Brain-derived GLP-1 – understanding the physiological function and anti-obesity potential of PreProGlucagon Neurons

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Financial support: Work in our laboratory is supported by the Medical Research Council UK (grants MR/X003604/1 and MR/Z50614X/1

Disclosure summary: The authors have no conflicts of interest to disclose.

Keywords: GCG, brainstem, PPG, food intake, obesity, transgenic

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Abstract

Glucagon-like Peptide-1 (GLP-1) is produced within the central nervous system by preproglucagon (PPG) neurons. This brain-derived GLP-1, rather than that released from the gut, is the physiological agonist for brain GLP-1 receptors. With brain GLP-1 receptors being a major target for eating suppression, understanding the physiology and the translational potential of PPG neurons is of pivotal importance, particularly since PPG neuron activation is also strongly associated with stress. This review critically summarizes the current knowledge of PPG neuron anatomy, physiology and molecular make-up together with insight into the relevant research tools, and consideration of the different PPG neuron populations within the CNS, in order to provide an appraisal of the potential of these neurons as drug targets and the associated risks and benefits.

Introduction

The most extensively studied population of PreProGlucagon (PPG) neurons comprises a few thousand neurons located in the lower brainstem. As their name indicates, these cells transcribe the product of the glucagon gene. Proglucagon is then processed post-translationally to create glucagon-like peptide-1 and -2 (GLP-1/2) as well as glicentin that is further processed to oxyntomodulin (1). Often, immunocytochemical detection of GLP-1 (or GLP-2) is used to identify PPG neurons in brain tissue (2) and consequently they are also called GLP-1 neurons. Whilst they have been studied most intensively in mouse and rat, this brainstem population of PPG neurons has been identified in other mammals including man (3,4), and in non-mammalian vertebrates, namely bird (5,6). The observation that this CNS source of GLP-1 is conserved, at least in vertebrates, suggests functional importance. However, this functional importance has not been clarified unequivocally to date. Interestingly, in addition to the global preservation of these cells across species, there are also reports of functional differences, primarily between rat and mouse, e.g. (7), the only two species with a reasonable number of functional studies that can be compared. Hence, it is currently unclear whether functional aspects differ more between non-rodent species, or not.

Neuroanatomy

Brainstem PPG neurons and their projections were first described as GLP-1 immunoreactive neurons in rat in the late 1980s (8). Individual cell bodies were found in the caudal nucleus tractus solitarii (NTS) and

in the intermediate reticular nucleus (IRT), whilst axons were detected in multiple brain regions, with highest abundance in hypothalamus (2,8). Immunostaining of axon terminals with a GLP-1 antibody clearly demonstrated that GLP-1 neurons store GLP-1 as neurotransmitter or neuromodulator at the release sites for the peptide. This finding was later confirmed by electron microscopy localising GLP-1 to dense core vesicles (9), and also by the observation that large amounts of active GLP-1 are located in the hypothalamus which has dense innervation from these neurons but contains no PPG cell bodies (10).

Expression of preproglucagon in brainstem cell bodies was confirmed by *in situ* hybridisation (11), and generation of *Gcg*-transgenic mice expressing yellow fluorescent protein (YFP) under control of the glucagon promoter allowed ever more detailed analysis of the projection pattern of these neurons (12,13). For example, these transgenic mice enabled the discovery that a substantial proportion of PPG neurons project into the spinal cord (14). In addition to the populations in NTS and IRT small numbers of PPG neurons were also found along the midline ventral from the hypoglossal nucleus in a position overlapping with the raphe obscurus. These were also detected by *in situ* hybridisation in rat (11) and recently confirmed in a new transgenic rat model (15).

Additionally, the mouse expressing YFP under the glucagon promoter revealed PPG cell bodies in the olfactory bulb, the piriform cortex and the lumbar-sacral spinal cord, but their physiological function remains unclear to date. Whilst those in spinal cord (14) have not been described in other species, PPG neurons in piriform cortex and olfactory bulb have also been reported in rat (11,15). Furthermore, functional characterisation of the olfactory bulb PPG neurons has begun in mouse (16-19). These studies suggest that olfactory bulb PPG neurons, which are located in the granule cell layer, are either local interneurons or short axon cells which do not have far reaching projections outside the olfactory bulb.

There is consequently a consensus that at least the vast majority of GLP-1 containing axonal projections originate from the brainstem population of PPG neurons. Several lines of evidence support this notion. Firstly, injection of retrograde tracers into various forebrain areas (20-22), as well as injection into the spinal cord (14), label brainstem PPG neurons. Secondly, viral transduction of brainstem PPG neurons with fluorescent markers labels axons in the forebrain (23,24). Thirdly, knockdown of PPG expression in the NTS reduced GLP-1 immunoreactivity in the PVN in rat (25), and finally, virally mediated selective ablation of PPG neurons in the NTS in mouse significantly reduced the amount of active GLP-1 detected not only in brainstem, but also in hypothalamus and spinal cord (10). Based on these observations it seems very plausible that the widespread PPG axon projections throughout most of the brain originate from the brainstem.

The PPG neuron cell bodies in NTS and IRT are in spaces partially overlapping with the location of dorsal vagal neurons and parasympathetic nucleus ambiguous neurons, respectively (12). However, unlike these efferent vagal neurons, axons from PPG neurons do not leave the CNS (14). Whether their physical location close to these vagal efferents is of functional significance is presently unclear, however several studies in rat suggest that dorsal vagal neurons are sensitive to GLP-1 (26,27). It is also unclear whether NTS and IRT populations of PPG neurons form distinct groups or a continuum along the arc from NTS to ventrolateral IRT. Retrograde tracing from projection targets such as the dorsomedial hypothalamus (DMH) or the spinal cord label both NTS and IRT PPG neurons (14,21,24). Overall, to date there is no indication of distinct populations.

However, the neuroanatomy of the central GLP-1 system is highly suggestive of an organisation where GLP-1 is stored within axon terminals or varicosities along the length of axons, with release of GLP-1 into the brain parenchyma synaptically or perisynaptically in response to electrical signals within each individual axon. Thus, GLP-1 released at one specific brain site could have a different effect to GLP-1 released at a different site. On the other hand, Card et al (2018) showed close appositions between NTS PPG neurons in mouse suggestive of potential crosstalk between individual PPG neurons (28). Additionally, another study in rat demonstrated that PPG neurons innervate the paraventricular nucleus of the thalamus (PVT), the paraventricular nucleus of the hypothalamus (PVN) and the bed nucleus of the stria terminalis (BNST) collectively, and in light of the anatomical connectivity of NTS PPG neurons described before, it proposed that PPG neurons form a tightly coordinated network where activation of a subset of neurons will lead to concerted activation of the entire population (24). However, this proposed model of synchronised action is only based on anatomical observations and it is currently unclear whether brainstem PPG neurons form a homogeneous population that produces synchronous effects at diverse projection targets or a highly diverse population of individual PPG neurons that have divergent functions. These functional considerations are discussed below under physiological roles.

Transgenic animal models as essential tools to investigate and understand PPG neurons

Many of the investigations into the physiology of PPG neurons would not have been possible without the use of transgenic animal models. Whilst these are invaluable, essential tools, it is also important to be aware of both the opportunities and the limitations that these present.

The first mouse model that allowed identification of PPG neurons by fluorescent marker was the Glu-Venus mouse (29). In this model, the yellow fluorescent protein Venus was introduced as a simple

transgene consisting of a bacterial artificial chromosome (BAC) containing the glucagon (*Gcg*) promoter, followed by Venus in an open reading frame. The transgene was randomly inserted into the mouse genome and copy number was undefined. With Venus under the direct control of the strong *Gcg* promoter, highly fluorescent PPG neurons were detected without the requirement for amplification with immunofluorescence (30). The resulting pattern of fluorescently labelled cell bodies primarily in the lower brainstem as well as axon terminals in various forebrain regions (13), including the limbic system (23), matched well the previously reported distribution of GLP-1 immunoreactivity in rat (2). Additionally, the distribution of Venus through all cell compartments enabled visualisation of the entire cells, including dendritic trees and axons in passage (13), which are not detected by GLP-1 antibody staining, because GLP-1 is mainly found in cell bodies and axon terminals. The untargeted genetic approach ensured that the native *Gcg* gene in these mice was not affected but came with the risk that random insertion could disrupt another important gene. Subsequently, two more mouse models were generated independently with a similar approach to create mice that express cre-recombinase under control of the *Gcg* promoter (31,32). These mice allowed targeted expression of foreign genes, such as DREADD receptors or Channelrhodopsin, selectively in PPG neurons by stereotaxic injection of AAVs that encode these proteins in a Cre-dependent fashion. This enabled functional manipulation of PPG neurons in order to elucidate their function, as discussed in more detail below (10,32). Whilst no adverse effects have been reported for these strains using random insertion, there could potentially be issues. This would be avoided by a targeted approach such as a knock-in. In fact, another mouse line was created with a targeted approach where improved Cre recombinase (iCre) was inserted into exon 2 of the *Gcg* gene (33). Interestingly, however, in this mouse line the *Gcg* gene locus was accidentally duplicated, leading to the targeted allele possessing both the transgene and the unaltered wildtype *Gcg* gene. These mice have been used to assess NTS PPG neuron activity in vivo with Ca^{2+} fibre photometry and to optogenetically stimulate the same cells (34).

The latest addition to the transgenic animal toolbox available to study PPG neurons is a transgenic rat (15). The advantages of this addition are not only to confirm PPG action/neuroanatomy in a different rodent model, but rat models have distinct advantages in research in general due to the similarity in genetics to humans, the advantages of a larger size in some experimental techniques and the difference in baseline behaviour compared to mice (35).

This rat model was generated with CRISPR technology to target an IRES-Cre construct to the end of the open reading frame of the *Gcg* gene. By targeting the *Gcg* gene this approach avoids the potential

complications caused by random insertion of the transgene. The use of an IRES construct ensured that the Cre-recombinase is translated independently of the proglucagon peptide and should not interfere with the expression of the native *Gcg* gene product. Crossing this rat with a tdTomato reporter strain, revealed an expression pattern for the tdTomato fluorescence very similar to that reported for the cre-reporter mice, but GLP-1 levels in the blood and PPG mRNA levels in brain were severely reduced particularly in homozygous rats (15), suggesting that targeting the construct to the native *Gcg* gene affected transcription and translation level despite the use of an IRES construct. As such limitations were not observed in the knock-in mouse (34), the accidental duplication of the allele in the mouse might have been rather beneficial for the overall phenotype.

These observations highlight both the opportunities as well as the limitations of different transgenic approaches. Firstly, activity of the *Gcg* gene within the CNS varies during development, but if cre-recombination is utilised to switch on expression of a reporter gene by crossing the cre-expressing mouse strain with a flox'd reporter mouse strain, then the reporter is permanently expressed in all cells that exhibited Cre expression at some point during development (36). Hence, reporter expression in a specific neuron in an adult mouse does not necessarily mean that this neuron is expressing GLP-1 at the point in time when a study is performed. In accordance with this caveat, more widespread expression of the reporter has been reported for the *Gcg*-cre mice compared to the expression of Venus directly under the *Gcg* promoter (32). In both the rat and mouse *Gcg*-cre model this has been shown by reporter expression in areas such as paraflocculus of cerebellum, inferior colliculus, the basolateral amygdala (BLA) and orbital cortex (15). Investigating the areas further, the BLA was confirmed to show *Gcg* expression in neonates, but not adult age rats (15). Secondly, there might be the question of physiologically-relevant expression levels. If an individual cell aberrantly expresses a few molecules of GLP-1 or glucagon, this has no physiological consequences. If the cell however expresses a few molecules of Cre-recombinase, this is sufficient to elicit cre-dependent expression of virally or transgenically delivered exogenous genes. Thus Cre-driver mice should always be used with caution to ensure that those cells that are manipulated functionally in a cre-dependent fashion are indeed those that comprise the physiologically-relevant population of cells intended to be targeted.

PPG neurons of the olfactory bulb

With development of transgenic models, the capability of studying PPG neurons increased substantially, and this also provided the impetus to investigate PPG neurons described in brain regions beyond the lower brainstem. Whilst the presence of PPG mRNA in the olfactory bulb of rat was first reported 25 years ago

(11), attempts to functionally study these cells only began 10 years ago with *ex vivo* studies demonstrating the presence of a microcircuit within the olfactory bulb, where GLP-1 is produced by short axon cells in the granule cell layer and mitral cells being responsive to GLP-1 (16,17). Importantly, these functional studies demonstrated that olfactory bulb PPG neurons are glutamatergic like their counterparts in the lower brainstem, and not GABAergic as was expected for interneurons located within the olfactory bulb granule cell layer. Building on these studies, more recently a couple of studies by Montaner et al demonstrated with shRNA knockdown of GLP-1 expression in the olfactory bulb, that GLP-1 signalling from these PPG neurons is necessary for odour-evoked cephalic phase insulin release and that this cephalic response was dependent on the sympathetic nervous system, rather than the vagal nerve (19,37). Additionally, they demonstrated that local administration of the GLP-1R antagonist exendin-9 extended the time taken for mice to find buried food, indicating that GLP-1 signalling in the olfactory bulb increases either the ability to find food or the interest in food (37). A striking finding about PPG neurons from these studies is the observation that GLP-1 in the olfactory bulb increases rather than decreases the interest in, or ability to detect, food. In the brainstem and in relation to GLP-1R agonists, it is usually the postprandial situation that is considered, whilst here it is a pre-prandial role that GLP-1 fulfils. Regarding the effects on insulin release, it clearly works like peripheral GLP-1. These observations raise the question whether it would be counterproductive if 'postprandial' GLP-1 could spill over from the circulation or from brainstem PPG neuron release into the olfactory bulb? Would this enhance, rather than suppress, food intake?

One could argue that this emphasises the need to keep the different GLP-1 systems in the body separate (38,39). If it is assumed that the different PPG neuron populations (Fig 1), namely an olfactory bulb system, a piriform cortex system and the main system originating from the lower brainstem, reflect three distinct CNS GLP-1 systems, the requirement arises to keep these separated. Working on the assumption that GLP-1 is released synaptically within each of these systems and that its spread is limited to the synaptic and peri-synaptic space, this separation is easily achieved. However, GLP-1 is both a neurotransmitter produced within the brain and a peptide hormone secreted as an incretin from the L-cells of the gut epithelium into the blood stream and potentially distributed throughout the body. Hence, to ensure temporally and spatially precise signalling in different projection targets of neuron populations within the CNS, it is essential that the central GLP-1 system is isolated from hormonally-acting circulating GLP-1. This has recently been demonstrated in mouse and has been discussed in detail elsewhere (38,39). In fact, that study not only indicated a clear separation between the targets for gut-derived GLP-1 and GLP-1 from PPG neuron but also suggested that systemically-administered exogenous GLP-1 receptor agonists act on different targets to PPG neurons in order to reduce food intake. Whether this separation also means that

GLP-1 receptor agonists categorically do not cross the blood-brain barrier (BBB) has not been comprehensively clarified and its discussion, whilst important, is beyond the scope of this review.

Since it seems to be imperative to prevent spillover of GLP-1 between the olfactory bulb and the brainstem PPG neurons, would a highly brain-permeant GLP-1R agonist be counterproductive? We will come back to this question after focussing on the putative physiological role(s) of brainstem PPG neurons.

Physiological role of PPG neurons

The precise physiological function of PPG neurons has remained largely unresolved to date and has often been shaped by our preconceptions and assumptions rather than unbiased observation. We are also regularly conflating the physiological role with the potentially achievable effect upon pharmacological or chemogenetic stimulation.

A longstanding question in GLP-1 physiology was that of whether PPG neurons are simply an 'extension' of the gut GLP-1 system into the brain. This hypothesis has been refuted by several lines of independent evidence. Firstly, PPG neurons themselves do not express GLP-1 receptors and do not respond to GLP-1 (28,30). Additionally, systemic application of GLP-1RAs does not elicit cFos in PPG neurons (39,52), and GLP-1-expressing vagal afferents do not innervate PPG neurons (39). Furthermore, Holt et at 2019 (10) demonstrated that ablation of NTS PPG neurons, strongly reduces the amount of GLP-1 detectable in brainstem, hypothalamus and spinal cord. Finally, concomitant systemic administration of a GLP-1RA and chemogenetic activation of PPG neurons produces stronger hypophagia than either treatment alone (39). Thus, PPG neurons and peripheral GLP-1 do not appear to be functionally linked. This concept has been discussed in detail recently (38) and will not be revisited here.

Studies investigating the physiology of PPG neurons fall into two groups. They either address the question of what stimuli activate PPG neurons or they ask what the consequence of PPG neuron activation is. The most commonly reported consequence of PPG neuron activation is a reduction in food intake and the most commonly reported stimuli of PPG neurons are gastric or systemic satiety or satiation signals, both electrical and hormonal, and stress, both visceral and physical/psychological (30,34,40,41,53,54).

Until transgenic mouse models allowed unequivocal identification as well as manipulation of the brainstem PPG neurons, only post-hoc analysis of cFos expression, as surrogate marker for neuronal activity, coupled with GLP-1 immunocytochemistry provided insight into what stimuli activate these cells (40). Direct recording of the electrical activity of PPG neurons only became feasible with transgenic mice

that fluorescently labelled PPG neurons enabling their identification in brain(stem) slices for patch-clamp recordings (30,41). Another line of investigations has used the intracranial administration of GLP-1 (receptor agonists and antagonists) to glimpse an insight into the role of GLP-1 within the CNS. Particularly, brain administration of antagonist only has demonstrated that native GLP-1 has physiological effects within the brain (20,48-51). The various effects of GLP-1 receptor agonists in multiple parts of the CNS have been reviewed comprehensively (42,43) and will thus only be touched upon in this review. Activation of GLP-1 receptors inside the BBB could potentially replicate either the action of gut-derived GLP-1 gaining access to the brain, or the action of PPG neurons. However, various studies have indicated that postprandial gut derived GLP-1 is extremely unlikely to reach the brain (44-46), making it most likely that GLP-1Rs inside the BBB are the physiological target of PPG neurons rather than GLP-1 released from enteroendocrine L-cells. This view is further substantiated by the observation that knockdown of gut GLP-1 affects glucose tolerance but not food intake and bodyweight (47). However, this does not preclude the possibility that systemic administration of pharmacological doses of GLP-1R agonists, particularly under pathophysiological conditions that compromise the BBB, might be able to elicit some of the responses attributed physiologically to PPG neuron activation. Here, we'll first focus on what stimuli activate PPG neurons and then examine the consequences of PPG neuron activation, both at physiological and pharmacological level.

Stimuli that activate PPG neurons

It has been suggested that PPG neurons are primarily activated by adverse effects, be these stress, severe gastric distension, or enteric malaise, rather than physiological signals. For example, a study comparing cFos expression in PPG neurons and catecholaminergic A2 neurons in response to voluntary meals, found only the very largest meals elicited cFos in PPG neurons, whilst A2 neuron cFos tracked meal size (55). A second study with meal-entrained rats demonstrated a strong positive correlation between the amount of liquid diet consumed and the percentage of cFos expressing PPG neurons (53). Similarly, a study in mouse found that only unusually large meals increased subsequent food intake after chemogenetic inhibition of NTS PPG neurons, suggesting that normal meals do not activate NTS PPG neurons sufficiently to suppress subsequent food intake (10). Whilst these studies suggest that only unusually large intake stimulates PPG neurons, a recent study in mouse monitoring Ca^{2+} dynamics of individual PPG neurons in vivo, demonstrated an initial response of these cells to food intake within seconds. This was followed by increasing activity over minutes, reflecting the tracking of cumulative intake, and is suggestive of a physiological response to food intake (34). Similarly, when liquid food was infused directly into the

stomach at a rate of 1ml in 10 min, the Ca^{2+} signal in PPG neurons ramped up over this period rather than only occuring once strong gastric distension was achieved with the entire volume. Also, interestingly infusion of 1ml of saline did not elicit a response despite its volume. Whilst these observations strongly suggest that the PPG neuron activation is elicited by gastric signalling, and not only under drastic gastric distension, this does not seem to be gastric distension alone, though infusion of 1ml of air did produce some response. It is currently unclear how these observations can be reconciled and whether they are due to different experimental paradigms in these studies. Possibly only strong stimulation reaches the threshold for cFos expression whilst smaller stimulation already elicits measurable Ca^{2+} signals.

It might be worth taking a closer look at the activation of PPG neurons by gastric distension. Whilst this aligns with the old observation that PPG neurons receive inputs from vagal afferents (30), recent data might suggest subtle nuances in this picture. Firstly, in rat, only strong gastric distension activated these neurons, whilst moderate distension activated catecholaminergic NTS neurons (55). Secondly, GLP-1 receptor expressing vagal afferents are not a significant input to PPG neurons, despite these neurons being shown to carry gastric distension signals (39). Thirdly, a recent study has demonstrated that ilial GLP-1 or the systemically-administered GLP-1 receptor agonist exendin-4 induce gastric distension (46), whilst the same compound and other GLP-1 receptor agonists fail to induce cFos in PPG neurons (52). Whilst it could still be argued that exendin-4 might not produce sufficient gastric distension to induce PPG neuron activation, it might be pertinent to consider a different interpretation. Oxytocin-receptor expressing vagal afferents have been shown to be a strong vagal input to PPG neurons (39). Additionally, oxytocin-induced hypophagia was strongly attenuated when NTS PPG neurons were ablated (39), indicating the physiological importance of this pathway. Considering that oxytocin receptor expressing vagal afferents receive inputs from the duodenum, rather than the stomach (56), this might suggest that PPG neurons are not activated by gastric distension, but rather by duodenal extension. The findings from strong gastric distension might be explained by the suggestion that extreme gastric distension actually causes duodenal distension, which is then signaled to the PPG neurons. In this context it is interesting to note that systemically administered exendin-4 delays gastric emptying, which would reduce any duodenal distension, and this effect is prevented by knockdown of vagal afferent GLP-1Rs (57).

Restraint stress induced hypophagia requires endogenous GLP-1 signalling within the brain (23,42,49,50), and subsequently a few studies have demonstrated directly a strong activation of PPG neurons by restraint stress (10,58). This is not linked to (postprandial) vagal afferent signals and indicates that PPG neurons must receive input from other sources as well. Holt et al revealed a multitude of synaptic inputs

to PPG neurons from throughout the central nervous system (59). They demonstrated that PPG neurons in the cNTS receive direct descending inputs from forebrain regions implicated in stress processing, including the PVN. Additionally, Leon et al identified serotonin signalling via 5-HT_{2C} and 5-HT_3 receptors as necessary for cFos expression in PPG neurons in response to LiCl or restraint stress in rat (58). Interestingly, mouse PPG neurons do not respond to 5-HT_3 receptor agonists in brain slices *ex vivo* but are responsive to 5-HT_{2C} and 5-HT_{1A} receptor activation (60,61), a finding that was further validated by the observation that the 5-HT_{2C} receptor agonist lorcaserin, elicits cFos expression in mouse PPG neurons (61). These studies indicate both parallels as well as differences between rat and mouse for brainstem serotonin signalling.

As stated above, systemic and psychogenic stressors activate brainstem PPG neurons and are hypothesized to activate the HPA axis (62), based on increases of both plasma corticosterone and adrenocorticotropic hormone after intracerebroventricular injection of GLP-1 in rat (63). Zhang et al demonstrated this involvement from a different angle, where a decrease in *Gcg* mRNA but an increase of *Gcg* heteronuclear RNA (hnRNA) in the NTS and reduced GLP-1 immunoreactivity in the PVN was observed in response to restraint stress and to LiCl, both of which were accompanied by an increase in plasma corticosterone (64). Injection of corticosterone also led to downregulation of PPG mRNA, suggesting that the lower PPG mRNA amount is due to glucocorticoid secretion. The concurrent increase of hnRNA, an indicator of recent gene transcription, and decrease of mRNA suggests that the loss of mRNA is not due to reduced transcription, but rather a feedback-loop to regulate GLP-1 availability after rapid PPG neuron activation to limit the GLP-1 induced activation of the HPA-axis (64). These data suggest that PPG neurons are not only stimulated by the acute stress response but also limit the response. Whether activation of PPG neurons is dampened during chronic stress remains to be investigated.

Although the study above did observe a decrease in *Gcg* mRNA with the injection of LiCl, there was no measurement of actual PPG neuron activation with the stimulus (64). It has previously been reported that pharmacological stressors such as LPS and LiCl elicit a cFos response in PPG neurons when tested in rat (40). However, a recent study recording Ca^{2+} dynamics of PPG neurons with fiber photometry failed to observe immediate activation of PPG neurons with LiCl and LPS (34). This discrepancy raises the question of whether there is a context-dependence in PPG engagement during stress, or alternatively whether activation of PPG neurons by stress differs between rat and mouse.

Additionally, electrophysiological recordings from PPG neurons *ex vivo* have demonstrated their direct activation by leptin, glutamate, vagal afferent stimulation, and presynaptic modulation of glutamatergic

inputs by CCK-8 and epinephrine, but no direct or indirect effects of GLP-1, PYY3-36, ghrelin and melanotan-II (30,41). Similarly, Ca^{2+} recordings from PPG neurons in *ex vivo* brainstem slices demonstrated responsiveness to oxytocin (39), interleukin-6 (65), leptin, CCK-8 and 5-HT_{2C} receptor agonists (60), but not GLP-1 (28) or 5-HT_3 receptor agonists (60). Further, systemic administration of peptides linked to the physiological regulation of satiety or satiation, such as CCK, elicited cFos immunoreactivity in PPG neurons (40). A further step-change in the abilities to interrogate the regulation of PPG neuron activity was the demonstration that fibre photometry recordings of NTS PPG neurons in awake mice are feasible, despite the amount of movement in the caudal NTS. This first study provided exciting insight into the time course of activation by ingestive signals as well as confirmation of the pharmacology determined by the earlier *ex vivo* studies (34). It will be exciting to see whether this can be pushed technically even further to enable endoscopic recordings from individual PPG neurons *in vivo*.

In summary, PPG neurons receive diverse inputs ranging from signals reflecting adiposity, via satiation signals to those indicating malaise or stress, as well as multiple inputs from the forebrain, suggesting that PPG neurons balance various inputs to generate an output that determines the appropriate level of food intake for the given situation. As such, these neurons do not seem to determine energy balance but are informed by energy balance and other inputs to optimize decisions to ensure survival. E.g. in a situation of sufficient energy reserves and a full stomach, it is not necessary to take the risk of seeking food, and hence their activity suppresses food intake, whilst in negative energy balance their activity is suppressed and circuits that promote energy intake are not overridden by the activity of PPG neurons (66). Consequently, neuronal GLP-1 knockout has no substantial effect on body weight, particularly in *ad libitum*-fed laboratory animal (10,67), because the homeostatic regulation of food intake remains intact. In contrast, pharmacological activation of the central GLP-1 system has the capacity to strongly suppress food intake (10,32,39,68). This is also seen with systemically administered GLP-1R agonists which are highly effective in reducing body weight, whilst removal of the receptors has minimal impact on body weight (69).

Such physiological regulation would make the PPG neurons highly desirable as a target for weight loss medication, unless their activation is linked to effects beyond food intake suppression, that is detrimental to health. To evaluate this, the next section focusses on the effects elicited by pharmacological, chemogenetic or optogenetic stimulation of PPG neurons.

Effects elicited by PPG neuron activation

When considering effects of PPG neuron stimulation, it is critical to ensure that the observed physiological effects in response to e.g. pharmacological stimulation of PPG neurons are indeed downstream effects and not due to the substance used also activating parallel, PPG neuron independent, pathways that cause the observed effects. For example, whilst stress undoubtedly activates PPG neurons, it is less clear which of the consequences of stress, such as hypophagia, activation of the HPA axis, tachycardia, etc are dependent on PPG neuron activation. In pharmacological experiments, this is usually addressed by demonstrating that the response is blocked by administration of a GLP-1R antagonist. However, this has two potential caveats: Firstly, PPG neurons are also glutamatergic, therefore some of their physiological effects could be mediated by glutamatergic transmission, secondly, the GLP-1R antagonist would also block potential effects mediated by independent release of GLP-1 from enteroendocrine L-cells, and thirdly, it is doubtful of whether the antagonist when given systemically would cross the BBB (70). Consequently, the most reliable approaches to ascertain functions selective fulfilled by PPG neuron activation is their chemogenetic or optogenetic activation. Additionally, selective chemogenetic or optogenetic inhibition or ablation of the PPG neurons enables determination of which physiological or pharmacological effects require functional PPG neurons.

Acute chemogenetic activation of brainstem PPG neurons has consistently yielded a substantial reduction in food intake in both mouse and rat (10,15,32,39) and so has optogenetic stimulation of PPG neuron terminals in the PVN (68). This study additionally demonstrated that the optogenetic stimulation of these PPG neuron terminals reduced food intake in a GLP-1 dependent manner, because it persisted in vGlut2-KO mice, thus confirming that the co-transmitter glutamate is not the driver for reduced food intake. Finally, that study revealed an increase in food intake upon optogenetic inhibition of PPG neuron terminals in the PVN, suggesting ongoing endogenous release of GLP-1 in the PVN under their experimental conditions (68). This is interesting, because chemogenetic inhibition or ablation of NTS PPG neurons only produced hyperphagia when either restraint stress or an abnormally large meal had activated these PPG neurons (10). In contrast to the consistent effect on food intake, a glucose-lowering effect of chemogenetic PPG neuron stimulation was observed in one study (71), but not in another (10). Finally, chemogenetic activation of NTS PPG neurons increases heart rate both in anaesthetised and awake mice (52). This could be indicative of an involvement in a fight-and-flight response.

A number of studies link GLP-1/GLP1R and stress response (63,72), but there is little data to demonstrate direct involvement of PPG neurons. A recent study employing chemogenetic activation of PPG neurons

resulted in female mice reducing both the time spent in the centre of the open field arena and the total distance travelled (73). These results were interpreted as a stress or anxiety-like response and this interpretation was further supported in an acoustic startle test, although in a sex-independent manner. In contrast, Gaykema et al (2017) failed to observe a difference in stress-response measured by open-field or elevated plus maze and also did not observe a rise in plasma corticosterone in response to chemogenetic activation of NTS PPG neurons (32). However, that study was performed in male rats, so no sex dependent effect would have been noted.

Although evidence supports a modulatory role for PPG neurons and GLP-1 in stress-related behaviour, several questions remain to be answered. Firstly, while GLP1R activation induces anxiety-like behaviours and activates the HPA axis (74-77), it is still unclear to what extent endogenous GLP-1 release from PPG neurons drives these effects under physiological conditions. Secondly, it is also unclear what drives the difference in the response in females compared to males and whether there is a stressor-specific PPG neuron response (with potential different outcome downstream)?

Consequently, one might ask the question whether these are all individual aspects of a concerted physiological response that involves activation of all PPG neurons, or are there a variety of PPG neuron groups with different projection patterns and different inputs that reflects a range of physiological functions that are controlled/modulated by central GLP-1?

Are there PPG neuron subpopulations?

Release of GLP-1 at one place and time within the CNS might reduce food intake in response to peripheral signals such as gastric distension, whilst GLP-1 release at a different site, for example onto sympathetic preganglionics in the spinal cord produces tachycardia, e.g. as part of a fight or flight response to stress. We would argue that such a mode of action reflects much more the variety of responses that are observed upon local application of GLP-1 or its analogues into specific brain areas, where effects on food intake, on reward and addictive behaviour as well as on blood glucose, thermoregulation and cardiovascular regulation are reported dependent upon the precise site of local application.

The obvious question that arises from these various quite disparate effects of GLP-1 within the brain is whether these effects represent simply different aspects of an integrated response to PPG neuron activation. Or do these diverse effects reflect the fact that several PPG neuron subpopulations exist with each tuned to a specific function? Randolph et al demonstrated that PPG neurons innervate the PVT, PVN and BNST collectively, and proposed similar groups of circuits could be possible for other part of the

forebrain (24). At some overarching level GLP-1R activation within the CNS (and by extension PPG neuron activation) is linked to hypophagia, but when examining details, this hypophagia might occur in response to various reasons. It could be in response to restraint stress or to ingesting a very large meal or linked to a reduced reward assigned to the intake, or the hypophagia might occur for even different reasons. Given the extent of axonal projections from the PPG neurons in NTS and IRT towards the limbic system, combined with the abundancy of GLP1R expressing neurons in this area, one might expect a role of PPG neurons in reward-related behaviour. Some insights have emerged through direct stimulation of PPG neurons. Activation of PPG neurons in NTS decreases nicotine intake, suggesting that PPG neurons have a direct effect on addiction/reward circuits (78). Applebey et al (2025) described the brainstem GLP1 system as reducing motivation/rewards-seeking behaviour (79). These findings align with reduced reward- and food seeking behaviour in female, but not male, rats following both optogenetic and chemogenetic stimulation (80). Additionally, the hypoactive or reduced locomotor behaviour observed in various studies of chemogenetic PPG activation (32,39,73) may be attributed to reduced food-seeking behaviour or reduced motivation via the reward-based circuit. However, to confirm such hypotheses further investigation is necessary.

Transcriptomic profiling

A large dataset of transcriptomes of cells from the dorsal vagal nucleus might give a first insight into PPG neuron expression profiles. Ludwig et al 2021 (81) demonstrated that the *Gcg* expressing neurons in the NTS represent a distinct population of glutamatergic neurons, characterized by high expression of *Lepr (30)* and lower expression of *Cck*. This data set also confirmed that PPG neurons do not express e.g. *Glp1r, Gipr or Mc4r (28,30,41)*. The expression of the *Lepr* on PPG neurons has also been demonstrated by us using Nuc-Seq (61). However, the expression of *Lepr* was observed to be lower than the expression of *Htr2c.* In comparison to Ludwig et al. we also observed a slight, and possible negligible, expression of *Mc4r* both with Nuc-seq and FISH (61). In line with these findings, a 5-HT_{2c}R agonist activated PPG neurons, but they were not required for MC4R agonist-induced hypophagia (61). This alignment with functional *in vivo* and *ex vivo* data supports the validity of transcriptomics data as a useful source for interrogating PPG neurons, with the potential to reveal other targetable pathways within these cells.

A comparison between transcriptomic data and histological analysis in the dorsal vagal complex, described PPG neurons as glutamatergic NTS neurons that do not express *Phox2b* nor *Lmx1b*, an unusual feature of NTS-located neuron according to the authors, since most glutamatergic neurons in the NTS are positive for at least one of the two transcription factors (82). This finding validated the earlier observation

that lentiviruses delivering constructs under the control of the artificial Phox2b promoter PRSx8 are not active in PPG neurons (10). Importantly, this finding impacts the interpretation of results from a few publications that assumed Phox2b expression in PPG neurons (78,83,84). The finding that most, if not all, PPG neurons are glutamatergic also confirms immunocytochemical demonstration of *Slc17a6* (vGlut2) expression by PPG neurons of both rat and mouse (9,85). Additionally, GLP-1 and vGlut2 are located in the same varicosities within the brainstem (9). The functional importance of glutamate as co-transmitter was also tested by Liu et al (68). Knocking out vGlut2 abolished light-evoked glutamate release in the PVN but did not affect the concomitant PPG neuron induced food-intake suppression, demonstrating that food-intake suppression by PPG neurons projecting to the PVN is independent of glutamate release.

Transcriptomic datasets of brainstem and/or dorsal vagal complex helped to identify and characterize the PPG neurons as a distinct neuronal population, but not enough information is available from current datasets to resolve whether PPG neurons constitute a homogenous or heterogenous population.

Are PPG neurons of potential translational interest?

Whilst most questions about the precise physiological role of PPG neurons remain unanswered, the aforementioned chemogenetic and optogenetic experiments have demonstrated the strong capacity of these neurons to reduce food intake when activated. Yet, as discussed above, removal of GLP-1 or GLP-1 receptors does not elicit strong hyperphagia and excessive weight gain. This finding could be interpreted as a positive, because it implies that activation of these neurons leads to a reduction in food intake that is in addition to that achievable with targeting core appetite or satiation pathways. From this observation follows one pertinent question to be answered when considering the potential for obesity treatment. Is the significant reduction in food intake the only substantive consequence to be considered, or does activation of PPG neurons elicit adverse effects such as nausea, or even more severe effects such as anxiety and activation of the HPA axis? Whilst these possibilities have not been conclusively excluded, studies from at least two laboratories found either no conditioned taste avoidance or no disrupted behavioural satiety sequence upon chemogenetic activation of NTS PPG neurons in combination with the observed reduction in acute food intake, arguing against obligatory induction of nausea (32,39). Similarly, one of these studies found no evidence for anxiety using elevated plus maze and open field paradigms (32). On the other hand, a recent study activating NTS PPG neurons chemogenetically reported mild anxiety-like behaviour, particularly in female mice (73).

Translational exploitation of GLP-1 release from PPG neurons could be achieved in two ways. The first would be to activate these neurons *in vivo* without the use of transgenic technologies and thereby elicit release of GLP-1 and co-transmitter from these neurons. A couple of recent studies serve as proof-of-principle that this might be possible. Peripherally administered oxytocin acutely reduces food intake, and this effect is greatly diminished when PPG neurons are ablated (39). Similarly, the 5-HT_{2C} receptor agonist lorcaserin requires PPG neurons for its food intake suppressive effect (61).

The second approach would be to selectively target brain GLP-1 receptors, or specifically those that are reached by PPG neurons, but not those that are accessible to current peripherally-administered GLP-1R agonists. This approach would not utilize any co-transmitter released by the PPG neurons. At present it is not clear how relevant this difference is, and studies thus far, have concluded that GLP-1 release is the crucial component of PPG neuron action for those instances investigated (68).

It is clear that a variety of pathways converge onto the reduction in food intake and ultimately bodyweight as the final desired outcome. All of these different pathways have components of CNS signalling. Peripherally-administered GLP-1R agonists reduce food intake by engaging central nervous pathways (86-88), though most likely not via GLP1Rs located inside the BBB (89). In contrast, PPG neurons, which do not project to the periphery (14), act by engaging GLP1Rs within the brain. Additionally, activation of other pathways, such as the central melanocortin system, reduces food intake. Whilst the ultimate target of food intake reduction is the same for all these circuits, there are differences in the overlap in the actual circuits. For example, food intake suppression by the 5-HT_{2C} receptor agonist lorcaserin is prevented by the loss of either MC-4 receptors or PPG neurons (61,90,91). In contrast, neither of these two manipulations affects the hypophagia elicited by GLP-1R agonists (39,61). Consequently, lorcaserin and liraglutide can be given as a combination treatment to achieve greater reduction in food intake than either drug alone (61).

Conclusions

Recent years have seen a renewed interest in PPG neurons, since their effect on eating is distinctly separate from that of clinical GLP-1 therapy and the latest studies evaluating their activity in freely behaving mice finally enables detailed examination of their precise role under various physiological conditions. Such investigations will be crucial to determine whether PPG neurons and their GLP-1 release

will become a valuable tool in clinical practice for obesity treatment or is linked to too many adverse effects such as stress or anxiety.

Data sharing: Data sharing is not applicable to this manuscript, as no original data was collected or analyzed during this study

References

1. Muller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, Fritsche A, Gribble F, Grill HJ, Habener JF, Holst JJ, Langhans W, Meier JJ, Nauck MA, Perez-Tilve D, Pocai A, Reimann F, Sandoval DA, Schwartz TW, Seeley RJ, Stemmer K, Tang-Christensen M, Woods SC, DiMarchi RD, Tschop MH. Glucagon-like peptide 1 (GLP-1). *Molecular metabolism*. 2019;30:72-130.
2. Larsen PJ, Tang-Christensen M, Holst JJ, Orskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience*. 1997;77(1):257-270.
3. Zheng H, Cai L, Rinaman L. Distribution of glucagon-like peptide 1-immunopositive neurons in human caudal medulla. *Brain structure & function*. 2015;220(2):1213-1219.
4. Vrang N, Grove K. The brainstem preproglucagon system in a non-human primate (Macaca mulatta). *Brain Res*. 2011;1397:28-37.
5. Tachibana T, Hiramatsu K, Furuse M, Hasegawa S, Yoshizawa F, Sugahara K. Distribution of proglucagon mRNA and GLP-1 in the brainstem of chicks. *Comp Biochem Physiol A Mol Integr Physiol*. 2005;140(2):203-207.
6. Honda K. Glucagon-related peptides and the regulation of food intake in chickens. *Anim Sci J*. 2016;87(9):1090-1098.
7. Lachey JL, D'Alessio DA, Rinaman L, Elmquist JK, Drucker DJ, Seeley RJ. The role of central glucagon-like peptide-1 in mediating the effects of visceral illness: differential effects in rats and mice. *Endocrinology*. 2005;146(1):458-462.
8. Jin SL, Han VK, Simmons JG, Towle AC, Lauder JM, Lund PK. Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J Comp Neurol*. 1988;271(4):519-532.
9. Zheng H, Stornetta RL, Agassandian K, Rinaman L. Glutamatergic phenotype of glucagon-like peptide 1 neurons in the caudal nucleus of the solitary tract in rats. *Brain structure & function*. 2014.
10. Holt MK, Richards JE, Cook DR, Brierley DI, Williams DL, Reimann F, Gribble FM, Trapp S. Preproglucagon Neurons in the Nucleus of the Solitary Tract Are the Main Source of Brain GLP-1, Mediate Stress-Induced Hypophagia, and Limit Unusually Large Intakes of Food. *Diabetes*. 2019;68(1):21-33.
11. Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J Comp Neurol*. 1999;403(2):261-280.
12. Llewellyn-Smith IJ, Gnanamanickam GJ, Reimann F, Gribble FM, Trapp S. Preproglucagon (PPG) neurons innervate neurochemically identified autonomic neurons in the mouse brainstem. *Neuroscience*. 2013;229:130-143.
13. Llewellyn-Smith IJ, Reimann F, Gribble FM, Trapp S. Preproglucagon neurons project widely to autonomic control areas in the mouse brain. *Neuroscience*. 2011;180:111-121.

14. Llewellyn-Smith IJ, Marina N, Manton RN, Reimann F, Gribble FM, Trapp S. Spinally projecting preproglucagon axons preferentially innervate sympathetic preganglionic neurons. *Neuroscience*. 2015;284:872-887.
15. Zheng H, Lopez-Ferreras L, Krieger JP, Fasul S, Cea Salazar V, Valderrama Pena N, Skibicka KP, Rinaman L. A Cre-driver rat model for anatomical and functional analysis of glucagon (Gcg)-expressing cells in the brain and periphery. *Molecular metabolism*. 2022;66:101631.
16. Thiebaud N, Gribble F, Reimann F, Trapp S, Fadool DA. A unique olfactory bulb microcircuit driven by neurons expressing the precursor to glucagon-like peptide 1. *Sci Rep*. 2019;9(1):15542.
17. Thiebaud N, Llewellyn-Smith IJ, Gribble F, Reimann F, Trapp S, Fadool DA. The incretin hormone glucagon-like peptide 1 increases mitral cell excitability by decreasing conductance of a voltage-dependent potassium channel. *J Physiol*. 2016;594(10):2607-2628.
18. Montaner M, Denom J, Jiang W, Magnan C, Trapp S, Gurden H. The local GLP-1 system in the olfactory bulb is required for odor-evoked cephalic phase of insulin release in mice. *Molecular metabolism*. 2023;73:101738.
19. Montaner M, Denom J, Simon V, Jiang W, Holt MK, Brierley DI, Rouch C, Foppen E, Kassis N, Jarriault D, Khan D, Eygret L, Mifsud F, Hodson DJ, Broichhagen J, Van Oudenhove L, Fioramonti X, Gault V, Cota D, Reimann F, Gribble FM, Migrenne-Li S, Trapp S, Gurden H, Magnan C. A neuronal circuit driven by GLP-1 in the olfactory bulb regulates insulin secretion. *Nature communications*. 2024;15(1):6941.
20. Dossat AM, Lilly N, Kay K, Williams DL. Glucagon-like peptide 1 receptors in nucleus accumbens affect food intake. *J Neurosci*. 2011;31(41):14453-14457.
21. Vrang N, Hansen M, Larsen PJ, Tang-Christensen M. Characterization of brainstem preproglucagon projections to the paraventricular and dorsomedial hypothalamic nuclei. *Brain Res*. 2007;1149:118-126.
22. Alhadeff AL, Rupprecht LE, Hayes MR. GLP-1 Neurons in the Nucleus of the Solitary Tract Project Directly to the Ventral Tegmental Area and Nucleus Accumbens to Control for Food Intake. *Endocrinology*. 2012;153(2):647-658.
23. Williams DL, Lilly NA, Edwards IJ, Yao P, Richards JE, Trapp S. GLP-1 action in the mouse bed nucleus of the stria terminalis. *Neuropharmacology*. 2018;131:83-95.
24. Randolph AB, Zheng H, Rinaman L. Populations of Hindbrain Glucagon-Like Peptide 1 (GLP1) Neurons That Innervate the Hypothalamic PVH, Thalamic PVT, or Limbic Forebrain BST Have Axon Collaterals That Reach All Central Regions Innervated by GLP1 Neurons. *J Neurosci*. 2024;44(31).
25. Barrera JG, Jones KR, Herman JP, D'Alessio DA, Woods SC, Seeley RJ. Hyperphagia and increased fat accumulation in two models of chronic CNS glucagon-like peptide-1 loss of function. *J Neurosci*. 2011;31(10):3904-3913.
26. Wan S, Browning KN, Travagli RA. Glucagon-like peptide-1 modulates synaptic transmission to identified pancreas-projecting vagal motoneurons. *Peptides*. 2007;28(11):2184-2191.
27. Holmes GM, Browning KN, Tong M, Qualls-Creekmore E, Travagli RA. Vagally mediated effects of glucagon-like peptide 1: in vitro and in vivo gastric actions. *J Physiol*. 2009;587(Pt 19):4749-4759.
28. Card JP, Johnson AL, Llewellyn-Smith IJ, Zheng H, Anand R, Brierley DI, Trapp S, Rinaman L. GLP-1 neurons form a local synaptic circuit within the rodent nucleus of the solitary tract. *J Comp Neurol*. 2018;526(14):2149-2164.
29. Reimann F, Habib AM, Tolhurst G, Parker HE, Rogers GJ, Gribble FM. Glucose sensing in L cells: a primary cell study. *Cell Metab*. 2008;8(6):532-539.
30. Hisadome K, Reimann F, Gribble FM, Trapp S. Leptin directly depolarizes preproglucagon neurons in the nucleus tractus solitarius: electrical properties of glucagon-like Peptide 1 neurons. *Diabetes*. 2010;59(8):1890-1898.

31. Parker HE, Adriaenssens A, Rogers G, Richards P, Koepsell H, Reimann F, Gribble FM. Predominant role of active versus facilitative glucose transport for glucagon-like peptide-1 secretion. *Diabetologia*. 2012;55(9):2445-2455.
32. Gaykema RP, Newmyer BA, Ottolini M, Raje V, Warthen DM, Lambeth PS, Niccum M, Yao T, Huang Y, Schulman IG, Harris TE, Patel MK, Williams KW, Scott MM. Activation of murine pre-proglucagon-producing neurons reduces food intake and body weight. *J Clin Invest*. 2017;127:1031-1045.
33. Shiota C, Prasadan K, Guo P, Fusco J, Xiao X, Gittes GK. Gcg (CreERT2) knockin mice as a tool for genetic manipulation in pancreatic alpha cells. *Diabetologia*. 2017;60(12):2399-2408.
34. Ly T, Oh JY, Sivakumar N, Shehata S, La Santa Medina N, Huang H, Liu Z, Fang W, Barnes C, Dundar N, Jarvie BC, Ravi A, Barnhill OK, Li C, Lee GR, Choi J, Jang H, Knight ZA. Sequential appetite suppression by oral and visceral feedback to the brainstem. *Nature*. 2023;624(7990):130-137.
35. Bryda EC. The Mighty Mouse: the impact of rodents on advances in biomedical research. *Mo Med*. 2013;110(3):207-211.
36. Zac-Varghese S, Trapp S, Richards P, Sayers S, Sun G, Bloom SR, Reimann F, Gribble FM, Rutter GA. The Peutz-Jeghers kinase LKB1 suppresses polyp growth from intestinal cells of a proglucagon-expressing lineage in mice. *Disease models & mechanisms*. 2014;7(11):1275-1286.
37. Montaner M, Denom J, Jiang W, Magnan C, Trapp S, Gurden H. The local GLP-1 system in the olfactory bulb is required for odor-evoked cephalic phase of insulin release in mice. *Molecular metabolism*. 2023:101738.
38. Trapp S, Brierley DI. Brain GLP-1 and the regulation of food intake: GLP-1 action in the brain and its implications for GLP-1 receptor agonists in obesity treatment. *Br J Pharmacol*. 2022;179(4):557-570.
39. Brierley DI, Holt MK, Singh A, de Araujo A, McDougle M, Vergara M, Afaghani MH, Lee SJ, Scott K, Maske C, Langhans W, Krause E, de Kloet A, Gribble FM, Reimann F, Rinaman L, de Lartigue G, Trapp S. Central and peripheral GLP-1 systems independently suppress eating. *Nat Metab*. 2021;3(2):258-273.
40. Rinaman L. Interoceptive stress activates glucagon-like peptide-1 neurons that project to the hypothalamus. *Am J Physiol*. 1999;277(2 Pt 2):R582-590.
41. Hisadome K, Reimann F, Gribble FM, Trapp S. CCK Stimulation of GLP-1 Neurons Involves {alpha}1-Adrenoceptor-Mediated Increase in Glutamatergic Synaptic Inputs. *Diabetes*. 2011;60(11):2701-2709.
42. Williams DL. The diverse effects of brain glucagon-like peptide 1 receptors on ingestive behaviour. *Br J Pharmacol*. 2021.
43. Kabahizi A, Wallace B, Lieu L, Chau D, Dong Y, Hwang ES, Williams KW. Glucagon-like peptide-1 (GLP-1) signalling in the brain: From neural circuits and metabolism to therapeutics. *Br J Pharmacol*. 2022;179(4):600-624.
44. Orskov C, Poulsen SS, Moller M, Holst JJ. Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes*. 1996;45(6):832-835.
45. Punjabi M, Arnold M, Geary N, Langhans W, Pacheco-Lopez G. Peripheral glucagon-like peptide-1 (GLP-1) and satiation. *Physiol Behav*. 2011;105(1):71-76.
46. Zhang T, Perkins MH, Chang H, Han W, de Araujo IE. An inter-organ neural circuit for appetite suppression. *Cell*. 2022;185(14):2478-2494 e2428.
47. Song Y, Koehler JA, Baggio LL, Powers AC, Sandoval DA, Drucker DJ. Gut-Proglucagon-Derived Peptides Are Essential for Regulating Glucose Homeostasis in Mice. *Cell Metab*. 2019;30(5):976-986 e973.

48. Turton MD, O'Shea D, Gunn I, Beak SA, Edwards CM, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JP, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature*. 1996;379(6560):69-72.
49. Terrill SJ, Holt MK, Maske CB, Abrams N, Reimann F, Trapp S, Williams DL. Endogenous GLP-1 in lateral septum promotes satiety and suppresses motivation for food in mice. *Physiol Behav*. 2019;206:191-199.
50. Terrill SJ, Maske CB, Williams DL. Endogenous GLP-1 in lateral septum contributes to stress-induced hypophagia. *Physiol Behav*. 2018;192:17-22.
51. Dossat AM, Diaz R, Gallo L, Panagos A, Kay K, Williams DL. Nucleus accumbens GLP-1 receptors influence meal size and palatability. *Am J Physiol Endocrinol Metab*. 2013;304(12):E1314-1320.
52. Holt MK, Cook DR, Brierley DI, Richards JE, Reimann F, Gourine AV, Marina N, Trapp S. PPG neurons in the nucleus of the solitary tract modulate heart rate but do not mediate GLP-1 receptor agonist-induced tachycardia in mice. *Molecular metabolism*. 2020;39:101024.
53. Kreisler AD, Rinaman L. Hindbrain glucagon-like peptide-1 neurons track intake volume and contribute to injection stress-induced hypophagia in meal-entrained rats. *Am J Physiol Regul Integr Comp Physiol*. 2016;310(10):R906-916.
54. Vrang N, Phifer CB, Corkern MM, Berthoud HR. Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. *Am J Physiol Regul Integr Comp Physiol*. 2003;285(2):R470-478.
55. Kreisler AD, Davis EA, Rinaman L. Differential activation of chemically identified neurons in the caudal nucleus of the solitary tract in non-entrained rats after intake of satiating vs. non-satiating meals. *Physiol Behav*. 2014;136:47-54.
56. Bai L, Mesgarzadeh S, Ramesh KS, Huey EL, Liu Y, Gray LA, Aitken TJ, Chen Y, Beutler LR, Ahn JS, Madisen L, Zeng H, Krasnow MA, Knight ZA. Genetic Identification of Vagal Sensory Neurons That Control Feeding. *Cell*. 2019;179(5):1129-1143 e1123.
57. Krieger JP, Arnold M, Pettersen KG, Lossel P, Langhans W, Lee SJ. Knockdown of GLP-1 Receptors in Vagal Afferents Affects Normal Food Intake and Glycemia. *Diabetes*. 2016;65(1):34-43.
58. Leon RM, Borner T, Stein LM, Urrutia NA, De Jonghe BC, Schmidt HD, Hayes MR. Activation of PPG neurons following acute stressors differentially involves hindbrain serotonin in male rats. *Neuropharmacology*. 2021;187:108477.
59. Holt MK, Pomeranz LE, Beier KT, Reimann F, Gribble FM, Rinaman L. Synaptic inputs to the mouse dorsal vagal complex and its resident preproglucagon neurons. *J Neurosci*. 2019.
60. Holt MK, Llewellyn-Smith IJ, Reimann F, Gribble FM, Trapp S. Serotonergic modulation of the activity of GLP-1 producing neurons in the nucleus of the solitary tract in mouse. *Molecular metabolism*. 2017;6(8):909-921.
61. Wagner S, Brierley DI, Leeson-Payne A, Jiang W, Chianese R, Lam BYH, Dowsett GKC, Cristiano C, Lyons D, Reimann F, Gribble FM, Martinez de Morentin PB, Yeo GSH, Trapp S, Heisler LK. Obesity medication lorcaserin activates brainstem GLP-1 neurons to reduce food intake and augments GLP-1 receptor agonist induced appetite suppression. *Molecular metabolism*. 2023;68:101665.
62. Maniscalco JW, Rinaman L. Interoceptive modulation of neuroendocrine, emotional, and hypophagic responses to stress. *Physiol Behav*. 2017;176:195-206.
63. Kinzig KP, D'Alessio DA, Herman JP, Sakai RR, Vahl TP, Figueiredo HF, Murphy EK, Seeley RJ. CNS glucagon-like peptide-1 receptors mediate endocrine and anxiety responses to interoceptive and psychogenic stressors. *J Neurosci*. 2003;23(15):6163-6170.
64. Zhang R, Packard BA, Tauchi M, D'Alessio DA, Herman JP. Glucocorticoid regulation of preproglucagon transcription and RNA stability during stress. *Proc Natl Acad Sci U S A*. 2009;106(14):5913-5918.

65. Anesten F, Holt MK, Schele E, Palsdottir V, Reimann F, Gribble FM, Safari C, Skibicka KP, Trapp S, Jansson JO. Preproglucagon neurons in the hindbrain have IL-6 receptor-alpha and show Ca2+ influx in response to IL-6. *Am J Physiol Regul Integr Comp Physiol*. 2016;311(1):R115-123.
66. Maniscalco JW, Zheng H, Gordon PJ, Rinaman L. Negative Energy Balance Blocks Neural and Behavioral Responses to Acute Stress by "Silencing" Central Glucagon-Like Peptide 1 Signaling in Rats. *J Neurosci*. 2015;35(30):10701-10714.
67. Cheng W, Ndoka E, Hutch C, Roelofs K, MacKinnon A, Khoury B, Magrisso J, Kim KS, Rhodes CJ, Olson DP, Seeley RJ, Sandoval D, Myers MG, Jr. Leptin receptor-expressing nucleus tractus solitarius neurons suppress food intake independently of GLP1 in mice. *JCI Insight*. 2020;5(7).
68. Liu J, Conde K, Zhang P, Lilascharoen V, Xu Z, Lim BK, Seeley RJ, Zhu JJ, Scott MM, Pang ZP. Enhanced AMPA Receptor Trafficking Mediates the Anorexigenic Effect of Endogenous Glucagon-like Peptide-1 in the Paraventricular Hypothalamus. *Neuron*. 2017.
69. Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat Med*. 1996;2(11):1254-1258.
70. Williams DL, Baskin DG, Schwartz MW. Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety. *Endocrinology*. 2009;150(4):1680-1687.
71. Shi X, Chacko S, Li F, Li D, Burrin D, Chan L, Guan X. Acute activation of GLP-1-expressing neurons promotes glucose homeostasis and insulin sensitivity. *Molecular metabolism*. 2017;6(11):1350-1359.
72. Kinzig KP, D'Alessio DA, Seeley RJ. The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. *J Neurosci*. 2002;22(23):10470-10476.
73. Holt MK, Valderrama N, Polanco MJ, Hayter I, Badenoch EG, Trapp S, Rinaman L. Modulation of stress-related behaviour by preproglucagon neurons and hypothalamic projections to the nucleus of the solitary tract. *Molecular metabolism*. 2024;91:102076.
74. Lopez-Ferreras L, Eerola K, Shevchouk OT, Richard JE, Nilsson FH, Jansson LE, Hayes MR, Skibicka KP. The supramammillary nucleus controls anxiety-like behavior; key role of GLP-1R. *Psychoneuroendocrinology*. 2020;119:104720.
75. Anderberg RH, Richard JE, Hansson C, Nissbrandt H, Bergquist F, Skibicka KP. GLP-1 is both anxiogenic and antidepressant; divergent effects of acute and chronic GLP-1 on emotionality. *Psychoneuroendocrinology*. 2016;65:54-66.
76. Ghosal S, Packard AEB, Mahbod P, McKlveen JM, Seeley RJ, Myers B, Ulrich-Lai Y, Smith EP, D'Alessio DA, Herman JP. Disruption of Glucagon-Like Peptide 1 Signaling in Sim1 Neurons Reduces Physiological and Behavioral Reactivity to Acute and Chronic Stress. *Journal of Neuroscience*. 2017;37(1):184-193.
77. Ghosal S, Myers B, Herman JP. Role of central glucagon-like peptide-1 in stress regulation. *Physiol Behav*. 2013;122:201-207.
78. Tuesta LM, Chen Z, Duncan A, Fowler CD, Ishikawa M, Lee BR, Liu XA, Lu Q, Cameron M, Hayes MR, Kamenecka TM, Pletcher M, Kenny PJ. GLP-1 acts on habenular avoidance circuits to control nicotine intake. *Nat Neurosci*. 2017;20(5):708-716.
79. Applebey SV, Xiao AG, Harris EP, Levine C, Belser DL, Geisler CE, Parent MB, Bangasser DA, Crist RC, Reiner BC, Hayes MR. Characterizing Brainstem GLP-1 Control of Sensory-Specific Satiety in Male and Female Rats Across the Estrous Cycle. *Biological psychiatry*. 2025;98(3):249-259.
80. Lopez-Ferreras L, Asker M, Krieger JP, Skibicka KP. Sex-divergent effects of hindbrain GLP-1-producing neuron activation in rats. *Frontiers in neuroscience*. 2023;17:1265080.
81. Ludwig MQ, Cheng W, Gordian D, Lee J, Paulsen SJ, Hansen SN, Egerod KL, Barkholt P, Rhodes CJ, Secher A, Knudsen LB, Pyke C, Myers MG, Jr., Pers TH. A genetic map of the mouse dorsal vagal complex and its role in obesity. *Nat Metab*. 2021;3(4):530-545.

82. Gasparini S, Almeida-Pereira G, Munuzuri ASP, Resch JM, Geerling JC. Molecular Ontology of the Nucleus of Solitary Tract. *J Comp Neurol*. 2024;532(12):e70004.
83. Scott MM, Williams KW, Rossi J, Lee CE, Elmquist JK. Leptin receptor expression in hindbrain Glp-1 neurons regulates food intake and energy balance in mice. *J Clin Invest*. 2011;121(6):2413-2421.
84. Wang XF, Liu JJ, Xia J, Liu J, Mirabella V, Pang ZP. Endogenous Glucagon-like Peptide-1 Suppresses High-Fat Food Intake by Reducing Synaptic Drive onto Mesolimbic Dopamine Neurons. *Cell Rep*. 2015;12(5):726-733.
85. Trapp S, Cork SC. PPG neurons of the lower brain stem and their role in brain GLP-1 receptor activation. *Am J Physiol Regul Integr Comp Physiol*. 2015;309(8):R795-804.
86. Gabery S, Salinas CG, Paulsen SJ, Ahnfelt-Ronne J, Alanentalo T, Baquero AF, Buckley ST, Farkas E, Fekete C, Frederiksen KS, Helms HCC, Jeppesen JF, John LM, Pyke C, Nohr J, Lu TT, Polex-Wolf J, Prevot V, Raun K, Simonsen L, Sun G, Szilvasy-Szabo A, Willenbrock H, Secher A, Knudsen LB. Semaglutide lowers body weight in rodents via distributed neural pathways. *JCI Insight*. 2020;5(6).
87. Salinas CBG, Lu TT, Gabery S, Marstal K, Alanentalo T, Mercer AJ, Cornea A, Conradsen K, Hecksher-Sorensen J, Dahl AB, Knudsen LB, Secher A. Integrated Brain Atlas for Unbiased Mapping of Nervous System Effects Following Liraglutide Treatment. *Sci Rep*. 2018;8(1):10310.
88. Secher A, Jelsing J, Baquero AF, Hecksher-Sorensen J, Cowley MA, Dalboge LS, Hansen G, Grove KL, Pyke C, Raun K, Schaffer L, Tang-Christensen M, Verma S, Witgen BM, Vrang N, Bjerre Knudsen L. The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J Clin Invest*. 2014;124(10):4473-4488.
89. Adams JM, Pei H, Sandoval DA, Seeley RJ, Chang RB, Liberles SD, Olson DP. Liraglutide Modulates Appetite and Body Weight Through Glucagon-Like Peptide 1 Receptor-Expressing Glutamatergic Neurons. *Diabetes*. 2018;67(8):1538-1548.
90. D'Agostino G, Lyons D, Cristiano C, Lettieri M, Olarte-Sanchez C, Burke LK, Greenwald-Yarnell M, Cansell C, Doslikova B, Georgescu T, Martinez de Morentin PB, Myers MG, Jr., Rochford JJ, Heisler LK. Nucleus of the Solitary Tract Serotonin 5-HT2C Receptors Modulate Food Intake. *Cell Metab*. 2018;28(4):619-630 e615.
91. Burke LK, Ogunnowo-Bada E, Georgescu T, Cristiano C, de Morentin PBM, Valencia Torres L, D'Agostino G, Riches C, Heeley N, Ruan Y, Rubinstein M, Low MJ, Myers MG, Rochford JJ, Evans ML, Heisler LK. Lorcaserin improves glycemic control via a melanocortin neurocircuit. *Molecular metabolism*. 2017;6(10):1092-1102.

Figure Legend

Figure 1

Anatomical distribution of the preproglucagon (PPG) neuron populations, their known projections, regulation and functions. The cell bodies of PPG neurons are located in the olfactory bulb (OB, upper left), the piriform cortex (Pir, lower left) and the caudal nucleus tractus solitarii (NTS) and intermediate reticular nucleus (IRT) of the brainstem (right), here visualised in the Glu-Venus mouse model (13,29,30). In the OB, PPG neurons reside in the granule cell layer, and when stimulated increase foraging in mice. Little is known about the PPG neurons in the Pir, neither stimulus nor outputs have been characterized, yet. Most PPG neurons are found in the lower brainstem from where they project to a plethora of forebrain areas. This population is activated by gastric distension and various stimulus of stress, with hypophagia as the main physiological outcome. While the mechanisms driving the hypophagia have not been fully

elucidated, it has been suggested that activation of these PPG neurons supresses reward circuitry contributing to the reduction in food intake. The involvement of brainstem PPG neurons, particularly NTS PPG neurons, has been suggested to induce a stress response, and at least an increase in heart rate has been demonstrated upon direct activation of these neurons. AP, area postrema; Arc, arcuate nucleus; Barr, Barrington's nucleus; BNST, bed nucleus of the stria terminalis; DMH, dorsomedial hypothalamus; DMX, dorsal motor nucleus of the vagus; IRT, intermediate reticular nucleus; LC, locus coeruleus; LS, lateral septum; MN, mammillary nucleus; NAc, nucleus accumbens; NTS, nucleus tractus solitarii; OB, olfactory bulb; OVLT, organum vasculosum of the lamina terminalis; PAG, periaqueductal grey; PBN, parabrachial nucleus; Pir, Piriform cortex; PVH, paraventricular nucleus of the hypothalamus; PVT, paraventricular thalamus; RPa, raphe pallidus; SFO, subfornical organ; VLM, ventrolateral medulla; VMH, ventromedial hypothalamus. Scale bars: 250μm for main images; 40 μm for inserts.

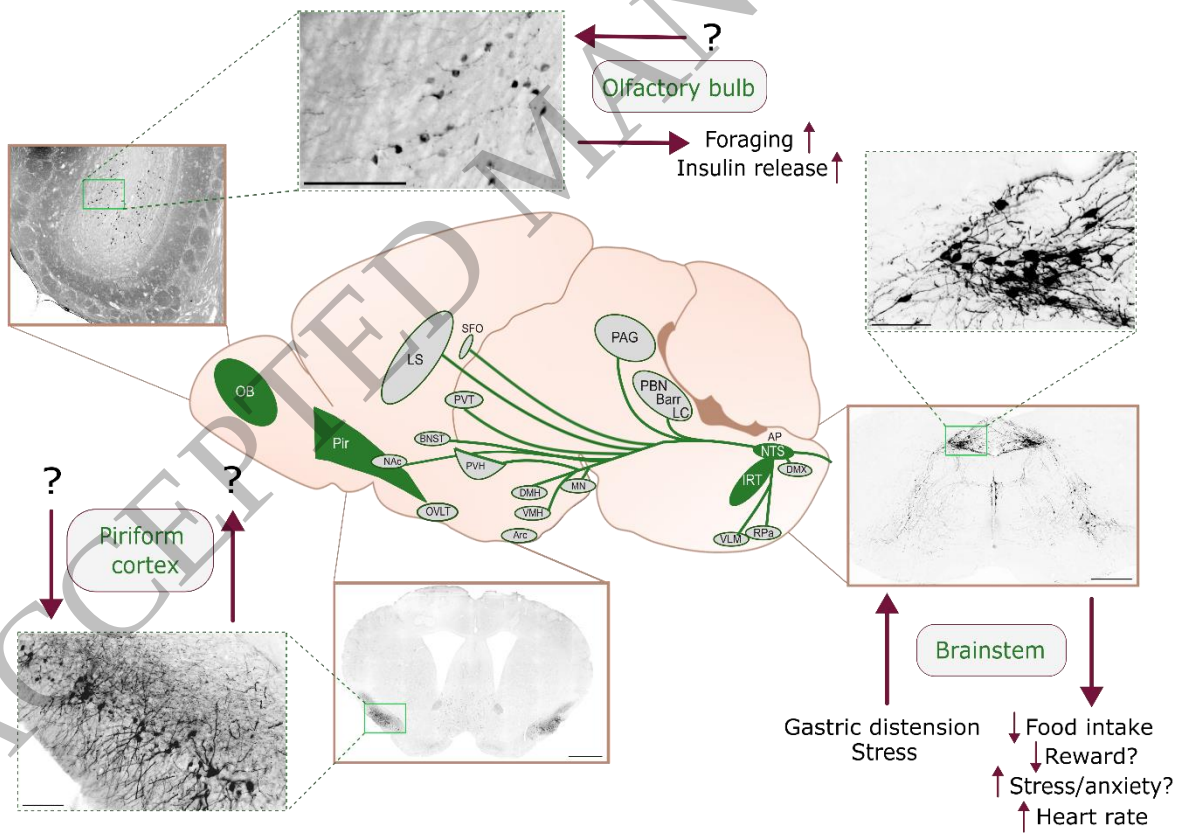

Figure 1
159x138 mm (DPI)