released within the brain. While there are known oxytocin-containing efferents from some parvocellular PVN neurones (21), these projections are generally sparse and so most research in recent years has focussed on the potential for somato-dendritic release to function as a long-range signalling system within the brain (413). However, the recent identification of axon collaterals from oxytocin MNCs that project to areas of the brain involved in behaviour (24) has identified a new avenue for MNCs to communicate with the rest of the brain.

Concluding remarks

Here, we have highlighted the interaction of afferent inputs with local and intrinsic mechanisms in the regulation of magnocellular neurosecretory activity responses to physiological challenges. Many peripheral inputs converge on the same afferent inputs. However, it is not clear whether this convergence is upon the same neurones within the afferent input population, or upon different individuals within the population and this question remains one of the challenges for the field.

Hypothalamic MNCs are among the most studied and best characterised neurones in the brain, yet each passing year opens new perspectives on the control and function of these neurones. The wide-ranging peripheral effects of circulating oxytocin and vasopressin (1) are mirrored in the increasingly diverse central actions of these peptides that must result from central release. Many of the central (neuropeptide) and peripheral (hormonal) effects of these peptides might represent two arms of a co-ordinated whole-organism physiological and behavioural response to challenges to homeostasis. Whether achieved by co-ordinated somato-dendritic and axon terminal release, or by release from centrally-projecting axon collaterals, MNCs are ideally placed to respond physiological and environmental conditions and, as the endocrine system has long been known to do, orchestrate the whole body response to maintain homeostasis.

Acknowledgments

CHB was supported by the Health Research Council of New Zealand and the Marsden Fund of the Royal Society of New Zealand; JSB by Alberta Innovates and the Canadian Institutes of Health Research: ML by the Biotechnology and Biological Sciences Research Council; JES by R01 HL112225 and R21 HL113801 from the National Institutes of Health.

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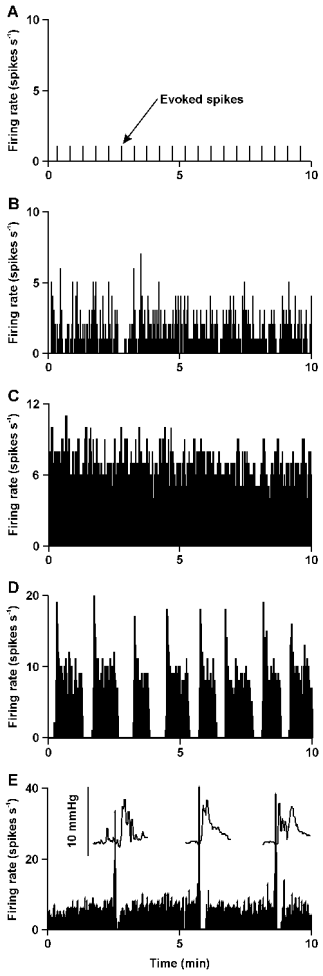

Figure 1. Activity patterning in magnocellular neurosecretory cells *in vivo*

The ratemeter records show individual examples of the spontaneous activity (averaged in 1 s bins) of MNCs displaying each of the activity patterns typical of magnocellular neurosecretory cells (MNCs) in urethane anaesthetised rats. Under basal conditions, MNCs exhibit a range of activity patterns from silence (**A**), through irregular activity (**B**) to continuous activity (**C**) and, in vasopressin MNCs only, phasic activity (**D**). In **A**, action potentials were evoked in a silent MNC by electrical stimulation of the posterior pituitary gland every 30 s (evoked spikes). The recording in **E** is from an oxytocin MNC in a urethane-anesthetised rat being suckled during lactation and shows the typical high frequency bursts evident in oxytocin MNCs during parturition and suckling. The insets in **E** show the intramammary pressure increase for milk ejection that follows each burst of activity in the oxytocin MNC. Data for **E** was kindly provided by Prof J.A. Russell, University of Edinburgh.

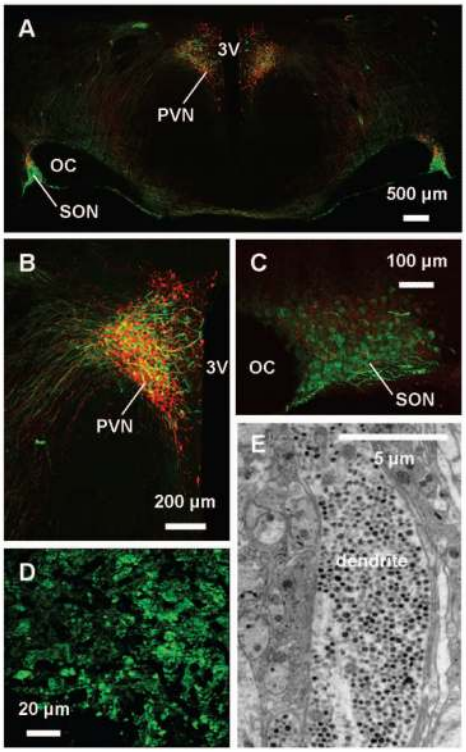

Figure 2. The magnocellular neurosecretory system

A – C, Photomicrographs of coronal sections of rat hypothalamus (**A**), in which vasopressin magnocellular neurosecretory cells (MNCs) are immunostained with fluorescent green and oxytocin MNCs with fluorescent red. MNC 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 MNCs that project to the posterior pituitary gland, whereas the PVN also contains parvocellular oxytocin and vasopressin MNCs (as well as other parvocellular neurones) that project elsewhere in the brain. **D**, Photomicrograph of vasopressin axon terminals in the posterior pituitary gland. **E**, Electron micrograph showing MNC dendrites densely packed with dense-core vesicles (small black dots). We thank Dr Vicky Tobin, University of Edinburgh, for generation of the immunohistochemistry images.

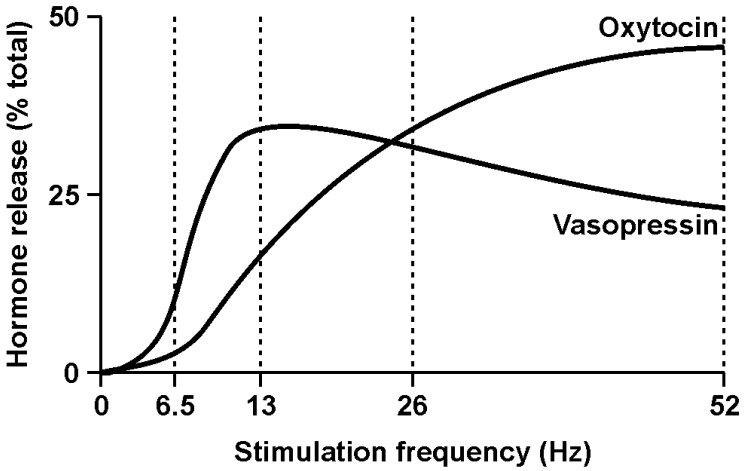

Figure 3. Frequency facilitation of oxytocin and vasopressin release from magnocellular neurosecretory cells terminals

Isolated posterior pituitary glands were electrically stimulated with 156 pulses delivered at each of the four frequencies indicated in a balanced order of presentation. Evoked hormone release is expressed as a percentage of the total release evoked by the four stimulations. Note that hormone release is facilitated at higher frequencies, that little hormone is released at frequencies of < 4 Hz and that frequency facilitation of vasopressin release peaks at a lower frequency than for oxytocin release. Modified from (33), with permission.

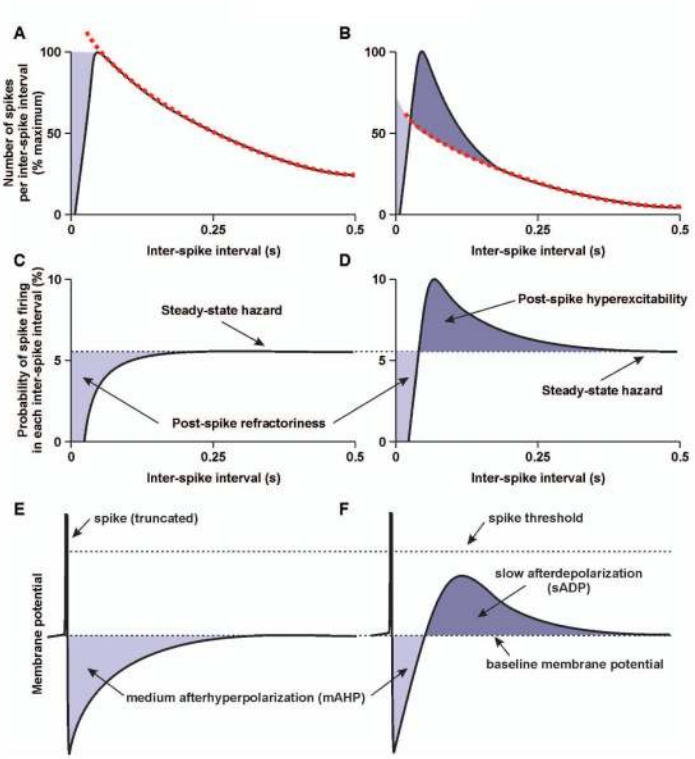

Figure 4. Post-spike excitability and post-spike potentials in magnocellular neurosecretory cells

A and **B**, Schematic representations of inter-spike interval distribution of oxytocin (**A**) magnocellular neurosecretory cells (MNCs) and vasopressin MNCs (**B**). The tail of the distribution is fit by a single exponential decay (dashed line); for vasopressin MNCs, but not oxytocin MNCs, the exponential does not fit the peak of the distribution. **C** and **D**, Schematic representations of hazard functions for oxytocin MNCs (**C**) and vasopressin MNCs (**D**). Hazard functions are the probability of the next spike firing with time after the preceding spike and represent the post-spike excitability of neurones. Hazards are calculated from the inter-spike distributions using the formula: $h_{[i-1, i]} = n_{[i-1, 1]} / (N - n[0, i-1])$, where $h_{[i-1, i]}$ is the hazard at inter-spike interval i, $n_{[i-1, 1]}$ is the number of spikes in inter-spike interval, i, $n[0, i-1]$ is the total number of spikes preceding the current inter-spike interval and N is the total number of spikes in all inter-spike intervals. **E** and **F**, Schematic representations of individual spikes from an oxytocin MNC (**E**) and a vasopressin MNC (**F**), with the associated changes in membrane potential caused by the medium afterhyperpolarization (mAHP) and slow afterdepolarization (sADP) (not to scale). MNCs exhibit a prominent post-spike mAHP that initially hyperpolarises the cell after each spike, making it less likely to reach spike threshold (post-spike refractoriness). Vasopressin MNCs also exhibit a prominent post-spike sADP that is lower amplitude and longer-lasting than the mAHP; the sADP increases causes post-spike hyperexcitability by bringing the membrane potential closer to spike threshold, making it more likely that on-going (stochastic) synaptic input will reach spike threshold to trigger a further spike. If a spike does not fire, the probability of spike firing returns a steady-state hazard that is inferred to reflect the baseline membrane potential and the on-going synaptic input activity.

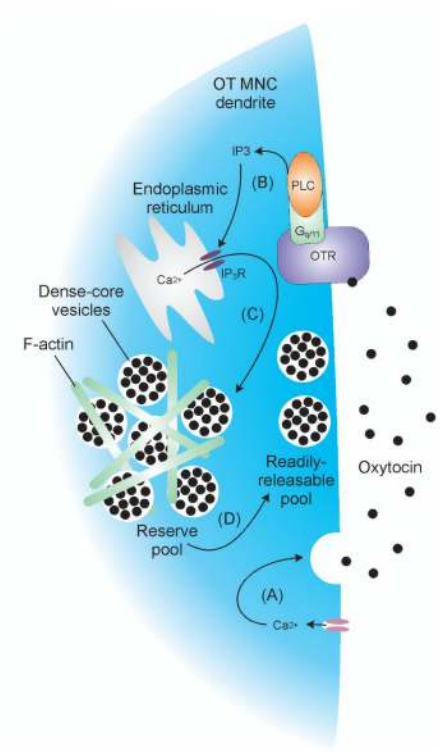

Figure 5. Priming somato-dendritic oxytocin release

Calcium influx triggers somato-dendritic oxytocin release (**A**). Oxytocin activates oxytocin receptors on the plasma membrane to increase intracellular inositol triphosphate (IP3) concentrations (**B**). IP3 increases calcium release from the endoplasmic reticulum (**C**) to mobilise dense-core vesicles from the reserve pool to the readily-releasable pool (**D**), 'priming' oxytocin MNCs to release increased amounts of oxytocin in response to subsequent stimuli.

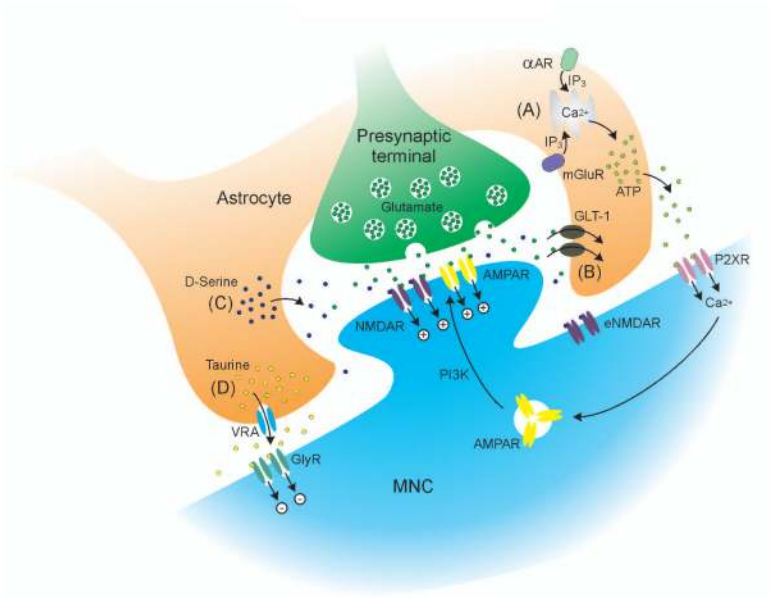

Figure 6. Glial regulation of magnocellular neurosecretory cell activity under basal conditions

A, α1-adrenoreceptor (αAR) or group 1 and 5 metabotropic glutamate receptor (mGluR) activation increases intracellular calcium in astrocytes via inositol triphosphate (IP_3) to trigger ATP release. ATP activates magnocellular neurosecretory cell (MNC) P2X receptors (P2XR) to increase calcium influx, which activates phosphotidyl inositol 3-kinase (PI3K) to increase AMPA receptor (AMPAR) insertion into the postsynaptic membrane, mediating long-term potentiation of glutamate synapses (synaptic scaling). **B**, Astrocyte glutamate transporters (GLT-1) remove glutamate from the extracellular space to limit activation of extrasynaptic NMDA receptors (eNMDAR). **C**, Astrocytes release D-serine to act as a co-agonist with glutamate at postsynaptic NMDARs on MNCs. **D**, Astrocytes release taurine through volume-regulated anion channels (VRA) to activate extrasynaptic glycine receptors (GlyR) to hyperpolarise the MNC via chloride influx.

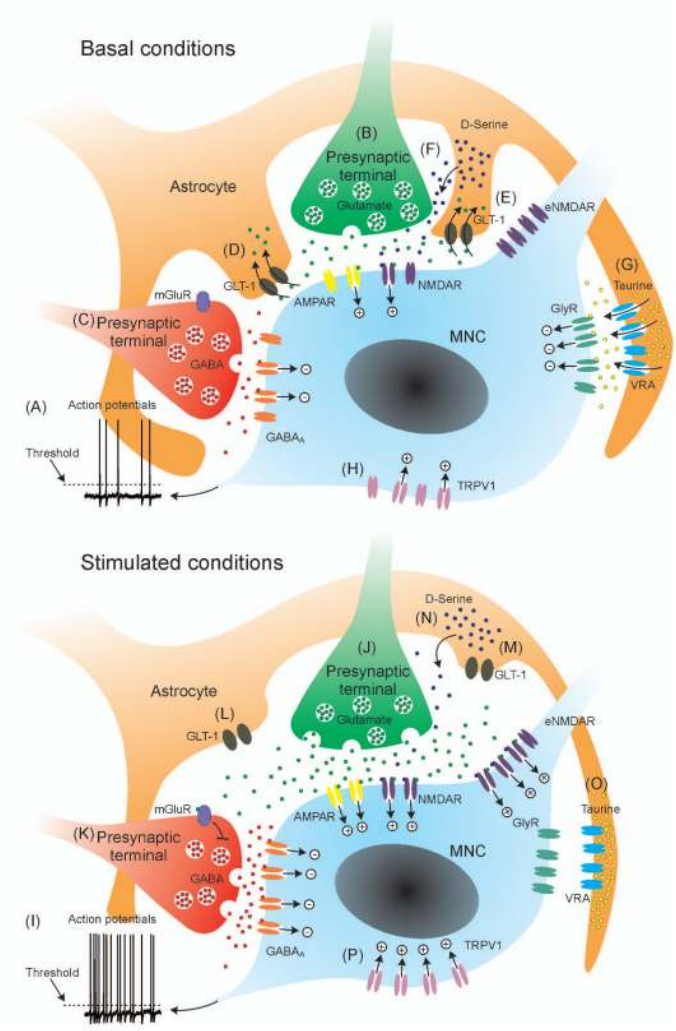

Figure 7. Integrated physiological modulation of magnocellular neurosecretory cell activity

Under basal conditions (top panel) various excitatory and inhibitory intrinsic, local and afferent mechanisms integrate to generate low frequency action potential discharge (**A**) in magnocellular neurosecretory cells (MNC) to maintain relatively low and constant circulating oxytocin and vasopressin concentrations. Excitatory inputs, principally but not exclusively from glutamate neurones (**B**) depolarise the MNC for a short time after each synaptic event; if threshold is reached, post-spike potentials (not shown) will modulate membrane potential. In parallel with excitatory drive, inhibitory inputs, principally but not exclusively from GABA neurones (**C**) will transiently hyperpolarise the MNC. Astrocytes express glutamate transporter-1 (GLT-1), which prevents synaptically-released glutamate reaching metabotropic glutamate receptors (mGluR) on other afferent inputs (**D**) and extrasynaptic NMDA receptors (NMDAR) on MNCs (**E**). Astrocytes release D-Serine that acts as a co-agonist at NMDARs to permit glutamate activation (**F**) and taurine that activates extrasynaptic glycine receptors to tonically hyperpolarise MNCs (**G**). Some stretch-inactivated TRPV1 channels are active to tonically depolarise MNCs. Under stimulated conditions, each of these mechanisms is altered to increase the probability of action potential discharge (**I**). Glutamate release is increased to increase EPSC frequency (**J**). GABA release is also increased to increase IPSC frequency (**K**) to dampen the gain of the excitatory drive. Withdrawal of astrocytic processes (glial retraction) reduces the physical barrier to

neurotransmitter diffusion and reduces the efficacy of GLT-1 to allow spillover of glutamate to activate mGluR on neighbouring GABA synaptic terminals (**L**), presumably to prevent over-inhibition. Glial retraction also allows glutamate to activate extrasynaptic NMDARs (eNMDAR) to induce a tonic depolarisation (**M**). A further consequence of glial retraction is to reduce the efficacy of D-serine released from astrocytes (**N**), presumably to limit excitation. When astrocytes shrink (e.g in hyperosmotic conditions), volume-regulated anion (VRA) channels close to reduce glycine release and reverse the tonic hyperpolarization mediated by GlyR (**O**). MNC shrinkage opens TRPV1 channels to increase the tonic depolarisation. The net effect of these changes is to drive baseline membrane potential towards threshold and increase noise in the baseline membrane potential, increasing the probability that action potential threshold will be reached. See text for details of the specific physiological conditions that modulate each of the mechanisms.

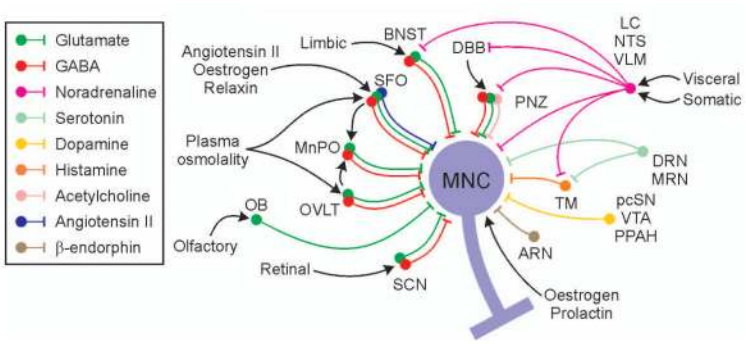

Figure 8. Schematic representation of some of the major peripheral and afferent inputs to magnocellular neurosecretory cells

See text for details of the physiological functions of each of the inputs. Abbreviations: ARN: arcuate nucleus; BNST: bed nucleus of the stria terminalis; DBB: diagonal band of Broca; DRN: dorsal raphe nucleus; LC: locus coeruleus; MnPO: median preoptic nucleus; MRN: median raphe nucleus; NTS: nucleus tractus solitarius; OB: olfactory bulb; OVLT: organum vasculosum of the lamina terminalis; pcSN: pars compacta of the substantia nigra; PNZ: perinuclear zone; PPAH: preoptic periventricular /anterior hypothalamic region; SCN: suprachiasmatic nucleus; SFO: subfornical organ; TM: tuberomammillary nucleus; VLM: ventrolateral medulla; VTA: ventral tegmental area.