Mind affects matter: hindbrain GLP1 neurons link stress, physiology, and behaviour

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1. What is the topic of this review?

This Lecture covers the role of caudal brainstem GLP1 neurons in acute and chronic stress responses.

2. What advances does it highlight?

This Lecture focusses on the recent advances in our understanding of GLP1 neurons and their physiological role in many aspects of stress. Particular focus is given to the recently partly elucidated anatomical basis for recruitment of GLP1 neurons in response to acute stress. Finally, the potential, but at this time somewhat speculative, role of GLP1 neurons in chronic stress is discussed.

Abstract

The brain responds rapidly to stressful stimuli by increasing sympathetic outflow, activating the hypothalamic-pituitary-adrenal (HPA) axis, and eliciting avoidance behaviours to limit risks to safety. Stress responses are adaptive and essential but can become maladaptive when the stress is chronic, causing autonomic imbalance, HPA axis hyperreactivity, and a state of hypervigilance. Ultimately, this contributes to the development of cardiovascular disease and affective disorders, including major depression and anxiety. Stress responses are often thought to be driven mainly by forebrain areas, however the brainstem nucleus of the solitary tract (NTS) is ideally located to control both autonomic outflow and behaviour in response to stress. Here I review the preclinical evidence that the NTS and its resident glucagon-like peptide-1 (GLP1)-expressing neurons are prominent mediators of stress responses. This Lecture introduces the reader to the idea of good and bad stress and outlines the types of stress that engage the NTS and GLP1 neurons. I describe in particular detail recent studies by us and others aimed at mapping sources of synaptic inputs to GLP1 neurons and consider the implications for our understanding of the role of GLP1 neurons in stress. This is followed by a discussion of the contribution of brain GLP1 and GLP1 neurons to behavioural and physiological stress responses. The evidence reviewed highlights a potentially prominent role for GLP1 neurons in the brain's response to acute stress and reveals important unanswered questions regarding their role in chronic stress.

1. Introduction and scope of review

Our brain continuously monitors the health and function of our body and rapidly responds to potential threats by eliciting changes in physiology and behaviour (Holt & Trapp, 2016; Ulrich-Lai & Herman, 2009). These processes are driven by corticolimbic, hypothalamic, and brainstem circuits, with the caudal nucleus of the solitary tract (cNTS) in the medulla playing a key role (Herman, 2018). Within the cNTS, a relatively small population of neurons, characterised by their expression of glucagon-like peptide-1 (GLP1), has potentially wide-reaching impact on the brain's response to stress (Ghosal et al., 2013; Holt & Trapp, 2016). In this Lecture I first outline how we may understand the term 'stress' and how the brain orchestrates physiological and behavioural responses to stressful stimuli. I then introduce the reader to the cNTS and GLP1 neurons. Building on that I consider three key questions:

1) What is the pre-clinical evidence that the cNTS and GLP1 neurons are integral to the modulation of stress responses?
2) What are the upstream circuits that drive GLP1 neuron activation in response to stress?
3) Finally, is there any evidence that GLP1 neuron function may be altered under conditions of chronic stress?

Naturally, our aim is to understand stress-related disorders in humans, often elicited not by exposure to a single, severely stressful event, but rather constant exposure to mild stressors. While some animal studies use chronic stress paradigms (and some of these reasonably recapitulate human stress, see section 5), most animal studies involve a single, severe stressor. One might pose that researchers are mistaken in focussing on *acute* stress that elicits protective, adaptive changes, rather than *chronic* stress, which more accurately mimics the human condition. However, I argue, it is essential we understand normal adaptive changes in response to stress, in the same way that we must study normal cellular signalling to better understand the dysregulation of cell proliferation that occurs in cancer or the failure of insulin signalling in type II diabetes. Furthermore, there is a rich literature on human responses to stress, but so far circuit level, causal evidence can only be obtained using animal models. Indeed, novel neuroscience techniques, including opto- and chemogenetics as well as virally mediated transsynaptic tracing have greatly facilitated cell-type and circuit-specific investigations of stress-responsive circuits in rodents. Some of these advances will be discussed below.

2. Stress: the good, the tolerable, and the toxic

The word “stress” carries, for most, a heavy negative connotation. However, it is a muddled term and before delving into the intricacies of neural stress modulation, we do best to attempt to define and qualify it. ‘Stress’ is the bodily and emotional response to adverse stimuli, so-called stressors, and can be divided into three, more easily digestible, subcategories [see (McEwen, 2017; Yoshikawa et al., 2007)]. First, e*ustress*, or good stress, which includes all the little challenges we experience throughout our lives, that make us stronger and more resilient and able to face novel challenges in the future. We face these challenges head on and they have, generally, positive outcomes. Second, *tolerable stress* is the type of stress that occurs when bad things happen, but we cope and recover, often with the help of friends and loved ones. Finally, *toxic stress* covers the stress we are unable to cope with and which has detrimental effects on our health and well-being. Importantly, the same stressor may elicit eustress, tolerable stress, or toxic stress in different individuals or in the same individual when circumstances change. The level of stress experienced by the individual depends on innate (genetic), learned (determined by previous experiences), and external social factors (your available buffers and your overall feeling of security and safety) (McEwen, 2017). This is recapitulated in animal models: from preclinical animal studies we know that prairie voles, a highly social species, are better able to cope with stress in the presence of their pair-bonded mate (Smith & Wang, 2014). Similarly, in the same way that intake of comfort foods (high in fat and sugar) can alleviate negative emotions in humans (Dubé et al., 2005), intake of palatable foods effectively buffers responses to stress in rats (Ulrich-Lai et al., 2010).

In addition to classifying stress based on the perceived severity, we must also distinguish between *acute* and *chronic* stressors as briefly mentioned above. Acute stress constitutes the organism's response to an immediate actual or perceived threat to homeostasis, while chronic stress is elicited by repeated and unrelenting stressors, and although they can be mild in principle, the cumulative impact is severe. Whereas the organism's response to *acute* stressors is adaptive and promotes survival, *chronic* stress is often *toxic* when adaptive responses become either insufficient or maladaptive.

Finally, we can categorise acute stressors according to their origin and primary signalling pathways. *Interoceptive* stressors are actual threats to homeostasis, the carefully maintained internal state of the body. Examples include blood loss, exposure to toxic substances, and acute illness. Interoceptive stress responses originate outside the brain and are driven initially by the immune, endocrine, and peripheral nervous systems, which act both locally and relay signals of stress to the brain (Berntson & Khalsa, 2021). Psychogenic stressors, on the other hand, comprise *perceived* (real or imagined) threats to the well-being of the organism. Examples include predator exposure and social rejection. In contrast with interoceptive stressors, psychogenic stressors originate externally and are initially processed by the brain. While these starting points are distinct, both types of stressors engage partially overlapping neural circuits to achieve adaptive changes in physiology and behaviour: an increase in sympathetic outflow, activation of the hypothalamic pituitary adrenal (HPA) axis, and an increase in avoidance behaviours (McEwen et al., 2015). These rapid processes allow the body to prioritise defensive behaviours and mobilise glucose while also initiating slower endocrine feedback. This slower endocrine response driven by glucocorticoids limits the duration of the stress response to facilitate recovery and also serves to drive genetic and structural changes in the brain's stress circuits (McEwen et al., 2015).

In the next sections of this Lecture, I focus on how the brain orchestrates responses to *acute* stressors, and how GLP1-producing neurons contribute. This will be followed by a brief discussion on how these adaptive processes may become maladaptive under chronic stress conditions.

3. The caudal nucleus of the solitary tract – an integrative hub for multimodal sensory inputs

The behavioural and physiological responses to acute stress are modulated by distributed circuits within the brain. In addition to corticolimbic and subcortical hypothalamic structures, acute stressors engage regions in the lower brainstem, including the rostroventrolateral medulla, the raphe magnus, and the cNTS (Cullinan et al., 1995). For an integrative physiologist, the cNTS is particularly interesting due to its anatomy: it is the entry point of the afferent vagus nerve and receives sensory input from the spinal cord, mechanosensory input from baroreceptor afferents via the vagus and glossopharyngeal nerves, as well as direct and indirect hormonal input (Housley et al., 1987; Maniscalco & Rinaman, 2018). In addition, the cNTS receives input from a range of brain regions, some of which are activated in response to acute stress (Holt, Pomeranz, et al., 2019). The cNTS in turn sends projections throughout the brain to regions involved in autonomic regulation and motivated behaviour, including the paraventricular nucleus of the hypothalamus (PVN), bed nucleus of the stria terminalis, central amygdala, parabrachial nucleus, and dorsomedial hypothalamus (Rinaman, 2010). This anatomical configuration makes the cNTS ideally positioned to integrate multimodal signals relating to the physiological and emotional state of the organism. Indeed, the cNTS is robustly activated following both interoceptive stressors [lithium chloride (Rinaman, 1999), lipopolysaccharide (Rinaman, 1999), hypoxia (Teppema et al., 1997), loss of blood volume (Dun et al., 1993)] and psychogenic stressors [forced swimming (Cullinan et al., 1995), restraint (Cullinan et al., 1995), and predator odour (Day et al., 2004)]. However, the cNTS is heterogenous, made up of diverse cell types, and not all of these cell populations can reasonably be assumed to mediate stress responses. With modern neuroscience tools we could now take an unbiased approach to identify cell types involved in stress responding. The cFOS-TRAP mouse model (DeNardo et al., 2019) would allow genetic 'capturing' of cells activated in response to a stressful stimulus. In this model, tamoxifen-inducible Cre recombinase is expressed under the control of the *Fos* promoter. When a cell is activated in response to stress in the presence of tamoxifen, Cre is expressed and enters the nucleus where it facilitates the expression of a fluorescent protein through excision of a stop site. This permanently labels any cell activated in response to stress. Trapped cells from the NTS could then be isolated and characterised using single-nucleus or single-cell RNAseq revealing all the cell types in the NTS that are activated following stress. Until these data become available, traditional double-labelling for specific cell markers and cFOS following stress exposure can get us some of the way. These types of investigations have led to identification of two distinct populations of stress-responsive cNTS neurons: tyrosine hydroxylase-expressing A2 neurons and GLP1-expressing neurons (Holt & Rinaman, 2021). The latter population is the focus of this review and the next sections will introduce the reader to the anatomy and function of GLP1 neurons and review evidence that they drive multiple aspects of acute stress responses.

3.1 The cNTS is the main source of GLP1 in the brain

GLP1 is expressed from the glucagon (*Glu*) gene along with a number of other peptides (Fig. 1A). Tissue-specific post-translational processing of the proglucagon peptide leads to expression of either glucagon (in α cells in the pancreas) or GLP1 (in neural tissue and L-cells of the gut) (Fig. 1A) (Müller et al., 2019). Within the brain, GLP1 is expressed in the cNTS and intermediate reticular nucleus (IRT) of the caudal brainstem as well as in the olfactory bulb (Fig.

1B-D) (Merchenthaler et al., 1999). GLP1 neurons in the olfactory bulb are interneurons and as such do not project outside the bulb (Merchenthaler et al., 1999). GLP1 derived from L-cells is generally accepted to not reach brain GLP1 receptors under normal physiological conditions (Trapp & Cork, 2015). Peripheral GLP1 levels may be elevated following Roux-en-Y gastric bypass, allowing it to reach receptors in the brain, but the normal rise in blood GLP1 levels following a meal appears not to be sufficient to reach the brain before it is broken down in the bloodstream (Punjabi et al., 2014). Based on this it has been presumed that, under physiological conditions, any GLP1 found in the brain (not including the olfactory bulb) originates from the brainstem population of GLP1 neurons. We recently confirmed that assumption experimentally: when GLP1 neurons in the cNTS were selectively ablated, the concentration of GLP1 was dramatically reduced in the hypothalamus (Holt, Richards, et al., 2019). Any remaining GLP1 most likely originates in the IRT, although this has yet to be confirmed experimentally.

3.2 The afferent and efferent connections of GLP1 neurons – a case for more than vagal relay?

GLP1 neurons are glutamatergic (Zheng, Stornetta, et al., 2015), suggesting their effect on downstream neurons is at least partially excitatory. The brainstem GLP1 system is highly conserved between species with largely similar distribution in mice (Hisadome et al., 2010; Llewellyn-Smith et al., 2011), rats (Larsen et al., 1997; Merchenthaler et al., 1999), non-human primates (Vrang & Grove, 2011), and humans (Zheng, Cai, et al., 2015). They project widely throughout the brain and spinal cord (Llewellyn-Smith et al., 2011, 2013, 2015), with target regions largely overlapping with the distribution of GLP1 receptor expression (Fig 1D) (Cork et al., 2015). Many of these regions are pre-autonomic, suggesting that GLP1 neurons may have the ability to modulate autonomic activity. The PVN is particularly densely innervated by GLP1 neurons in mice (Llewellyn-Smith et al., 2011) and rats (Larsen et al., 1997; Zheng, Stornetta, et al., 2015) and corticotropin-releasing hormone (CRH)-expressing neurons, the archetypal stress neurons, receive direct excitatory input from GLP1 neurons (Liu et al., 2017). When injected directly into the brain, GLP1 induces cFOS expression in many of the same brain regions, including the area postrema, NTS, parabrachial nucleus, arcuate nucleus, PVN, central amygdala, and bed nucleus of the stria terminalis (Rowland et al., 1997; Van Dijk et al., 1996).

As is typical for cNTS neurons, GLP1 neurons receive direct vagal input (Hisadome et al., 2010; Holt, Pomeranz, et al., 2019) and are robustly activated to express cFOS, a marker of recent neuronal activity, following stimuli which presumably stimulate afferent vagal fibres, including intraperitoneal injection of cholecystokinin and distension of the stomach (Rinaman, 1999; Vrang et al., 2003). As such, one might argue that these cells are ideally positioned to respond postprandially to meal-related signals derived from the gastrointestinal tract and induce satiation accordingly. This remains a prevailing idea and is likely to be part of their function (Brierley et al., 2021). However, work has shown that these cells are ‘reluctant’ to activate in response to ad libitum feeding and lack of GLP1 receptors or GLP1 signalling in the brain has limited impact on bodyweight and food intake in mice (Cheng et al., 2020; Holt, Richards, et al., 2019; Kreisler et al., 2014; Sisley et al., 2014), suggesting they are not crucial for appetite regulation under ad libitum feeding conditions in rodents, although this remains controversial with conflicting evidence available (Barrera et al., 2011; Hayes et al., 2009; Kreisler & Rinaman, 2016; Liu et al., 2017; Turton et al., 1996). On the other hand, large unanticipated meals (particularly in liquid form) do activate GLP1 neurons in mice and rats (Brierley et al., 2021; Holt, Richards, et al., 2019; Kreisler et al., 2014) with much larger intakes required to activate GLP1 neurons than the neighbouring A2 neurons (Kreisler et al., 2014). One interpretation of this is that GLP1 neurons are part of a secondary satiation pathway that only becomes important following large meals. Another, perhaps more controversial notion, is that GLP1 neurons are mainly engaged following stress and that intake of a very large, unanticipated meal is a form of interoceptive stress. This latter point has been argued based on the sudden disruption in glucose homeostasis and body temperature following intake of large meals (Woods, 1992). Based on the available evidence, can we reasonably conclude that GLP1 neurons may not modulate normal ad libitum feeding, but *only* become physiologically important when the body meets a stressor? Perhaps not, but there is some evidence to support the idea. The most robust activation of GLP1 neurons (up to 80% of the population expressing cFOS) is seen following exposure to a range of more archetypal stressful stimuli: lithium chloride, immune challenge, and restraint (Holt & Rinaman, 2021; Holt & Trapp, 2016).

It seems reasonable to assume that this stress-induced activation of GLP1 neurons is triggered by multiple upstream circuits, however the identity of those inputs remains mostly unknown. We recently set out to map the inputs to the mouse dorsal vagal complex (DVC, comprising the area postrema, the dorsal motor nucleus of the vagus, and the NTS), along with the specific circuits terminating on GLP1 neurons in the cNTS (Holt, Pomeranz, et al., 2019). To this end we used conventional and virally-mediated retrograde tracing (Fig 2A,B). We created a map of poly- and monosynaptic inputs to GLP1, which can now be used to generate hypotheses regarding the modulation of GLP1 neuron activity. Areas of direct input to GLP1 neurons are shown in Fig 2C. Many of those same areas are activated to express cFOS following acute stress (Holt, Pomeranz, et al., 2019). By combining conventional retrograde tracing with cFOS immunolabelling we were able to show that DVC-projecting neurons in the PVN, the lateral hypothalamus, and Barrington’s nucleus are activated in mice following restraint stress (Fig 2D), suggesting these regions could be responsible for stress-induced activation of GLP1 neurons. However, we did not confirm that those stress-activated DVC-projecting neurons synapse directly onto GLP1 neurons and it remains to be experimentally confirmed that input from those regions is *necessary* for stress-induced activation of GLP1 neurons. A recent study by Leon and colleagues confirmed that multiple types of stressors (interoceptive and psychogenic) activate GLP1 neurons in rats and that this activation is mediated partly by 5-hydroxytryptamine (5-HT) receptor signalling (Leon et al., 2021). Intriguingly, the authors identify the raphe magnus as a potential driver of stress-induced activation of GLP1 neurons and also confirm our previous finding that GLP1 neurons receive monosynaptic input from the raphe magnus (Holt, Pomeranz, et al., 2019). Interestingly, different types of stressors were differentially dependent on 5-HT signalling for GLP1 neuron activation: while LiCl-induced activation of GLP1 neurons was abolished following blockade of 5-HT signalling, restraint stress-induced activation was only attenuated, suggesting that multiple pathways contribute to psychogenic stress-induced activation of these neurons (Leon et al., 2021). The origin of these additional pathways will be the subject of future investigations with potential candidates selected based on our current knowledge of the anatomical organisation of the cNTS and GLP1 neurons. As an example, the cNTS is densely innervated by oxytocin- and CRH-expressing terminals (Sawchenko & Swanson, 1982; Wang et al., 2018), both prominent stress-modulating neuropeptides. Interestingly, we did not find evidence that oxytocin-expressing neurons in the PVN provide input to GLP1 neurons (Holt, Pomeranz, et al., 2019). Conversely, CRH-expressing neurons in Barrington’s nucleus did project directly to GLP1 neurons in the cNTS and DVC-projecting Bar neurons were activated following acute stress (Fig 2D) (Holt, Pomeranz, et al., 2019), highlighting this neuronal population as a potential driver of stress-induced activation of GLP1 neurons, a hypothesis that should be tested experimentally.

Based on the evidence reviewed thus far, we can reasonably conclude the following: 1) GLP1 neurons are anatomically positioned to receive and modulate responses to stress; 2) GLP1 neurons are activated in response to multiple stressful stimuli; 3) the origin of those signals of stress remains largely unknown but may include serotonergic raphe magnus neurons and CRH-expressing neurons in Barrington’s nucleus. In the following section I briefly review the evidence that GLP1 drives behavioural and physiological responses to stress. For a detailed discussion of the evidence readers can refer to our recent review on the topic (Holt & Rinaman, 2021).

4. The wide-ranging effects of central GLP1 – a modulator of stress responses?

GLP1 acts on the GLP1 receptor, dense numbers of which are expressed throughout the brain (Cork et al., 2015). As a class B G-protein coupled receptor, the GLP1 receptor couples intracellularly to a stimulatory G protein leading to activation of adenylate cyclase and an increase in intracellular cAMP and calcium (Müller et al., 2019), although the exact intracellular signalling cascade has not been explored in detail in neurons. Stimulation of brain GLP1 receptors through delivery of either GLP1 or a GLP1 analogue, such as Exendin-4 or liraglutide, has wide-ranging behavioural and physiological impact. In two parallel landmark papers, the first behavioural effect of direct delivery of GLP1 to the rat brain was demonstrated: an immediate and robust suppression in feeding (Tang-Christensen et al., 1996; Turton et al., 1996). Since this initial discovery GLP1 or GLP1 analogues delivered directly to the brain have been found in rodents to increase heart rate, increase body temperature, activate the HPA axis, elicit nausea, promote gastric emptying, drive neuroprotection, decrease locomotion, and increase avoidance behaviours (Müller et al., 2019). Supraphysiological activation of GLP1 neurons using either chemo- or optogenetics recapitulates at least some of these effects (Fig. 3): decreased food intake (Brierley et al., 2021; Cheng et al., 2020; Gaykema et al., 2017; Holt, Richards, et al., 2019; Liu et al., 2017), increased heart rate (Holt et al., 2020), improved glucose handling (Shi et al., 2017), and decreased drug-induced reward (Tuesta et al., 2017). Interestingly, acute activation of GLP1 neurons had no effect on either anxiety-like behaviours or corticosterone levels in one study (Gaykema et al., 2017), an unexpected finding given the wealth of evidence that central GLP1 drives anxiety-like behaviours (Kinzig et al., 2003; López-Ferreras et al., 2020; Maniscalco et al., 2015; Zheng et al., 2019).

It may seem puzzling at first that a neuropeptide released from only a small population of neurons should have such wide-ranging physiological and behavioural effects (Fig 3). However, if we again consider GLP1 neurons as mediators of stress responses, this array of effects is perhaps less surprising. Indeed, we might expect a stress-modulating neuropeptide to drive avoidance behaviour, HPA axis activation, and sympathoexcitation. We might also put forward that for GLP1 to be physiologically relevant for stress modulation, responses to stressors should be attenuated by inhibiting GLP1 signalling, either through suppression of GLP1 neuron activity or through disruption of GLP1 receptor signalling. This is indeed the case: central delivery of a GLP1 receptor antagonist as well as chemogenetic inhibition of GLP1 neurons attenuates stress-induced hypophagia (Holt, Richards, et al., 2019; Maniscalco et al., 2015; Terrill et al., 2019), and knockout of GLP1 receptors in *Sim1* neurons in the PVN of mice prevents stress-induced increases in corticosterone release, anxiety-like behaviour, and sympathetic activity (Ghosal et al., 2017). We were surprised when we failed to find any effect on heart rate, blood pressure, and locomotion in mice with ablated GLP1 neurons, but importantly we did not test them under stressful conditions (Holt et al., 2020).

Taken together the evidence suggests that GLP1 neurons are activated in response to a wide range of stressful stimuli and in response elicit avoidance behaviours, sympathoexcitation, and HPA axis activation. What sets these cells apart from other neuronal populations with similar functional profiles (e.g. CRH neurons in the PVN and CeA, tyrosine hydroxylase neurons in the locus coeruleus) is their direct input from vagal afferents, allowing them to continuously monitor the energy balance state of the body and integrating this information with signals of stress (Brierley et al., 2021; Holt, Pomeranz, et al., 2019). Indeed, activation of GLP1 neurons in response to both interoceptive and psychogenic stress is dependent on the internal state of the animal. Following prolonged food deprivation (16 hours) restraint fails to activate GLP1 neurons above baseline and behavioural anxiety-like behaviours are attenuated (Maniscalco et al., 2015). At times of neutral or positive energy balance, stressful stimuli activate GLP1 neurons, which in turn promote avoidance behaviours, sympathetic activation, and HPA axis activation. On the other hand, when energy stores are low, GLP1 neurons are silenced, possibly as a result of increased levels of circulating ghrelin (Maniscalco et al., 2020), and are therefore unable to elicit stress responses. These findings could place GLP1 neurons at the centre of sensing competing needs.

Rapid and accurate prioritisation of the needs of the organism is essential to survival: when faced with an acute threat to well-being, the body must elicit effective adaptive responses. However, when faced with repeated, unrelenting stress, the brain's response may become maladaptive. In the next sections, I explore how chronic stress may impact the central GLP1 system.

5. Maladaptation of neural circuits following chronic stress

Chronic stress in humans contributes to the development of cardiovascular disease and affective disorders, including anxiety and major depression (McEwen & Akil, 2020). In rodents, chronic stress is induced through repeated delivery of stressors. Recently, a translationally relevant form of chronic stress is gaining traction as a research model: chronic social defeat stress (Golden et al., 2011). In this model, rodents are exposed to intermittent defeat sessions by placing them temporarily in a cage with a larger, dominant mouse. This results in profound changes in physiology and behaviour, including increased avoidance and sympathetic hyperactivity, reminiscent of changes observed in humans exposed to toxic stress. The translational validity of the chronic social defeat stress model is further supported by two observations, which recapitulate observations in humans with affective disorders: 1) the HPA axis continues to be activated in response to social defeat after weeks of stress; 2) animals undergoing social defeat are responsive to chronic, not acute, administration of antidepressants. The existence of translationally relevant rodent models for toxic stress allows us to delineate the underlying neural mechanisms in detail.

Neurobiologically, chronic stress leads to changes in receptor and transmitter expression as well as neuronal excitability and connectivity (McEwen, 2017). From a translational point of view, these changes harbour potential therapeutic targets for the prevention and treatment of affective disorders. In fact, the aim should not be to prevent adaptive responses to acute stressors, but rather to alleviate maladaptive changes to chronic stress. There is some, albeit limited, evidence that central GLP1 plays a role in chronic stress responses. Chronic delivery of a GLP1 receptor antagonist, dHG-Exendin, attenuates the chronic stress-induced facilitation of corticosterone release in response to a novel stressor, suggesting that central GLP1 contributes to hypersensitivity of the HPA axis following chronic stress (Tauchi et al., 2008). In addition, genetic knockout of GLP1 receptors in *Sim1* PVN neurons, attenuates bodyweight loss following chronic stress and rescues HPA axis hypersensitivity (Ghosal et al., 2017). Interestingly, GLP1 neurons are targets of feedback mechanisms following repeated exposure to stress: expression of GLP1 is downregulated following chronic stress in a corticosterone-dependent manner (Zhang et al., 2010).

6. Summary

The cNTS plays a key role in integrating multimodal sensory inputs, including signals of energy balance and stress. Within the cNTS, GLP1-expressing neurons are likely important mediators of both interoceptive and psychogenic stress responses (Fig 4). Their anatomical organisation, stress responsiveness, as well as the effects of central delivery of GLP1 on avoidance behaviour, cardiovascular parameters, and HPA axis activity indicate a potentially prominent role in stress response modulation. Limited, but tantalising evidence, points to a role for central GLP1 in chronic stress responses (Fig 4). However, these data do not address whether chronic stress-induced changes in GLP1 signalling are adaptive (promoting eustress or tolerable stress) or maladaptive (inducing toxic stress). Considering the ability of central GLP1 to elicit tachycardia as well as anxiety-like behaviour, future studies should investigate the role of GLP1 in avoidance behaviour and cardiovascular disruption following chronic stress. These investigations will be essential foundation building blocks for the development of GLP1-targeted therapies for stress-related behaviours.

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Figure legends

Fig 1. Expression of GLP1 and anatomical organisation of GLP1 neurons. A) Tissue-specific post-translational cleavage of proglucagon. The propeptide proglucagon is expressed from the *Glu* gene in pancreatic α-cells, enteroendocrine L-cells, and GLP1 neurons. Tissue-specific post-translation cleavage of proglucagon by prohormone convertase 2 (PC-2) releases glucagon, glicentin-related pancreatic polypeptide (CGRP), intervening peptide-1 (IP1), and major proglucagon fragment in pancreatic α-cells, while prohormone convertase 1/3 (PC-1/3) liberates GLP1, intervening peptide-2 (IP2), oxyntomodulin, GLP2, and CGRP in L-cells and GLP1 neurons. B) Immunolabelling for GLP1 in mouse NTS and IRT. Scale bar: 100µm. CC: central canal. C) Distribution of GLP1 neurons in the medulla. D) Distribution of GLP1 expression (OB, NTS, and IRT) and targets of efferent projections from brainstem GLP1 neurons in the mouse brain. OB: olfactory bulb, NAc: nucleus accumbens, LS: lateral septum, BST: bed nucleus of the stria terminalis, PVT: paraventricular nucleus of the thalamus, PVN: paraventricular nucleus of the hypothalamus, CeA: central amygdala, Arc: arcuate nucleus, DMH: dorsomedial hypothalamus, SuMM: supramammillary nucleus, VTA: ventral tegmental area, PAG: periaqueductal grey, Bar: Barrington’s nucleus, VLM: ventrolateral medulla, RPa: raphe pallidus, IRT: intermediate reticular nucleus, cNTS: caudal nucleus of the solitary tract, CAA: central autonomic area of the spinal cord, IML: intermediolateral cell column of the spinal cord.

Fig 2. Viral-mediated mapping of synaptic inputs to GLP1 neurons. A) Pseudorabies virus-mediated circuit mapping. PRV-introvert-GFP is injected into the NTS of Glu-Cre mice. The virus is taken up by locally projecting GLP1 neurons and Cre-mediated recombination allows for expression of GFP and propagation of the virus. PRV-Introvert-GFP then propagates retrogradely to upstream neurons with the number of synaptic crossings only limited by the post-infection interval. B) Rabies-mediated monosynaptic tracing. The EnvA-pseudotyped G-deleted rabies virus is unable to infect cells and propagate. Injection of helper AAVs leading to Cre-dependent expression of TVA:mCherry and RabiesG into Glu-Cre mice allows for infection of and propagation from GLP1 neurons only. Since first-order neurons do not express RabiesG, the virus is unable to spread to neurons further upstream, thus restricting the spread monosynaptically. C) Sources of monosynaptic input to GLP1 neurons in the mouse. BST: bed nucleus of the stria terminalis, PVN: paraventricular nucleus of the hypothalamus, CeA: central amygdala, LH: lateral hypothalamus, PSTh: parasubthalamic nucleus, lPAG: lateral periaqueductal grey, KF: Kölliker-Fuse nucleus Bar: Barrington's nucleus, RPa: raphe pallidus, RMg: raphe magnus, Gi: gigantocellular nucleus, IRT: intermediate reticular nucleus, AP: area postrema, NG: nodose ganglion, DH: dorsal horn of the spinal cord. D) Restraint stress activates DVC-projecting neurons in the lateral hypothalamus (LH), paraventricular nucleus of the hypothalamus (PVN), and Barrington's nucleus (Bar) in the mouse.

Figure 3. Physiological and behavioural effects of activating GLP1 neurons. Opto- or chemogenetic activation of GLP1 neurons increases neuronal firing and release of GLP1 and glutamate. A number of effects on physiology and behaviour have been reported. In addition, Gaykema et al. failed to detect any difference in glucose tolerance, anxiety-like behaviour, conditioned flavour avoidance, and corticosterone release (Gaykema et al., 2017). ChR2: Channelrhodopsin (excitatory optogenetic tool); hM3Dq: modified form of Gq-coupled human M3 muscarinic receptor (excitatory chemogenetic tool). Relevant references can be found in the text.

Figure 4. Proposed model for the role of GLP1 neurons in acute and chronic stress. Acute stressors activate serotonergic neurons in the RMg as well as other currently unknown neural populations upstream of GLP1 neurons. This leads to activation of GLP1 neurons, release of GLP1 into distributed brain regions, and a resulting activation of the HPA axis, increase in activity of the sympathetic nervous system (SNS), and avoidance behaviours, including decreased feeding, reward, and anxiety-like behaviours. Under chronic stress conditions, GLP1 neurons contribute to hyperreactivity of the HPA axis and body weight loss. Glucocorticoids (GC) feed back to the cNTS to decrease expression of GLP1. Relevant references can be found in the text.