HHS Public Access

Author manuscript

Peptides. Author manuscript; available in PMC 2022 June 01.

Published in final edited form as:

Peptides. 2021 June ; 140: 170534. doi:10.1016/j.peptides.2021.170534.

Demystifying Functional Role of Cocaine- and Amphetamine-Related Transcript (CART) Peptide in Control of Energy Homeostasis: A Twenty-Five Year Expedition

Arashdeep Singh[1,2,*], **Alan Moreira de Araujo**[1,2,*], **Jean-Philippe Krieger**[3], **Macarena Vergara**[1,2], **Chi Kin Ip**[4,5], **Guillaume de Lartigue**[1,2]

[1]Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, Florida, USA

[2]Center for Integrative Cardiovascular and Metabolic Disease, University of Florida, Gainesville, Florida, USA

[3]Department of Metabolic Physiology, Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

[4]Neuroscience Division, Garvan Institute of Medical Research, Darlinghurst, Sydney, Australia

[5]Faculty of Medicine, University of New South Wales, Sydney, Australia

Abstract

Cocaine- and amphetamine-related transcript (CART) is a neuropeptide first discovered in the striatum of the rat brain. Later, the genetic sequence and function of CART peptide (CARTp) was found to be conserved among multiple mammalian species. Over the 25 years, since its discovery, CART mRNA (*Cartpt*) expression has been reported widely throughout the central and peripheral nervous systems underscoring its role in diverse physiological functions. Here, we review the localization and function of CARTp as it relates to energy homeostasis. We summarize the expression changes of central and peripheral *Cartpt* in response to metabolic states and make use of available large data sets to gain additional insights into the anatomy of the *Cartpt* expressing vagal neurons and their expression patterns in the gut. Furthermore, we provide an overview of the role of CARTp as an anorexigenic signal and its effect on energy expenditure and body weight control with insights from both pharmacological and transgenic animal studies. Subsequently, we discuss the role of CARTp in the pathophysiology of obesity and review important new developments towards identifying a candidate receptor for CARTp signalling. Altogether, the field of CARTp research has made rapid and substantial progress recently, and we review the case for considering CARTp as a potential therapeutic target for stemming obesity epidemic.

Address for correspondence: Guillaume de Lartigue, PhD, Department of Pharmacodynamics, College of Pharmacy, University of Florida, 1345 Center Drive, Gainesville, FL 32610, Tel: +1-352-273-7693, gdelartigue@ufl.edu.

*These two authors contributed equally to this work.

Disclosures: The authors have nothing to report.

Keywords

energy balance; bodyweight; feeding; neuropeptide; GPR160; obesity; gut-brain axis; high-fat diets; hypothalamus; vagus nerve

1. Introduction

This year marks the 25th year anniversary since the discovery of the neuropeptide cocaine- and amphetamine-regulated transcript (CART). In 1995, Douglass and colleagues discovered a transcript in the rat ventral striatum that was differentially displayed in response to intraperitoneal injection of the psychostimulation drugs, cocaine and amphetamine [1]. A fragment of CART peptide (CARTp) matched with a previously isolated somatostatin-like peptide from the ovine hypothalamus [2] confirming that the transcript was translated into protein. Further studies identified that mRNA splicing and polyadenylation of CART mRNA (*Cartpt)* in rats give rise to two different pro-peptides of 116 or 129 amino acids that are post-translationally processed by pro-hormone convertases in two stable biologically-active forms CART (55–102) and CART (62–102) [1, 3, 4]. Importantly, CARTp was found to be highly conserved between species [1, 3–13], with the amino acid sequence of both the short-form CART (49–89) and the long-form CART (40–89) in humans sharing 95% amino acid sequence homology with the respective rodent forms [1, 13–15]. Past studies have reported that CARTp is involved in a wide range of physiological roles including stress, feeding behavior, immune function, autonomic regulation, fluid balance, metabolic processes, sexual function and endocrine control as reviewed in [16–19], with increasing evidence for a role in the pathophysiology of obesity. This is supported by polymorphisms in human populations that show an association between CARTp and obesity. Most studies reported only a few specific single nucleotide polymorphisms (SNPs) in *Cartpt* gene, for instance, higher allele frequency of the 1475A>G SNP in the individuals with obesity [20–23]. While there are various challenges on the translation of observations from animal studies to human system, mutations in *Cartpt* have been documented to promote weight gain and thus endorsing the anorectic role of the CARTp. This review will focus on a growing literature highlighting an important modulatory role for CARTp in energy homeostasis.

Firstly, we review the localization of *Cartpt* in the brain and periphery, focusing on areas with a known role in controlling energy homeostasis. Specifically we highlight recent RNA profiling data that indicate heterogeneity of *Cartpt* expressing neurons and review the stimuli that modulate *Cartpt* expression. Secondly, we discuss important new developments that identify a putative candidate receptor for CARTp signaling. Finally, we summarize the function of CARTp as an anorexigenic signal and its effect on energy expenditure and body weight control based on studies relying on pharmacological or genetic approaches. In each section, we conclude with a summary of the state of the field and identified gaps in knowledge.

2. CART Expression patterns

Early studies identifying the expression patterns of *Cartpt* provided insight into its potential function. Since its original discovery as a differentially displayed transcript, it has been

found to be widely expressed in an evolutionarily conserved manner. Immunohistochemical and *in situ* hybridization studies in rodents [1, 3–6], amphibians [7], marsupials [8], primates [9–11] and human [12, 13] brains have revealed the presence of CARTp and *Cartpt* mRNA throughout the brain. Recently, a full comprehensive expression pattern of *Cartpt* has been identified throughout the entire mouse brain in the Allen brain atlas [24]. In this section, we summarize the *Cartpt* expression patterns along the rostro-caudal axis of the brain (Figure 1), and peripheral organs. In each section, we highlight metabolic stimuli capable of modulating *Cartpt* expression levels, supporting a role for CARTp in energy homeostasis.

2.1 Forebrain

In the frontal part of the mouse brain, *Cartpt* expressing neurons are distributed across different nuclei including the inner plexiform layer of the olfactory bulb and olfactory tubercle [25] (Figure 1) and the prefrontal cortex (PFC) [24]. Furthermore, *Cartpt* expression in the somatosensory, piriform, and insular cortices was also reported in rats [26] and humans [12]. These regions are actively involved in olfaction, integration of sensory information, as well as decision making [25, 27]. To date little evidence of CART protein has been confirmed in these brain regions.

Work in fish suggests that *Cartpt* expression in these forebrain regions may be sensitive to feeding cues. Prolonged fast or food deprivation decreased *Cartpt* levels in the telencephalon-preoptic region and olfactory bulb of goldfish [28] and in the forebrain of Atlantic cod fish [29], while an increase in *Cartpt* levels was reported in the olfactory bulb of goldfish postprandially [28]. Further work is required to determine if this can translate to mammals, however studies in genetically obese CCKA receptor deficient (OLEFT) rats suggests that neither CCK nor obesity can influence *Cartpt* expression in rodents in these forebrain regions [30].

2.2 Midbrain

Cartpt transcript was originally identified in the striatum of rats. It has since been reported to be expressed in a number of other midbrain nuclei important in the control of feeding behavior. Specifically this includes nuclei that influence 1) valence and motivation, including the ventral tegmental area (VTA) and nucleus accumbens (NAc) [6, 15, 24], as well as the central (CeA) and basomedial nucleus (BMA) of the amygdala [3, 5, 6], 2) learning and memory, including the hippocampus (dentate gyrus, CA1, CA2, CA3 and indusium griseum) [26] and very high expression in the Edinger–Westphal nucleus as well as the pontine gray [7, 31]; and 3) energy homeostasis, including the arcuate nucleus (Arc), lateral hypothalamus (LHA), dorsal medial hypothalamus (DMH), ventral medial hypothalamus (VMH) and paraventricular nuclei (PVN).

In the amygdala changes in CARTp or *Cartpt* expression patterns in both CeA and BMA have recently been suggested to modulate stress response, hedonic feeding and energy homeostasis [32–36]. Under normal physiological conditions, CARTp levels in amygdala of rats were lower in the light cycle (inactive, sleeping) compared to dark cycle (active, eating) suggestive that CARTp in the amygdala is upregulated in response to feeding. In support of this, 24 h food deprivation in rats, prevents this diurnal variation of CARTp [37]. Future

work further assessing the postprandial stimuli responsible for CARTp upregulation will be important, while in contrast, 48 h food deprivation upregulated *Cartpt* in non-preganglionic Edinger–Westphal nucleus in male but not female rats [31] indicative of CNS region specific responses.

The original finding that *Cartpt* is upregulated in response to drugs of abuse [1], highlight a potential role for CARTp in reward and reinforcement. Although later, *Cartpt* expression in response to these psychostimulants was found to be gender-specific [38]. Since the original finding *Cartpt* expression in NAc has been associated with food consumption. In rats, fasting for 24 h or 48 h suppressed *Cartpt* levels in NAc shell [39]. Furthermore, *ad libtum*-fed rats had lower CARTp levels in the inactive light period compared to active dark period. However, in 24 h fasted animals, this diurnal variation of CARTp was not present [37]. Interestingly, an increase in CARTp immunoreactivity in NAc shell was also observed in a binge eating model behavior (restricted access to high fat and sugar diet for 4 weeks) animals [40]. It is interesting to note that *Cartpt* appears to be predominantly involved in the mesolimbic reward system and less in the nigrostriatal pathway based on mouse data available in the Allen brain atlas [24].

In the hippocampus, the exact functions of CARTp remain unclear. No studies have yet reported changes in *Cartpt* or CARTp levels in response to feeding related cues. Furthermore, i.c.v. injection of leptin in goldfish also upregulated the expression of *Cartpt* in the optic tectum [28]. Further work is necessary to assess whether CARTp in these brain regions is affected by feeding or metabolic states or is involved in sensory processing, reward and/or memory formation.

2.3 Hypothalamus

The arcuate nucleus is often described as having two separate populations of neurons that express either *Cartpt* and pro-opiomelanocortin (CART/POMC) or neuropeptide Y and agouti-related peptide (NPY/AgRP), with these two neuronal populations having opposing metabolic functions [41, 42]. However, single-cell sequencing in the Arc reveals that there exists far greater diversity of Arc neuronal populations than originally assumed [43, 44]. Three separate clusters of POMC neurons were identified, of these *Cartpt* is most abundantly found in the POMC/Anxa2 cluster, with little to no co-expression in the POMC/Ttr and POMC/Glipr1 clusters [44]. Interestingly, *Cartpt* was also identified in two separate clusters, Tbx19 or Trh/Cxcl12, that lack POMC. The functional significance of these individual clusters of Arc neurons in feeding remain unknown, but these data highlight a previously unsuspected heterogeneity of *Cartpt* expressing Arc-neurons [44]. Similarly, scRNA sequencing of the LHA revealed *Cartpt* expression in several different clusters including *Calcr*, *Tac1*, *Gal*, and *Nts,* but not *NPY* [45]. In contrast to rodents, human *Cartpt* expression in the Arc has been demonstrated to colocalize the orexigenic NPY/AgRP and segregated from the anorexigenic POMC neurons [46]. Thus, it remains unclear whether the rodent data translates to humans especially in relation to its anorectic function given the primary orexigenic properties of NPY/AgRP neurons.

Early studies reported that in whole hypothalamus of *ad-libitum* chow-fed rats, CARTp levels were increased in the active dark period compared to the light period, and this

pattern was not observed under fasting conditions, suggesting that hypothalamic CARTp levels are regulated by food intake [37]. Within individual hypothalamic nuclei of the Arc, PVN, perifornical region, and DMH similar decreases in *Cartpt* expression was reported in response to 24–48 h fasts in rats [39, 47, 48]. Fasting had little-to-no reduction in *Cartpt* expression in the either the DMH or Arc of lean mice [49]. Notably, in some studies either feeding a high-fat diet for 1–3 weeks (50% kcal fat) [50] or 15–16 weeks (19% kcal fat) [51], increased *Cartpt* expression in the whole hypothalamus or its subregions (PVN, Arc, and LHA) in rats. However, other studies reported increase in Arc *Cartpt* expression in diabetic rats fed chow or 50% kcal fat diet for 2 weeks [52] or decreased CARTp expression after feeding a high-fat (30% kcal fat) diet to obesity-prone rats for 14 weeks while the expression between high-fat diet fed obesity-resistant or low-fat fed control rats was not different [53]. Despite using similar 50% kcal fat diets in above studies [50, 52], it is plausible that the reason for the differential response could be the differences in the fasting period before sacrifice (1–2 h in [50] vs 12 h in [52]). In diet-induced obese mice, *Cartpt* increased after 10 weeks of fed high fat diet (40–60% kcal fat) in the DMH, but not the Arc [49, 54]. *Cartpt* expression in LHA was also reported to be increased along with decreased in Arc and PVN of obesity-prone mice compared to obesity-resistant mice fed high-fat diets [54, 55]. As these effects were not observed in pair-fed or energy-restricted mice [54, 55], it plausible that the effects are mainly weight-dependent and partly diet-dependent. Furthermore, hypothalamic *Cartpt* expression and protein levels were also reported to be downregulated in other models of obesity such as gold thioglucose-treated mice [56], VMH-lesioned rats [57] and some [47] but not all studies using streptozotocin-diabetic rats [52]. There is also evidence that cold exposure, which promotes weight loss mainly due to increase in thermogenesis, was associated with an increase in *Cartpt* mRNA abundance in the Arc of cold-acclimatized, compared to warm-maintained rats [58]. These data suggest that *Cartpt* expression is sensitive to metabolic changes, although the discrepancy between rats and mice is poorly understood, and further work in mice is warranted.

In the Arc, *Cartpt* expression is regulated by peripheral hormones. Leptin, which is released from adipose tissue, activates Arc CART/POMC neurons and upregulates *Cartpt* expression but inhibits NPY/AgRP neurons expression and activity [41, 48, 59, 60]. *Cartpt* expression is almost absent from the Arc of genetically obese Zucker (*fa/fa*) rats (have dysfunctional leptin receptor) and *ob/ob* mice (have disrupted leptin gene) and downregulated in DMH of *ob/ob* mice [48, 57]. Treatment of *ob/ob* mice with leptin injected intraperitoneally restored *Cartpt* levels in the Arc and DMH [48]. When hyperleptinemia was induced by adenovirus-mediated overexpression of leptin gene or feeding high-fat diet (60% kcal fat) to normal rats for 8 weeks, *Cartpt* expression was increased in the whole hypothalamic tissue [57]. Furthermore, the co-injection of leptin with satiety peptide cholecystokinin (CCK) also increased hypothalamic *Cartpt* expression [61] and CCK might play a role mediating effects of CARTp on other hypothalamic neurons and in influencing energy balance [62]. This is supported by lower *Cartpt* expression in diet induced obese animals that also have blunted vagal CCK sensitivity [63] and CCK induced satiation[64]. In contrast, ghrelin activates Arc NPY/AgRP neurons and inhibit POMC/CART neurons [65]. It should be noted that the reason for discrepancy in *Cartpt* upregulation in some studies could be in-part due to

different experimental paradigms in which animals do not have alterations in CCK or leptin levels.

These recent findings highlight the possibility that CARTp may have diverse physiological functions through distinct circuits. Interestingly, CARTp is reported to co-express within hypothalamic neurons known to have opposing effects on feeding anorexigenic POMC neurons in Arc [66] and orexigenic NPY neurons in DMH [49]. CARTp has been demonstrated to stimulate the release of NPY and AgRP but not α-MSH from hypothalamic explants [67]. Furthermore, while *Cartpt* is expressed in POMC neurons in rodents, it is also colocalized with other orexigenic neuropeptides, including melanin-concentrating hormone neurons in the LHA [66]. Therefore, it is likely that hypothalamic CARTp not only has anorectic properties, rather plays a multifaceted role in controlling energy homeostasis. In the future, it would be important to decipher the function of each of these individual clusters and map downstream projections to assess how CARTp neurons function in both the Arc and LHA region to regulate diverse physiological responses.

Taken together, *Cartpt* is expressed in a number of midbrain nuclei that are associated with various aspects of feeding behavior. Most of the data suggest that *Cartpt* is upregulated in response to metabolic stimuli that decrease food intake, and down regulated in response to fasting and obesity. Different patterns of expression have been described in specific nuclei. Further deciphering of the functional relevance of these individual neuronal populations is still needed in order to fully understand the function of hypothalamic *Cartpt+* neurons under different experimental conditions.

2.4 Hindbrain

In situ hybridization indicates that there are significant *Cartpt* expressing neurons in the nucleus tractus solitarius (NTS) and area postrema (AP), with few soma in the Parabrachial nucleus (PBN) [1, 24] (Figure 1). At the protein level, high to moderate density of CARTp stained fibers were also observed in the NTS, AP, PBN, locus coeruleus, and raphe nucleus [6]. In the NTS, CARTp fibers were found to originate from vagal sensory neurons, a few neurons in the reticular formation, NTS itself, AP and hypothalamic nuclei [68]. CARTp expression in these hindbrain nuclei may suggest a role in integration of visceral and gustatory sensory information, however, to date no studies have assessed the role of *Cartpt* expressing neurons in the hindbrain. In obese OLETF rats, hindbrain CARTp expression was selectively reduced in the NTS compared to lean LETO rats [30], although this effect was likely a consequence of blunted CARTp expression in vagal sensory fibers that terminate in the NTS [69].

2.5 Peripheral sensory neurons

In addition to the presence of *Cartpt* in various central regions critical in controlling energy homeostasis, *Cartpt* is also expressed in the nodose ganglion (NG) of the vagus nerve [42]. Using *in situ* hybridization and immunohistochemistry, we and others have demonstrated that nearly half of the neurons in the NG express CARTp and *Cartpt* [42, 68, 70]. Retrograde tracing experiments indicate that vagal *Cartpt* expressing neurons innervate the gastrointestinal tract [68] and this is supported by the observation that vagal

Cartpt abundance changes depending on caloric intake. CARTp abundance in the NG and the number of *Cartpt* expressing NG neurons are significantly greater postprandially compared to fasted conditions [69–72]. Interestingly, we observed lateral asymmetry in NG *Cartpt* expression, with the change in CARTp expression between feeding and fasting conditions being more pronounced in the right NG [69]. CCK is both necessary and sufficient to increase *Cartpt* expression in NG neurons [70]. CCK-induced *Cartpt* expression is potentiated by leptin and blunted by ghrelin *in vivo* [71, 73] and in primary cultures of sensory vagal neurons [70]. Recent findings have provided more nuance about the physiological conditions which promote *Cartpt* expression in NG neurons. It was found that in animals fed *ad libitum*, *Cartpt* expression in NG neurons remains low in the light phase, when food is available, but the animals opt not to eat [69, 74]. These data suggest that nutrient-induced postprandial signals, rather than food availability or the absence of hunger alone, is required to increase NG *Cartpt* expression.

Recent profiling of vagal afferent neurons has provided a deeper understanding of their molecular, anatomical and functional diversity [75–77]. We took advantage of the available targeted single-cell RNA sequencing data originally published by Bai et al. [77] (GSE138651) to sub-characterize *Cartpt* expressing vagal sensory neurons based on the organs they innervate, the genes they express, and the type of sensory information that they likely communicate. The datasets were analysed using the Seurat package 3.1.1 [78] for R. We first investigated whether the vagal afferent neurons that terminate in different anatomical organs show a specific *Cartpt* expression pattern. Interestingly, we observed that the neurons retrogradely labelled from the stomach or various intestinal segments, show a wide range of *Cartpt* expression (Figure 2a), indicating that *Cartpt* positive (*Cartpt+*) vagal sensory neurons ubiquitously innervate the length of the gastrointestinal tract.

Second, we determined whether the molecular subtypes of subdiaphragmatic vagal afferents (as defined by target single-cell sequencing from [77]) show a specific pattern of *Cartpt* expression. *Cartpt*+ neurons are found among all 12 clusters of genetically defined subdiaphragmatic vagal afferent populations, but at different expression levels (Figure 2b). Among the anatomically-characterized clusters (colored in Figure 2b), *Cartpt* expression is found at low levels in *Oxtr*+/*Ctxn2*+ vagal afferents that form gastrointestinal intraganglionic laminar endings (IGLEs – cluster 1), and in *Sst*+ vagal afferents that form mucosal endings in the stomach antrum (cluster 4). *Cartpt* expression is found at higher levels in *Calca*+ vagal afferents that form mucosal endings in the stomach corpus (cluster 5) as well as in *Vip*+/*Uts2b*+ vagal afferents that terminate in the intestinal mucosa (cluster 6).

Finally, as *Cartpt* expression in vagal afferents encodes calories and mediate the effects of the intestinal peptide CCK [69], we further investigated whether vagal *Cartpt* is co-expressed with the CCK receptor (*Cckar*) and other known receptors for gut peptides. We found high levels of co-expression of *Cartpt* with *Cckar*, *Glp1r*, and *Npy2r* (Figure 2c) and importantly some of these neurons specifically innervate into the intestinal mucosal endings (*Vip*+/*Uts2b*+ neurons - cluster 6; Figure 2c). These findings are in support of the studies reporting that *Cartpt* expression is present in approximately half of the vagal neurons and the majority of *Cartpt* expressing vagal neurons co-express the CCK receptor [42, 73].

In obesity, re-feeding fails to increase CARTp concentration in NG or the percent of CARTp responsive neurons in rats [69]. Reduced sensitivity to CCK in obesity, as characterized by reduced CCK-induced c-Fos labeling in the NTS and reduced satiation [64] results in blunted CCK-induced CARTp expression in NG compared to lean controls [69]. A number of putative mechanisms for reduced CCK sensitivity have been identified. Leptin increases sensitivity of vagal sensory neurons to CCK in lean animals, and leptin resistance develops in vagal sensory neurons within the first few weeks of high fat diet consumption coinciding with the development of hyperphagia [79]. Conditional knockout of leptin receptor is sufficient to abolish anorexigenic CCK signaling as well as postprandial CARTp expression [80], suggesting that reduced leptin signaling is at least partly responsible for blunted CCK-induced CARTp expression. Furthermore, diet-induced obesity is associated with a 51% reduction in *Cckar* expression in high-fat high-sugar-fed compared to chow-fed rats, suggesting reduced *Cckar* expression may be at least partially responsible for blunted NG CARTp expression in diet induced obesity [69]. Finally, we have demonstrated that CARTp upregulation in response to meal remains blunted in NG even after switching obese rats from a high-fat high-sugar diet to chow for 6 months [69], suggesting that pharmacological intervention may be required to reverse the effects of diet induced obesity.

Taken together, *Cartpt* is extensively expressed in sensory vagal neurons that innervate the length of the gastrointestinal tract. CARTp expression changes depending on caloric intake indicative of a role in meal termination. Further supporting an anorexigenic role for vagal CARTp is the observation that CARTp expression is blunted in diet-induced obesity associating reduced CARTp and overeating. More direct evidence of the anorexic properties of CARTp are discussed in the sections below. Based on profiling data, vagal afferents that highly express *Cartpt* are primarily chemosensitive and form mucosal endings in the stomach and intestine. Additionally, gastrointestinal mechanoceptors also have low levels of *Cartpt* expression. However, a more-detailed anatomical and functional characterization of vagal *Cartpt*+ neuronal subtypes is still warranted to determine the cell-type specific or innervation-dependent functions of CARTp and its role in the control of energy homeostasis. Furthermore, whether there exist asymmetrical profiles of *Cartpt* expressing NG neurons or the impact of diet/pathophysiology on CARTp neurons profiles remains unclear.

2.6 Gastrointestinal tract

Cartpt is also expressed in cells of the pancreatic islets as well as visceral and subcutaneous adipocytes in both humans and rodents [81]. In the gut, *Cartpt* is expressed in enteric neurons of the myenteric plexus from rat ileum [82, 83] as well as enteroendocrine cells in human duodenum and jejunum [84]. In cultured enteroendocrine cell lines, fat but not sugar increases *Cartpt* expression [84]. Whether CARTp from peripheral organs has any impact on circulating levels of CARTp or if CARTp expression in peripheral organs is affected in condition like obesity and diabetes needs further investigation.

3. CARTp receptor

Since the discovery of CARTp in 1995, several attempts have been made to find a receptor for this protein. Radiolabelled CARTp failed to identify a binding partner in

brain tissue despite evidence that exogenous CARTp was capable of activating discrete neuronal populations [85]. The first direct evidence for the existence of the CARTp receptor was reported in hippocampal primary cell cultures, in which exogenous CART (55–102) inhibited voltage-dependent intracellular Ca^{2+}signaling. This effect was blocked by pertussis toxin, suggesting that signalling occurs through Gi/o proteins [86]. In AtT20 cells (mouse pituitary tumor cell-line), CARTp activated extracellular signal-regulated kinase (ERK) signalling, and this activation could similarly be blocked by pertussis toxin [87]. Radiolabelled CARTp applied to AtT20 cells was found to be displaced by CART (55–102), but not non-active forms of CARTp and binding was reduced in the presence of a GTP analog, confirming that the CARTp receptor is a GPCR [85].

To date, only one plausible candidate G-protein coupled receptor (GPR) has been identified as a putative CARTp receptor, GPR160 [88]. In cell culture, GPR160 is found to be necessary for CARTp mediated cell-activation. RNA interference of GPR160 prevents CARTp induced cFos expression or ERK phosphorylation in two different cell-lines (KATO III and PC12). Furthermore, CARTp co-localizes and co-immunoprecipitates with GPR160 in these cell-lines. *In vivo*, CARTp mediated mechano-allodynia acting through phosphorylation of ERK and phosphoCREB in the spinal cord is blunted using a GPR160 antibody [88]. Excitingly, recent data suggests that GPR160 is involved in controlling feeding [89]. GPR160 antiserum administered in the 4^{th} ventricle to passively immunoneutralize endogenous GPR160 ligand binding significantly increases *ad libitum* food intake when administered in the dark, but not in the light phase, suggesting that GPR160 plays a physiological role in satiation. Importantly, pre-injection with the GPR160 antibody into the 4^{th} ventricle completely prevented the anorexic effects of subsequent 4^{th} ventricle injection of CARTp [89]. Thus, GPR160 is required for CARTp-induced decrease in food intake at least in the hindbrain. Notably, the GPR160 antibody administered in the 4^{th} ventricle also potently reduced water intake, thus further work is required to determine the extent to which GPR160's effect on food intake is mediated by water intake.

In situ hybridization data from the Allen Brain Atlas identifies little GPR160 expression anywhere in the mouse brain [24]. However, Haddock and colleagues identified that GPR160 immunostaining is widely expressed in rat brain regions associated with feeding [89]. Specifically, GPR160 was found in the hindbrain (AP, NTS, hypoglossal, and lateral parabrachial nucleus), the hypothalamus (ARC and PVN), hippocampus, amygdala, and limbic regions (SNc, VTA, NAc). These expression patterns are similar to those of *Cartpt* and suggests a putative role for GPR160 in the integration of visceral inputs, satiation, aversion, taste, learning, memory, and/or reward. Thus, further work is required to fully assess the role of GPR160 signalling in feeding in each of these different brain regions and in different animal models.

In cell culture, GPR160 was found to be expressed in all major cell classes in the brain and spinal cord of rodents and humans [90, 91]. In rat NTS brain sections, GPR160 was most extensively localized in microglia, followed by neurons, and was sparsely reported in astrocytes. The expression of GPR160 in different cell-types in the brain makes it more difficult to define the specific function of this receptor in a physiological state. However, this also opens new possibilities for the functions and mechanism of action of

CARTp. It should be noted that early reports suggest that there may be multiple CARTp receptors. Different concentrations of the long and short active fragments of CARTp were required to produce behavioral responses and in rare cases even produce different behavioral responses. In Bannon et al.[92], i.c.v. injection of the long-form was five times more potent than the short form at reducing food intake, while only the short form produced an acoustic startle response. Furthermore, as discussed in more detail below, microinjection of CARTp into different brain nuclei can have opposing effects on food intake. Whether this is caused by activation of separate receptors or activation of different circuits requires further investigation. The idea that separate receptors could mediate CARTp signaling is furthermore supported by evidence that CARTp can activate different cell types via separate intracellular pathways in the paraventricular nucleus of the hypothalamus [93]. These separate lines of evidence suggest that GPR160 may not be the only CARTp receptor. In a GPR88 knockout mouse, *Cartpt* mRNA is among the highest upregulated gene [94], which may be indirect evidence of a ligand compensating for the loss of its receptor. However, direct evidence identifying GPR88 as another candidate receptor is currently lacking.

In summary, the CARTp receptor has characteristics of G-proteins coupled receptor, probably signalling via a Gi/o coupled mechanism. A major recent development in the CARTp field has been the discovery of GPR160 as a candidate CARTp receptor. GPR160 is widely expressed throughout the brain in regions associated with feeding, and passive neutralization of GPR160 abolishes 4V CARTp induced hypophagia. However, evidence that CARTp anorectic effects are directly mediated by GPR160 are still missing. Characterization and genetic manipulation of the neuronal subtypes that express this receptor may help gain better understanding of GPR160 function. Currently, whether GPR160 can mediate all the known physiological effects of CARTp or if there are other CARTp receptors remains unknown.

4. Role of CARTp in controlling Energy Balance- Pharmacological Interventions

Considerable evidence has accumulated for the role of CARTp in modulating metabolism. As described above, *Cartpt* is abundantly expressed in brain regions associated with energy homeostasis [5, 43, 95, 96]. In this section, we will review pharmacological evidence that CARTp influences various aspects of energy homeostasis – food intake, energy expenditure, and body weight.

4.1 Food Intake

Central administration of CART (55–102) into the third (3V), fourth (4V) or lateral ventricles (LV) greatly reduces food intake in mice and rats, suggesting that exogenous CART (55–102) has anorexigenic properties. In contrast, neutralizing endogenous CARTp with antibody exerts an opposite effect [48, 68, 69, 97–102], suggesting that endogenous CARTp is necessary and sufficient for inhibiting food intake. Moreover, studies using various active fragments of CARTp were able to reproduce the results on food intake reduction in rats, mice [92, 101–107], and even in fish models [108], highlighting a conserved anorexigenic function.

To define the site of i.c.v. CARTp action, the marker of neuronal activity, c-Fos, was assessed throughout the brain in multiple studies. The increased c-Fos activity was observed in the Arc, PVN and DMH of the hypothalamus as well as the NTS, AP, and PBN of the hindbrain, in rats [68, 101, 109], suggesting that i.c.v CARTp primarily activates neural substrates that are in contact with the 3V and 4V ventricle. Similarly, i.c.v. CART (61–102) administration increased the transcription factor c-jun in NTS and DMH of mice [103]. Injection of CART (55–102) in the 4V strongly induced cfos in the AP and NTS but failed to increase c-Fos in more proximal hypothalamic structures [109]. Importantly, although CART (55–102) injection into each brain ventricles reduced food intake, the effects were more pronounced after CARTp administration into the 4V compared to the LV [99]. The hypophagic effect of i.c.v. CART (55–102) was postponed and significantly attenuated by blocking the flow of the cerebrospinal fluid that prevents 3V CARTp from reaching the 4V [97]. Taken together, the data indicate that hindbrain regions mediate the major anorectic effect in response to CARTp.

While the effects of CARTp i.c.v. were extensively investigated and greatly reproduced, more targeted studies investigating the action of CART (55–102) in specific brain nuclei had more variable outcomes. CART (55–102) microinjection in Arc, DMH, or LHA hypothalamic nuclei increased food intake in both *ad libitum* fed and fasted rats [52, 58, 110]. Moreover, twice daily intra-Arc nucleus injection of 0.2 nmol CART (55–102) for 7 days was associated with a 60% higher daytime food intake [58]. Incubation of hypothalamic explants with CART (55–102) resulted in increased NPY [52, 111] and AgRP release, with no alteration in α-MSH levels [52]. Since in rodents, CARTp is largely colocalized with α-MSH-producing POMC neurons associated with inhibiting food intake, it is plausible, that CARTp release from these neurons acts as positive feedback that promotes NPY and AgRP release. This could explain the unexpected role of CARTp in the hypothalamus, although this hypothesis has not yet been tested.

In another hypothalamic nucleus, the PVN, the role of exogenous CARTp in feeding has been variable. Abbott et al. observed an increase in food intake 2–4 hours after intra-PVN injections of CART (55–102) in 24-h fasted rats [110]. In another study, intra-PVN CART (55–102) injection was not able to significantly alter food intake in 18-h fasted rats but significantly reduced NPY-induced hyperphagia [112]. Stanley et al. found that the administration of CARTp into the PVN resulted in a significant reduction in food intake in rats [111]. The dose of 0.2 nmol was able to acutely reduce food intake in the first-hour post-injection, whereas 0.6 nmol CARTp resulted in a sustained reduction in food intake, but this was accompanied with motor disturbances (that also occurred with high doses of i.c.v. CARTp) [111]. Discrepancies in those findings may be related to the volume of injection or duration of fast. Further studies are required to clarify the effects of CARTp in the PVN and its consequences on feeding behavior.

CART-expressing neurons in the NAc shell are sensitive to variations in the metabolic state [39]. Injection of CART (55–102) into the NAc or NAc shell decreased food intake in rats with no change in locomotor function [39, 113]. It has been hypothesized that anorexia nervosa may involve altered signaling events in the NAc, however, a direct association between CARTp and anorexia remains elusive.

Based on studies described above indicating that 1) the effects of i.c.v. CARTp on feeding are dampened by blocking the aqueduct [97], and 2) 3V and 4V injection of CARTp triggered c-Fos expression in the NTS and AP regions, it has been hypothesized that CARTp acts mainly in the hindbrain to suppress food intake. Indeed, although exogenous CARTp in the NTS of rats did not significantly reduce sucrose or chow intake 30 min after injection, or total 24-h cumulative food intake [68, 69, 100], intranuclear injection of CART (55–102) into the NTS was capable of transiently reduce chow diet intake in fasted rats 60- and 90-minutes post-injection [69]. In addition, NTS injection of CARTp correlated with increased c-Fos expression in the NTS and AP, 90 min after infusion [69]. Interestingly, exogenous CARTp failed to reduce food intake in lean fed rats, and infusion of anti-CARTp antibody in the NTS was capable of increasing food intake in fed rats but not fasted rats [69], suggesting that the anorexigenic effect of CARTp depends on the feeding state and on the level of endogenous CARTp.

CARTp positive terminals are extensively present in the commissural, dorsomedial and gelatinous subnuclei of the NTS and in the AP [68, 69]. The NTS is the central site of sensory vagal afferent termination, and it has been shown that approximately 50% vagal sensory neurons that reside in the NG express *Cartpt* [42, 99]. In addition to the NG, CART+ neuron cell bodies residing in the NTS itself and AP, the medullary reticular formation and the hypothalamus may all contribute the pool of CART+ fibers in the NTS [68]. The fact that the knockdown of CARTp in the NG prevented antibody-induced hyperphagia [69] suggests that, the NG provide the main source of endogenous NTS CARTp that inhibit food intake [69].

The effects of CARTp in regulating appetite is not only limited to lean animals. For instance, obese rats fed HFD decreased food intake and body weight after chronic CART (55–102) i.c.v. infusion [51], which is consistent with the results observed in a study with obese rats and i.c.v. injection of a viral vector encoding *Cartpt* gene [114]. In a study with Zucker (*fa/fa*) rats, a rat model of genetic obesity, chronic i.c.v infusion of human CART (42–89) reduced food intake [104]. Recently, Lee and colleagues showed that endogenous NTS CARTp expression is blunted in diet-induced obese rats and that the levels remained low even after switching to a regular chow diet [69]. In addition, the obese rats reduced their food intake after NTS injection of endogenous CARTp in both fed and fasted conditions [69, 104]. Altogether the data discussed above suggest a potential therapeutic role for targeting the CARTp signaling for the treatment of obesity.

4.2 Energy Expenditure

To date, no pharmacological study has reported energy expenditure measurements following CARTp injection in the brain. However, a few published studies have indirectly associated CARTp pharmacology with energy expenditure in rats. Indeed, the chronic infusion of CART (55–102) into the LV increased reduced RQ value associated with increased lipid oxidation measured by indirect calorimetry in both lean and obese rats [51]. In the hypothalamus, upregulation of brown adipose tissue UCP-1 mRNA was observed after intra-Arc and intra-PVN CART (55–102) injection, which is associated with hyperthermic action [58, 112]. Moreover, twice daily intra-Arc injection of CART (55–102) for seven days

resulted in 85% increase in thermogenic response to β3 adrenergic agonist [58]. Conversely, injection of CARTp into the 4V or NTS parenchyma caused long-lasting hypothermic response, with a maximum drop of 1.6 °C in core temperature [100]. Thus hypothalamic CARTp injection results in opposite thermic effects to NTS/hindbrain injection [58, 100, 112], indicating that CARTp responsive neurons within both brain regions are separately involved in the control of body temperature.

4.3 Body weight

There is converging evidence that central administration of CARTp has a role in the control of body weight. In both lean and obese animals, the chronic i.c.v. infusion of CART (55–102) has sustained inhibitory effects on body weight [51][100]. Similarly, chronic i.c.v infusion of human CART (42–89) resulted in a significant reduction in body weight in Zucker (*fa/fa*) rats [104]. Significant body weight reduction was also observed 24-h after CART (55–102) injection into the 4V in Sprague Dawley rats [100]. Together, these results show that the CARTp pathway is an important determinant of body weight homeostasis in lean and obese animals.

4.4 Peripheral administration of CARTp

Intraperitoneal (i.p.) injection of CART (55–102) did not reduce food intake, energy expenditure, or bodyweight after a prolonged fast (>12 hours) in rats [71, 100]. However, i.p. CARTp had a small inhibitory effect on food intake by prolonging the effects of CCK when co-administered in rats that underwent short-duration fasting (2.5 hours) [71]. These observations indicate that CARTp might be acting through a receptor located in the periphery (eg. nodose ganglia) or in brain regions that lack a proper blood-brain barrier, like the AP on the hindbrain.

CART (55–102) administered either through i.p. or subcutaneous routes do not affect gastric secretion or motility. In contrast, central injections of CART (55–102) into the 3V and 4V inhibited gastric emptying in mice and rats [115, 116]. Moreover, inhibition of gastric acid secretion and acceleration in colon motility was observed in rats after intracisternal and LV injection of CART (55–102), respectively [117, 118]. Therefore, although CARTp is found in cholinergic neurons of the myenteric plexus, the alterations in GI function seem to be mostly mediated by the CNS.

In summary, pharmacological studies support the idea that CARTp plays a role in energy balance regulation. Globally, CARTp inhibits food intake, and this is thought to be primarily mediated by the hindbrain. Yet, studies in which CARTp is locally administered in the hypothalamus repeatedly identify an orexigenic role for CART. This dichotomy in CARTp control of food intake has not yet been explained, however it is further supported by genetic and molecular studies discussed in detail below. Addressing this gap in knowledge will be important for developing therapeutic treatments of obesity that target the CARTp system. Specifically, future work focused on a) clearly defining the spatial and temporal boundaries of CARTp induced hyperphagia within the hypothalamus, b) determining whether multiple CARTp receptors mediate different feeding effects of CARTp, c) identifying the expression patterns of CARTp receptor(s) at the cellular level throughout

the brain, and d) measuring whether different active CARTp fragments are produced by different neuronal subpopulations or under different physiological conditions, may provide insights into how CARTp can cause opposing effects on feeding.

5. Role of CART in controlling Energy Balance: Gene Overexpression or Transgenic Models

Over the last 20 years, multiple transgenic models have been generated and characterized to understand the role of CARTp in energy balance. In vivo rodent models that either had *Cartpt* gene knockout (CART KO), viral-mediated targeted knockdown (CART KD) or overexpression of *Cartpt* in adult animals, enabled researchers to precisely phenotype the role of CARTp in various physiological functions [69, 113, 119–125]. The phenotypic effects resulting from *Cartpt* gene manipulation in transgenic rodent studies have in-part shown inconsistency; however, they displayed a general trend supporting with the idea of CARTp acting as a satiety signal that can cause long-term weight loss with minimal effect on energy expenditure.

5.1 CART knockout studies

5.1.1 Food Intake—Given the pharmacological studies discussed above indicating the anorexigenic role of CARTp, it would be anticipated that a global CART KO model would increase food intake compared to wild-type (WT). This expected hyperphagic response was observed in high-fat diet fed [122] but not in chow-fed CART KO mice [121–124]. In fact, in one of these chow-fed studies, CART KO mice unexpectedly decreased food intake compared to WT mice after fasting at room temperature, and in both *ad libitum* or fasted conditions when maintained at thermoneutrality [121]. This highlights different roles of endogenous CARTp in food intake control depending on diet/metabolic condition (i.e. lean vs obese), physiological state (i.e. fed vs fasted), or external factors (i.e. room temperature, light cycle, etc). However, it should be noted that at least three different CART KO mouse lines have been used in the different studies described above. Each mouse lines may result in different compensatory mechanisms during development, and/or variability in *Cartpt* gene deletion in a heterogeneous neuronal population with opposing (orexigenic or anorexigenic) roles.

To address the issues of compensation and competition of different *Cartpt* expressing neurons, viral mediated approaches targeting specific nuclei have provided important insight into the functions of CARTp. Knockdown of CARTp by injection of short interfering RNA (siCART) in the NAc decreased CARTp levels and increased food intake in fed, but not food deprived KD mice compared with control mice [113]. siCART in the NAc also inhibits the anorectic effects of a serotonin-4 receptor agonist, MDMA (3,4-methylenedioxy-N-methylamphetamine) [113] and CB1 antagonist (rimonabant) [126]. These data suggest that CARTp may be acting as a downstream mediator of either anorexigenic or orexigenic effects of NAc neurons depending on its interactions with serotonin-4 receptor or CB1 receptor respectively.

We recently demonstrated that lentiviral-mediated knockdown of *Cartpt* in the NG of vagus nerve markedly increased food intake, ingestion rate, and meal size in chow-fed rats [69]. Importantly, unilateral nodosectomy or CART KD selectively in the NG neurons abolished hyperphagic effects of the CARTp antibody in the NTS [69], suggesting that endogenous vagal CARTp is necessary to inhibit food intake. Vagal CART KD abolished CCK-mediated reduction in food intake indicative that CARTp expressed in vagal sensory neurons is necessary to mediate CCK-induced satiation [69]. These findings demonstrate that vagal CARTp release into the NTS control food intake and supports the idea that CARTp is most potently anorexigenic in the hindbrain.

5.1.2 Energy Expenditure—Studies in which *Cartpt* is knocked out globally in mice found no significant difference in total energy expenditure or respiratory quotient compared to WT controls regardless of diet [122, 123]. In a recent study using a different CART KO mouse line, similar results were observed in mice maintained in thermoneutral conditions, but at room temperature CART KO promoted energy expenditure and BAT activity with lower respiratory quotient compared to WT mice [121]. The reason for the differences between these studies may be attributable to the use of different mouse lines and the fact that energy expenditure studies were performed at different ages (4–5 weeks of age in [122, 123] vs 14–18 weeks of age in [121]).

There is evidence that CARTp locally in the NAc may play a role in energy expenditure. Activation of serotonin receptors (5-HTR4) increases *Cartpt* expression in the NAc [127]. CART KD in the NAc not only suppressed 5-HTR4 induced hypophagia but also motor hyperactivity. Thus, hypophagic and motor hyperactivity responses may be dependent upon activation of a NAc 5-HTR4-CART pathway. Taken together, data from the whole-body CART KO mice suggest that *Cartpt* expression is not necessary for the control of energy expenditure or respiratory quotient. Notably, the limitation of the CART KO model is that it does not delineate opposing effects of CARTp on energy expenditure from neurons in different brain nuclei thus might cancel each other out.

5.1.3 Body weight—Unsurprisingly, given the anorexigenic role of CARTp in feeding, long-term disruption in CARTp signaling causes increases in body weight which occur after a significant delay (8–40 weeks) [121–124]. This large variability may in-part be due to innate physiological differences in three different CART KO mouse lines studied [121–124]. Interestingly, although body weight gain and percent fat mass both increased in chow-fed CART KO mice when the animals were housed at room temperature (22 °C) but a decreased lean mass compensated for increased fat mass was observed when the mice were kept under thermoneutral conditions (30 °C) [121]. Thus, most studies reported elevated body weight and fat mass gain in CART KO mice regardless of dietary options, with an even more prominent effect shown with a high-calorie diet [121–124].

5.2 CART Overexpression Studies

5.2.1 Food Intake—To date, no global *Cartpt* gene overexpression mouse models have been developed. Based on multiple studies reporting that *Cartpt* expression in central and peripheral neurons is blunted in metabolic conditions such as obesity [48, 69], it would be

hypothesized that restoring *Cartpt* expression in these neurons might correct dysfunctional energy balance. In support of this hypothesis, viral delivery of recombinant *Cartpt* i.c.v. decreased daily food intake and fasting-induced hyperphagia in diet-induced obese rats fed high-fat high-sugar diet [114]. These findings suggest that blunted central *Cartpt* expression in obesity is sufficient for hyperphagia since restoring/overexpressing central *Cartpt* was effective in reducing food intake.

In contrast to i.c.v. delivery, local overexpression of *Cartpt* gene in the PVN of the hypothalamus using recombinant adeno-associated viral vector (AAV) increased food intake in rats fed either chow or high-fat diet, with more pronounced effects under high-fat conditions [128]. Furthermore, selectively re-introducing *Cartpt* gene into the LHA in a CART-cre mouse that lack endogenous *Cartpt* gene, using AAV to constitutively express CARTp, trended to increase food intake compared to CART KO mice [120]. This finding was also supported in another study where in CART-cre mice, chemogenetic stimulation with cre-dependent DREADD construct (hM3Dq) in *Cartpt* expressing LHA neurons increased food intake. However, stimulating the same population of LHA neurons in a CART KO mouse attenuated the hyperphagia [119], suggesting that CARTp release is necessary for hyperphagia and further supports the orexigenic effects of CARTp in the hypothalamus. In contrast, re-introducing *Cartpt* gene in Arc CART neurons of CART-cre knock in mouse that lack *Cartpt* gene did not alter food intake [120]. Also, chemogenetic stimulation of Arc *Cartpt* expressing neurons in CART-cre mice did not alter food intake, instead increased food intake when *Cartpt* gene was knocked out [119], suggesting a possible inhibitory role of CARTp in the Arc. These studies reinforce pharmacological studies described above, indicating opposing feeding outcomes of CARTp depending on the site of action. Taken together, endogenous CARTp is necessary for inhibition of food intake in the Arc but its overexpression or neuronal stimulation in the LHA or PVN increases food intake.

5.2.2 Energy Expenditure—Similar to feeding, overexpression studies suggests that CARTp may have differential effects on energy expenditure depending on the site of expression [58, 114, 120]. Overexpression of central *Cartpt* by i.c.v. administration of *Cartpt* using AAV in high-fat high-sugar diet-fed obese rats did not affect energy expenditure or locomotion compared to vehicle-treated controls [114]. A reduction in respiratory quotient was reported during the dark period, under both *ad libitum* feeding and fasting-refeeding conditions, indicating a role of CARTp in promoting fat oxidation and limiting fat storage [50, 114].

In CART KO mice, selective re-introduction of *Cartpt* into the LHA *Cartpt* expressing neurons resulted in a decrease in energy expenditure was reported while respiratory quotient was unaffected [120]. Furthermore, chemogenetic activation of *Cartpt* expressing neurons in the LHA stimulated energy expenditure and physical activity in the presence of *Cartpt* but respiratory quotient was unchanged [119]. In contrast, chronic overexpression of *Cartpt* through intra-arcuate targeted gene transfer increased brown adipose thermogenesis and this was accompanied by exaggerated weight loss in both fasted and food restricted conditions [58]. Similarly, in CART KO mice, the selective re-introduction of *Cartpt* in Arc CART neurons increased energy expenditure with no change in respiratory quotient

[120]. Unexpectedly, chemogenetic stimulation of Arc *Cartpt* expressing neurons suppressed energy expenditure with no change in respiratory quotient or bodyweight [119].

Altogether, these studies indicate that CARTp signalling in the Arc is essential and directly involved in mediating energy expenditure. However, *Cartpt* expressing neurons appears to mediate opposing effects on energy expenditure in the LHA and Arc. These data underscore the nuclei-specific functions of hypothalamic *Cartpt* expressing neurons in controlling energy expenditure but not respiratory quotient. Moreover, the effect of CARTp in controlling energy expenditure does not appear to be ubiquitous as we recently demonstrated that lentiviral-mediated KD of CARTp in the NG did not affect energy expenditure in chow-fed rats [69]. Therefore, more studies are still necessary to establish the role of central CARTp in modulating thermogenesis.

5.2.3 Body weight—The chronic overexpression of central *Cartpt* via administration of i.c.v. *Cartpt* AAV to high-fat high-sugar diet-fed obese rats significantly attenuated their body weight gain throughout a 7-month study duration primarily by decreasing lean mass gain without affecting fat mass [114]. Virally-mediated overexpression of *Cartpt* in hypothalamic PVN neurons increased body weight in chow fed rats, supporting an orexigenic role for CARTp in the hypothalamus. This effect was more pronounced in rats fed high-fat diet [72]. Virally-mediated overexpression of *Cartpt* in either the Arc or LHA of chow-fed mice did not affect weight gain [120]. In another study, chemogenetic stimulation of LHA *Cartpt* expressing neurons resulted in increased body weight of chow-fed mice [119]. In contrast, selective re-introduction of *Cartpt* in Arc in CART KO mice decreased body weight gain and fat mass compared to CART KO mice [120]. Furthermore, presence of endogenous *Cartpt* was also reported to inhibit weight gain in response to chemogenetic Arc CART neuron activation [119]. Overall, these findings are suggestive of the nuclei-specific role of *Cartpt* expressing neurons in controlling weight gain.

6. Conclusion

In summary, it is evident that CARTp plays a key role in controlling food intake, however, its function may be context-dependent, region-specific and nuclei-specific. Hypothalamic CARTp appears to be involved in both orexigenic (PVN and LHA *Cartpt* expressing neurons) and anorexigenic (Arc *Cartpt* expressing neurons) functions whereas vagal, NTS, and NAc CARTp exclusively inhibits food intake, and thus likely are key sites for mediating the global anorexigenic effect of CART. Furthermore, exogeneous CARTp has similar anorectic effects in obese models, suggesting that restoring blunted CARTp signaling in obese subjects could be a viable therapeutic strategy. Additional experiments identifying central and peripheral *Cartpt* expressing neural circuits using electrophysiology, optogenetic and viral vector strategies will be crucial to explain how, and under which physiological conditions central CARTp controls food intake. The findings from whole-body *Cartpt* knockout mice suggest that CARTp plays a critical role in long-term weight homeostasis. Disruption of *Cartpt* gene promotes weight gain while overexpression of *Cartpt* in the brain attenuates weight gain or promotes weight loss. Notably, while *Cartpt* expressing neurons are present in multiple brain regions and co-localize with other neuropeptides (or their receptors) that can modulate body weight, the effect of nuclei-specific *Cartpt* expressing

neuron stimulation on body weight changes in rodents might not be CARTp-specific. Therefore, more precise studies characterizing the neurochemical properties of different *Cartpt* expressing neurons and their projections to other brain regions are required in the future. Nevertheless, like central *Cartpt* expressing neurons, conditional KD of CARTp in peripheral neurons in rats also promotes weight gain. We recently demonstrated that lentiviral-mediated targeted KD of CARTp in the NG of vagus nerve markedly increased body weight gain 2-weeks post-surgery in chow-fed rats [69]. These data suggest that targeting CARTp can be a viable therapeutic strategy for reducing body weight and demand more studies to support the hypothesis.

The current literature provides evidence for the expression of CARTp throughout the gut-brain axis and supports a role for CARTp in controlling energy homeostasis. The results from the literature providing anatomical and functional evidence that central and peripheral CARTp is an anorexigenic signal. Pharmacological studies using CARTp demonstrate a role at least in the short-term on bodyweight, and are reinforced in genetic mouse models resulting in elevation in bodyweight attributed to the increased fat mass, suggest a critical role for CARTp in weight regulation. With the recent identification of a putative CARTp receptor, through de-orphanization of GPR160, there is hope that development of specific antagonists may be developed for the treatment of obesity. Using these types of pharmacological tools alongside knockouts of the receptor should yield greater mechanistic understanding of CARTp signaling, site of action, and role in energy homeostasis. While evidence provided in this review highlights the multifaceted role of CARTp expressing neurons in controlling energy homeostasis, it appears to be dependent on the location or cell-type, and many questions still remain. What are the temporal dynamics of activity these different neuronal populations? Do signals from these disparate populations and how are they integrated under physiological conditions? What are neuro-phenotypical characteristics of these distinct central and vagal CART-expressing neurons? Is activation or inhibition of these neurons sufficient to reverse the disturbed energy balance as observed under different physiological and metabolic states? To address these gaps in knowledge, we suggest that CARTp related pathways should be further investigated using novel tools that can specifically and precisely target different *Cartpt* expressing neuronal subpopulations. Notably, disrupting genetic models and clinical reports suggest that disruption of CARTp signaling is sufficient for the onset of obesity, and thus is a viable target for the development of future therapeutic treatment against metabolic disorders such as obesity.

Acknowledgments:

We thank Ling Bai and Zachary Knight (UCSF) for sharing single-cell sequencing data (GSE138651).

Grant Support: This work was supported by National Institutes of Health Grant to GDL (R01 DK116004) and Swiss National Science Foundation Postdoc Mobility Grant to JPK (183899)

References:

[1]. Douglass J, McKinzie AA, Couceyro P, PCR differential display identifies a rat brain mRNA that is transcriptionally regulated by cocaine and amphetamine, J Neurosci15(3 Pt 2) (1995) 2471–81. [PubMed: 7891182]

[2]. Spiess J, Villarreal J, Vale W, Isolation and sequence analysis of a somatostatin-like polypeptide from ovine hypothalamus, Biochemistry20(7) (1981) 1982–8. [PubMed: 7225368]

[3]. Koylu EO, Couceyro PR, Lambert PD, Ling NC, DeSouza EB, Kuhar MJ, Immunohistochemical localization of novel CART peptides in rat hypothalamus, pituitary and adrenal gland, J Neuroendocrinol9(11) (1997) 823–33. [PubMed: 9419833]

[4]. Thim L, Kristensen P, Nielsen PF, Wulff BS, Clausen JT, Tissue-specific processing of cocaine- and amphetamine-regulated transcript peptides in the rat, Proceedings of the National Academy of Sciences96(6) (1999) 2722–2727.

[5]. Couceyro PR, Koylu EO, Kuhar MJ, Further studies on the anatomical distribution of CART by in situ hybridization, J Chem Neuroanat12(4) (1997) 229–41. [PubMed: 9243343]

[6]. Koylu EO, Couceyro PR, Lambert PD, Kuhar MJ, Cocaine- and amphetamine-regulated transcript peptide immunohistochemical localization in the rat brain, J Comp Neurol391(1) (1998) 115–32. [PubMed: 9527537]

[7]. Lázár G, Calle M, Roubos EW, Kozicz T, Immunohistochemical localization of cocaine- and amphetamine-regulated transcript peptide in the central nervous system of the frog Rana esculenta, J Comp Neurol477(3) (2004) 324–39. [PubMed: 15305368]

[8]. Ashwell KW, Mai JK, Distribution of CART (cocaine- and amphetamine-regulated transcript) peptide in mature and developing marsupial brain, Brain Behav Evol76(2) (2010) 101–15. [PubMed: 20881368]

[9]. Dall Vechia S, Lambert PD, Couceyro PC, Kuhar MJ, Smith Y, CART peptide immunoreactivity in the hypothalamus and pituitary in monkeys: analysis of ultrastructural features and synaptic connections in the paraventricular nucleus, J Comp Neurol416(3) (2000) 291–308. [PubMed: 10602089]

[10]. Smith Y, Kieval J, Couceyro PR, Kuhar MJ, CART peptide-immunoreactive neurones in the nucleus accumbens in monkeys: ultrastructural analysis, colocalization studies, and synaptic interactions with dopaminergic afferents, J Comp Neurol407(4) (1999) 491–511. [PubMed: 10235641]

[11]. Smith Y, Koylu EO, Couceyro P, Kuhar MJ, Ultrastructural localization of CART (cocaine- and amphetamine-regulated transcript) peptides in the nucleus accumbens of monkeys, Synapse27(1) (1997) 90–4. [PubMed: 9268069]

[12]. Hurd YL, Fagergren P, Human cocaine- and amphetamine-regulated transcript (CART) mRNA is highly expressed in limbic- and sensory-related brain regions, Journal of Comparative Neurology425(4) (2000) 583–598.

[13]. Douglass J, Daoud S, Characterization of the human cDNA and genomic DNA encoding CART: a cocaine- and amphetamine-regulated transcript, Gene169(2) (1996) 241–5. [PubMed: 8647455]

[14]. Kuhar MJ, Adams LD, Hunter RG, Vechia SD, Smith Y, CART peptides, Regul Pept89(1–3) (2000) 1–6. [PubMed: 10771306]

[15]. Dallvechia-Adams S, Kuhar MJ, Smith Y, Cocaine- and amphetamine-regulated transcript peptide projections in the ventral midbrain: colocalization with gamma-aminobutyric acid, melanin-concentrating hormone, dynorphin, and synaptic interactions with dopamine neurons, J Comp Neurol448(4) (2002) 360–72. [PubMed: 12115699]

[16]. Kuhar MJ, Dall Vechia SE, CART peptides: novel addiction- and feeding-related neuropeptides, Trends in Neurosciences22(7) (1999) 316–320. [PubMed: 10370256]

[17]. Ahmadian-Moghadam H, Sadat-Shirazi MS, Zarrindast MR, Cocaine- and amphetamine-regulated transcript (CART): A multifaceted neuropeptide, Peptides110 (2018) 56–77. [PubMed: 30391426]

[18]. Lau J, Herzog H, CART in the regulation of appetite and energy homeostasis, Front Neurosci8 (2014) 313. [PubMed: 25352770]

[19]. Subhedar NK, Nakhate KT, Upadhya MA, Kokare DM, CART in the brain of vertebrates: circuits, functions and evolution, Peptides54 (2014) 108–30. [PubMed: 24468550]

[20]. del Giudice EM, Santoro N, Cirillo G, D'Urso L, Di Toro R, Perrone L, Mutational screening of the CART gene in obese children: identifying a mutation (Leu34Phe) associated with reduced resting energy expenditure and cosegregating with obesity phenotype in a large family, Diabetes50(9) (2001) 2157–60. [PubMed: 11522684]

[21]. Yamada K, Yuan X, Otabe S, Koyanagi A, Koyama W, Makita Z, Sequencing of the putative promoter region of the cocaine- and amphetamine-regulated-transcript gene and identification of polymorphic sites associated with obesity, Int J Obes Relat Metab Disord26(1) (2002) 132–6. [PubMed: 11791158]

[22]. Rigoli L, Munafo C, Di Bella C, Salpietro A, Procopio V, Salpietro C, Molecular analysis of the CART gene in overweight and obese Italian children using family-based association methods, Acta Paediatr99(5) (2010) 722–726. [PubMed: 20151954]

[23]. Guerardel A, Barat-Houari M, Vasseur F, Dina C, Vatin V, Clement K, Eberle D, Vasseur-Delannoy V, Bell CG, Galan P, Hercberg S, Helbecque N, Potoczna N, Horber FF, Boutin P, Froguel P, Analysis of sequence variability in the CART gene in relation to obesity in a Caucasian population, BMC Genet6 (2005) 19. [PubMed: 15823203]

[24]. Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, Boe AF, Boguski MS, Brockway KS, Byrnes EJ, Chen L, Chen L, Chen TM, Chin MC, Chong J, Crook BE, Czaplinska A, Dang CN, Datta S, Dee NR, Desaki AL, Desta T, Diep E, Dolbeare TA, Donelan MJ, Dong HW, Dougherty JG, Duncan BJ, Ebbert AJ, Eichele G, Estin LK, Faber C, Facer BA, Fields R, Fischer SR, Fliss TP, Frensley C, Gates SN, Glattfelder KJ, Halverson KR, Hart MR, Hohmann JG, Howell MP, Jeung DP, Johnson RA, Karr PT, Kawal R, Kidney JM, Knapik RH, Kuan CL, Lake JH, Laramee AR, Larsen KD, Lau C, Lemon TA, Liang AJ, Liu Y, Luong LT, Michaels J, Morgan JJ, Morgan RJ, Mortrud MT, Mosqueda NF, Ng LL, Ng R, Orta GJ, Overly CC, Pak TH, Parry SE, Pathak SD, Pearson OC, Puchalski RB, Riley ZL, Rockett HR, Rowland SA, Royall JJ, Ruiz MJ, Sarno NR, Schaffnit K, Shapovalova NV, Sivisay T, Slaughterbeck CR, Smith SC, Smith KA, Smith BI, Sodt AJ, Stewart NN, Stumpf KR, Sunkin SM, Sutram M, Tam A, Teemer CD, Thaller C, Thompson CL, Varnam LR, Visel A, Whitlock RM, Wohnoutka PE, Wolkey CK, Wong VY, Wood M, Yaylaoglu MB, Young RC, Youngstrom BL, Yuan XF, Zhang B, Zwingman TA, Jones AR, Genome-wide atlas of gene expression in the adult mouse brain, Nature445(7124) (2007) 168–76. [PubMed: 17151600]

[25]. Nogi Y, Ahasan MM, Murata Y, Taniguchi M, Sha MFR, Ijichi C, Yamaguchi M, Expression of feeding-related neuromodulatory signalling molecules in the mouse central olfactory system, Scientific Reports10(1) (2020) 890. [PubMed: 31964903]

[26]. Rodrigues BC, Cavalcante JC, Elias CF, Expression of cocaine- and amphetamine-regulated transcript in the rat forebrain during postnatal development, Neuroscience195 (2011) 201–214. [PubMed: 21903152]

[27]. Nagayama S, Homma R, Imamura F, Neuronal organization of olfactory bulb circuits, Front Neural Circuits8 (2014) 98. [PubMed: 25232305]

[28]. Volkoff H, Peter RE, Characterization of two forms of cocaine- and amphetamine-regulated transcript (CART) peptide precursors in goldfish: molecular cloning and distribution, modulation of expression by nutritional status, and interactions with leptin, Endocrinology142(12) (2001) 5076–88. [PubMed: 11713200]

[29]. Kehoe AS, Volkoff H, Cloning and characterization of neuropeptide Y (NPY) and cocaine and amphetamine regulated transcript (CART) in Atlantic cod (Gadus morhua), Comp Biochem Physiol A Mol Integr Physiol146(3) (2007) 451–61. [PubMed: 17254820]

[30]. Abraham H, Covasa M, Hajnal A, Cocaine- and amphetamine-regulated transcript peptide immunoreactivity in the brain of the CCK-1 receptor deficient obese OLETF rat, Exp Brain Res196(4) (2009) 545–56. [PubMed: 19533109]

[31]. Xu L, Bloem B, Gaszner B, Roubos EW, Kozicz T, Sex-specific effects of fasting on urocortin 1, cocaine- and amphetamine-regulated transcript peptide and nesfatin-1 expression in the rat Edinger-Westphal nucleus, Neuroscience162(4) (2009) 1141–9. [PubMed: 19426783]

[32]. Douglass AM, Kucukdereli H, Ponserre M, Markovic M, Grundemann J, Strobel C, Alcala Morales PL, Conzelmann KK, Luthi A, Klein R, Central amygdala circuits modulate food consumption through a positive-valence mechanism, Nat Neurosci20(10) (2017) 1384–1394. [PubMed: 28825719]

[33]. Ip CK, Zhang L, Farzi A, Qi Y, Clarke I, Reed F, Shi YC, Enriquez R, Dayas C, Graham B, Begg D, Bruning JC, Lee NJ, Hernandez-Sanchez D, Gopalasingam G, Koller J, Tasan R, Sperk G, Herzog H, Amygdala NPY Circuits Promote the Development of Accelerated Obesity under Chronic Stress Conditions, Cell Metab30(1) (2019) 111–128.e6. [PubMed: 31031093]

[34]. Lukaszewska I, Korczynski R, Kostarczyk E, Fonberg E, Food-motivated behavior in rats with cortico-basomedial amygdala damage, Behav Neurosci98(3) (1984) 441–51. [PubMed: 6732925]

[35]. Cai H, Haubensak W, Anthony TE, Anderson DJ, Central amygdala PKC-delta(+) neurons mediate the influence of multiple anorexigenic signals, Nat Neurosci17(9) (2014) 1240–8. [PubMed: 25064852]

[36]. Hardaway JA, Halladay LR, Mazzone CM, Pati D, Bloodgood DW, Kim M, Jensen J, DiBerto JF, Boyt KM, Shiddapur A, Erfani A, Hon OJ, Neira S, Stanhope CM, Sugam JA, Saddoris MP, Tipton G, McElligott Z, Jhou TC, Stuber GD, Bruchas MR, Bulik CM, Holmes A, Kash TL, Central Amygdala Prepronociceptin-Expressing Neurons Mediate Palatable Food Consumption and Reward, Neuron102(5) (2019) 1037–1052.e7. [PubMed: 31029403]

[37]. Vicentic A, Lakatos A, Hunter R, Philpot K, Dominguez G, Kuhar MJ, CART peptide diurnal rhythm in brain and effect of fasting, Brain Res1032(1–2) (2005) 111–5. [PubMed: 15680948]

[38]. Fagergren P, Hurd YL, Mesolimbic gender differences in peptide CART mRNA expression: effects of cocaine, Neuroreport10(16) (1999) 3449–52. [PubMed: 10599860]

[39]. Yang SC, Shieh KR, Li HY, Cocaine- and amphetamine-regulated transcript in the nucleus accumbens participates in the regulation of feeding behavior in rats, Neuroscience133(3) (2005) 841–51. [PubMed: 15908130]

[40]. Bharne AP, Borkar CD, Subhedar NK, Kokare DM, Differential expression of CART in feeding and reward circuits in binge eating rat model, Behav Brain Res291 (2015) 219–231. [PubMed: 26008155]

[41]. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG, Identification of targets of leptin action in rat hypothalamus, J Clin Invest98(5) (1996) 1101–6. [PubMed: 8787671]

[42]. Broberger C, Holmberg K, Kuhar MJ, Hokfelt T, Cocaine- and amphetamine-regulated transcript in the rat vagus nerve: A putative mediator of cholecystokinin-induced satiety, Proc Natl Acad Sci U S A96(23) (1999) 13506–11. [PubMed: 10557351]

[43]. Chen R, Wu X, Jiang L, Zhang Y, Single-Cell RNA-Seq Reveals Hypothalamic Cell Diversity, Cell Rep18(13) (2017) 3227–3241. [PubMed: 28355573]

[44]. Campbell JN, Macosko EZ, Fenselau H, Pers TH, Lyubetskaya A, Tenen D, Goldman M, Verstegen AM, Resch JM, McCarroll SA, Rosen ED, Lowell BB, Tsai LT, A molecular census of arcuate hypothalamus and median eminence cell types, Nat Neurosci20(3) (2017) 484–496. [PubMed: 28166221]

[45]. Mickelsen LE, Bolisetty M, Chimileski BR, Fujita A, Beltrami EJ, Costanzo JT, Naparstek JR, Robson P, Jackson AC, Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons, Nat Neurosci22(4) (2019) 642–656. [PubMed: 30858605]

[46]. Menyhért J, Wittmann G, Lechan RM, Keller E, Liposits Z, Fekete C, Cocaine- and amphetamine-regulated transcript (CART) is colocalized with the orexigenic neuropeptide Y and agouti-related protein and absent from the anorexigenic alpha-melanocyte-stimulating hormone neurons in the infundibular nucleus of the human hypothalamus, Endocrinology148(9) (2007) 4276–81. [PubMed: 17525122]

[47]. Li H-Y, Hwang H-W, Hu Y-H, Functional characterizations of cocaine- and amphetamine-regulated transcript mRNA expression in rat hypothalamus, Neuroscience Letters323(3) (2002) 203–206. [PubMed: 11959420]

[48]. Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S, Hypothalamic CART is a new anorectic peptide regulated by leptin, Nature393(6680) (1998) 72–6. [PubMed: 9590691]

[49]. Lee SJ, Verma S, Simonds SE, Kirigiti MA, Kievit P, Lindsley SR, Loche A, Smith MS, Cowley MA, Grove KL, Leptin Stimulates Neuropeptide Y and Cocaine Amphetamine-Regulated Transcript Coexpressing Neuronal Activity in the Dorsomedial Hypothalamus in Diet-Induced Obese Mice, The Journal of Neuroscience33(38) (2013) 15306–15317. [PubMed: 24048859]

[50]. Wortley KE, Chang GQ, Davydova Z, Fried SK, Leibowitz SF, Cocaine- and amphetamine-regulated transcript in the arcuate nucleus stimulates lipid metabolism to control body fat accrual on a high-fat diet, Regul Pept117(2) (2004) 89–99. [PubMed: 14700744]

[51]. Rohner-Jeanrenaud F, Craft LS, Bridwell J, Suter TM, Tinsley FC, Smiley DL, Burkhart DR, Statnick MA, Heiman ML, Ravussin E, Caro JF, Chronic central infusion of cocaine- and amphetamine-regulated transcript (CART 55–102): effects on body weight homeostasis in lean and high-fat-fed obese rats, Int J Obes Relat Metab Disord26(2) (2002) 143–9. [PubMed: 11850744]

[52]. Hou J, Zheng DZ, Zhou JY, Zhou SW, Orexigenic effect of cocaine- and amphetamine-regulated transcript (CART) after injection into hypothalamic nuclei in streptozotocin-diabetic rats, Clin Exp Pharmacol Physiol37(10) (2010) 989–95. [PubMed: 20626417]

[53]. Tian DR, Li XD, Shi YS, Wan Y, Wang XM, Chang JK, Yang J, Han JS, Changes of hypothalamic alpha-MSH and CART peptide expression in diet-induced obese rats, Peptides25(12) (2004) 2147–53. [PubMed: 15572204]

[54]. Yu Y, South T, Wang Q, Huang XF, Differential expression of hypothalamic CART mRNA in response to body weight change following different dietary interventions, Neurochem Int52(8) (2008) 1422–30. [PubMed: 18455834]

[55]. Yu Y, Deng C, Huang XF, Obese reversal by a chronic energy restricted diet leaves an increased Arc NPY/AgRP, but no alteration in POMC/CART, mRNA expression in diet-induced obese mice, Behav Brain Res205(1) (2009) 50–6. [PubMed: 19616032]

[56]. Robson AJ, Rousseau K, Loudon AS, Ebling FJ, Cocaine and amphetamine-regulated transcript mRNA regulation in the hypothalamus in lean and obese rodents, J Neuroendocrinol14(9) (2002) 697–709. [PubMed: 12213131]

[57]. Wang Z-W, Zhou Y-T, Kakuma T, Lee Y, Higa M, Kalra SP, Dube MG, Kalra PS, Unger RH, Comparing the hypothalamic and extrahypothalamic actions of endogenous hyperleptinemia, Proceedings of the National Academy of Sciences96(18) (1999) 10373–10378.

[58]. Kong W, Stanley S, Gardiner J, Abbott C, Murphy K, Seth A, Connoley I, Ghatei M, Stephens D, Bloom S, A role for arcuate cocaine and amphetamine-regulated transcript in hyperphagia, thermogenesis, and cold adaptation, Faseb j17(12) (2003) 1688–90. [PubMed: 12958177]

[59]. Elias CF, Lee C, Kelly J, Aschkenasi C, Ahima RS, Couceyro PR, Kuhar MJ, Saper CB, Elmquist JK, Leptin activates hypothalamic CART neurons projecting to the spinal cord, Neuron21(6) (1998) 1375–85. [PubMed: 9883730]

[60]. Elias CF, Aschkenasi C, Lee C, Kelly J, Ahima RS, Bjorbaek C, Flier JS, Saper CB, Elmquist JK, Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area, Neuron23(4) (1999) 775–86. [PubMed: 10482243]

[61]. Akieda-Asai S, Poleni PE, Date Y, Coinjection of CCK and leptin reduces food intake via increased CART/TRH and reduced AMPK phosphorylation in the hypothalamus, Am J Physiol Endocrinol Metab306(11) (2014) E1284–91. [PubMed: 24735891]

[62]. Pirnik Z, Maixnerová J, Matysková R, Koutová D, Železná B, Maletínská L, Kiss A, Effect of anorexinergic peptides, cholecystokinin (CCK) and cocaine and amphetamine regulated transcript (CART) peptide, on the activity of neurons in hypothalamic structures of C57Bl/6 mice involved in the food intake regulation, Peptides31(1) (2010) 139–44. [PubMed: 19818819]

[63]. Daly DM, Park SJ, Valinsky WC, Beyak MJ, Impaired intestinal afferent nerve satiety signalling and vagal afferent excitability in diet induced obesity in the mouse, J Physiol589(Pt 11) (2011) 2857–70. [PubMed: 21486762]

[64]. Covasa M, Ritter RC, Rats maintained on high-fat diets exhibit reduced satiety in response to CCK and bombesin, Peptides19(8) (1998) 1407–15. [PubMed: 9809656]

[65]. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S, A role for ghrelin in the central regulation of feeding, Nature409(6817) (2001) 194–198. [PubMed: 11196643]

[66]. Vrang N, Larsen PJ, Clausen JT, Kristensen P, Neurochemical characterization of hypothalamic cocaine- amphetamine-regulated transcript neurons, J Neurosci19(10) (1999) Rc5. [PubMed: 10234051]

[67]. Dhillo WS, Small CJ, Stanley SA, Jethwa PH, Seal LJ, Murphy KG, Ghatei MA, Bloom SR, Hypothalamic interactions between neuropeptide Y, agouti-related protein, cocaine- and amphetamine-regulated transcript and alpha-melanocyte-stimulating hormone in vitro in male rats, J Neuroendocrinol14(9) (2002) 725–30. [PubMed: 12213133]

[68]. Zheng H, Patterson LM, Berthoud HR, CART in the dorsal vagal complex: sources of immunoreactivity and effects on Fos expression and food intake, Brain Res957(2) (2002) 298–310. [PubMed: 12445972]

[69]. Lee SJ, Krieger JP, Vergara M, Quinn D, McDougle M, de Araujo A, Darling R, Zollinger B, Anderson S, Pan A, Simonnet EJ, Pignalosa A, Arnold M, Singh A, Langhans W, Raybould HE, de Lartigue G, Blunted Vagal Cocaine- and Amphetamine-Regulated Transcript Promotes Hyperphagia and Weight Gain, Cell Rep30(6) (2020) 2028–2039.e4. [PubMed: 32049029]

[70]. de Lartigue G, Dimaline R, Varro A, Dockray GJ, Cocaine- and amphetamine-regulated transcript: stimulation of expression in rat vagal afferent neurons by cholecystokinin and suppression by ghrelin, J Neurosci27(11) (2007) 2876–82. [PubMed: 17360909]

[71]. De Lartigue G, Dimaline R, Varro A, Raybould H, De la Serre CB, Dockray GJ, Cocaine- and amphetamine-regulated transcript mediates the actions of cholecystokinin on rat vagal afferent neurons, Gastroenterology138(4) (2010) 1479–90. [PubMed: 19854189]

[72]. de La Serre CB, de Lartigue G, Raybould HE, Chronic exposure to low dose bacterial lipopolysaccharide inhibits leptin signaling in vagal afferent neurons, Physiol Behav139 (2015) 188–94. [PubMed: 25446227]

[73]. Heldsinger A, Lu Y, Zhou SY, Wu X, Grabauskas G, Song I, Owyang C, Cocaine- and amphetamine-regulated transcript is the neurotransmitter regulating the action of cholecystokinin and leptin on short-term satiety in rats, Am J Physiol Gastrointest Liver Physiol303(9) (2012) G1042–51. [PubMed: 22936273]

[74]. de Lartigue G, Putative roles of neuropeptides in vagal afferent signaling, Physiol Behav136 (2014) 155–69. [PubMed: 24650553]

[75]. Kupari J, Haring M, Agirre E, Castelo-Branco G, Ernfors P, An Atlas of Vagal Sensory Neurons and Their Molecular Specialization, Cell Rep27(8) (2019) 2508–2523 e4. [PubMed: 31116992]

[76]. Egerod KL, Schwartz TW, Gautron L, The Molecular Diversity of Vagal Afferents Revealed, Trends Neurosci42(10) (2019) 663–666. [PubMed: 31421944]

[77]. 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, Cell179(5) (2019) 1129–1143 e23. [PubMed: 31730854]

[78]. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM 3rd, Hao Y, Stoeckius M, Smibert P, Satija R, Comprehensive Integration of Single-Cell Data, Cell177(7) (2019) 1888–1902 e21. [PubMed: 31178118]

[79]. de Lartigue G, Barbier de la Serre C, Espero E, Lee J, Raybould HE, Leptin resistance in vagal afferent neurons inhibits cholecystokinin signaling and satiation in diet induced obese rats, PLoS One7(3) (2012) e32967. [PubMed: 22412960]

[80]. de Lartigue G, Ronveaux CC, Raybould HE, Deletion of leptin signaling in vagal afferent neurons results in hyperphagia and obesity, Mol Metab3(6) (2014) 595–607. [PubMed: 25161883]

[81]. Banke E, Riva M, Shcherbina L, Wierup N, Degerman E, Cocaine- and amphetamine-regulated transcript is expressed in adipocytes and regulate lipid- and glucose homeostasis, Regul Pept182 (2013) 35–40. [PubMed: 23318496]

[82]. Ekblad E, Kuhar M, Wierup N, Sundler F, Cocaine- and amphetamine-regulated transcript: distribution and function in rat gastrointestinal tract, Neurogastroenterol Motil15(5) (2003) 545–57. [PubMed: 14507354]

[83]. Couceyro P, Paquet M, Koylu E, Kuhar MJ, Smith Y, Cocaine- and amphetamine-regulated transcript (CART) peptide immunoreactivity in myenteric plexus neurons of the rat ileum and co-localization with choline acetyltransferase, Synapse30(1) (1998) 1–8. [PubMed: 9704875]

[84]. Shcherbina L, Lindqvist A, Thoren Fischer AH, Ahlqvist E, Zhang E, Falkmer SE, Renstrom E, Koffert J, Honka H, Wierup N, Intestinal CART is a regulator of GIP and GLP-1 secretion and expression, Mol Cell Endocrinol476 (2018) 8–16. [PubMed: 29627317]

[85]. Vicentic A, Lakatos A, Jones D, The CART receptors: background and recent advances, Peptides27(8) (2006) 1934–7. [PubMed: 16713658]

[86]. Yermolaieva O, Chen J, Couceyro PR, Hoshi T, Cocaine- and amphetamine-regulated transcript peptide modulation of voltage-gated Ca2+ signaling in hippocampal neurons, J Neurosci21(19) (2001) 7474–80. [PubMed: 11567037]

[87]. Lakatos A, Prinster S, Vicentic A, Hall RA, Kuhar MJ, Cocaine- and amphetamine-regulated transcript (CART) peptide activates the extracellular signal-regulated kinase (ERK) pathway in AtT20 cells via putative G-protein coupled receptors, Neurosci Lett384(1–2) (2005) 198–202. [PubMed: 15908120]

[88]. Yosten GL, Harada CM, Haddock C, Giancotti LA, Kolar GR, Patel R, Guo C, Chen Z, Zhang J, Doyle TM, Dickenson AH, Samson WK, Salvemini D, GPR160 de-orphanization reveals critical roles in neuropathic pain in rodents, J Clin Invest130(5) (2020) 2587–2592. [PubMed: 31999650]

[89]. Haddock CJ, Almeida-Pereira G, Stein LM, Hayes MR, Kolar GR, Samson WK, Yosten GLC, Signaling in Rat Brainstem via Gpr160 is Required for the Anorexigenic and Antidipsogenic Actions of Cocaine- and Amphetamine-Regulated Transcript Peptide, Am J Physiol Regul Integr Comp Physiol (2020).

[90]. Sathyamurthy A, Johnson KR, Matson KJE, Dobrott CI, Li L, Ryba AR, Bergman TB, Kelly MC, Kelley MW, Levine AJ, Massively Parallel Single Nucleus Transcriptional Profiling Defines Spinal Cord Neurons and Their Activity during Behavior, Cell Rep22(8) (2018) 2216–2225. [PubMed: 29466745]

[91]. Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, O'Keeffe S, Phatnani HP, Guarnieri P, Caneda C, Ruderisch N, Deng S, Liddelow SA, Zhang C, Daneman R, Maniatis T, Barres BA, Wu JQ, An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex, J Neurosci34(36) (2014) 11929–47. [PubMed: 25186741]

[92]. Bannon AW, Seda J, Carmouche M, Francis JM, Jarosinski MA, Douglass J, Multiple behavioral effects of cocaine- and amphetamine-regulated transcript (CART) peptides in mice: CART 42–89 and CART 49–89 differ in potency and activity, J Pharmacol Exp Ther299(3) (2001) 1021–6. [PubMed: 11714891]

[93]. Sarkar S, Wittmann G, Fekete C, Lechan RM, Central administration of cocaine- and amphetamine-regulated transcript increases phosphorylation of cAMP response element binding protein in corticotropin-releasing hormone-producing neurons but not in prothyrotropin-releasing hormone-producing neurons in the hypothalamic paraventricular nucleus, Brain Res999(2) (2004) 181–92. [PubMed: 14759497]

[94]. Quintana A, Sanz E, Wang W, Storey GP, Güler AD, Wanat MJ, Roller BA, La Torre A, Amieux PS, McKnight GS, Bamford NS, Palmiter RD, Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors, Nature Neuroscience15(11) (2012) 1547–1555. [PubMed: 23064379]

[95]. Vrang N, Kristensen P, Tang-Christensen M, Larsen PJ, Effects of leptin on arcuate pro-opiomelanocortin and cocaine-amphetamine-regulated transcript expression are independent of circulating levels of corticosterone, J Neuroendocrinol14(11) (2002) 880–6. [PubMed: 12421341]

[96]. Schwartz MW, Woods SC, Porte D, Seeley RJ, Baskin DG, Central nervous system control of food intake, Nature404(6778) (2000) 661–671. [PubMed: 10766253]

[97]. Aja S, Sahandy S, Ladenheim EE, Schwartz GJ, Moran TH, Intracerebroventricular CART peptide reduces food intake and alters motor behavior at a hindbrain site, Am J Physiol Regul Integr Comp Physiol281(6) (2001) R1862–7. [PubMed: 11705771]

[98]. Aja S, Schwartz GJ, Kuhar MJ, Moran TH, Intracerebroventricular CART peptide reduces rat ingestive behavior and alters licking microstructure, Am J Physiol Regul Integr Comp Physiol280(6) (2001) R1613–9. [PubMed: 11353662]

[99]. Zheng H, Patterson C, Berthoud HR, Fourth ventricular injection of CART peptide inhibits short-term sucrose intake in rats, Brain Res896(1–2) (2001) 153–6. [PubMed: 11277984]

[100]. Skibicka KP, Alhadeff AL, Grill HJ, Hindbrain cocaine- and amphetamine-regulated transcript induces hypothermia mediated by GLP-1 receptors, J Neurosci29(21) (2009) 6973–81. [PubMed: 19474324]

[101]. Vrang N, Tang-Christensen M, Larsen PJ, Kristensen P, Recombinant CART peptide induces c-Fos expression in central areas involved in control of feeding behaviour, Brain Res818(2) (1999) 499–509. [PubMed: 10082837]

[102]. Lambert PD, Couceyro PR, McGirr KM, Dall Vechia SE, Smith Y, Kuhar MJ, CART peptides in the central control of feeding and interactions with neuropeptide Y, Synapse29(4) (1998) 293–8. [PubMed: 9661247]

[103]. Nagelova V, Pirnik Z, Zelezna B, Maletinska L, CART (cocaine- and amphetamine-regulated transcript) peptide specific binding sites in PC12 cells have characteristics of CART peptide receptors, Brain Res1547 (2014) 16–24. [PubMed: 24378198]

[104]. Larsen PJ, Vrang N, Petersen PC, Kristensen P, Chronic intracerebroventricular administration of recombinant CART(42–89) peptide inhibits and causes weight loss in lean and obese Zucker (fa/fa) rats, Obes Res8(8) (2000) 590–6. [PubMed: 11156435]

[105]. Thim L, Nielsen PF, Judge ME, Andersen AS, Diers I, Egel-Mitani M, Hastrup S, Purification and characterisation of a new hypothalamic satiety peptide, cocaine and amphetamine regulated transcript (CART), produced in yeast, FEBS Lett428(3) (1998) 263–8. [PubMed: 9654146]

[106]. Maletinska L, Maixnerova J, Matyskova R, Haugvicova R, Sloncova E, Elbert T, Slaninova J, Zelezna B, Cocaine- and amphetamine-regulated transcript (CART) peptide specific binding in pheochromocytoma cells PC12, Eur J Pharmacol559(2–3) (2007) 109–14. [PubMed: 17292884]

[107]. Kask A, Schioth HB, Mutulis F, Wikberg JE, Rago L, Anorexigenic cocaine- and amphetamine-regulated transcript peptide intensifies fear reactions in rats, Brain Res857(1–2) (2000) 283–5. [PubMed: 10700578]

[108]. Volkoff H, Peter RE, Effects of CART peptides on food consumption, feeding and associated behaviors in the goldfish, Carassius auratus: actions on neuropeptide Y- and orexin A-induced feeding, Brain Res887(1) (2000) 125–33. [PubMed: 11134597]

[109]. Smedh U, Scott KA, Moran TH, Fourth ventricular CART peptide induces c-fos in the area postrema and nucleus of the solitary tract via a CRF-receptor dependent mechanism, Neurosci Lett609 (2015) 124–8. [PubMed: 26475505]

[110]. Abbott CR, Rossi M, Wren AM, Murphy KG, Kennedy AR, Stanley SA, Zollner AN, Morgan DG, Morgan I, Ghatei MA, Small CJ, Bloom SR, Evidence of an orexigenic role for cocaine- and amphetamine-regulated transcript after administration into discrete hypothalamic nuclei, Endocrinology142(8) (2001) 3457–63. [PubMed: 11459791]

[111]. Stanley SA, Small CJ, Murphy KG, Rayes E, Abbott CR, Seal LJ, Morgan DG, Sunter D, Dakin CL, Kim MS, Hunter R, Kuhar M, Ghatei MA, Bloom SR, Actions of cocaine- and amphetamine-regulated transcript (CART) peptide on regulation of appetite and hypothalamo-pituitary axes in vitro and in vivo in male rats, Brain Res893(1–2) (2001) 186–94. [PubMed: 11223006]

[112]. Wang C, Billington CJ, Levine AS, Kotz CM, Effect of CART in the hypothalamic paraventricular nucleus on feeding and uncoupling protein gene expression, Neuroreport11(14) (2000) 3251–5. [PubMed: 11043558]

[113]. Jean A, Conductier G, Manrique C, Bouras C, Berta P, Hen R, Charnay Y, Bockaert J, Compan V, Anorexia induced by activation of serotonin 5-HT4 receptors is mediated by increases in CART in the nucleus accumbens, Proc Natl Acad Sci U S A104(41) (2007) 16335–40. [PubMed: 17913892]

[114]. Qing K, Chen Y, Central CART gene delivery by recombinant AAV vector attenuates body weight gain in diet-induced-obese rats, Regul Pept140(1–2) (2007) 21–6. [PubMed: 17182122]

[115]. Asakawa A, Inui A, Yuzuriha H, Nagata T, Kaga T, Ueno N, Fujino MA, Kasuga M, Cocaine-amphetamine-regulated transcript influences energy metabolism, anxiety and gastric emptying in mice, Horm Metab Res33(9) (2001) 554–8. [PubMed: 11561216]

[116]. Smedh U, Moran TH, Peptides that regulate food intake: separable mechanisms for dorsal hindbrain CART peptide to inhibit gastric emptying and food intake, Am J Physiol Regul Integr Comp Physiol284(6) (2003) R1418–26. [PubMed: 12468444]

[117]. Okumura T, Yamada H, Motomura W, Kohgo Y, Cocaine-amphetamine-regulated transcript (CART) acts in the central nervous system to inhibit gastric acid secretion via brain corticotropin-releasing factor system, Endocrinology141(8) (2000) 2854–60. [PubMed: 10919272]

[118]. Tebbe JJ, Ortmann E, Schumacher K, Monnikes H, Kobelt P, Arnold R, Schafer MK, Cocaine- and amphetamine-regulated transcript stimulates colonic motility via central CRF

receptor activation and peripheral cholinergic pathways in fed, conscious rats, Neurogastroenterol Motil16(4) (2004) 489–96. [PubMed: 15306004]

[119]. Farzi A, Lau J, Ip CK, Qi Y, Shi YC, Zhang L, Tasan R, Sperk G, Herzog H, Arcuate nucleus and lateral hypothalamic CART neurons in the mouse brain exert opposing effects on energy expenditure, Elife7 (2018).

[120]. Lau J, Farzi A, Qi Y, Heilbronn R, Mietzsch M, Shi YC, Herzog H, CART neurons in the arcuate nucleus and lateral hypothalamic area exert differential controls on energy homeostasis, Mol Metab7 (2018) 102–118. [PubMed: 29146410]

[121]. Lau J, Shi YC, Herzog H, Temperature dependence of the control of energy homeostasis requires CART signaling, Neuropeptides59 (2016) 97–109. [PubMed: 27080622]

[122]. Asnicar MA, Smith DP, Yang DD, Heiman ML, Fox N, Chen YF, Hsiung HM, Koster A, Absence of cocaine- and amphetamine-regulated transcript results in obesity in mice fed a high caloric diet, Endocrinology142(10) (2001) 4394–400. [PubMed: 11564703]

[123]. Moffett M, Stanek L, Harley J, Rogge G, Asnicar M, Hsiung H, Kuhar M, Studies of cocaine- and amphetamine-regulated transcript (CART) knockout mice, Peptides27(8) (2006) 2037–45. [PubMed: 16762458]

[124]. Wierup N, Richards WG, Bannon AW, Kuhar MJ, Ahren B, Sundler F, CART knock out mice have impaired insulin secretion and glucose intolerance, altered beta cell morphology and increased body weight, Regul Pept129(1–3) (2005) 203–11. [PubMed: 15927717]

[125]. Couceyro PR, Evans C, McKinzie A, Mitchell D, Dube M, Hagshenas L, White FJ, Douglass J, Richards WG, Bannon AW, Cocaine- and amphetamine-regulated transcript (CART) peptides modulate the locomotor and motivational properties of psychostimulants, J Pharmacol Exp Ther315(3) (2005) 1091–100. [PubMed: 16099925]

[126]. Osei-Hyiaman D, Depetrillo M, Harvey-White J, Bannon AW, Cravatt BF, Kuhar MJ, Mackie K, Palkovits M, Kunos G, Cocaine- and amphetamine-related transcript is involved in the orexigenic effect of endogenous anandamide, Neuroendocrinology81(4) (2005) 273–82. [PubMed: 16131814]

[127]. Jean A, Laurent L, Bockaert J, Charnay Y, Dusticier N, Nieoullon A, Barrot M, Neve R, Compan V, The nucleus accumbens 5-HTR(4)-CART pathway ties anorexia to hyperactivity, Transl Psychiatry2 (2012) e203. [PubMed: 23233022]

[128]. Smith KL, Gardiner JV, Ward HL, Kong WM, Murphy KG, Martin NM, Ghatei MA, Bloom SR, Overexpression of CART in the PVN increases food intake and weight gain in rats, Obesity (Silver Spring)16(10) (2008) 2239–44. [PubMed: 18719668]

Highlights

- The localization of *Cartpt* in the brain and periphery, focusing on areas with a known role in controlling energy homeostasis.
- Recent RNA profiling data that indicate heterogeneity of *Cartpt* expressing neurons and the stimuli that modulate *Cartpt* expression.
- New developments that identify a putative candidate receptor for CARTp signalling.
- The function of CARTp as an anorexigenic signal and its effect on energy expenditure and body weight control based on studies relying on pharmacological or genetic approaches.

Brain region	Peptide or mRNA	Fasting	Fed	Obesity
Forebrain				
OB	*Cartpt*	↓	↑	?
Valence				
NAc	CARTp	↓	↑	?
CeA	CARTp	↓	↑	?
Hypothalamus				
PVN	*Cartpt*	↓	↑	↑
LHA	*Cartpt*	?	?	↑
DMH	*Cartpt*	↓	↑	↓
Arc	*Cartpt*	↓	↑	↓
Vagal inputs				
NTS	CARTp	↓	↑	↓
AP	CARTp	↓	↑	↓

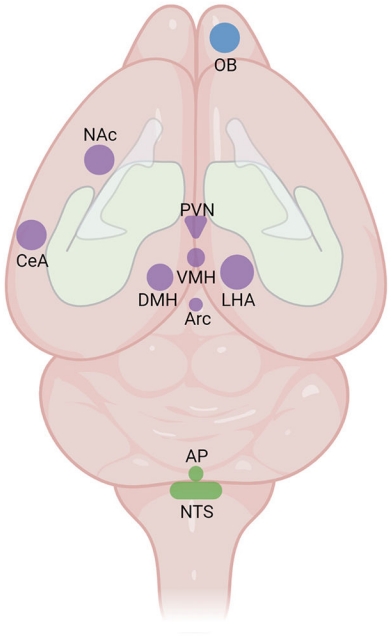

Figure 1.
Graphical summary of changes in *Cartpt* or CARTp levels in response to feeding related cues in different regions of the brain.

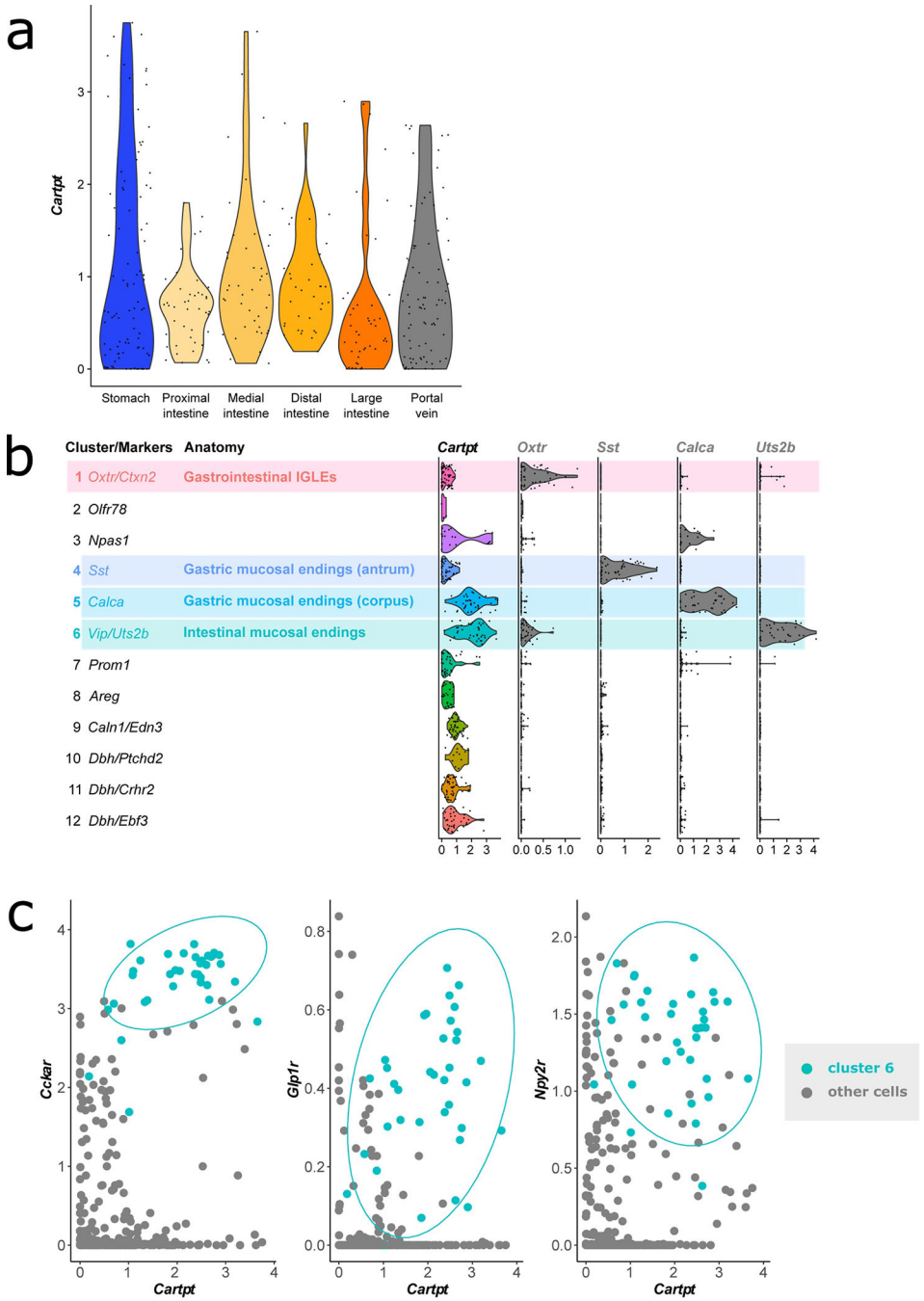

Figure 2: *Cartpt*+ vagal afferents project to the entire gastrointestinal tract but show a specific expression pattern

(re-analysis of target single-cell sequencing data from Bai et al. [77] and deposited on GEO (GSE138651)).

a. Single-cell expression of *Cartpt* in vagal afferents retrogradely labelled from segments of the gastrointestinal tract (target single-cell sequencing).

b. Single-cell expression pattern of *Cartpt* across vagal afferent subtypes. IGLEs: Intraganglionic laminar endings

c. Single-cell co-expression of *Cartpt* with *Cckar* (left), *Glp1r* (center) and *Npy2r* (right) in vagal afferents innervating visceral organs with 95% confidence ellipses around cluster 6 neurons.