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From sensory circumventricular organs to cerebral cortex: neural pathways controlling thirst and hunger.

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Abstract

Much progress has been made during the past thirty years elucidating neural and endocrine pathways by which bodily needs for water and energy are brought to conscious awareness through the generation of thirst and hunger. One way that circulating hormones influence thirst and hunger is by acting on neurons within sensory circumventricular organs (CVOs). This is possible because the subfornical organ and organum vasculosum of the lamina terminalis (OVLT), the sensory CVOs in the forebrain, and the area postrema in the hindbrain, lack a normal blood-brain barrier so that neurons within them are exposed to blood-borne agents. The neural signals generated by hormonal action in these sensory CVOs are relayed to several sites in the cerebral cortex to stimulate or inhibit thirst or hunger. The subfornical organ and OVLT respond to circulating angiotensin II, relaxin and hypertonicity to drive thirst-related neural pathways; whereas circulating amylin, leptin and possibly GLP-1, act at the area postrema to influence neural pathways inhibiting food intake.

As a result of investigations using functional brain imaging techniques, the insula and anterior cingulate cortex, as well as several other cortical sites, have been implicated in the conscious perception of thirst and hunger in humans. Viral tracing techniques show that the anterior cingulate cortex and insula receive neural inputs from thirst related neurons in the subfornical organ and OVLT, hunger-related neurons in the area postrema have polysynaptic efferent connections to these cortical regions. For thirst, the median preoptic nucleus initially, and thereafter the thalamic paraventricular nucleus and lateral hypothalamus have been identified as likely sites of synaptic links in pathways from subfornical organ and OVLT to the cortex. The challenge remains to identify the links in the neural pathways that relay signals originating in sensory CVOs to cortical sites subserving either thirst or hunger.

Introduction

Thirst and hunger are basic homeostatic emotions (i.e. subjective states subserving water and energy homeostasis) that drive the intake of nutrients necessary for survival. Unlike many autonomic and endocrine mechanisms that are also essential for survival, these homeostatic emotions generate a conscious perception of a particular bodily need and provide the motivation

to repair the specific deficit. During the 30 years since the inception of the Journal of Neuroendocrinology, significant progress has been made in elucidating the neural and endocrine pathways by which bodily deficits in water and energy content are brought to the level of conscious awareness. Much of this progress has come about because of an explosion of new techniques that were developed during these years thereby enabling investigators to delve into areas of enquiry that had hitherto seemed impenetrable. In the 30 years prior to 1988, we had relied on tried and true physiological, pharmacological and behavioural methods of enquiry that included lesions, microelectrode recordings, intracerebral micro-injections, radioimmunoassay, immunohistochemistry, in situ hybridization histochemistry, receptor autoradiography, neuroanatomical pathway tracing and combinations of the above. However, in the years subsequent to 1988 we have been gifted many new techniques to add to the methodological arsenal. These techniques include c-Fos labelling, functional brain imaging, calcium imaging, patch clamp recording, pathway tracing with neurotropic viruses, gene knockouts, knock-ins and conditional knockouts, optogenetic and chemogenetic techniques, and single-cell PCR. This review focuses on our increased understanding of how hormonal and other circulating factors, acting on the sensory CVOs, generate the conscious sensation of thirst and influence hunger.

Initially, we will consider the factors in the circulation, both hormonal and osmotic, that act on neurons within sensory CVOs to stimulate or inhibit thirst and hunger. Second, the cortical regions, identified from imaging studies, that may subserve the conscious perception of thirst and hunger will be addressed. Finally, neural circuitry will be proposed by which thirst- and hunger-related signals, generated initially in sensory CVOs, might reach appropriate sites in the cerebral cortex to participate in the generation or inhibition of these homeostatic emotions.

The sensory circumventricular organs

There are three sensory circumventricular organs in the mammalian brain, the subfornical organ, OVLT and area postrema. They are small protuberances situated at strategic sites along the midline wall of the cerebral ventricles. The subfornical organ and OVLT are located in the anterior wall of the third ventricle; the former at the junction of the two interventricular foramina, while the latter is more ventrally located, sitting immediately dorsal to the optic chiasm. The area postrema in the medulla oblongata is located in the wall of the fourth ventricle at the level of the entrance of the central canal (1). Unlike either the secretory circumventricular organs (the median eminence and pineal gland), or ependymal circumventricular organs (the

subcommissural organ and choroid plexus), the sensory CVOs contain neuronal cell bodies that receive afferent neural signals from, and send efferent projections to, other brain regions (1). The circumventricular organs are among the most highly vascularized structures in the brain. The property that distinguishes the vasculature of circumventricular organs (with the exception of the subcommissural organ) from other parts of the brain is the lack of a normal blood brain barrier due mainly to the presence of fenestrations in the endothelial cells of capillaries in CVOs; as well there are fewer tight junctions between these cells (1, 2). This property enables circulating hydrophilic molecules such as ions, amino acids, monamine transmitters and larger molecular weight peptides and proteins to pass rapidly from the vasculature into the interstitium of CVOs. However, tight junctions between ependymal cells and tanycytes prevents passage of such molecules within circumventricular organs to adjacent brain tissue or cerebrospinal fluid, thereby preserving the integrity of the blood-brain barrier in brain regions adjacent to the CVOs (2). Thus, the three sensory CVOs that house neuronal cell bodies and their dendrites, are the only sites in the brain where neural activity can be directly and rapidly influenced by changes in the ionic and humoral milieu of the circulating blood. Efferent neural pathways from the sensory CVOs are then able to transmit the altered neural activity to effector regions in many other cerebral regions.

The sensory CVOs are not uniform structures; all three have subdivisions within them based on morphology, neurochemistry and neural connectivity (1, 3-5). As well, the altered blood-brain barrier within the sensory CVOs is not uniform in nature; while fenestrated capillary endothelium is found throughout the sensory CVOs, capillary tight junctions are absent in core regions but plentiful in more peripheral parts of these CVOs (3). Thus, studies in mice show penetration of circulating markers such as fluorescein isothiocyanate, or higher molecular weight bovine serum albumin or dextrans permeated core regions more rapidly than peripheral subdivisions of sensory CVOs (2, 3).

Thirst

Neural pathways generating thirst may originate in forebrain CVOs

Angiotensin-induced thirst

Following the demonstration by James Fitzsimons and others that angiotensin II (Ang II) was a dipsogenic hormone that mediated (in part) water drinking associated with hypovolemia (6), John Simpson and his colleagues in the 1970s identified the subfornical organ as the site of action at which circulating Ang II stimulated thirst in rats (7, 8). One of the clues that led them to identify a role for the subfornical organ in thirst was the fact that blood-borne peptides like Ang II normally do not cross the blood brain barrier in any appreciable quantity, so the question arose as to how Ang II could stimulate neurons within the brain to cause thirst. The sensory CVOs, lack a normal blood brain barrier, so neurons within them are exposed to circulating hormones such as Ang II, making them likely targets of Ang II action (1). Ang II AT_1 receptors are expressed strongly in the subfornical organ and OVLT of rodents (9, 10) and other species including humans (11), and circulating Ang II has an excitatory action on neurons within the subfornical organ (12-14). Evidence from studies in rats convincingly shows that the subfornical organ is the main site of the dipsogenic action of circulating Ang II. This evidence includes observations that (i) microinjection of fentamole amounts of Ang II into the subfornical organ stimulates drinking (7), (ii) ablation of the subfornical organ abolishes drinking stimulated by systemic Ang II (7), (iii) injection of Ang II antagonists into the subfornical organ blocks systemic Ang II-induced drinking (7).

Relaxin-induced drinking

Relaxin is a peptide hormone that is released from the ovary into the circulation during pregnancy (15). Initially, it was shown that relaxin stimulated water drinking (and vasopressin secretion) when it was infused intracerebroventricularly in rats (16). The relaxin molecule is a larger molecule than Ang II, containing a total of 53 amino acid residues arranged in two chains connected by disulphide bridges. Since a polar molecule of this size is unlikely to pass across the blood brain barrier, we investigated whether systemically infused relaxin could induce water drinking. Intravenous infusion causes a dose dependent increase in water intake (17, 18), interestingly, in both female and male rats. While relaxin receptors have been identified in both the subfornical organ and OVLT (19), ablation of the subfornical organ, but not the OVLT, abolished the dipsogenic response to intravenously infused relaxin (18). Intravenously infused relaxin causes strong stimulation of many neurons in the periphery of the rat subfornical organ as shown by the expression of c-Fos (18). Furthermore, electrophysiological recordings from neurons in the periphery of rat subfornical organ slices showed that both relaxin and Ang II

stimulated the same neurons (18). These results strongly suggest that circulating relaxin acts on the subfornical organ as a dipsogenic hormone in a similar manner to Ang II. Water intake in rats and mice is maintained during the course of pregnancy in spite of reduced plasma osmolality (15, 20). It is likely that circulating relaxin and Ang II, acting in combination at sensory CVOs, play a role in maintain water intake during pregnancy (15, 17).

Osmoregulatory thirst

It has long been held that hypertonicity of body fluids is a major physiological stimulus of thirst and vasopressin secretion, and that cerebral osmoreceptors detect such hypertonicity (21-25). The first clue that sensory CVOs may be the site of such osmoreceptors came from observations of differential drinking responses in sheep to hyperosmolar saline or sucrose versus hyperosmolar urea, despite evidence that all three solutions dehydrated the brain behind the blood brain barrier (26). These data suggested that osmoreceptors were located in a brain region(s) lacking a blood brain barrier; specifically in the OVLT and subfornical organ (26). Consistent with this notion was evidence that ablation of the OVLT severely impaired water drinking in response to acute systemic infusion of hypertonic saline in sheep and dogs (27, 28). While ablation of the subfornical organ did not impair drinking responses to systemic infusion of hypertonic saline in rats and sheep (7, 29), if it was ablated together with the OVLT in sheep, drinking to this stimulus was impaired in comparison to animals in which the OVLT alone was ablated (29). Subsequently, many studies in rats and mice, using either c-Fos labelling (30-32), electrophysiology (33-35) or calcium imaging (36) have shown that systemic hypertonicity and/or dehydration activates populations of neurons in both the subfornical organ and OVLT. These neurons express several genes that include neuronal nitric oxide synthase (nNOS), Ca/calmodulin-dependent protein kinase II (Cam-KII), the transcription factor ETS translocation variant-1 (ETV-1) and the angiotensin AT_{1a} receptor. In addition, they are excitatory glutamatergic neurons because they express the glutamate transporter Vglut2 (36-39). Furthermore, recent experiments in mice show that optogenetic stimulation of osmoresponsive neurons in the subfornical organ or OVLT (shown by their expression of c-Fos in response to hypertonicity or dehydration), immediately begin to drink water (37). Thus, subfornical organ and OVLT neurons expressing nNOS/Glut2, from hereon termed SFO^{Glut} and $OVLT^{Glut}$ respectively, appear to be the origin of neural signals that drive osmotic and hormonally mediated thirst.

Circadian thirst

Each day, rats and mice engage in bouts of water drinking in the hours immediately prior to their sleep period; this circadian drinking behavior is dependent on an intact suprachiasmatic nucleus (40), is not associated with any fluid deficit and is considered to be anticipatory so that dehydration due to future fluid loss during sleep is prevented (41). Recent investigations of this anticipatory water drinking have shown that it is initiated by suprachiasmatic vasopressin-expressing clock neurons that project to the OVLT. Optogenetic stimulation of this neural pathway in mice initiates drinking, and blocking its activation prior to the sleep period prevents circadian drinking (41). Specific pharmacological blockade of vasopressin V1a receptors with SR49059 blocked drinking induced by stimulation of the suprachiasmatic to OVLT pathway, and V1a receptor knockout mice do not exhibit anticipatory circadian drinking prior to sleep (41). These data show that while the suprachiasmatic nucleus initiates this behavior, a vasopressinergic synapse within the OVLT is an essential relay in the pathway mediating anticipatory water drinking; its downstream components from the OVLT remain to be determined.

Mapping cortical sites that are activated in thirsty subjects

Intuition suggests that conscious feelings such as hunger and thirst should involve parts of the cerebral cortex. While earlier studies of patients with cortical damage resulting from brain injuries, or electrical stimulation of various sites within the human cerebral cortex, were able to assign a number of physiological functions (e.g. somatosensory, taste and motor function) to specific cortical regions, little information on thirst was forthcoming (42). However, a wide-ranging mapping study of the cerebral cortex of rhesus monkeys, using localized electrical stimulation, did show that stimulus-bound drinking of water could be obtained consistently from stimulation of the anterior cingulate cortex (43). Following the monumental discovery that changes in brain activity in conscious human subjects could be detected by positron emission topography (PET) (44) or magnetic resonance imaging (45), it became evident that the regions of the cerebral cortex that played a role in subjective interoceptive sensations such as hunger and thirst might be identified by using such technology. Crucially, human brain imaging of thirst

has an advantage over studies in laboratory animals because the subjective intensity of thirst can be reported during experiments by human subjects and correlated with changes in regional blood flow in the brain.

PET was utilized for the first functional brain imaging of thirsty humans. These subjects were infused intravenously with 0.5 mol/l hypertonic saline to stimulate thirst, their mouths were rinsed with water to remove the effect of dry mouth and they were also allowed to drink water to satiation. At each of those steps, PET images were obtained. Several cortical regions showed activation if the subjects were thirsty without a dry mouth e.g cingulate cortex (anterior, mid and posterior divisions), insula, claustrum, temporal lobe and parahippocampal region (46). Later investigations, using functional magnetic resonance imaging which provides greater spatial and temporal resolution than PET, confirmed that the anterior and medial cingulate cortices, insula, and parahippocampal regions were consistently activated bilaterally in thirsty human subjects (Fig. 1), along with sites in the frontal, temporal and parietal lobes (47). Within minutes, many of these sites were no longer activated when thirst was satiated by drinking (Fig. 1). The lamina terminalis, which contains both subfornical organ and OVLT, was also activated in some subjects (47). Using the OVLT as a seed for examining functional connectivity, activities in the anterior cingulate cortex and insula of thirsty subjects were significantly correlated to that in the lamina terminalis (48). While a number of cortical sites were implicated by the aforementioned studies to be involved in the generation of thirst, two regions were stand-outs. These were the anterior cingulate cortex and the posterior insula; these two regions were consistently identified in these studies (46-49).

Thirst-related neural pathways connecting the subfornical organ and OVLT to the cingulate or insular cortex

A synaptic relay in the median preoptic nucleus

Efferent neural pathways emanating from both the subfornical organ and OVLT were initially mapped using conventional neuroanatomical tracing techniques in rats (50, 51). Several different efferent targets were revealed (e.g. supraoptic and paraventricular nuclei in the hypothalamus, lateral hypothalamic area, thalamic paraventricular nucleus (PVT), and median preoptic nucleus (MnPO)). Which of these efferent pathways shown to project from the subfornical organ or OVLT was responsible for transmitting thirst-related signals could not be

determined from these tracing studies, however, a relay via the MnPO seemed likely. Evidence in rats supporting this contention is (i) ablation of the MnPO disrupts water intake in response to Ang II or osmotic stimulation (52), (ii) so too does destruction of neurons within the MnPO (but not fibres of passage) using microinjection of ibotenic acid into the MnPO (53), (iii) neurons within the MnPO are activated by dehydration, systemic hypertonicity and Ang II, all dipsogenic stimuli (13, 30-32, 54), and (iv) direct optogenetic stimulation of glutamatergic neurons within the MnPO ($MnPO^{Glut}$) stimulates water drinking in mice (55, 56).

Recent investigations using combinations of optogenetic and viral tracing methods in mice have established unequivocally that water drinking is driven by glutamatergic pathways projecting from neurons within both subfornical organ and OVLT to glutamatergic neurons in MnPO. If SFO^{Glut} neurons that project directly to neurons in the MnPO are engineered to express Channel-rhodopsin, when the Channel-rhodopsin within their terminals in the MnPO is stimulated by light, drinking occurs immediately (14, 38). As well, when such MnPO neurons that receive input from SFO^{Glut} neurons are genetically modified to express caspase3, thereby resulting in their ablation, water drinking caused by stimulation of SFO^{Glut} or glutamatergic neurons within OVLT ($OVLT^{Glut}$) is greatly reduced (56), as is drinking caused by dehydration (56). However, as long as these MnPO neurons are intact, ablation of SFO^{Glut}, either individually or in combination with $OVLT^{Glut}$ neurons, does not affect the drinking response to photo-stimulation of MnPO neurons (54). Furthermore, ablation of $OVLT^{Glut}$ neurons has little effect on subfornical organ-stimulated drinking; vice-versa, knocking out subfornical organ neurons does not block drinking induced by $OVLT^{Glut}$ stimulation (56). These data indicate that neural signals, arising from osmotic, angiotensin or relaxin stimulation of the subfornical organ and OVLT, are relayed onward through a glutamatergic synapse within the MnPO to other sites in the brain including cortical regions (Fig. 2). It should also be pointed out that many neurons within the MnPO are sodium sensitive (57) which may explain the profound effects that changes in cerebrospinal fluid Na^+ concentration have on thirst and water intake in sheep and goats (23, 26, 58, 59). It seems likely that the ambient Na concentration may influence MnPO neurons relaying dipsogenic neural signals that arise in CVOs in response to circulating tonicity and Ang II.

As well, notwithstanding the possibility of plasticity occurring within these circuits following damage, the recent data in mice are consistent with the suggestion that there is considerable redundancy of function between the subfornical organ and OVLT in generating thirst (29).

Connecting thirst-related pathways radiating from the lamina terminalis to the cerebral cortex

To determine whether the MnPO, subfornical organ and OVLT were polysynaptically connected to cortical sites such as the anterior cingulate cortex and insula (that had been implicated from imaging studies in the generation of thirst), we injected the transsynaptic, retrogradely transported pseudorabies virus, into these cortical regions. Three days after its injection into either the cingulate cortex or insula of rats, pseudorabies virus had been retrogradely transported from both sites back to neurons in the MnPO, as well as to the subfornical organ and the OVLT (60). These were not direct neural links, but polysynaptic connections because the monosynaptic retrograde tracer cholera toxin B injected into cingulate cortex or insula was not transported back to MnPO, subfornical organ or OVLT (60). However, cholera toxin B was retrogradely transported back from these cortical regions to the lateral hypothalamic area and its perifornical region, to a number of midline thalamic sites that included the PVT and mediodorsal thalamic nucleus, and to the periaqueductal gray, all sites that were also retrogradely labelled by pseudorabies virus after its injection into either the cingulate or insular cortex (60). Such data show the existence of neural chains linking subfornical organ and OVLT neurons with the cerebral cortex (Fig. 2). If the first link in the chain is via a synapse in the MnPO, what are the subsequent steps in the neural connections to the cortex?

Neurons within the PVT that project to the insula, as shown by cholera toxin B tracing in rats, express c-Fos in response to the dipsogenic stimulus of hypertonicity. So too, do MnPO neurons projecting to the PVT (58) - a result consistent with the PVT relaying thirst-related signals from MnPO to insula. However, the most telling evidence that PVT neurons relay thirst-related signals from MnPO to the cerebral cortex, is the finding in mice that they receive efferent nerve fibres from $MnPO^{Glut}$ neurons (55, 56); if terminals in the PVT coming from these fibres are photo-stimulated, immediate, copious drinking results (37, 56). Similarly, lateral hypothalamic and hypothalamic paraventricular neurons receive input terminals from $MnPO^{Glut}$ neurons, which when stimulated by light cause mice to drink (37, 56). As lateral hypothalamic neurons have efferent projections to both insula and cingulate cortex (60), they could also have a role in mediating thirst. These data show that there are at least two neural pathways from sensory CVOs to the cerebral cortex driving thirst.

Activation of multiple pathways to various cortical sites during the generation of thirst may be the mechanism by which different components of the sensation of thirst arise; indeed,

several other cortical sites e.g. parahippocampus, medial temporal lobe, located outside the insula and cingulate cortex, are activated in thirsty humans. Thirst is unpleasant. It has been characterized as having a negative valence; that is, it is a disagreeable sensation and becomes more and more distressing as its intensity increases so that a motivation to be rid of the condition is engendered (61). Avoidance of the place in a cage where photo-stimulation of the MnPO in mice drives thirst, suggests that thirst's negative valence is driven via neural circuitry arising in the lamina terminalis (37). In this regard, light-activation of the dipsogenic MnPO to lateral hypothalamic area pathway, but not the dipsogenic MnPO to PVT pathway, causes mice to lever-press to stop the photo-stimulation or avoid the place where it is triggered (38). These data are consistent with the idea that different neural paths projecting from the lamina terminalis may mediate the various subjective components of thirst.

Arousal, bringing about a conscious awareness of the disagreeable nature of thirst is another of its components; as well, there is also a vague somatosensory perception associated with the throat and upper esophagus. The cognitive appreciation that water is the object of desire, and memories of water sources that will facilitate drive reduction are also associated with this basic homeostatic emotion. Besides the lateral hypothalamic area and PVT, dipsogenic $MnPO^{Glut}$ neurons project to many subcortical sites in mice, such as the perifornical area, periaqueductal gray, locus ceruleus, lateral parabrachial nucleus, and medullary sites (55). Future research may reveal if some or any of these pathways are also involved in relaying the various components that may be amalgamated to produce the sensation of thirst.

Inhibitory signals from sensory CVOs mediating the satiation of thirst

When dehydrated animals are given access to water, they accurately calibrate the amount they ingest to match physiological need. Intriguingly, the signal to stop drinking occurs tens of minutes in advance of ingested water reaching the circulation, suggesting that there are 'preabsorptive' inputs that arise from the oropharynx, oesophagus and/or upper gastrointestinal tract which anticipate the amount of fluid required to restore fluid homeostasis (62, 63). These signals are likely to be carried via the vagal (X) nerve to the nucleus of the solitary tract (NTS) and lateral parabrachial nucleus, including oxytocin receptor-expressing neurons in the lateral parabrachial nucleus which project to the OVLT and MnPO (64).

Developments in calcium imaging have enabled the visualization of real-time neuronal activity firing patterns within neuronal subgroups of the lamina terminalis during behavioural tasks. These studies have revealed that activity in thirst-promoting neurons in the subfornical organ, i.e. SFO^{Glut}, and MnPO declines the moment thirsty mice start licking water. This declining activity continues over several minutes until the water deficit has been repaired, at which time it reaches a basal level that is maintained until another thirst stimulus arises (14, 37). Using lever presses for water to prolong the time spent ingesting fluid (37), Allen et al. elegantly demonstrated that the activity in $MnPO^{Glut}$ neurons reached a minimum when the rate of licking and lever pressing by mice ceased, suggesting that decreasing calcium activity in thirst-responsive CVO neurons corresponds to satiation of thirst.

Recent studies in mice reveal that glucagon–like peptide-1 (GLP-1) receptor-expressing neurons in the MnPO, that are probably GABAergic, inhibit the thirst promoting glutamatergic neurons in the subfornical organ (56), suggesting that peripheral signals for quenching thirst reach the subfornical organ indirectly via brainstem nuclei and the MnPO. Although the precise phenotype of the relevant neuronal populations involved in inhibiting thirst within each brain region remains to be identified, GABAergic neuronal populations within the subfornical organ, as well as those in the MnPO, have been shown to decrease water consumption when activated (36, 55). In addition to their projections to the subfornical organ, GABAergic neurons within the MnPO also project to other sites that have been implicated in driving thirst such as the PVT and lateral hypothalamic area (55). These data suggest that a complex interplay between excitatory and inhibitory neurons within the lamina terminalis ultimately determines the cortically directed output signals coming from the lamina terminalis to signal satiety of thirst.

Although the neuronal pathways to the cortex involved in fluid satiation are currently unknown, PET and functional magnetic resonance imaging in human subjects demonstrate that activity in the mid-cingulate gyrus, which increases during thirst, disappears below an arbitrary threshold within three minutes after drinking to satiation (46, 48); by contrast there is increased activity in the mid-cingulate area in Brodmann's area 24 (BA24), the periaqueductal gray and the pons by 14 min after drinking to satiation (48). Whether the cortical signals that correlate with satiety of thirst are relayed from sensory CVOs via thalamic nuclei is not known but likely will be the subject of future investigations.

Hunger

Hunger, like thirst, relies on inputs from the periphery to shift the motivational state required to bring energy stores into balance. In the case of hunger, signals to the brain that reflect metabolic status may be neural or blood borne and lead to coordinated responses involving CNS circuits to seek and ingest food. The humoral route, in particular, might be expected to engage the ready access to the brain parenchyma offered by the sensory CVOs in a similar fashion to that employed in the coordinated thirst response to hormonal and osmolality changes in the blood. In fact, this notion has been proposed and tested over recent decades (65). The following provides an evaluation of the evidence that CVOs, specifically the sensory CVOs, are involved in the hunger, feeding and satiety responses to the changing metabolic milieu. Of the sensory CVOs, there has been a focus in the forebrain on the subfornical organ and in the hindbrain on the area postrema with regard to the mediation of energy homeostasis.

Subfornical Organ

As described in the section above on thirst, there are many studies involving the ablation of the subfornical organ which result in profound effects on fluid balance; however, none of these report lasting and appreciable effects on food intake. Despite this fact, there is a persistent reference to the importance of the subfornical organ in metabolic control (65, 66). This is based largely on the ready access of circulating indicators of metabolic status to this region, the presence of receptors to key metabolic hormones, and the nature of neuronal projections to other brain areas involved in the generation of hunger and food intake (66-68). Of those circulating factors, the two that are most prominent are amylin and ghrelin (Figure 3).

Amylin action in the SFO

Amylin is a peptide hormone, co-secreted with insulin from the pancreas (69, 70), which has been shown to have anorectic effects when introduced peripherally or directly into the CNS (71-73). While there is evidence for facilitated transport of the pancreatic-ß cell-derived peptide across the blood brain barrier (74), the possibility exists that it exerts its centrally-mediated anorectic effects via the CVOs. Binding sites for amylin are present in the subfornical organ and area postrema (75). Moreover, peripherally-introduced amylin results in increased c-Fos expression in the subfornical organ which is co-located with mRNA coding for the functional RAMP receptors for amylin (76). Amylin has also been shown to depolarize neurons in the subfornical organ (66). In terms of the effector neurons that may drive the hunger or feeding response to the actions of metabolic marker hormones such as amylin in this CVO, the point

has been made that the subfornical organ is “well connected” to hypothalamic feeding related nuclei (65) and these may at least partially mediate the satiety-promoting effects of amylin.

Ghrelin action in the SFO

Ghrelin is an orexigenic peptide derived predominantly from the stomach and is the endogenous ligand for the growth hormone secretagogue receptor (GHSR) (77). Its signaling from the gut to the brain is at least partially mediated via gastric vagal afferents (78), but also involves an action of the circulating hormone on the CNS. This is widely acknowledged to occur in the mediobasal hypothalamus, specifically on GHSRs in the arcuate nucleus (79). The focus on the arcuate nucleus does not rule out actions of ghrelin elsewhere in the CNS and, like amylin, the presence of its cognate receptor in the subfornical organ may provide insight into potential actions in the CVOs (66). In fact, at least one report indicates that intravenously-administered ghrelin increases neural activity in the subfornical organ (80). In this vein, ghrelin has been shown to increase calcium fluxes in dissociated rat subfornical organ neurons, an effect abolished by co-administration of a GHSR antagonist (66). Depolarisation of subfornical organ neurons by ghrelin in these studies was concentration dependent and occurred over a range of concentrations that overlapped those detected in rats extending from fasted to sated conditions (81). Significantly, those subfornical neurons that were depolarised by ghrelin were always different to those depolarized by amylin (see above), consistent with the notion that there are two distinct populations of subfornical neurons that are activated by the gut derived peptides, ghrelin and amylin, that have opposing action on food intake. If these were to ultimately impact food intake, such populations would presumably project to different eating-promoting and -inhibiting relays elsewhere in the CNS.

The functional evidence for a role of the subfornical organ in mediating hunger and feeding

Despite the demonstrable actions of metabolic hormones in the subfornical organ described above, the evidence that these translate into effects on hunger and feeding behavior is limited at best. To our knowledge, there are no loss of function experiments that impact food intake either in traditional lesion/pharmacological approaches involving the subfornical organ or more current mutagenesis, knock down, chemogenetic or optogenetic experiments targeting specific elements within the subfornical organ, or pathways emanating from it, that effect metabolic parameters. Each of these approaches has featured prominently in the recognition of

neighboring hypothalamic regions as important metabolic hubs (see (82) for review). In fact, at the most basic level, ablation of the subfornical organ in rats does not result in any significant and consistent impact on food intact and body weight (83). The simplest explanation for this result is that the subfornical organ does not mediate, to any appreciable extent, an effect of circulating metabolic hormones on hunger and feeding, despite the presence of cognate receptors and neuronal activation in the subfornical organ following peripheral infusion of such hormones. The alternative, which is consistent with data showing *activation* of distinct populations of subfornical organ neurons with either the orexigenic agent ghrelin, or the anorectic peptide amylin, is that there is no net effect on food intake of ablation of the subfornical organ. Considering the weight of evidence however it seems likely that the most parsimonious explanation is that the subfornical organ is not a key regulator of metabolic function.

Area postrema

Unlike the subfornical organ there is ample evidence, based on lesion studies, to support a role for the area postrema in the control of feeding and body weight. Some of the early work in this area by Ritter and colleagues (84) showed that ablation of the area postrema and the subjacent NTS resulted in increased ingestion, particularly of palatable food – an effect that was not abolished by subdiaphragmatic vagotomy. The latter result obviates a role for vagal sensory input leaving open the possibility that this enhanced feeding response was mediated by circulating factors accessing the area postrema. There are a number of potential blood-borne candidates that could elicit this effect via the area postrema. Foremost amongst these are amylin, leptin and GLP-1 (Figure 3).

Amylin action in the AP

Circulating levels of the peptide hormone amylin are increased robustly with short latency after meals in both humans (70) and experimental animals (69). As well, physiologically-appropriate levels of exogenous amylin inhibit eating (85) while conversely, amylin antagonists increase food intake and meal size (86). Considered together, these observations have led to this pancreatic beta cell derived hormone being entrenched as a satiation signal (87). A

manifestation of this regulatory role is the observation that sustained peripheral administration of amylin leads to a reduction in food intake through a decrease in average meal size; as a result, a reduction in body weight occurred (88).

Early studies using c-Fos as a marker of neural activation showed that peripheral administration of amylin activated several regions of the brain that included the area postrema, but also the subjacent NTS, the lateral parabrachial nucleus, central nucleus of the amygdala and lateral aspects of the bed nucleus of the stria terminalis (89-91). The primacy of the area postrema in this centrally coordinated response was illustrated by attenuation of the activations in each of the other brain areas above after area postrema lesions (89, 92). It is clear however, that while the area postrema is sufficient to mediate the actions of amylin, it is not, in itself, necessary. Amylin receptors are widespread in the CNS (75, 93) and, as noted above, amylin readily crosses the blood brain barrier (94). In fact, there is good evidence that amylin receptors in the ventral tegmental area, an integral part of the mesolimbic reward pathway, are responsive to exogenous amylin and possibly important in the motivation to ingest highly palatable foods (95). Moreover, amylin of hypothalamic origin is likely to act on leptin receptor-expressing neurons in the lateral hypothalamus; these two anorectic peptides acting in concert to downregulate feeding in a way that could explain the efficacy of leptin (metreleptin)-amylin (pramlitide) combinations for the treatment of obesity (96).

Leptin actions in the AP

The identification of the adipocyte-derived hormone, leptin, nearly 25 years ago, has had an unparalleled impact on our understanding of the neurocircuitry of energy balance (97). The concentration of the long isoform of the leptin receptor (ObRb) in the arcuate nucleus of the hypothalamus, helped to explain the long established importance of the mediobasal hypothalamus as a satiation centre (see (98)). Only more recently has the arcuate-centric view of the importance of leptin been expanded by the identification of ObRb more widely in the CNS and the elucidation of its function in these extra-hypothalamic regions - one of these regions being the area postrema. A number of converging lines of evidence including the presence of ObRb protein (99), mRNA (100) and its downstream regulators of the ObRb (101), together with the established actions of leptin in this region on metabolic outcomes has shown the importance of leptin acting in the area postrema to influence appetite and energy expenditure (101). It should be appreciated that identification of elements of the leptin signaling pathway that

elucidate the presence of the ObRb receptor and leptin-mediated functions are generally common to both the area postrema and the underlying NTS; they are often considered as a unit, with similar functional and neuronal projections (see (102)). For example, the brain derived neurotrophic factor (BDNF) receptor TrkB, is highly expressed throughout these two regions, suggesting that these areas are jointly responsible for the anorexigenic effects of brainstem BDNF (103). Moreover, knockdown of ObRb using sh-RNA in the area postrema and commissural NTS causes hyperphagia and increased adiposity highlighting a role for endogenous CNS ObRb signaling in the control of energy balance (101). Importantly, this study also showed that knockdown of leptin receptor signaling in the area postrema-NTS axis dramatically decreased the sensitivity to the anorectic effect of peripheral CCK administration, consistent with an interaction of these two anorectic peptide hormones or their intracellular signaling mechanisms (101). This type of interaction is also evident in the synergy between leptin signaling and sensory (stretch) fibres terminating in the area postrema-NTS region where leptin is shown to potentiate the effects of gastric distension (104). In a similar vein there are reports of synergistic actions of amylin and leptin where acute central administration of leptin enhanced the food – inhibitory actions of peripherally – administered amylin (Osto M et al., 2007 Physiol Behav 91,566 – 572). Moreover, co-administration of amylin and subthreshold levels of leptin cooperatively induced an elevation of pSTAT3, a marker of leptin activation (Turek v et al., 2010, Endo 151, 143 -152). The latter effects were in the arcuate nucleus of the hypothalamus but the evidence for similar foci of leptin/amylin interactivity in the CVOs including the area postrema is less extensive. In this respect, while acute leptin administration did not augment amylin–induced neuronal activation in the area postrema, as indicated by cFos, *sustained* pretreatment with amylin in diet induced obese leptin resistant rats increased both basal and leptin-activated pSTAT within the area postrema (Roth JD et al., 2008 PNAS 105, 7257-7262). A substrate consistent with this interaction is shown to exist using single cell PCR and laser capture microscopy whereby approximately half of the neurons in the area postrema expressing amylin receptors also expressed the mRNA for the leptin receptor (Lepr-b) (Liberini CG Eur J Neurosci 43 653-. . Such reports are also consistent with the hyperphagia and subsequent weight gain following RNA interference of Lepr-b in the area postrema ((Hayes MR Cell Metab 11,77-83). The detail of the interaction between amylin and leptin, particularly in the area postrema under normal and high fat feeding remains to be elucidated.

Glucagon–like peptide-1 (GLP-1) actions in the Area Postrema

GLP-1 is released from intestinal L cells in response to nutrient loads and, first and foremost, results in insulin-mediated reductions in circulating glucose (see (105)). It is this effect that has seen GLP-1 agonists adopted as frontline treatments for type 2 diabetes and more recently, due to their anorexigenic properties, as anti-obesity pharmacotherapies (106). The latter effects may be mediated by local actions of GLP-1 on vagal afferent neurons within the intestinal mucosa (106, 107). Alternatively, higher levels of circulating GLP-1 or analogs with longer half-lives may saturate the available DPP-IV, an enzyme in the walls of capillaries that would normally degrade the peptide, and as such increase the availability of GLP-1 in the circulation to enable its actions directly in the CNS (108, 109) or at CVOs including the subfornical organ and area postrema (110, 111).

There is evidence that GLP-1 receptors are present at high density in the area postrema (112) and that peripheral infusions of GLP-1 analogs activate neurons in the area postrema (113) which project to sites implicated in the control of energy balance including the parabrachial nucleus and NTS (110). However, compelling data that would implicate the area postrema in GLP-1-mediated changes in food intake or appetite, outside of emesis (114), are scarce (115, 116). This leaves open the question as to whether GLP-1 in the area postrema exerts a specific satiating effect or impacts on food intake through causative action on nausea (117) or conditioned taste aversion (118).

What is clear is that GLP-1, whether it be infused peripherally or centrally, exerts an effect on food intake that is independent of supracollicular levels of the neuraxis (119). Moreover, GLP-1 receptor antagonism is only effective in blocking lipopolysaccharide-mediated anorexia if the antagonist is infused into the ventricular space overlying the caudal brainstem and not the forebrain ventricles (120). In the same vein, additive anorexic effects of GLP-1 and leptin occur only when these peptides are co-administered into the 4th ventricle (121). At face value, most of these data fail to discriminate effects of GLP-1 in the area postrema vs the closely subjacent NTS; however, the implication of the latter co-administration experiment is that, because leptin receptors are restricted to the caudal brainstem to the NTS, the effect in this case, is mediated through this site and not the area postrema.

Taken as a whole, the observations described above, provide an intriguing but poorly resolved involvement of the satiating effects of GLP-1 in the area postrema that is distinct from that of the NTS and is independent of the impact of nausea.

Potential interaction of CVOs with sites in the cerebral cortex

In analogous experiments to those described above in relation to thirst, we injected different isoforms of the retrogradely-transported virus, pseudorabies, into regions of the cerebral cortex, namely the insular and anterior cingulate cortex (122). These regions have been aligned with motivation to eat based on imaging studies (123, 124). In addition, viruses were injected into the terminal endpoints of mesolimbic pathways in the shell of the nucleus accumbens with a view to elucidate the overall inter-connectedness of homeostatic, reward and executive control pathways that relate to feeding. In the context of the present discussion, centered around the involvement of CVOs in integrated feeding circuits, the only circumventricular site that showed polysynaptic axonal projections to the cortical loci for the motivation to eat was the area postrema (122). While it is important not to over-interpret this anatomical data, it is tempting to (theoretically) link this brainstem CVO, which is well positioned to sample the circulating nutrient and metabolic hormonal milieu, with cognitive or motivated feeding control in the cerebral cortex via synaptic relays highlighted by the passage of virus through other brainstem, midbrain, hypothalamic, amygdaloid and thalamic sites.

Concluding Remarks

In the thirty years since the inception of the Journal of Neuroendocrinology we have witnessed a considerable escalation of insight into the neural pathways that could link subcortical sensory sites, such as CVOs, with regions of the cerebral cortex that participate in generating or inhibiting conscious sensations of thirst and hunger. However, along with this new knowledge comes a greater appreciation of the complexity of the neural systems involved. Fortunately, the expanding armamentarium of innovative methods and approaches that have recently been developed by neuroscientists, both within and outside these specific fields, will ensure that insights into the neural systems subserving thirst and hunger will continue to escalate at an extraordinary rate.

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916 **Legends to Figures**

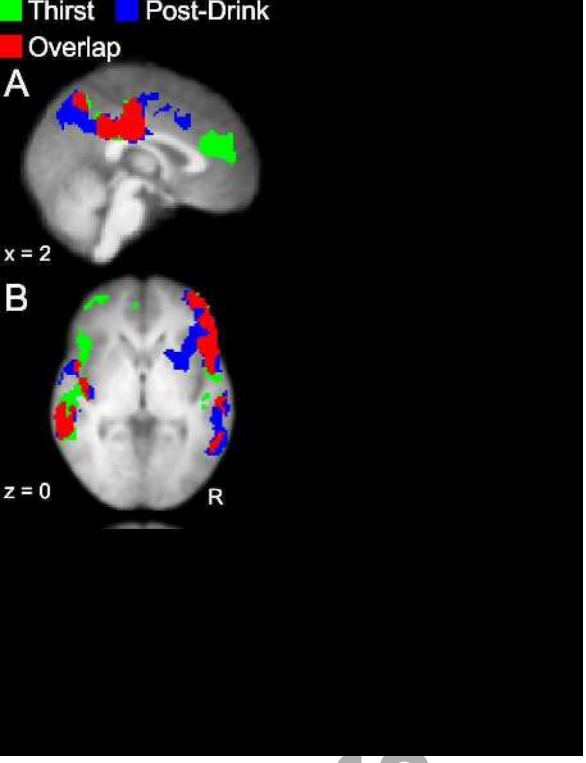
917 Fig. 1. Functional magnetic imaging of thirsty subjects before and after drinking water to
918 satiation. Green areas indicate regions activated only when subjects were thirsty, blue areas
919 only when satiated following drinking, and red areas were activated during both conditions.
920 Panel A (top) shows activation of anterior and posterior cingulate regions in thirsty subjects.
921 Panel B (middle) shows activation of left posterior insula and bilateral activation of frontal
922 operculi and middle temporal gyrus in thirsty subjects. From Farrell et al. 2011; Am J Physiol
923 301: R623-R631, (Reference 44) with permission.

924

925 Fig 2. Diagram of proposed neural pathways that link thirst-related neurons in the subfornical
926 organ and OVLT with cortical sites associated with the conscious sensation of thirst.
927 Abbreviations: LHA, lateral hypothalamic area; LPBN, lateral parabrachial nucleus; MDT, medial
928 dorsal thalamic nucleus; MnPO, median preoptic nucleus; NTS, nucleus of the solitary tract;
929 OVLT, organum vas culaosum of the lamina terminalis; PVN, hypothalamic paraventricular
930 nucleus; SCN, suprachiasmatic nucleus; SFO, subfornical organ.

931

932 Fig 3. Schematic diagram showing the major hormonal and other major sensory inputs to the
933 subfornical organ and area postrema together with the primary efferent outflows of these
934 circumventricular structures (see (67, 68, 107, 125). Abbreviations: AP, area postrema; BNST,
935 bed nucleus of the stria terminalis; CeA, central amygdala; DMN-X, dorsal motor nucleus of the
936 vagus; GLP-1, glucagon like peptide-1; ILC, infralimbic cortex; LHA, lateral hypothalamic area;
937 LPBN, lateral parabrachial nucleus; MnPO, median preoptic nucleus; NTS, nucleus of the
938 solitary tract; OVLT, organum vas culaosum of the lamina terminalis; PeF, perifornical area;
939 PVN, hypothalamic paraventricular nucleus; SFO, subfornical organ ; SON, supraoptic nucleus.



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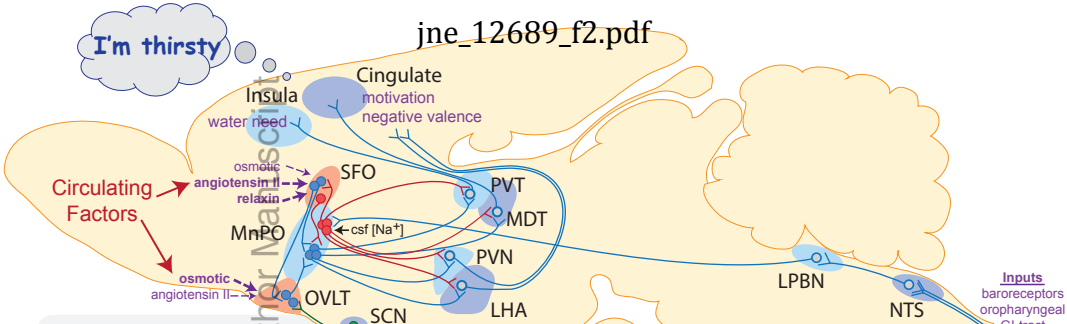

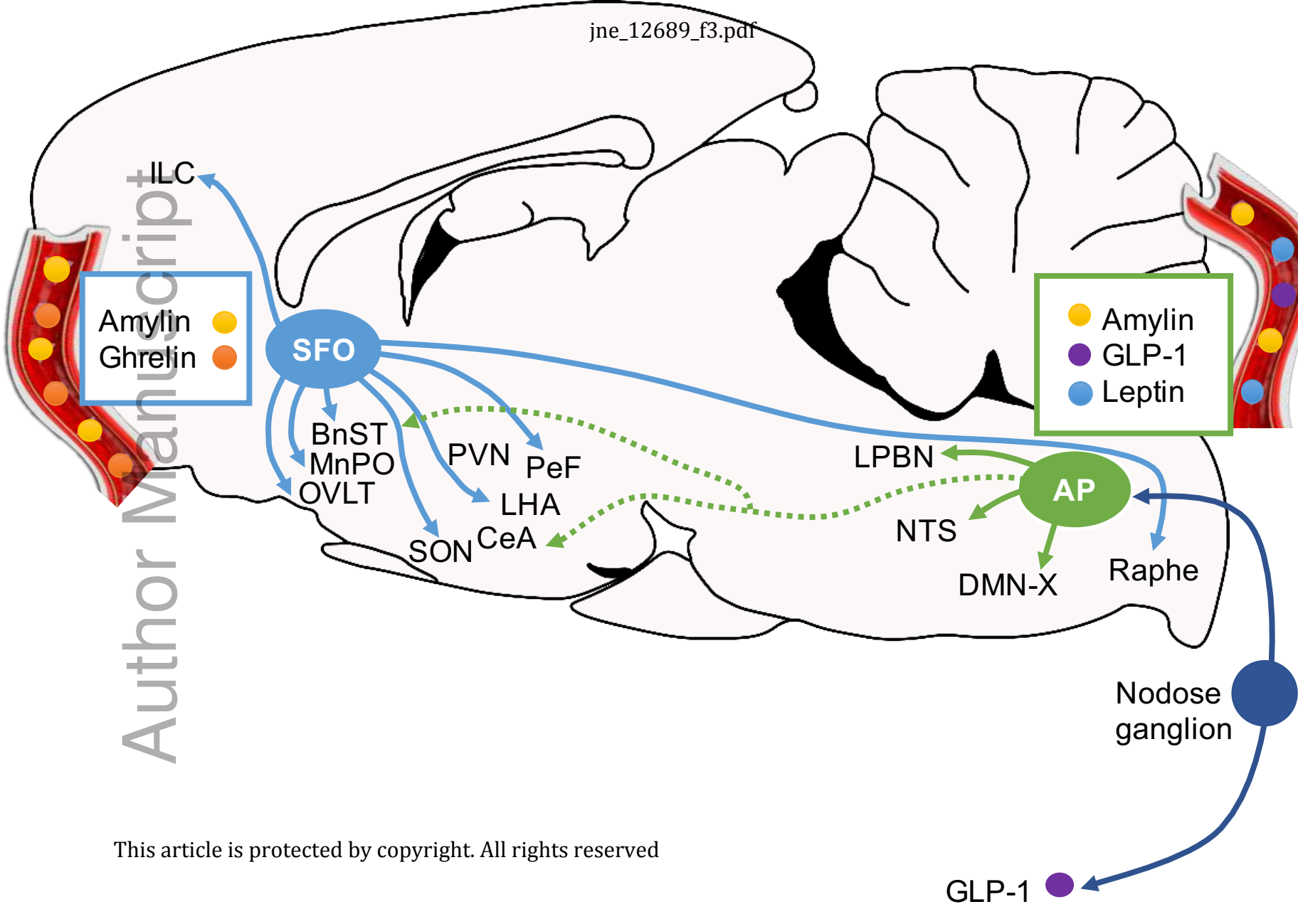

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