The multiple faces of the oxytocin and vasopressin systems in the brain

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Abstract

Classically, hypothalamic neuroendocrine cells that synthesize oxytocin and vasopressin were categorized in two major cell types, the magnocellular and parvocellular neurones. It was believed that magnocellular neurones project exclusively to the pituitary gland where they release oxytocin and vasopressin into the systemic circulation. The parvocellular neurones, on the other hand, project within the brain to regulate discrete brain circuitries and behaviours. Within the last few years it has become evident that the classical view of these projections is outdated. It is now clear that oxytocin and vasopressin in the brain are released extrasynaptically from dendrites and from varicosities in distant axons. The peptides act principally to modulate information transfer through conventional synapses (such as glutamate synapses) by actions at respective receptors that may be preferentially localised to synaptic regions (on either side of the synapse) to alter the 'gain' of conventional synapses.

The oxytocin and vasopressin system and its projections

Classically oxytocin- and vasopressin-synthesising neurones have been categorized as two major types: magnocellular and parvocellular cells(1-3). They differ in size, shape, anatomical location, function, projection sites, mode of release and electrophysiological properties(4, 5). While there has recently been speculation about the potential existence of additional oxytocinergic cell types, based on genetic cluster analysis(6), so far, no concrete functional evidence has been provided to corroborate these findings. Moreover, due to the fact that concrete genetic profiles for magno- and parvocellular oxytocin neurones are currently missing, it is not possible to genetically target and reliably manipulate these two oxytocinergic cell types.

Oxytocin neurones in the rodent brain are located almost exclusively located in the PVN, SON and accessory nuclei of the hypothalamus, with a few scattered in the bed nucleus of stria terminalis (BNST)(Fig. 1A)(7).

Recent publications on oxytocinergic transmission in the CNS suggest that the classical view of projections from magno- and parvocellular oxytocin, as well as the presumed modes of release from these axons, is outdated and may have to be abandoned. Using viral tracing and whole-brain imaging, Zhihua Gao's lab has recently reconstructed the three-dimensional architecture of the hypothalamo-neurohypophysial system(8). It confirms our own work (9, 10) and shows that most – if not all – magnocellular oxytocin neurones project collaterals from axons of the *hypoth*alamic-neurohypophysial tract to various forebrain regions. To this day, more than 50 forebrain regions have been identified as targets for magnocellular oxytocin

neurones(9, 10), which modulate contextual and social fear responses, emotional transfer between conspecifics and even inter-female aggression(11-13).

Parvocellular oxytocin neurones project to the midbrain, brainstem and spinal cord and are involved in food intake regulation(14), autonomic functions such as breathing(15), erection and copulation(16), cardiovascular reactions(17), gastric reflexes(18) and pain perception(19). All of these projections arise from a small population of parvocellular oxytocin neurones residing within the PVN. While it is well established that parvocellular oxytocin neurones synapse onto magnocellular oxytocin neurones located in the SON to control activity-dependent release of oxytocin into the systemic circulation (Fig. 1E) (20, 21), it was recently demonstrated that parvocellular oxytocin neurones control magnocellular oxytocin activity within the PVN as well (20). While parvocellular oxytocin neurones have been underappreciated for most of the 20th century, they recently emerged as key regulators of the oxytocin system. In fact, the latest research suggests that somatosensory information first converges on parvocellular oxytocin neurones, which, on activation, subsequently activate the much larger population of magnocellular oxytocin neurones. This mode of action allows fine-tuned and effective global activation of the oxytocin system, with coordinated release and context-dependent activity patterns of magnocellular oxytocin PVN subdivisions (20).

Within the last 5 years, parvocellular oxytocin neurones have emerged as new players in modulation of the oxytocin system and it has become evident that this small subpopulation of cells plays a vital role in somatosensory signal integration during social interaction(22), coordination of nociceptive response at both a central and a peripheral level(20) and context-dependent activation of fear-sensitive oxytocinergic engram cells in the hypothalamus(21). In fact, these studies suggest that parvocellular oxytocin neurones may be master regulators that synaptically control and orchestrate magnocellular oxytocin neuron activity under various conditions(22). Given the types of scenarios described (fear, pain and social interaction), it seems reasonable to suspect that the coordination of magnocellular oxytocin release by parvocellular oxytocin neurones may be the general rule rather than the exception.

In addition to the PVN and SON, vasopressin neurones are also found in various extra-hypothalamic forebrain nuclei, including the suprachiasmatic nucleus, the bed nucleus of stria terminalis (BNST), the medial nucleus of the amygdala(23) and the entorhinal cortex (24).

In transgenic rats expressing a vasopressin-GFP fusion protein under the control of the vasopressin promoter(25), a GFP signal was also found in neurones of the olfactory system, where vasopressin modulates the processing of olfactory social signals (Fig. 1B) (26, 27). Very recently, it has also been shown that a small fraction of ganglionic cells in the retina expresses

vasopressin and, through its projections to the suprachiasmatic nucleus (which also contains vasopressin cells) modulates circadian rhythmicity(28).

Similarly, to magnocellular oxytocin neurones, magnocellular vasopressin neurones project not only to the posterior pituitary gland but also to multiple extrahypothalamic sites, especially limbic regions, influencing emotional responses during stress coping and motivational behaviours(24, 29, 30). Our own ongoing studies, employing new vasopressin transgenic rats and viral vectors support these studies, indicating that magnocellular vasopressin system, at least in rats, is not so elaborated as the oxytocin system. This can be probably explained by more divergent vasopressin sub-systems in the brain and the specialization of distinct vasopressin groups in modulation of certain types of behaviours, while magnocellular vasopressin neurons are primarily involved in neurohormonal regulation of water homeostasis.

While the projections, properties and functions of parvocellular oxytocin neurones have been extensively studied, very little is known about the respective role of parvocellular vasopressin neurones. Early studies identified vasopressin as a regulator of the hypothalamic-pituitary-adrenocortical axis and showed that vasopressin can potentiate the stimulatory effect of corticotropin-releasing hormone (CRH) on adrenocorticotropin (ACTH) cells of the anterior pituitary(31). Later, Greti Aguilera and her colleagues showed that synthesis of vasopressin in CRH neurones is triggered by chronic stress, which coincides with downregulation of CRH expression in these cells. It was proposed that vasopressin substitutes for CRH as the main factor maintaining the release of adrenal corticosteroids under chronic stress and inflammatory conditions(32-34). A similar mechanism has been observed in lactating rats, which exhibit a blunted CRH response that is partly compensated by enhanced synthesis of vasopressin in CRH neurones, which results in increased neuronal sensitivity(35). While it seems possible that parvocellular CRH/vasopressin cells are involved in the stress-induced regulation of the CRH system, especially under chronic inflammatory stress, no concrete evidence confirming this theory has yet been presented. In addition, this particular line of research has been discontinued and thus the role of CRH neurones co-expressing vasopressin should be re-visited, with implementation of novel genetic and functional techniques developed during the last two decades.

Taken together, the current body of knowledge does not provide evidence for a clear magno-/parvocellular distinction based on projections, functions and input for vasopressin neurones. In fact, the unique interaction of parvo- and magnocellular oxytocin neurones seems to be a unique feature of the oxytocin system. A reliable discrimination of parvo- and magnocellular oxytocin neurones based on their genetic profiles has not yet been achieved.

Genetic analysis of oxytocin neurones resulted in four different clusters, although it is not clear whether parvo- and magnocellular oxytocin neurones are exclusively represented within those genetic subgroups of oxytocin neurones (6). In a recent study(36), the laboratory of Gul Dölen reported autism risk genes to be enriched in parvocellular oxytocin neurones, which have been genetically dissected based on anatomical location and Fluorogold (FG) labelling. Intriguingly, the group reported that 34% of all oxytocin neurones are parvocellular (36), which is in stark contrast to 1-5%, as has been previously reported in rats (1). While this discrepancy can partially be attributed to a species-dependent difference in the composition of the oxytocinergic system and technical limitations in the use of Fluorogold as a marker of magnocellular (neuroendocrine) neurones, further identification of genetic markers discriminating oxytocin cell types will be essential to dissect phenotypes of oxytocin neurones, which are not limited only to parvocellular or magnocellular cells.

Modes of release of oxytocin and vasopressin

It has recently been reported that parvocellular oxytocin neurones form true synapses in various structures including the SON, PVN, brainstem and spinal cord(20). Is oxytocin released at synapses? It appears that a very few oxytocin vesicles are released into the synaptic cleft, however there is also exocytosis from axonal swellings and even occasionally from undilated axons (37, 38). It seems that the function of these synapses is to facilitate the release not of oxytocin, but rather of glutamate. The vesicular glutamate transporter 2 is expressed and glutamate is co-released with oxytocin (9, 21, 39).

There is very little evidence that magnocellular oxytocin neurones form true, functionally-relevant synapses with other neurones. Although magnocellular oxytocin neurones project axons to almost the entire rodent forebrain, there has been no report of actual synapse formation by magnocellular oxytocin axons, except the synaptic contact found in the central nucleus of the amygdala(9). Optogenetic experiments from our group suggests that magnocellular neurones make true synapses in the amygdala, but oxytocin vesicles are not located in the active zone of the synapse which, like the true synapses in the brainstem and elsewhere formed by parvocellular neurones, release mainly glutamate(24).

Oxytocin and vasopressin are released from the somata and dendrites of magnocellular neurones(40, 41), as well as from synapses. This non-axonal release does not necessarily parallel release from axonal terminals in the neurohypophysis (41,42): although it may be evoked by the same stimulus, it can occur on a different time scale and may be regulated separately (Fig. 1D) (39). Dendritic release can be evoked by certain peptides; most notably,

oxytocin itself is a potent stimulator of oxytocin release. Oxytocin mobilizes intracellular Ca^{2+} and triggers release from dendrites independently of electrical activity, and also primes dendritic stores of the peptide, making them available for activity-dependent release. Priming is an important phenomenon that underlies functional reorganization of the magnocellular system, leading to positive-feedback coupling between oxytocin cells and producing the intense synchronized bursts observed during parturition and suckling(42, 43).

In addition to the function of dendritically-released peptides in autoregulating the electrical activity of their cells of origin, paracrine actions of dendritically-released vasopressin from magnocellular neurosecretory neurones, stimulating neighbouring presympathetic neurones of different nature within a range of 100 μm and thereby mediating interpopulation crosstalk, have been described by Stern and colleagues(44). Moreover, the mechanisms described seem to play a pivotal role in the vasopressin-dependent polymodal neurohumoral response to a hyperosmotic challenge. Importantly, the interpopulation crosstalk between magnocellular vasopressin and parvo-presympathetic-V1a receptor-expressing neurones may also play an important role in prevalent cardiometabolic diseases, including hypertension, heart failure and diabetes, conditions in which an exacerbated neurohumoral activation state, characterized by elevated neurosecretory and sympathetic outflows, is known to influence prognosis, morbidity and mortality(45-47).

Magnocellular oxytocin neurones also engage in volume transmission or *en passant* release, which although not confirmed functionally, is likely to involve the synapse-independent, diffuse release of a small number of large dense core vesicles (LDCVs, see below), containing oxytocin, within a target region (Fig. 1D) (48). In this mode of release no synapse formation is required and the bulk of released neuropeptide diffuses to its target site down the concentration gradient. This phenomenon partly explains the occasionally observed delays (up to 90 seconds) of oxytocinergic action after evoking instantaneous release via optogenetics(9, 21).

The action of dendritically-released vasopressin and oxytocin may resemble that of hormones (44) and involve general diffusion of the neuropeptide through the extracellular space to a mixed population of functionally distinct neurones, with the specificity of signalling depending simply on the presence of receptors specific for the transmitter. The behavioural effects of oxytocin and vasopressin may be initiated at sites that are innervated by few peptide-containing projections, but still express receptors for these neurotransmitters. Dendritically-released transmitters may therefore produce long-lasting effects on behaviour by hormone-like actions on the brain, which they reach by simple diffusion through the extracellular space,

although this remains controversial. Both vasopressin and oxytocin are present in the cerebrospinal fluid (CSF), although the concentrations present within the brain areas do not always reflect those within the CSF(40, 41). While both neuropeptides have half-lives of ~20 min in the CSF, they are likely to be degraded more rapidly by extracellular proteinases in the parenchyma, and diffusion, either from release-sites or from the ventricles, will be restricted by the tortuosity of the parenchyma: (for example, this delays by ~2–5 s the effects of vasopressin on preautonomic neurones after its somato-dendritic release from sites some ~100–200 μm away). However, it is still possible that hormone-like signalling by these neuropeptides may occur over long distances and greater timescales, particularly in those regions of the brain that express neuropeptide receptors but have no neuropeptide axons, such as the olfactory bulb. Such hormone-like effects of vasopressin and oxytocin after somato-dendritic release could also modulate the excitability of target neurones that also receive axonal projections from the same (or parvocellular) neurones at a population level, upon which the short-term point-to-point modulation achieved by synaptic transmission is superimposed. Resolving this longstanding debate remains an ongoing challenge for the field.

Controversy surrounding oxytocin receptor activation at pre- and postsynaptic sites

For several decades it seemed clear that oxytocinergic activation of neuronal circuits follows the classical cascade of Ca^{2+}-mediated exocytosis and downstream oxytocin signalling(49). Briefly, oxytocin is packaged in large-dense core vesicles (LDCVs), each of which contains up to 85,000 molecules of the neuropeptide(50, 51). LDCVs are transported along the axonal terminals to the readily releasable pool of vesicles and SNARE-mediated fusion of vesicles with the presynaptic membrane, resulting in exocytosis, takes place in a Ca^{2+}-dependent manner. It was assumed that secreted oxytocin binds to postsynaptic oxytocin receptors, triggering a postsynaptic G-protein-dependent signalling cascade involving various G-protein subtypes/pathways (Gaq, Ga11, Gi/o and β-arrestin)(48).

Despite extensive information on its synthesis and release, the precise mechanisms by which oxytocin targets and activates cells still remain largely elusive. It is clear that oxytocin can act on oxytocin neurones themselves (both in the SON and PVN), in a postsynaptic manner. In the 1980's several papers showed that oxytocin acts in an autocrine manner(52-55) and that this process involves calcium release from intracellular thapsigargin-sensitive calcium stores(42). Finally, Brussaard and colleagues showed that within oxytocin neurones, oxytocin can also postsynaptically modulate the potency of GABAergic synapses(56). Although it has been demonstrated that oxytocin receptor immunoreactivity is preferentially associated with

synapses, indicating that oxytocin may modulate synaptic activity(10), this does not necessarily mean that oxytocin is released at those synapses. Mitre and colleagues demonstrated oxytocin receptors at both sides of synapses (presynaptic terminals and post synaptic densities) and showed that oxytocin, released extrasynaptically, may preferentially act on GABA and/or glutamate synapses at either (or both) sides of the synapse(10).

Some reports have been published that suggest presynaptic expression of oxytocin receptors(57-59). Their proposed mechanism of action includes activation of oxytocin receptors on presynaptic neurones by neighbouring (or the same) cells, which may lead a subsequent release of conventional neurotransmitters or neuromodulators (such as glutamate or serotonin), which in turn activate the postsynaptic cell in addition to or instead of direct 'postsynaptic' oxytocin action on oxytocin-sensitive neurones.

Conclusions

In conclusion, magnocellular oxytocin and vasopressin neurones in addition to their evolutionary-determined 'endocrine' specialization(60), also bear all the features of classical neurones operating with conventional transmitters as was also reported for other hypothalamic neuroendocrine neurones, producing releasing factors (for example corticotropin-releasing hormone, luteinizing hormone-releasing hormone, thyrotropin-releasing hormone, etc.) or peptidergic neurones expressing agouti-related peptide, neuropeptide Y, pro-opiomelanocortin, vasoactive intestinal peptide, galanin and other neuropeptides. The implications of such functional 'dualism' in hypothalamic neurones are far from clear, but this opens novel directions to reveal how neuropeptides and transmitters exert their mutual action on target cells ranging from neurones to glia cells(61). It is tempting to speculate that oxytocin and vasopressin act synergistically with a conventional transmitter. It is plausible that even a few action potentials may trigger synaptic glutamate release to initiate excitation of neurones, while prolonged excitation of magnocellular neurones(62, 63) triggers axonal release of oxytocin or vasopressin, which exerts long-lasting effects on the target neuronal networks and respective behaviours.

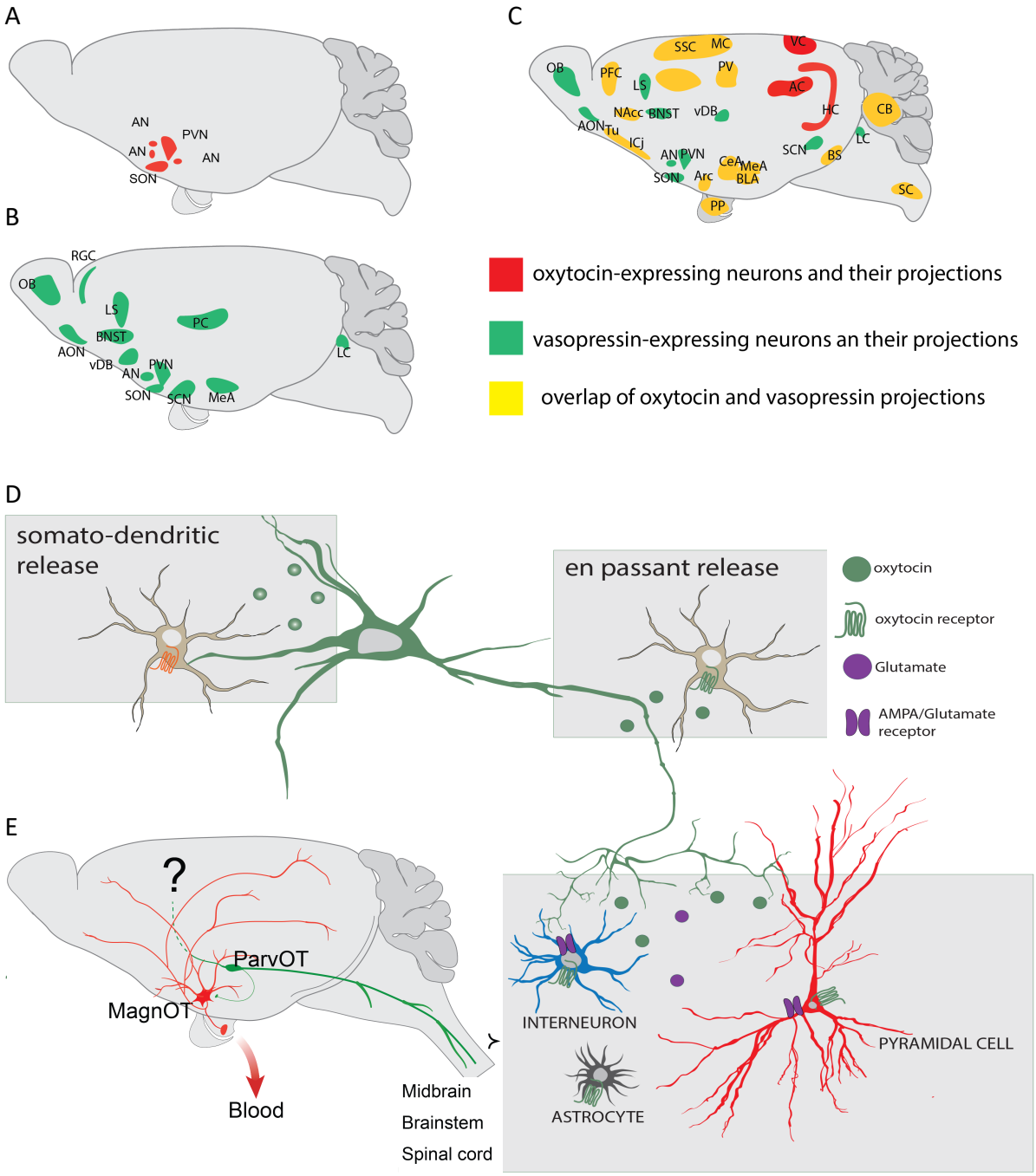
During the last decade, substantial progress has been made in dissection of the anatomy and functional role of subpopulations of parvocellular oxytocin neurones, which exist in lower(64) to advanced vertebrates including humans(65). This cell type is very distinct from magnocellular ones, which represent a 'classical' neuronal phenotype primarily operating by glutamate and thus the expression of oxytocin in these cells seems to be subordinate to oxytocin. However, the anatomical heterogeneity of these cells, the mode of oxytocin release (e.g.

dendritic or synaptic), and concomitant oxytocin and glutamate action on the midbrain, brainstem and spinal cord networks all await further extensive studies.

In comparison to parvocellular oxytocin neurones, the presence of 'pure' parvocellular vasopressin neurones in the PVN has not been clearly demonstrated. Although vasopressin is expressed in 90% of CRH neurones upon chronic stress or adrenalectomy, these cells cannot be distinguished from pre-sympathetic or classical neuroendocrine CRH-expressing neurones(66, 67). It is tempting to speculate, that that the vast majority of parvocellular neurones in the PVN that express vasopressin are CRH neurones.

In summary, magnocellular oxytocin and vasopressin neurones, in addition to their classic projection to the posterior pituitary, send axonal collaterals to forebrain regions and release the neuropeptides non-synaptically (e.g. by 'local micro-volume release') to subsequently modulate activity of target networks controlling various forms of behaviours. Parvocellular oxytocin neurones, from one side, coordinate the activity of magnocellular oxytocin neurones to elicit whole-body homeostatic adaption to occurring behavioural challenges. In contrast, vasopressin is co-expressed in CRH neurones only under insufficient glucocorticoid feed-back and thus these cannot be considered to be an independent parvocellular vasopressin cell type.

Following this updated view of multifaceted hypothalamic oxytocin and vasopressin neurones, further studies are required, particularly, to reveal the role of conventional transmitters (such as glutamate) co-expressed and potentially co-released from same axonal terminals of parvocellular vs. magnocellular oxytocin and vasopressin neurones, employing alternated ablation of glutamate or oxytocin in the same cells along with high resolution imaging and simultaneous recording of 'postsynaptic' cell responses. These and other studies addressing similarity and distinction between parvocellular and magnocellular neurones will be essential for understanding their mutual contribution to pathophysiology of metabolic, cardiovascular and especially mental diseases in human patients.



Magnocellular oxytocin release

- Somato-dendritic release
- *En passant* release
- Axonal/synaptic release (?)
- Glutamate (co)-release
- No evidence for true synapse formation
- Paracrine activation of astrocytes

Parvocellular oxytocin release

- Axonal/synaptic release
- Glutamate (co)-release
- Evidence for true synapse formation
- Activation of WDR neurons in the spinal cord
- Interaction with autonomic nervous system in the brainstem

Grinevich Ludwig Fig. 1

Figure 1: The oxytocin **(A)** and vasopressin **(B)** systems in the rodent brain, **(C)** synthesizing nuclei and distinct/overlapping projection sites. **D)** Projection sites and modes of release of

magno- and parvocellular oxytocin indicating that magnocellular neurones release oxytocin **(**and vasopressin) from somas and dendrites, from axons passing by (*en passant*,) and from long-range axons. Oxytocin receptors (OTR) have been found in various neuronal cell types, including GABAergic interneurones and pyramidal cells. **E)** Brain scheme depicts the currently known interconnectivity of magno- (red) and parvocellular oxytocin (green) neurones within the paraventricular (PVN) and supraoptic nucleus (SON) and their distinct projections to the pituitary, forebrain, midbrain, brainstem and spinal cord. The green dashed lines highlight potential but so-far unconfirmed parvocellular oxytocin projections to the forebrain. Parvocellular oxytocin neurones act as hypothalamic master cells and project onto magnocellular oxytocin neurones in the PVN and SON to coordinate their activity. Somato-dendritic release from magnocellular oxytocin neurones provides a feedback mechanism between magnocellular oxytocin neurones. Parvocellular oxytocin neurones form clear synapses with other neurones in the PVN and spinal cord, while secretion from magnocellular oxytocin neurones via volume transmission or *en passant* release activates nearby neurones and astrocytes.

Accessory nuclei: AN, Anterior olfactory nucleus: AON, Arcuate hypothalamic nucleus: Arc, Auditory cortex: AC, Bed nucleus of stria terminalis: BNST, Basolateral amygdala: BLA, Brainstem: BS, Central amygdala: CeA, Cerebellum: CB, Hippocampus: HC, Horizontal limb of diagonal band nucleus: HDB, Island of Calleja: iCj, Lateral septum: LS, Locus coeruleus: LC, magnocellular oxytocin neurones: magnOT neurones, Medial amygdala: MeA, Motor cortex: MC, Nucleus accumbens: NAcc, Olfactory bulb: OB, Olfactory tubercele: Tu, oxytocin: OT, Posterior pituitary: PP, Paraventricular nucleus: PVN, Paraventricular thalamus: PV, parvocellular oxytocin neurones: parvOT neuroen, Piriform cortex: PC, Prefrontal cortex: PFC, Prelimbic cortex: PLC, Raphe magnus nucleus: RMg, RVLM: Rostral ventrolateral medulla, Somatosensory cortex: SSC, Spinal cord: SC, Suprachiasmatic nucleus: SCN, Supraoptic nucleus: SON, Ventral diagonal band of Broca: vDB, Wide dynamic range neurones: WDR, (adapted from (1, 68)).

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