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Oxytocin neurons: integrators of hypothalamic and brainstem circuits in the regulation of macronutrient-specific satiety

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Hypothalamic oxytocin neurons are differentially regulated by the ingestion of different macronutrients, presumably through inputs from other hypothalamic and brainstem regions that are sensitive to food-associated signals from the periphery, including the gut. After integrating this information, the oxytocin system appears to target multiple hypothalamic and extra-hypothalamic regions to suppress food consumption in a macronutrient-specific manner, specifically decreasing preference for carbohydrate-containing foods.

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Introduction

To date, most research on the neural control of energy balance has focused on the hypothalamic arcuate nucleus, especially on the orexigenic agouti related peptide (AgRP) and anorexigenic pro-opiomelanocortin (POMC) neurons, due to their ability to directly respond to peripheral energy status signals and their powerful influence on feeding behaviors [1]. The oxytocin system is a key target of these neurons [2–4]. Early research on the oxytocin system and appetite focused on projections to the caudal brainstem from *parvocellular* oxytocin neurons in the hypothalamic paraventricular nucleus (PVN) in the regulation of gastric motility and meal size [3]. However, the *magnocellular* oxytocin neurons in the PVN and supraoptic nucleus (SON) are also critical regulators of food intake [2–4]. In this review, we focus on how the magnocellular oxytocin system interacts with the arcuate nucleus and nucleus of the solitary tract (NTS) to regulate the motivation for carbohydrate-containing foods.

Involvement of the magnocellular oxytocin system in feeding regulation

In the brain, oxytocin is exclusively synthesised in the hypothalamus, and the classical understanding of the anatomy of this system derived from immunohistochemical and tract-tracing studies in rats conducted in the 1980's. A review by Swanson and Sawchenko in 1983 [5] distinguished between *magnocellular* oxytocin neurons, whose 'principal, and perhaps only' projection was to the posterior pituitary, and *parvocellular* oxytocin neurons that projected densely to the caudal brainstem and spinal cord but not to the posterior pituitary. About half of the magnocellular neurons were located in the SON and PVN, but many were aggregated in various 'accessory magnocellular nuclei', and isolated magnocellular neurons could be found scattered in adjacent regions. By contrast, the parvocellular oxytocin neurons were exclusively located in the PVN amongst several other populations of pre-autonomic neurons.

Oxytocin is potently anorectic in rats [2–4]. It has often been assumed that this is reflected in the activity of centrally-projecting parvocellular oxytocin neurons while magnocellular neurons were assumed to be solely involved in regulating peripheral actions of oxytocin. That assumption was challenged first by the recognition that large amounts of oxytocin are released from the dendrites of magnocellular neurons [6–8]. That gave rise to the notion that oxytocin might act as a 'neurohormone', acting not at specific synapses as conventional neurotransmitters do, but at extrasynaptic receptors after bulk-flow of oxytocin following co-ordinated dendritic secretion. This seemed to fit with observations of dense expression of oxytocin receptors at sites that had apparently little if any innervation by oxytocin-containing fibres. Moreover, dendritic secretion can be regulated independently of electrical activity and axonal secretion, by peptides that act to mobilise calcium stores in the dendritic endoplasmic reticulum [7,8]. This opened up the possibility that magnocellular neurons might respond differentially to afferent inputs by releasing oxytocin either centrally from their dendrites, or peripherally from the axon terminals in the pituitary.

A second challenge came with the recognition that many magnocellular oxytocin neurons project not only to the posterior pituitary but also to diverse forebrain regions, including the amygdala and the nucleus accumbens [9]. Finally, came the recognition that, while the rat hypothalamus contains about 8000 oxytocin neurons, the

overwhelming majority of these project outside the blood-brain barrier (presumably to the posterior pituitary), as shown by their retrograde uptake of fluorogold injected intravenously. Only about 3% of all oxytocin neurons project *only* within the brain, and at least some of these seem to be involved in co-ordinating the activity of diversely located groups of magnocellular oxytocin neurons [10].

The apparently small population of parvocellular oxytocin neurons project densely to the caudal brainstem, where they regulate meal size through control of gastric reflexes [4]. However, oxytocin also has motivational effects on appetite, and these seem likely to be attributable to the forebrain projections of magnocellular oxytocin neurons, and/or to intrahypothalamic projections and dendritic oxytocin release. Certainly magnocellular neurons in both the PVN and SON are very strongly activated by food intake in rats [11], and are regulated by diverse appetite regulating factors released from the gut and peripheral fat stores, including leptin [12,13], secretin [14] and insulin [15•].

In rats, the active involvement of magnocellular neurons in feeding was clearly indicated by studies of the temporal profile of c-Fos expression during scheduled feeding [11]. In response to scheduled delivery of food, there was prompt and intense activation not only of the arcuate nucleus and the NTS, which relays signals from the gut sensed by the afferent vagus, but also of the magnocellular system — most unambiguously evident in the intense activation of the SON, which contains only magnocellular oxytocin and vasopressin neurons: the activation involved both populations. Importantly, in rats killed 30 min after the expected time of arrival of food but in which food was not delivered, there was strong activation in the arcuate nucleus, but no activation of either the NTS or of the magnocellular system — and no activation of the anorexigenic POMC neurons [11]. Thus it seemed that the orexigenic pathways of the arcuate nucleus are acutely activated by the imminent expectation of food even in the absence of any sensory cues, while anorexigenic pathways, including the POMC neurons, the NTS and the oxytocin system, are promptly activated by signals associated with food ingestion.

AgRP and POMC neurons target hypothalamic oxytocin neurons to regulate feeding

The arcuate nucleus contains a multitude of neuronal populations, and is a key site at which peripherally secreted hormones that control appetite, including leptin, peptide YY (PYY) and ghrelin, can influence activity in the brain. Amongst these, AgRP neurons and POMC neurons have particularly important roles [16]. In awake, behaving mice, the activity of these neurons is dominated by cues that signal food availability [17–19] — orexigenic AgRP neurons are activated by the imminent expectation of food while anorexigenic POMC neurons are inhibited by the same cues. During feeding, signals from the gut suppress the activity of AgRP neurons, and this inhibition involves the effects of two gut hormones, cholecystokinin (CCK) and PYY, and serotonin pathways in the brain [20].

Both AgRP and POMC populations are very heterogeneous [21,22] but most AgRP neurons co-express another orexigenic peptide, neuropeptide Y (NPY), and use GABA as an inhibitory neurotransmitter; all three of these secreted products are involved in the powerful ability of AgRP neurons to stimulate feeding behaviour. The POMC neurons are thought to mediate satiety mainly by release of alpha melanocyte stimulating hormone (α-MSH), acting at melanocortin 4 receptors (MC4R) — receptors at which AgRP is an inverse agonist, but they also seem to use glutamate and acetylcholine as conventional neurotransmitters and express other co-existing peptides [23,24].

Axons of these two populations of arcuate neurons project densely towards the PVN (dorsally) [25,26•,27] and towards the SON (rostrally) [27], in both cases terminating on or near oxytocin neurons. In the PVN, α-MSH, and AgRP act at MC4R [28] to inhibit or stimulate feeding behaviours respectively in both mice and rats [25,29,30]. MC4R signalling in the PVN is critical in the regulation of energy balance, as demonstrated by the generation of obesity-like phenotypes in transgenic mice with viral disruption of PVN MC4R expression [31].

In mice, optogenetic activation of AgRP neurons inhibits PVN oxytocin neurons and this action is necessary for activation of AgRP neurons to evoke feeding [25]. This effect can be blocked by the intra-PVN administration of a $GABA_A$ or a NPY Y1 receptor antagonist [25]; the effect that AgRP has on oxytocin neurons remains unknown. In rats, Y1 receptors are expressed on oxytocin neurons in the SON [32]; but Y1 agonists stimulate oxytocin release from explants of the neurohypophysial system, while NPY itself has little effect, possibly reflecting disparate actions at multiple receptor types [33].

In different neurons expressing the MC4R, melanocortin ligand binding can trigger distinct signalling pathways by recruiting different G proteins [34]. Specific PVN G_q knockout mice develop diet-induced obesity and are resistant to melanocortin agonist induced feeding inhibition [35]. In rats, α-MSH increases intracellular calcium concentrations in SON oxytocin neurons and this triggers activity-independent dendritic oxytocin secretion [36]. As the calcium-mobilising effect is seen in isolated oxytocin neurons and is blocked by specific MC4R antagonists, this is a direct action via MC4R on the oxytocin neurons. However, α-MSH administered directly to the SON *in vivo* by retrodialysis *inhibits* the electrical activity of

oxytocin neurons. This is a consequence of the mobilisation of intracellular calcium stores by α-MSH: this stimulates release of endocannabinoids from the oxytocin neurons, which act at CB1 receptors on the presynaptic endings of glutamatergic afferents to suppress these excitatory inputs [37]. By contrast, systemically applied MC4R agonists activate oxytocin neurons in the rat SON [38], presumably through activating other afferent pathways.

MC4R mRNA is densely expressed in both the SON and PVN of rats [39] and MC4R have been immunohistochemically quantified in the PVN of mice [40,41]. There are conflicting reports of whether MC4R are expressed in the mouse SON [40,42], but in mice, the SON comprises mainly vasopressin neurons. Studies using transgenic reporter expressing mice and immunohistochemistry suggest that, in mice, only some PVN oxytocin neurons express the MC4R [31,42]. In humans, oxytocin neurons in both the SON and PVN express MC4Rs [43].

In both rats and mice, SON and PVN oxytocin neurons appear to project to the arcuate nucleus [26•,44]. In mice, POMC neurons express the oxytocin receptor [26•] and arcuate dopamine neurons (which communicate with neighbouring AgRP and POMC neurons [45]), are sensitive to oxytocin *in vitro* [46]. In rats, oxytocin administration into the arcuate nucleus reduces food intake [44] on similar timescales to the effects of optogenetic stimulation of POMC neurons in mice [29], suggesting that oxytocin acts at the arcuate nucleus to potentiate anorexigenic signalling.

NTS neurons target hypothalamic oxytocin neurons to suppress feeding

The magnocellular oxytocin system is densely innervated by neurons of the NTS, a hindbrain region that relays signals from the gastrointestinal tract related to food intake and peripheral energy status [16]. Various NTS neuronal populations have been characterised, including noradrenaline neurons and neurons that express a diversity of peptides in both mice and rats [47–51]. In rats, noradrenergic NTS neurons innervate SON oxytocin neurons [49]. Peripheral administration of the CCK activates these NTS neurons [52], and this pathway mediates the excitatory effects of peripheral administration of CCK on oxytocin release [49,53].

The NTS also contains several other neuropeptides, including CCK in mice [47,50] and glucagon like peptide 1 (GLP-1) in both mice and rats [48,51]. In mice, CCK neurons in the NTS project to the PVN and optogenetic activation of their axon terminals within the PVN supresses feeding [47]. In rats, subsets of NTS GLP-1 and noradrenergic neurons also project to the PVN [48,54•], and prolactin-releasing peptide neurons of the NTS have been implicated in the activation of oxytocin neurons in response to food intake [55]. Intra-PVN administration of a GLP-1 receptor antagonist or lesioning noradrenergic projections to the PVN increases food intake in rats [48,54•], suggesting that these projections suppress food intake, consistent with evidence that these are excitatory to oxytocin neurons. A subpopulation of PVN oxytocin neurons respond to GLP-1 with increased intracellular calcium concentrations, and intra-PVN administration of a GLP-1 receptor antagonist increases food intake [48].

Conversely, in rats, parvocellular oxytocin neurons in the PVN project to the NTS [56,57]. Oxytocin release at this site is involved in regulating gastric emptying and meal size [3]. NTS neurons densely express the oxytocin receptor [58], and specific knockdown of NTS oxytocin receptors increases food intake in rats [59•], while chronic administration of oxytocin into the fourth ventricle induces weight loss in diet-induced obese rats through reduced food intake and increased energy expenditure [60].

Hypothalamic oxytocin neurons mediate macronutrient-specific satiety

In rats, both the consumption and intragastric delivery of a high-sugar food increases the activity of hypothalamic oxytocin neurons [61••]. Short-term sugar consumption also increases hypothalamic oxytocin mRNA expression in mice [62]. By contrast, oxytocin neurons are inhibited by the intragastric delivery of a high-fat food [61••]. Consistent with this, following the consumption of a fat-containing solution, mice have lower expression of c-Fos in PVN oxytocin neurons than those consuming a sucrose-containing solution, and administration of a oxytocin receptor antagonist increases preference for sucrose solutions [63]. Intragastric delivery of dietary amino acids also increases c-Fos expression in SON and PVN oxytocin neurons, increases hypothalamic oxytocin mRNA expression, and decreases food intake in mice — an effect that can be attenuated by the administration of an oxytocin receptor antagonist [64••,65].

These studies indicate that, whereas the suppression of AgRP neuron activity with food ingestion in mice appears to be proportional to caloric load rather than being macronutrient-specific [20], magnocellular oxytocin neurons are differentially regulated by the ingestion of different macronutrients. The mechanisms underlying this differential regulation are unknown. Recently it has been shown that sugar activates enteroendocrine cells of the gut of mice that directly synapse with vagal neurons [66]. In addition, mouse NTS CCK neurons are activated following the ingestion of sugar or amino acids [47]. Therefore, the excitatory response of oxytocin neurons to sugar or amino acids could be mediated by gut-brain signalling via the NTS.

Magnocellular oxytocin neurons may also be regulated directly by glucose and/or insulin. In mice, insulin increases c-Fos expression and intracellular calcium concentrations in PVN oxytocin neurons and increases plasma oxytocin concentrations, an effect abolished by deletion of the insulin-signalling molecule PDK1 (phosphoinositide-dependent protein kinase-1) within oxytocin neurons [15•]. A study using rat hypothalamic explant preparations has shown that glucose itself can also increase intracellular calcium concentrations in SON neurons and induces oxytocin secretion, this effect is potentiated by insulin and dependent on glucokinase (enzyme that metabolises glucose) activity [67]. In rats, blood glucose and plasma insulin concentrations virtually mirror increases in oxytocin neuron activity with the intragastric infusion of a high-sugar food [61••], indicating that increased glucose and insulin levels may contribute towards the effects of sugar ingestion on the regulation of oxytocin neurons. The activity of mouse arcuate nucleus and NTS neurons are also influenced by glucose and insulin [68,69], suggesting that the regulation of oxytocin neurons by glucose and/or insulin could be partially mediated through inputs from these regions. In diet-induced obese rats, chronic oxytocin administration onto the fourth ventricle reduces body weight by a combination of reduced food intake and brown adipose tissue thermogenesis [70•], effects probably mediated by actions of oxytocin in the caudal brainstem.

That magnocellular oxytocin neurons are sensitive to macronutrient ingestion suggests that they are involved in mediating macronutrient-specific satiety. Disrupting oxytocin signalling through administration of an oxytocin antagonist increases the consumption and preference for sugar in mice [62]. The enzyme SIRT1 (NAD-dependent deacetylase sirtuin-1, implicated in energy homeostasis as a metabolic sensor) may be involved in oxytocin-induced macronutrient preference, as overexpression of SIRT1 in oxytocin neurons decreases sucrose preference and increases fat preference in mice [71••]. In line with this, specific ablation of PVN catecholaminergic fibers speeds up the progression of diet-induced obesity through the increased consumption of a high-sugar food in rats [54•].

Together these studies suggest that oxytocin signalling dampens the drive to specifically consume carbohydrates. Controversially, a recent study has shown that specific virus-based knock down of PVN oxytocin expression has no effect on feeding behaviours in mice [31]. In addition, reintroduction of MC4R into oxytocin neurons in transgenic knockout mice does not appear to reverse MC4R deficiency induced hyperphagia [72]. However, these studies looked at overall food intake and body weight, but did not investigate macronutrient-specific preferences.

Oxytocin action at the ventromedial nucleus of the hypothalamus (VMN) and reward system in macronutrient-specific satiety

Peripheral oxytocin administration has no effect on the consumption or preference for carbohydrate solutions in rats [73], therefore, it is hypothesised that central oxytocin secretion mediates oxytocin-induced macronutrient-specific preferences. Accordingly, many studies have investigated the effects of oxytocin directly administered into potential target regions on subsequent food intake and macronutrient preference.

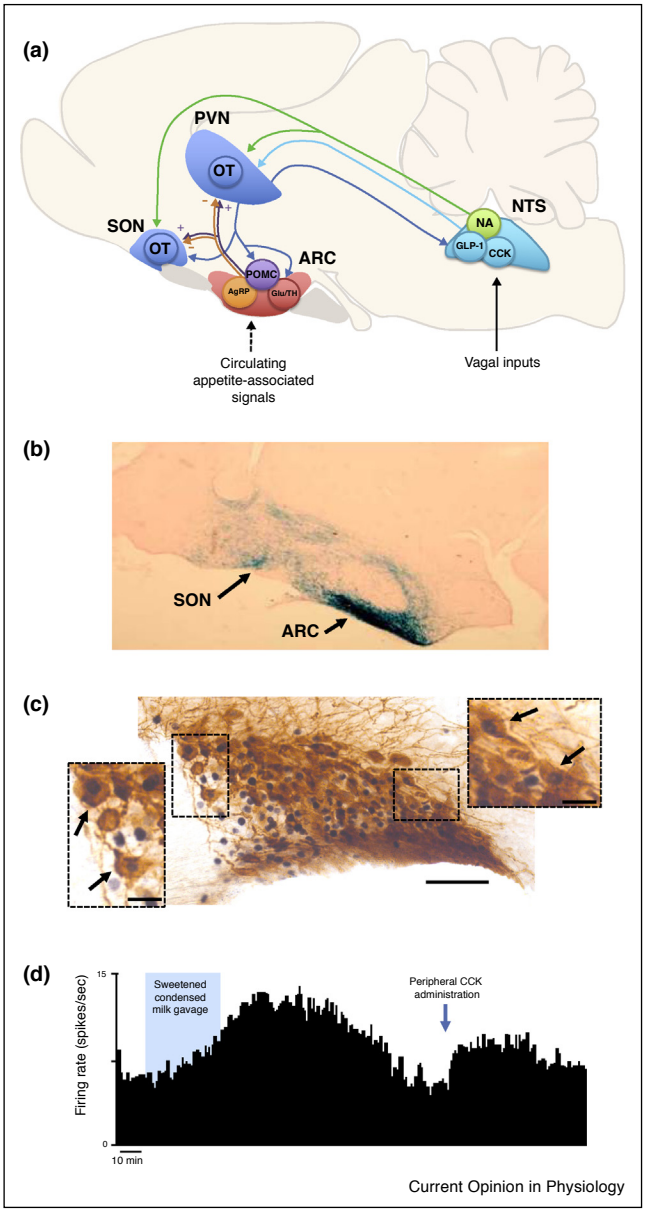
One potential target region is the VMN, a hypothalamic structure that densely expresses the oxytocin receptor in rats and mice [58]. Oxytocin infusion directly into the adjacent third ventricle influences the electrical activity of VMH neuron subpopulations in anaesthetised rats [3]. Furthermore, oxytocin administration into the VMN decreases feeding [74], but doesn't appear to influence preference between sweet carbohydrate (sucrose) and sweet low-carbohydrate (saccharin) solutions in rats [75••].

Oxytocin may also act at extra-hypothalamic regions to influence macronutrient preference and food motivation, including the reward system. In the ventral tegmental area (VTA) of mice, oxytocin receptors are expressed on dopaminergic and glutamatergic neurons that project to the nucleus accumbens (NAcc) [76••]. Intra-NAcc oxytocin administration increases c-Fos expression in NAcc neurons and decreases feeding in rats [77]. Furthermore, oxytocin administration into either the VTA or NAcc decreases sugar consumption in rats [77,78]. In mice, VTA neurons that express oxytocin receptors also project to the amygdala [76••]. In rats, oxytocin administration into the basolateral nucleus of the amygdala decreases overall feeding and sugar consumption [79••].

Conclusions and future directions

The oxytocin system integrates peripheral food-associated information, either directly conveyed by the arcuate nucleus and NTS and/or by other signalling mechanisms involving glucose and insulin (Figure 1), and communicates with other circuits, including those involved in motivation, to influence preference and subsequent food consumption in a macronutrient-specific manner. However, our understanding of this system remains incomplete. Specifically, the gut-brain signalling mechanisms that regulate oxytocin neuron activity and oxytocin secretion remain unclear; in particular, the different contributions of dendritic oxytocin release and axonal release at different sites have yet to be fully dissected. Future investigation requires the use of *in vivo* pathway-specific manipulations and complex food preference paradigms to pick apart the mechanisms by which the oxytocin system mediates macronutrient-specific satiety.

Figure 1



Overview of the connectivity of hypothalamic oxytocin neurons and their regulation by food ingestion.
(a) Diagram highlighting the connectivity of the arcuate nucleus (ARC) and NTS with SON and PVN oxytocin neurons. Glu, glutamate; NA, noradrenaline; OT, oxytocin; TH, tyrosine hydroxylase. **(b)** POMC projections (black/blue) to the SON in sagittal mouse brain slices. Image supplied by Drs BG Challis and AP Coll, University of Cambridge from studies in [80]. **(c)** c-Fos expression (black) in SON oxytocin neurons (brown) in coronal rat brain slices following the intra-gastric delivery of sweetened condensed milk [61••]. Main scale bar, 50 μm. Inset scale bars, 20 μm. **(d)** The increased firing rate (spikes/s) of a single SON oxytocin neuron during intra-gastric delivery of sweetened condensed milk, followed by the peripheral administration of CCK *in vivo* in rats [61••].

Conflict of interest statement

Nothing declared.

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