Hypothalamic cellular changes in neuroendocrine disorders

Human postmortem studies

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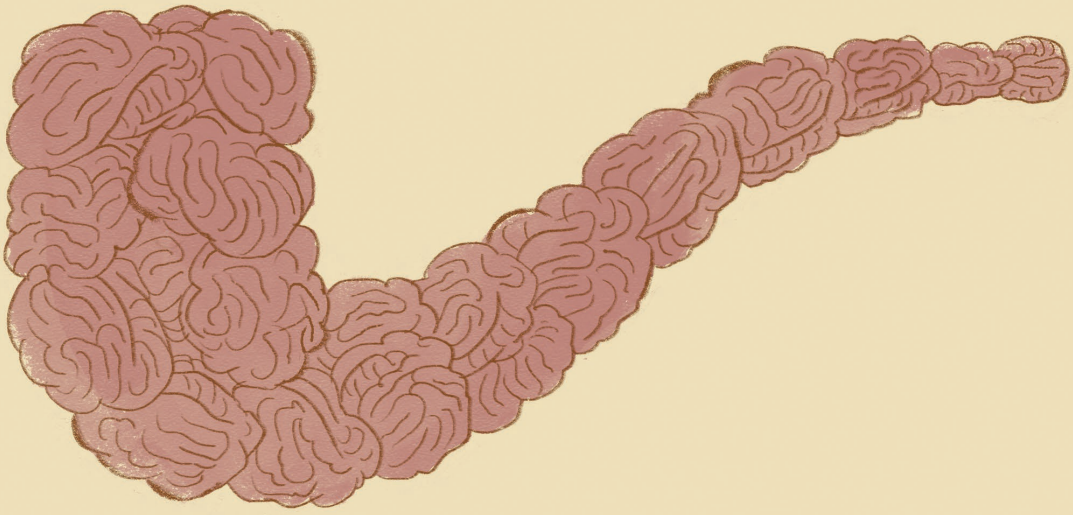

Chapter 8

General Discussion

The hypothalamus is a small diencephalic structure that is crucial for survival of the individual and regulates a multitude of physiological processes. Diverse hypothalamic nuclei act as fundamental integrators of vegetative and peripheral cues directly coordinating reproduction, osmotic balance, stress responses, energy homeostasis, glucose homeostasis and circadian rhythms (Sternson, 2013). Specialized hypothalamic neural populations are responsible for these outputs based on internal and endocrine inputs, finely regulating homeostasis through behavioral, autonomic, or neuroendocrine modulations (van den Beld et al., 2018). Extensive research from the past decades refined our comprehension of the participation of the hypothalamus in metabolic regulation. Hypothalamic dysfunction emerged as a mechanistic link between a number of neuroendocrine disorders, including obesity, type 2 diabetes mellitus (T2DM), and Prader-Willi Syndrome (PWS) (Bochukova et al., 2018; Yeo et al., 1998; Correa-da-Silva et al., 2023).

To date, obesity is one of the most well-studied neuroendocrine diseases for which many genetic, cellular, and environmental pathogenic components have been identified (Farooqi et al., 2006; Roberto et al., 2015; Clement et al., 1998). Large population studies show that genetic variants associated with body weight regulation are predominantly associated with the central nervous system (CNS) (Locke et al., 2015). Multiple neural pathways involved in the physiological and hedonic components of eating were unveiled, and prominent attention has been given to neuronal populations located in the arcuate nucleus of the hypothalamus (Arc; infundibular nucleus – INF – in humans) (Krude et al., 1998; Horvath et al., 1992). Two functionally distinct appetite-regulating neuronal populations of the Arc coordinate energy homeostasis in response to peripheral nutrient-state signals by modulating appropriate responses, such as food seeking behavior, temperature fluctuations, and insulin secretion (Gropp et al., 2005; Balthasar et al., 2005; Campbell et al., 2017).

Resident neurons in the Arc, such as the anorexigenic pro-opiomelanocortin-expressing (POMC-expressing) neurons, undergo a drastic circuitry remodeling upon chronic exposure to obesogenic cues. These alterations include synaptic reorganization, changed electrophysiological properties, and impaired neural function; however, ultimately these changes may result in neuronal death (Horvath et al., 2010; Moraes et al., 2009; Yi et al., 2017). Initial genetic studies revealed that mutations in the melanocortin 4 receptor (MC4R), one of the receptors for POMC-derived peptides, are the most

common cause of monogenic obesity in humans, resulting in a chronic orexigenic state (Farooqi et al., 2006; Yeo et al., 1998). Likewise, mutations in leptin and its receptor leads to early-onset morbid obesity (Krude et al., 1998). Those observations stimulated research in animal models, further exploring leptin- and POMC-related neural pathways in the framework of obesity (Maffei et al., 1995; Challis et al., 2004). Moreover, technical, and methodological advances allowed the identification of other hypothalamic neural populations beyond the Arc involved in metabolic regulation, with special highlights to the paraventricular nucleus of the hypothalamus (PVN) and the lateral hypothalamic area (LHA) (Atasoy et al., 2012). Neurons in these areas are responsive to both afferent signals from the Arc, as well as peripheral cues (Ionescu et al., 1989; Qualls-Creekmore and Munzberg, 2018; Slomp et al., 2023). Our current understanding is that obesity dampens the capacity of the CNS to integrate and react appropriately to circulating nutritional and endocrine cues (Patel and Abate, 2013; Berbari et al., 2013).

Under homeostatic conditions, neurons establish a constant and dynamic interaction with glial cells resulting in optimal CNS functioning. Astrocytes constitute one of the major glial populations and provide metabolic and trophic support to neurons (Banker, 1980). Abnormal neuron-glia interaction underlies disruption of the anorexic circuitry through a multitude of mechanisms, predominantly mediated by astrocytes and microglial cells (Kalin et al., 2015). Data from neuroimaging studies also point to an altered hypothalamic microstructure in obese humans compared to lean controls, and independent findings support the idea of hypothalamic reactive gliosis in human obesity (Thaler et al., 2012). Hypothalamic astrocytes control the interstitial levels of neurotransmitters and, more recently, astrocytic gliotransmission has emerged as a modulatory node in neuroendocrine networks in control of energy homeostasis (Fuente-Martin et al., 2012; Sa et al., 2023). Astrocytic-mediated gliovascular coupling is also disrupted upon consumption of energy-dense diets and contributes to the functional impairment of hypothalamic neural populations (Garcia-Caceres et al., 2016; Gruber et al., 2021; Delle et al., 2023). Neurovascular remodeling in obesity enables the entry of neurotoxic molecules form the periphery into the CNS, affecting neural responsiveness to nutritional cues and tissue waste clearance (Benoit et al., 2009; Banks et al., 2015; Delle et al., 2023). Lastly, a subset of hypothalamic astroglia also produces inflammatory mediators during overnutrition (BahniwalLittle and Klegeris, 2017), in synergism with microglia cells.

Microglia are the CNS resident immune cell population, and they coordinate a great range of homeostatic functions in the CNS, ranging from synaptic punning to tissue clearance (Kierdorf and Prinz, 2017). Microglia-associated changes are considered one of the major contributors and inductors of hypothalamic neuronal dysfunction (Reis et al., 2015; Gao et al., 2017a; Yi et al., 2017; Bocarsly et al., 2015). Under metabolic stress, dietary components and imbalanced endocrine signaling triggers an inflammatory response in microglia (Valdearcos et al., 2014; Gao et al., 2017a; Gao et al., 2018b). It is noteworthy that microglial inflammatory changes are persistent throughout body weight fluctuations and precede any phenotypic alterations associated with obesity (Thaler et al., 2012). Hampered microglial phagocytic capacity is also reported in murine models of obesity, together with elevated cytokine production (Gao et al., 2017b; Wang et al., 2021). This translates into accumulation of unwanted particles and cellular waste within the hypothalamic microenvironment. Moreover, recent evidence shows that obesity affects the function of other glial cell types in the hypothalamus, including tanycytes and oligodendrocytes. This may alter the secretion of neuroendocrine factors through defective tanycytic transport (Muller-Fielitz et al., 2017; Duquenne et al., 2021), and hamper the metabolic support that oligodendrocytes provide to neurons (Djogo et al., 2016; Ou et al., 2019). In summary, data from animal models show that prolonged changes in hypothalamic microstructure and cellular composition are intimately associated with obesity.

The large majority of research in the metabolism field has been conducted in murine models exposed to obesogenic diets. However, these models have intrinsic flaws and cannot fully recapitulate the human condition, especially regarding genetic heterogeneity. Pending questions such as whether the same neurobiological alterations associated with obesity can also be found in other metabolic disorders remain to be elucidated. Experimental limitations such as the influence of maternal metabolic imprinting, consistency of lifestyle choices (*i.e.*, exercises and dietary choices), the microbiome and genetic variation are usually not possible to be equated to the fullest in human studies (Lewis, 2002). In this context, studies using postmortem human material have proven to be representative for pathological stages of numerous diseases, including the ones of neuroendocrine nature. For instance, such studies have shown the association of the body max index (BMI) with number of hypothalamic neurons in the control of energy homeostasis, and confirmed animal findings of loss of appetite-curbing

neurons in obesity (Alkemade et al., 2012). Furthermore, hypothalamic microgliosis has also been observed in humans, yet little is understood about its temporal basis and causality (Baufeld et al., 2016). Whether neuron-glia interactions are a fundamental part of pathophysiological alterations in human metabolic diseases as seen in animal studies remains to be elucidated.

The aim of this thesis was to finely dissect the molecular and cellular drivers of hypothalamic abnormalities associated with these disorders, with a main focus on the alterations that take place in glial cells. We investigated molecular and cellular changes in human postmortem material, to better understand the translational relevance of evidence derived from animal studies.

1 THE NEUROENDOCRINE BASIS OF PRADER-WILLI SYNDROME

PWS was the first human imprinted disorder to be described and it is associated with lack of expression of genes in the paternally acquired chromosome 15 q11-q13 (Cassidy et al., 2012; Holm et al., 1993). Up to date, no animal model can fully recapitulate the PWS phenotype, and some of the attempts have generated contradictory phenotypes, such as accentuated leanness (Zahova and Isles, 2021). These data indicate some degree of synergism and interaction among the PWS-causative genes. This is supported by the fact that larger deletion genetic subtypes lead to worse clinical outcomes (Butler et al., 2004). Microdeletions in the PWS genomic area affecting single or a few of the PWS loci lead to a PWS-like phenotype (Schulze et al., 1996; Cao et al., 2017; McCarthy et al., 2018). PWS clinical manifestations are extensive, and include uncontrollable hyperphagia and morbid obesity, delayed puberty, abnormal growth and body composition, and cognitive impairment (Cassidy et al., 2012). PWS symptomatology is, at least partially, of hypothalamic etiology, and numerous examples of neuroendocrine pathway disruptions underlie the clinical manifestations of the disease, such as growth hormone (GH) deficiency, central adrenal insufficiency, and hypogonadism (Muller, 1997; Grugni et al., 2013; Aycan and Bas, 2014). To our knowledge, research of hypothalamic dysfunction in PWS has not yet explored in depth the role of glial cells in its pathogenesis, leaving a gap in our understanding of the disease.

To understand the potential role of glial malfunctioning in this disorder, we analyzed hypothalamic postmortem material of PWS individuals at molecular and cellular levels. First, in **Chapter 3** we focused on hypothalamic structures involved in the control of biological rhythm generation, based on observations of deteriorated circadian rhythm outputs in PWS individuals (Vgontzas et al., 1996; Manni et al., 2001; Butler et al., 2002). The aim of this study was to determine whether PWS pathophysiology affects the numbers and functionality of the hypothalamic pacemaker cells. Next, we aimed to understand the cellular basis that underlies the phenotypic severity differences described in PWS sub-genotypes. We evaluated numeric and functional changes in distinct hypothalamic cell types, including neurons, astrocytes, microglia, and oligodendrocytes.

Circadian rhythmicity comprises the cyclic behavioral and physiological variations that are needed to accommodate changes in the environmental states to which organisms are submitted to (Hurd and Ralph, 1998; Ouyang et al., 1998). It affects virtually all physiological systems, translating into oscillations in endocrine, metabolic, immune and neurological mediators (Logan and McClung, 2019). Temporal physiological and behavioral organization is conferred though synchronization of rhythms dictated by a central pacemaker, the suprachiasmatic nucleus (SCN), and its cellular molecular clocks (Reppert and Weaver, 2002). Circadian disturbances are observed in a plethora of neurological and psychiatric disorders and are often associated with abnormal SCN cytoarchitecture (Zhou et al., 2001; Liu et al., 2000; Aziz et al., 2010; De Pablo-Fernandez et al., 2018). Sleep disturbances are the most evident clinical outcome of circadian misalignment in PWS (Vgontzas et al., 1996; Manni et al., 2001; Butler et al., 2002; Lassi et al., 2016). Whether those misalignments result from disruption of the intrinsic clock machinery or emerged as a consequence of PWS symptomatology remains unknown.

To understand whether circadian desynchronization in PWS is driven by intrinsic SCN mechanisms, we evaluated the expression of PWS causative genes at single cell resolution in the murine SCN. Our results showed a strong neural signature for three PWS-related protein coding genes: Magel2, Necdin and Snrpn. Of those, Magel2 and Necdin were previously shown to be involved to some extent in circadian rhythmicity (Kozlov et al., 2007; Lu Dong and Li, 2020). To our knowledge, we are the first to report a potential role for Snrpn in biological rhythms. Overall, glial cells showed a more discreet expression level of genes in the PWS genomic region. Because PWS has distinct genetic subtypes, we included in our analysis genes related to PWS Type 1 (PWS T1) deletion, which compromises four extra genes in relation to the typical PWS Type 2 (PWS T2)

(Butler et al., 2004). We found no empirical suggestion for worsened circadian outcomes in PWS T1 subjects, which was consistent with low expression levels of PWS T1 exclusive genes in the SCN. These data are the first line of evidence indicating that PWS circadian abnormalities might be driven by intrinsic SCN factors.

Circadian adaptations are mediated by the SCN, which integrates environmental cues, such as the solar day, and transmits temporal representations to other physiological systems. It has been shown that the SCN timekeeping robustness is sustained even in ex vivo conditions, as demonstrated by organotropic SCN slices (Lehman et al., 1995; Jansen Van der Zee and Gerkema, 1999). Extensive research has examined the role of SCN neural machinery in adaptive and homeostatic circadian programming, especially through studies in animals where the SCN was ablated (Lehman et al., 1995; Marchant and Mistlberger, 1997). Recent state-of-the-art technology expanded the initial observations that AVP-containing neurons in the SCN are pivotal pacesetter cells (Mieda et al., 2015; Tsuno et al., 2023). Neurological conditions which include circadian disruption are often associated with reduced numbers of SCN AVP-containing cells (Liu et al., 2000; van Wamelen et al., 2013; Hogenboom et al., 2019). Interestingly, our data point to increased numbers of AVP-containing neurons in the SCN of PWS subjects, like what is observed in depression patients (Zhou et al., 2001). We found that vasoactive intestinal polypeptide-containing (VIP-containing) neurons were unaltered, suggesting a selective effect of PWS pathology on AVP-expressing cells. It is worth noting that both neuronal subtypes express similar levels of PWS causative genes in mice, as seen by our single cell RNA-sequencing analysis. As PWS neurons have defective production and secretion of neuropeptides (Chen et al., 2020), increased AVP-ir parameters may reflect impaired neuropeptidergic release. Our data also suggest at least a partial AVP neuronal dysfunction, as we observed increased AGEs content within the AVP domain in the SCN of PWS patients. Nevertheless, altered AVP content in the SCN implies an impaired capacity of the CNS to convey temporal programs to downstream tissues.

Glial cells have been shown to play a role in the maintenance of effective central clock machinery, especially astrocytes (Tso et al., 2017; Brancaccio et al., 2019; Brancaccio et al., 2017). These studies elegantly demonstrated that astroglia actively shape and participate in the generation of biological rhythms in mice. Reduced SCN astrocytic counts concomitant with circadian imbalance have been previously reported in a variety of human conditions (Hogenboom et al., 2019; Stopa et al., 1999). PWS is associated with a generalized hypothalamic astrocytic loss (Bochukova et al., 2018), and therefore this is

not limited to the SCN. Moreover, similar to our findings in the PVN of T2DM individuals, we found overrepresentation of components of the glymphatic system (GS). Taken together, these findings point to a greater AGEs SCN content in PWS, and we speculate that GS activity emerges as an adaptive response to metabolic waste. SCN microglia were unaltered in PWS, like previous reported data in T2DM (Hogenboom et al., 2019). This suggests that the human hypothalamus partitions waste clearance differently than shown in murine models, which are heavily microglia-centered (Podlesny-Drabiniok, Marcora and Goate, 2020). Thus, our results indicate an abnormal cytoarchitecture of the SCN of PWS subjects, affecting primarily AVP-containing neurons and astroglia.

Circadian timing is essential for neuroendocrine function, as widely demonstrated by diurnal periodicity of numerous hormones (Everett and Sawyer, 1950; Boyar et al., 1976; Plant, 1981). In fact, SCN neurons establish contact with neuroendocrine cells (van der Beek et al., 1993; Kalsbeek et al., 1996; Palm et al., 1999; Miller et al., 2006), evidencing the importance of circadian variation to endocrine function. Many of those reports focus on reproduction-controlling neurons, which are impaired in PWS (Hirsch et al., 2015). Moreover, rhythmicity is also sustained at the cellular level by a network of transcriptional factors, the clock genes, which creates cyclic waves of transcription in virtually every cell (Konopka and Benzer, 1971). Previously published data suggested increased levels of expression of the period circadian regulator 1, *Per1*, in the hypothalamus of PWS subjects (Bochukova et al., 2018). To our knowledge, our study is the first to demonstrate the neuroanatomical basis for circadian disruptions in PWS. Our results suggest that disruption in the SCN functionality might contribute to other phenotypic traits of PWS, beyond sleep abnormalities. In theory, these results open the door for novel therapeutic avenues in PWS, namely circadian-based therapies, such as timed exposure to light, time-restricted eating, and melatonin administration. Evidence from the clinical setting points to symptom attenuation in patients with Alzheimer's Disease (AD) patients upon some of those therapies (Figueiro et al., 2014; Riemersma-van der Lek et al., 2008).

Following our findings in **Chapter 3**, we concentrated our efforts on understanding the hypothalamic molecular and cellular basis of PWS phenotypic traits. In **Chapter 4**, we focused on the potential effect of PWS pathophysiology in hypothalamic perineuronal nets (PNNs), which are specialized structures of extracellular matrix in the CNS (Celio et al., 1998; Viggiano Ibrahim and Celio, 2000). Those structures are largely explored in the context of cortical and subcortical areas (Xu et al., 2003; Carulli et al., 2010; Vo et al., 2013; Gao et al., 2018a; Fawcett Oohashi and Pizzorusso, 2019), but emerging evidence

demonstrates their participation in hypothalamic energy homeostasis control (Alonge et al., 2020; Mirzadeh et al., 2019). PNNs are pivotal modulators of a multitude of neuronal functions, with special attention to synaptic organization and plasticity (Srinivasan Schachner and Catterall, 1998; Kochlamazashvili et al., 2010; Lensjo et al., 2017). Loss of PNNs is observed in several neuropathologies, such as Alzheimer's disease and epilepsy, and their diminished abundance is often connected to abnormal synaptogenesis and plasticity in circuitry specific to those disorders (Rogers et al., 2018; Alcaide et al., 2019; BaigWilcock and Love, 2005).

Transcriptional analysis of PWS hypothalami revealed downregulation of neuron-enriched genes compared to controls, with emphasis to transcripts involved in neurogenesis, neurotransmitter release and synaptic plasticity regulation (Bochukova et al., 2018). The presence and abundance of PNNs in the human hypothalamus is virtually unexplored, with exception of a few reports (Alonge et al., 2020; Mirzadeh et al., 2019; Zhang et al., 2021). Mirzadeh and colleagues demonstrate the presence of a subpopulation of orexigenic neurons that are enmeshed by the PNN component Wisteria floribunda (WFA). Namely, Neuropeptide Y-/Agouti Related Protein-containing neurons (NPY-/AgRP-) were described (Mirzadeh et al., 2019). Whether other neuronal populations are also surrounded by PNNs or if PNNs are affected in human pathological states remains undetermined. To understand if PNNs are affected by PWS pathophysiology and, potentially, can affect synaptic plasticity of neurons in control of energy homeostasis, we profiled WFA-positive structures in the INF of PWS and controls.

PNNs assembly occurs in postnatal development and is essential for restraining the maximal neural plasticity associated with embryonic and early stages of development of the CNS. Curiously, our data indicates that hypothalamic PNNs abundance is not age-dependent in controls in opposition to what has previously been described for other brain regions (Pizzorusso et al., 2002; Lensjo et al., 2017). This supports the notion that PNNs are highly heterogeneous structures, with distinct molecular composition and spatiotemporal patterns (Lupori et al., 2023). We observed a drastic reduction of PNNs in the INF of PWS subjects in comparison to controls, supporting previous findings of defective synaptic homeostasis in PWS (Bochukova et al., 2018; Soeda et al., 2023) . Conjectural analysis of our data points out that PWS INF neurons are structurally immature, with potential repercussions to their functionality. The precise contribution of hypothalamic PNNs to energy homeostasis is still poorly explored, but initial results suggest an active role in coordination of glucose homeostasis and feeding behavior

(Alonge et al., 2020; Mirzadeh et al., 2019). One of the limitations of our study is the undefined neuropeptidergic signature of INF WFA-positive neurons, hampering our ability to propose mechanisms by which loss of WFA-associated structures contribute to PWS symptomatology. Previous findings show that NPY/AgRP neurons are enmeshed by the cardinal PNN marker, WFA, in humans (Mirzadeh et al., 2019). Those neurons antagonize the anorexigenic neuronal population characterized by POMC expression and activation of NPY/AgRP neurons leads to promotion of feeding behavior and dampens energy expenditure (Baskin Breininger and Schwartz, 1999; Larsen et al., 1999). Thus, it is reasonable to speculate that reduction in hypothalamic PNNs might be linked to the severe hyperphagia that PWS individuals experience.

Next, we dwelled into the investigation of alterations that might underlie distinct phenotypic severity in PWS deletions subtypes. Notably, individuals with PWS T1 deletions as compared with PWS T2 deletions exhibit more pronounced physiological and cognitive abnormalities such as compulsive behaviors, obsessive thoughts, self-injury, cognitive impairment, and disruptions in visual processing (Butler et al., 2004; Milner et al., 2005; Hartley et al., 2005). In **Chapter 5**, we systematically analyzed the transcriptional profile and cellular composition of hypothalamic nuclei that compose the mediobasal hypothalamus (MBH). As the MBH harbors neurons in control of metabolism, various behaviors, and social cognition, we hypothesized that worsened phenotypic severity of PWS T1 compared with T2 is linked to more drastic cellular disruptions. Through comprehensive transcriptomic analyses and cell-specific protein expression profiling in neurons, glial cells, and white matter, we uncovered significant disparities in gene expression and cellular functional outcomes in PWS genetic subtypes. Notably, our findings highlight a dysfunctional microglial phagolysosome and altered neural communication as pivotal contributors to the heightened phenotypic severity observed in patients carrying PWS T1 deletion.

Neurodegenerative diseases are often associated with accumulation of neurotoxic proteins, which may lead to neural dysfunction and death (Menzies et al., 2017). Robust evidence from PWS animal models and postmortem human material show that neural populations in the MBH are heavily affected by PWS pathophysiology (Swaab Purba and Hofman, 1995; Goldstone et al., 2002; Mercer et al., 2013; Polex-Wolf et al., 2018; Bochukova et al., 2018). Despite the well-established role of glial cells in neurodegeneration, little is known about their role in the context of PWS. A recent report demonstrated increased microglia-related mediators in the hypothalamus of

PWS compared to controls consistent with non-genetic obesity (Bochukova et al., 2018). However, whether microglial activation differs between different PWS subgenotypes or it is associated with specific hypothalamic domains remains elusive. Our work demonstrates that PWS T2 individuals have classical features of microglial activation, such as microgliosis, similar to non-genetic obesity (Baufeld et al., 2016). Microglial activation seems to be restricted to the MBH, given our SCN findings in Chapter 3. Microglia of PWS T1 subjects show a generalized severe morphological disruption, characterized fragmentation of processes' and discontinuation of soma-ramifications, contrary to our observations in PWS T2 microglia. Those changes were not exclusive to the hypothalamus, they were also observed in the hippocampus of the same subjects. This morphological disruption is termed cytorrhexis and has been previously described in the context of AD and aging (Streit et al., 2004; Streit et al., 2009).Although it is not possible to establish whether microglial activation emerges as a consequence of the obese phenotype in PWS T2 individuals. Dysmorphic microglia are already present as early as 6 months of age in PWS T1, which precedes hyperphagia and obesity (Miller et al., 2011). Furthermore, comparisons of the transcriptional signature of PWS T1 and T2 hypothalami demonstrated a downregulation of cytoskeleton-related transcripts and process in PWS T1. Finally, our results in experimental animals demonstrate that microglial dimorphism can be rooted to the lack of the gene Cyfip1, one of the PWS T1 exclusive genes. Cyfip1-haploinsuficient rats and microglia-specific Cyfip1 knockout mice also present with microglial morphological disruptions.

Microglial morphological disruption translates into lesser immunosurveillance, as indicated by impaired autophagic flux in PWS T1. Once again, our molecular analyses were aligned with the morphometric characterization. Moreover, microglia cells are not only disease-associated. Beyond removal of threating agents, they actively release trophic factors, shape circuits, and provide protection against oxidative stress and excitotoxity (Kierdorf and Prinz, 2017). Cytorhexis is the ultimate state of cellular senesce (Neumann et al., 2023), indicating that the PWS T1 hypothalami are deprived of microglial trophic support. Diverse endogenous mechanisms promote CNS tissue clearance, both in microglia-dependent ways, such as phagocytosis, or extrusion of unwanted extracellular particles by the GS (Iliff et al., 2012; Podlesny-DrabiniokMarcora and Goate, 2020). Our examination of the GS in PWS T1 and T2 hypothalami showed enhanced GS activity in the MBH of PWS T1 subjects, which could be due to a compensatory mechanism for microglial malfunctioning. As mentioned earlier, increased GS activity as an adaptive

response to tissue injury seems to be region specific, since in the SCN (**Chapter 3**) we detected it irrespective of subgenotypes. Taken together, these findings suggest that the combination of dystrophic microglial profiles and accumulation of interstitial waste might amplify phenotypic severity in PWS T1.

Optimal CNS function relies on the ability of neurons to communicate with each other. Therefore, we assessed neuronal communication though evaluation of a synaptic marker, synaptophysin, evaluation of neuropeptidergic profiles and integrity of the fornix. Our observations point out that PWS T2 hypothalami have diminished content of energy homeostasis-controlling neuropeptides, but unchanged levels of expression of synaptophysin and unaltered fornicial microstructure compared to controls. In contrast, PWS T1 has a marked reduction in synaptophysin expression and abnormal microstructure of the fornix compared to controls. These data provide additional insights to the worsened PWS T1 phenotype, as synaptophysin knockout animals have behavioral inflexibility and cognitive defects (Schmitt et al., 2009; Tarpey et al., 2009). Moreover, the fornix is the major output tract from the hippocampus linking the limbic system to the hypothalamus (Ou et al., 2023). Abnormalities in the forniceal structure have been shown to be linked to memory defects and cognitive deviances (Cummings, 1994; Zhuang et al., 2013), which are exacerbated in PWS T1 in comparison to PWS T2 subjects (Butler et al., 2004). Cyfip1 has been previously implicated in myelination capacity and neuron-neuron communication (De Rubeis et al., 2013; Dominguez-Iturza et al., 2019; Silva et al., 2019), reinforcing the causative role of this gene in PWS T1 phenotypic determination.

Our findings carry potential implications for PWS therapeutic avenues. The distinct molecular and cellular signature in the hypothalami of PWS T1 and T2 advocate for the use of personalized medicine strategies targeting each subgenotype. Of note, fornicial deep brain stimulation has been extensively debated in other neurological disorders, such as AD (Leoutsakos et al., 2018). Outcomes from clinical trials have shown successful alleviation of AD symptomatology, such as cognitive decline (Laxton et al., 2010). However, competing reports suggest that this therapeutic route needs optimization, possibly explained by non-consistent electrode placement, and consequent targeted networks, in different studies (Li et al., 2020). Speculative interpretation of our results indicates that forniceal deep brain stimulation might ameliorate PWS T2 symptomatology, but not PWS T1, given the fornix structural deterioration observed in this group. T1-deletion personalized strategies might require restoring microglial homeostatic function. A

recent report suggested systemic hematopoietic cell transplantation as a possibility for microglial replacement in the context of AD (Yoo et al., 2023). The implementation of personalized interventions for distinct subgenotypes in PWS could lead to a more assertive targeting of the disease-modifying aspects of each subgenotype.

2 DISRUPTED NEUROENDOCRINE SYSTEMS IN T2DM

Obesity is a significant risk factor for development of T2DM (Khaodhiar McCowen and Blackburn, 1999). Thus, reasonable speculation points to at least a partial overlap of cellular and molecular mechanisms for both diseases. To this date, there is no animal model that can fully represent the human T2DM pathophysiology. Impaired glucose regulation in murine models is often achieved by introduction of obesogenic diets that mimic a human western-style hypercaloric diet (Speakman, 2019). Moreover, genetic rodent diabetic models are derived from mutations in the leptin signaling pathway (Lee et al., 1996; Takaya et al., 1996), that are shown not to be a primary cause in humans (Taylor, 2012). T2DM is characterized by disruptions in glucose homeostasis due to defective insulin signaling in metabolic-relevant organs, including the hypothalamus. (Taylor, 2012). Likewise, to what is observed in obese individuals, evidence from neuroimaging studies demonstrate brain structural and metabolic alterations in T2DM subjects (Rosenbaum et al., 2022). However, those studies fail to provide spatial resolution to these alterations, and whether these metabolic disruptions are rooted in selective neuronal populations or in a generalized hypothalamic malfunction is undetermined. To better understand the hypothalamic alterations that underlie T2DM pathogenesis, our group has resorted to the use of postmortem tissue (Alkemade et al., 2012; Yi et al., 2012; Hogenboom et al., 2019; Kalsbeek et al., 2020), allowing for spatial resolution, (*i.e.*, where potential alterations take place), and a retrograde analysis of medical records. Previous findings have pointed to a neuropeptidergic imbalance in the hypothalamus of T2DM subjects in a similar fashion to what is observed in obesity (Alkemade et al., 2012; Kalsbeek et al., 2020).

In this thesis we proposed to investigate the impact of T2DM pathophysiology on key glucoregulatory neural populations and homeostatic features of glia cells. In **Chapter 6**, we profiled hypothalamic lipid droplets (LD), inspired by previous reports demonstrating a strong connection between lipid metabolism, inflammation, and

insulin resistance in peripheral tissues (Sanjabi et al., 2015; van Dierendonck et al., 2022). We compared the distribution of the LD organelle in both mice and humans and found that LDs are preferentially present in the hypothalamic third ventricle tanycytic layer in homeostatic conditions. Previous studies point to a dysfunction in the lipid metabolism taking place in the CNS of metabolically diseased mice (Timper et al., 2018; Varela et al., 2021). Furthermore, a robust amount of evidence demonstrates that sutured lipid species trigger, at least partially, the hypothalamic remodeling observed in obesity (Benoit et al., 2009; Valdearcos et al., 2014; Melo et al., 2020).

We hypothesized that an obesogenic diet would prompt dynamic changes in the ventricular LD content, and impact regional lipid metabolism in the hypothalamus. Indeed, our results revealed that high fat fed mice showed increased hypothalamic content of LD, especially in cells that line the third ventricle. This tanycytic response to metabolic perturbations might indicate an adaptive mechanism buffering lipotoxicity, similar to what is observed in the periphery (Khatua et al., 2021). Our observations also suggest that the changes in the third ventricle wall might contribute to the cellular metabolic stress that appetite-curbing neurons suffer upon exposure to obesogenic cues (Timper et al., 2018). A study by Shen et al. showed that astrocytic lipid metabolism governs body weight gain and glucose homeostasis, and can impact POMC neurons function (Shen et al., 2008), supporting our findings. Interestingly we showed no correlation between BMI and hypothalamic ventricular LD content in humans. Of importance, our analysis was restricted to a limited number of individuals and increased sample size might refine our understanding on human hypothalamic lipid biology. In T2DM, LD content was reduced in comparison to controls, suggesting an opposite metabolic remodeling than the one observed in animal models of obesity (Sanjabi et al., 2015). Studies that focused on cortical areas demonstrate a role for LD accumulation in microglial cells, and a direct relationship with pathogenic outcomes, such as cognitive decline (Lee et al., 2023). In the hypothalamus, LD presence in the parenchyma was extremely low, and our analyses did not show LD presence in the human microglia. It is worth mentioning that the cells in the ventricular wall and in the brain parenchyma are interconnected through a vascular network, which allows cerebral blood and cerebrospinal fluid (CSF) flow (Eide et al., 2021; Göbel et al., 2020). The reduction in LD we observed in T2DM individuals might be indicative of a disruption in these gliovascular units since cellular metabolism heavily supports those functions. In fact, previous findings of our group reported hypothalamic hypervascularization in the INF of T2DM patients (Yi et al., 2012),

reinforcing this hypothesis. Independent observations demonstrated that T2DM is associated with increased protein levels in the CSF (Kobessho et al., 2008), suggesting that gliovascular remodeling also extends to the dynamics of CSF flow in this disease. The exact mechanism and physiological consequences behind those changes remain to be elucidated.

The PVN harbors key glucoregulatory and neuroendocrine neural populations and functions as an integrator of hypothalamic and extra-hypothalamic inputs (Ionescu et al., 1989; Biag et al., 2012). To better understand the impact of T2DM pathogenesis in the hypothalamus, we profiled the neuro-glio-vascular composition of the PVN of T2DM subjects. In Chapter 7, we report a drastic reduction of oxytocin-containing (Oxt-) neurons in the PVN of T2DM subjects. Oxt is a well-recognized neuroendocrine mediator, and it is released from neurohypophiseal terminals in response to a variety of stimuli. Central Oxt signaling regulates a multitude of physiological processes, and undoubtedly impacts feeding behavior and glycemic control (Atasoy et al., 2012). Evidence from murine models, non-human primates and humans consistently show that Oxt administration leads to reduction in glycaemia (Altszuler and Hampshire, 1981; Bjorkstrand Eriksson and Uvnas-Moberg, 1996; Lawson et al., 2015; Blevins et al., 2015). Oxt knockout mice are obese and glucose intolerant, even in standard chow-fed conditions (Camerino, 2009). Therefore, reduced numbers of Oxt neurons in T2DM individuals are coherent with the clinical outcomes of this disorder and support previous observations of lesser serum levels of Oxt in T2DM subjects (Qian et al., 2014; Al-Rawashdeh et al., 2017).

Our analysis also revealed that T2DM does not affect the numbers of AVP- and corticotrophin releasing hormone-containing (CRH-containing) neurons, suggesting a selective effect on the Oxt-expressing neuronal population. Dysregulations of the hypothalamic-pituitary axis (HPA) are extensively reported in animal models of T2DM, and a direct link with glucose intolerance and insulin resistance has been established (Andrews et al., 2002; Lucassen and Cizza, 2012; Di Dalmazi et al., 2012). Therefore, it is not unreasonable to conjecture that CRH neurons, which are central mediators of the HPA axis (Vale et al., 1981), may be affected by T2DM pathophysiology. Evidence from animal models show that the oxytocinergic secretory activity can diminish the response of the HPA axis to a given stressor (Lang et al., 1983; Legros, 1992). Thus, even though we found no numeric changes of CRH-containing neurons, reduced Oxt levels might indirectly contribute to persistent activation of the HPA in T2DM.

Imbalanced autonomic hypothalamic output is observed in T2DM subjects, partially attributed to hyperinsulinemia and/or insulin resistance (Rowe et al., 1981; Lembo et al., 1992; Vollenweider et al., 1993; Freeman, 2014; Gudbjornsdottir et al., 1994). These disruptions can lead to adverse clinical outcomes, for instance, in the gastrointestinal, cardiovascular, and endocrine systems (Ryder et al., 1990; Huggett et al., 2003). Since the pre-autonomic PVN neuronal populations modulate parasympathetic and sympathetic outflow (Busnardo et al., 2010; Buijs et al., 2003), our results also contribute to the understanding of autonomic-related endocrine abnormalities in T2DM. Despite immense efforts, the neuropeptidergic identity of autonomic neurons is still not known. Recently, a transneural network connecting PVN Oxt neurons to the endocrine pancreas was described and functionally characterized (Papazoglou et al., 2022). Moreover, previous studies have demonstrated that a subpopulation of PVN Oxt neurons contributes to the sympathetic innervation of the liver and adipose tissue (Stanley et al., 2010; Buijs et al., 2003). These results indicate that PVN Oxt cells may be involved in glucoregulation through autonomic innervations. Thus, reduced numbers of PVN Oxt neurons might underlie the increased sympathetic outflow observed in T2DM individuals.

Because of the neural changes observed, we also profiled the PVN glial and vascular composition. Insulin astrocytic signaling can dictate body weight gain and glucose homeostasis (Garcia-Caceres et al., 2016; Herrera Moro Chao et al., 2022). Therefore, it was expected that T2DM-related alterations are associated with alterations in astroglia. Interestingly, we observed reduced numbers of astrocytes and augmented vasculature in the PVN. Evidence from chronically diabetic rats shows a similar astroglial reduction (Lechuga-Sancho et al., 2006), suggesting lack of astrocytic trophic support to oxytocinergic cells, associated with disrupted glucose homeostasis. It is important to highlight that those findings are based on immunoreactivity of the cardinal astrocyte marker glial fibrillary acid protein (GFAP). Recent methodological advances in state-of-art technologies allowed the visualization of astrocytic transcriptional and molecular diversity at single cell resolution beyond GFAP (Khakh and Sofroniew, 2015; Endo et al., 2022). The comprehension of astrocytic subpopulations affected by T2DM pathophysiology might provide insightful information on T2DM-associated neurodegeneration, similar to recent discoveries in murine models of obesity (Lutomska et al., 2022). Lastly, augmented vascularization in the PVN suggests a generalized hypothalamic angiopathy in T2DM, potentially mediated by glial cells.

To understand hypothalamic astrocytic reactivity in diabetic subjects, we profiled aquaporin 4-positive (AQP4-positive) astroglia. These cells constitute the GS, and therefore form a network of cells that coordinate interstitial clearance through fluid interchange in perivascular spaces (Iliff et al., 2012; Mestre et al., 2018). The hypothalamic GS is virtually unexplored in metabolic disorders (Delle et al., 2023), and, to our knowledge, our work is the first to explore it in the human condition. Different neuropathologies have been associated with dysfunctions in the GS, especially in cortical areas (Aoki-Yoshino et al., 2005; Yang et al., 2011; Peng et al., 2023; SmithDuan and Verkman, 2019). In contrast to our results on GFAP-positive astrocytes, we found an increased representation of AQP4 astroglia. Speculative interpretation of those results indicates that in the T2DM condition there is accumulation of metabolic and interstitial waste in the hypothalamus. This is supported by previous reports demonstrating hypothalamic accumulation of advanced glycation end products (AGEs) in high fat diet-fed (HFD-fed) mice (Gao et al., 2017a). AGEs are macromolecules with pathogenic significance in T2DM and are used as a proxy for cellular dysfunction in diverse tissues (Reddy et al., 1995). Increased GS activity might emerge as a response to formation and accumulation of different AGEs. In fact, AGEs formation occurs through a non-enzymatic modification of macromolecules. As lipid species might participate of AGEs formation (Fu et al., 1996), it is possible that the dynamic changes we previously observed in LD content are also implicated in fueling the AGE pathways.

Extensive research has demonstrated the role of microglia-derived inflammatory mediators in hypothalamic dysfunction in obesity, both in animal models (De Souza et al., 2005; Thaler et al., 2012; Gao et al., 2014) and humans (Baufeld et al., 2016; Melo et al., 2020). It is important to stress that increased microglial activity in human obesity is not fully understood and is predominantly observed in the chronic stages of the disease. Therefore, there is a lack in temporal and causal basis, and speculative interpretation points to a mechanism similar to what is described in animal models, centered in the chronic production of neurotoxic mediators. Hypothalamic microgliosis is commonly used as a proxy for increased local proinflammatory tonus (Thaler et al., 2012; Berkseth et al., 2014; Gao et al., 2018b). Previous observations from our lab show that T2DM does not elicit microgliosis in the human hypothalamus (Hogenboom et al., 2019; Kalsbeek et al., 2020), despite comparable loss of key metabolism-controlling neurons in obesity (Kalsbeek et al., 2020; Lechner et al., 2023). This difference might be rooted in distinct immune phenotypes between rodents and humans (Vijayan et al., 2019; Medetgul-

Ernar and Davis, 2022), and divergence of neural pathophysiological mechanisms behind neurodegeneration in each condition. Strikingly, recent data from mice elegantly show that hypothalamic microglia participate in glucose metabolism in a body weight-independent manner (Douglass et al., 2023). In this study, the authors report that microglial inflammatory mediators act on glucose-sensing neurons, amplifying insulin secretion via parasympathetic circuitry, irrespective of HFD-feeding. Their results indicate that microglial reactivity is necessary for euglycemia, compatible with previous observations (Winkler et al., 2019). However, which factors orchestrate microglial activation to coordinate glycaemia are not yet fully understood. As microglial immunosurveillance and phagocytic capacity are unaltered in aglycemic conditions (Bernier et al., 2020), glucose itself might not be the primordial driver here. These findings seem to be consistent with our observations, where neither numeric nor functional changes in microglia were detected in the suprachiasmatic nucleus (SCN), INF and PVN of T2DM individuals (Kalsbeek et al., 2020; Hogenboom et al., 2019). Cautious interpretation of these data is necessary, as our analysis does not fully partition the impact of body weight in T2DM or control subjects, and it is impossible to determine whether microgliosis took place at earlier points of the disease. Our data on the hypothalamic GS suggest that the human brain might have a different functional organization of waste clearance than rodents. Associated with increased production of cytokines, murine microglia dampen their phagocytic capacity upon exposure to obesogenic cues (Gao et al., 2017b; Gao et al., 2018b; Wang et al., 2021; Milanova et al., 2022). This results in reduced ability for waste removal, which contributes to neuronal dysfunction (FrickerOliva-Martin and Brown, 2012). Our data show that microglial phagocytic function in the PVN is unaffected by T2DM pathology. However, enhancement of GS activity can be the primary mechanism for waste clearance in this pathogenic state or it can emerge in response to microglial hypoactivity. Further research is necessary for a full understanding of the cellular basis of hypothalamic dysfunction and its phenotypic consequences in T2DM.

Our results show that T2DM leads to defective neuroendocrine systems in T2DM, which are fundamental for insulin sensitivity and body weight maintenance. Our investigations revealed oxytocinergic alterations, and this thesis provides insights into the cellular alterations that might underlie neuroendocrine abnormalities in T2DM. Our data provides ample results in support of the hypothesis that disruption of the neuro-glia-vascular unit culminates in the pathophysiological alterations related to T2DM.

3 OXYTOCIN AS A THERAPEUTIC NODE IN T2DM AND PWS

Metabolic disorders have reached epidemic levels and have become a major cause of morbidity and mortality worldwide (Chew et al., 2023). Substantial efforts have been made in the past decades to combat obesity and, by consequence, its comorbidities like T2DM, beyond lifestyle and behavioral interventions. Until recent years, the development of pharmacotherapeutic agents for the treatment of obesity were modest and underperformed surgical interventions, such as gastric bypass (Boyers et al., 2021). Numerous efforts focused on endocrine pathways associated with the central control of metabolism, with special attention to leptin-, ghrelin- and melanocortin-related pathways (Barnett et al., 2010; Lee et al., 2016; Collet et al., 2017). Currently, therapies which target endogenous mechanisms of nutrient-stimulating hormones in control of energy homeostasis emerged as promising agents in suppressing hyperphagia and increase energy expenditure (Coskun et al., 2022; Jastreboff et al., 2023). One of the most promising medication classes now are the glucagon like peptide 1 receptor (GLP-1R) agonists, with several agents commercially available (Muller et al., 2019). Interestingly, some initial data indicate that those pharmacological agents also hold therapeutic value for monogenetic forms of obesity (Iepsen et al., 2018). Whether this is true for polygenic cases, such as PWS, is still poorly understood. It is noteworthy that those endocrine agonists are expected to compose a life-long treatment plan, potentially due to irreversibility of hypothalamic damage elicited by metabolic disorders (Wilding et al., 2021). However, it is possible to speculate that upon achievement of body weight stability and normoglycemic state, a comprehensive plan that includes behavioral and less potent pharmacological interventions should be considered.

Animal models have yielded robust evidence into the translational potential of Oxt in the treatment of a variety of pathologies. Diverse patient populations which have abnormal or reduced oxytocinergic signaling might benefit from exogenous Oxt administration. Given the magnitude of effects observed in mice, numerous interventions were posed to non-human primates and humans, targeting a broad range of pathologies such as autism spectrum disorder and metabolic diseases (Blevins et al., 2015; Thienel et al., 2016; Sikich et al., 2021). Our data demonstrated a clear deficiency of Oxt in two neuroendocrine disorders, T2DM and PWS, indicating a potential therapeutic avenue. In fact, Oxt therapy has been considered in both contexts previously (Zhang et al., 2013;

Correa-da-Silva et al., 2021), but great variability on administration route, number of doses and administration dosage hampered the significance and reproducibility of the findings (McCormackBlevins and Lawson, 2020). Moreover, the vast majority of studies do not equate the effect of circadian and/or ultradian rhythms in exogenous Oxt administration, despite increasing evidence of a marked circadian pattern for homeostatic Oxt secretion and signaling (AmicoSeif and Robinson, 1981; Zhang and Cai, 2011). Lastly, a number of trials with Oxt intervention for non-genetic metabolic disorders were performed exclusively in males (McCormackBlevins and Lawson, 2020), limiting the clinical significance of those findings.

Interventional studies of Oxt administration in humans demonstrate a clear anorectic and weight loss induction effect, in both control and obese subjects (Ott et al., 2013; Hsu et al., 2018; BurmesterHiggs and Terry, 2018; Plessow et al., 2018). These data confirm the large body of evidence from animal studies that showed the importance of oxytocinergic signaling in the physiological and hedonic control of feeding behavior (Blevins Schwartz and Baskin, 2004; Atasoy et al., 2012). Furthermore, a potent beneficial effect on glucose homeostasis was described, especially in young healthy men (Chiodera et al., 1984; Paolisso et al., 1988; Klement et al., 2017). Curiously, the effects of exogenous Oxt administration in glucoregulation seem to be restricted to normal weighted subjects (Zhang et al., 2013; Brede et al., 2019). Those results indicate that pronounced obesity might be associated with molecular resistance to the Oxt signal, similar to what has been observed for other energy-controlling hormones such as insulin, leptin and ghrelin (El-Haschimi et al., 2000; Saltiel and Kahn, 2001; CuiLopez and Rahmouni, 2017). Therefore, Oxt,-based therapies for T2DM might be a more suitable approach in the context of body weight and glucose levels maintenance.

Oxt has multiple physiological functions, ranging from lactation, partuirition, social cognition to global metabolism control (Baskaran et al., 2017). Oxt deficiency appeared as one of the fist targets for therapeutic interventions in PWS, following early reports of reduced numbers of Oxt-containing neurons in the PVN of PWS individuals (Swaab Purba and Hofman, 1995). Detailed work in PWS animal models and human postmortem hypothalamic material have confirmed those observations (Bochukova et al., 2018; Schaller et al., 2010; Meziane et al., 2015). Oxt-based interventions emerged as a promising therapeutic node in PWS, with potential beneficial effects on metabolism and behavioral deviances. To this date, five studies proposed to evaluate the clinical

potential of Oxt in PWS pathophysiology (Rice et al., 2018). All of them had the same administration route (nasal spray) which facilitates comparison among the findings. The studies, however, varied in administrated doses, age of the participants and the outcomes that were measured. Limited sample size and experimental design flaws, like the lack of control group (Tauber et al., 2017) or lack of statistical analysis (Miller et al., 2017), limit the interpretation of some of those findings. Exogenous Oxt improved social emotions and cognition in PWS individuals (Tauber et al., 2011; Kuppens Donze and Hokken-Koelega, 2016). Kuppens et al. report that Oxt administration has no effect in PWS individuals older than 11 years old, whereas Tauber and colleagues worked with adults (Tauber et al., 2011; KuppensDonze and Hokken-Koelega, 2016). Finally, no effects on hyperphagia or global metabolism were achieved (Tauber et al., 2011; Einfeld et al., 2014), except on children under 11 years old (Kuppens Donze and Hokken-Koelega, 2016). Together, these data do not provide sufficient evidence that intranasal Oxt administration may serve as an effective therapeutic avenue for PWS.

The lack of positive read outs in the aforementioned trials does not disprove the hypothesis that Oxt abnormalities underlie the PWS phenotypic traits. The conduction of a randomized, double-blind, placebo-controlled clinical trial of Oxt administration would provide robust information on the therapeutic value of exogenous Oxt administration for disease-modifying strategies in the context of PWS. The comprehension of the oxytocinergic anatomical projections that are defective in PWS might also contribute to potential designs of novel therapies involving Oxt (Althammer et al., 2022). In fact, increased serum levels of Oxt in PWS children (Johnson et al., 2016) suggests a compensatory production of this neuropeptide to some degree. It is important to mention that initial evidence suggests that Oxt can be also produced in peripheral structures, such as the gastrointestinal tract in humans (Ohlsson et al., 2006). Whether increased Oxt plasma levels are contributed by extra hypothalamic systems remains unexplored. Alternatively, Oxt signal transduction might be impaired in PWS, which is supported by recent findings of reduced Oxt receptor expression in the CNS of PWS individuals (Bittel et al., 2007). In this case, administration of Oxt would not mediate significant disease-modifying milestones. Further research is necessary to reach a precise determination of the therapeutic value of Oxt in PWS, and to evaluate the optimal conditions for such treatment (*i.e.*, most appropriate age, most effective combination of therapies).

LIMITATIONS OF THIS THESIS

To understand the molecular and cellular basis of neurological disorders, we took advantage of postmortem human brain tissue samples. The use of material of this nature allows for a great range of experimental techniques to be employed in consecutive sections of the same individual. This creates a potential for comprehensive determination of molecular and cellular changes that underlie a disease. However, there are inherent limitations to this model. Intrinsic differences between the living brain and postmortem material have been reported (Liharska et al., 2023). However, there are no options for non-invasive and/or safe acquisition of hypothalamic tissue in live subjects. Combined with the fact that animal models fail to fully recapitulate the diseases investigated in this thesis, the use of human postmortem material becomes the best model as a proxy to the living brain. Moreover, there is great variability in human studies driven by genetic heterogeneity, different lifestyles, and previous medical conditions. To address this, we impose a range of selection criteria that might act to reduce confounding factors in our groups. Those include a balanced gender proportion among controls and subjects of interest, and matched age and tissue processing parameters, such as fixation time. Future studies should consider larger sample sizes, pending on appropriate capacity of well-matched groups.

It is important to address that retrograde analysis of medical history of the individuals included in this thesis was possible, but the clinical complexity of PWS and T2DM still posed a significant challenge. T2DM is considered a progressive condition, suggesting that duration of the disease might influence our results. However, because symptoms can be minimal or unnoticed, a precise determination of this cofounder was not possible. Moreover, we were unable to fully address the impact of antidiabetic drugs in our studies, due to a limitation of sample size. In the context of PWS, we faced limitations on the number of samples due to lack of availability. This forced us to pool children and young adults, which might impact the measurement of our final outcomes. Moreover, our results must be interpreted in the light of PWS clinical complexities. Those include diversity of treatments that those patients might have undergone and the impact of failure of other physiological systems in this pathology other than in the CNS .

Histological analyses were conducted in sections adjacent to the region with the highest count of specific neuronal populations for each hypothalamic nucleus (*i.e.*, Oxt for the PVN, and NPY for the INF). Those sections were considered as a proxy for the whole nuclei, like previously published research (Alkemade et al., 2012; Yi et al., 2012; Hogenboom et al., 2019; Kalsbeek et al., 2020; Guo et al., 2022). However, emerging evidence points to great functional and molecular diversity of both neurons and glial cells (HealyZia and Plemel, 2022; Chen et al., 2017). To what extent this heterogeneity occurs in the human homeostatic and pathological condition is currently under investigation. Moreover, we took advantage of general markers for glial cells, analyzing pan astroglial and microglial markers (*i.e.*, Iba1 and GFAP). Therefore, we cannot fully exclude alterations in specific hypothalamic domains or the participation of discreet subpopulations in pathogenic events explored here. Concomitant research must be conducted in order to find suitable markers to further unravel human hypothalamic dysfunction.

REFERENCES

Al-Rawashdeh A, Kasabri V, Bulatova N, et al. (2017). The correlation between plasma levels of oxytocin and betatrophin in non-diabetic and diabetic metabolic syndrome patients: A cross sectional study from Jordan. Diabetes Metab Syndr 11: 59-67.

Alcaide J, Guirado R, Crespo C, et al. (2019). Alterations of perineuronal nets in the dorsolateral prefrontal cortex of neuropsychiatric patients. Int J Bipolar Disord 7: 24.

Alkemade A, Yi CX, Pei L, et al. (2012). AgRP and NPY expression in the human hypothalamic infundibular nucleus correlate with body mass index, whereas changes in alphaMSH are related to type 2 diabetes. J Clin Endocrinol Metab 97: E925-933.

Alonge KM, Mirzadeh Z, Scarlett JM, et al. (2020). Hypothalamic perineuronal net assembly is required for sustained diabetes remission induced by fibroblast growth factor 1 in rats. Nat Metab 2: 1025-1033.

Althammer F, Muscatelli F, Grinevich V & Schaaf CP (2022). Oxytocin-based therapies for treatment of Prader-Willi and Schaaf-Yang syndromes: evidence, disappointments, and future research strategies. Transl Psychiatry 12: 318.

Altszuler N & Hampshire J (1981). Intranasal instillation of oxytocin increases insulin and glucagon secretion. Proc Soc Exp Biol Med 168: 123-124.

Amico JA, Seif SM & Robinson AG (1981). Oxytocin in human plasma: correlation with neurophysin and stimulation with estrogen. J Clin Endocrinol Metab 52: 988-993.

Andrews RC, Herlihy O, Livingstone DE, et al. (2002). Abnormal cortisol metabolism and tissue sensitivity to cortisol in patients with glucose intolerance. J Clin Endocrinol Metab 87: 5587-5593.

Aoki-Yoshino K, Uchihara T, Duyckaerts C, et al. (2005). Enhanced expression of aquaporin 4 in human brain with inflammatory diseases. Acta Neuropathol 110: 281-288.

Atasoy D, Betley JN, Su HH & Sternson SM (2012). Deconstruction of a neural circuit for hunger. Nature 488: 172-177.

Aycan Z & Bas VN (2014). Prader-Willi syndrome and growth hormone deficiency. J Clin Res Pediatr Endocrinol 6: 62-67.

Aziz NA, Anguelova GV, Marinus J, et al. (2010). Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease. Parkinsonism Relat Disord 16: 345-350.

Bahniwal M, Little JP & Klegeris A (2017). High Glucose Enhances Neurotoxicity and Inflammatory Cytokine Secretion by Stimulated Human Astrocytes. Curr Alzheimer Res 14: 731-741.

Baig S, Wilcock GK & Love S (2005). Loss of perineuronal net N-acetylgalactosamine in Alzheimer's disease. Acta Neuropathol 110: 393-401.

Balthasar N, Dalgaard LT, Lee CE, et al. (2005). Divergence of melanocortin pathways in the control of food intake and energy expenditure. Cell 123: 493-505.

Banker GA (1980). Trophic interactions between astroglial cells and hippocampal neurons in culture. Science 209: 809-810.

Banks WA, Gray AM, Erickson MA, et al. (2015). Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. J Neuroinflammation 12: 223.

Barnett BP, Hwang Y, Taylor MS, et al. (2010). Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor. Science 330: 1689-1692.

Baskaran C, Plessow F, Silva L, et al. (2017). Oxytocin secretion is pulsatile in men and is related to social-emotional functioning. Psychoneuroendocrinology 85: 28-34.

Baskin DG, Breininger JF & Schwartz MW (1999). Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. Diabetes 48: 828-833.

Baufeld C, Osterloh A, Prokop S, et al. (2016). High-fat diet-induced brain region-specific phenotypic spectrum of CNS resident microglia. Acta Neuropathol 132: 361-375.

Benoit SC, Kemp CJ, Elias CF, et al. (2009). Palmitic acid mediates hypothalamic insulin resistance by altering PKC-theta subcellular localization in rodents. J Clin Invest 119: 2577-2589.

Berbari NF, Pasek RC, Malarkey EB, et al. (2013). Leptin resistance is a secondary consequence of the obesity in ciliopathy mutant mice. Proc Natl Acad Sci U S A 110: 7796-7801.

Berkseth KE, Guyenet SJ, Melhorn SJ, et al. (2014). Hypothalamic gliosis associated with high-fat diet feeding is reversible in mice: a combined immunohistochemical and magnetic resonance imaging study. Endocrinology 155: 2858-2867.

Bernier LP, York EM, Kamyabi A, et al. (2020). Microglial metabolic flexibility supports immune surveillance of the brain parenchyma. Nat Commun 11: 1559.

Biag J, Huang Y, Gou L, et al. (2012). Cyto- and chemoarchitecture of the hypothalamic paraventricular nucleus in the C57BL/6J male mouse: a study of immunostaining and multiple fluorescent tract tracing. J Comp Neurol 520: 6-33.

Bittel DC, Kibiryeva N, Sell SM, et al. (2007). Whole genome microarray analysis of gene expression in Prader-Willi syndrome. Am J Med Genet A 143A: 430-442.

Bjorkstrand E, Eriksson M & Uvnas-Moberg K (1996). Evidence of a peripheral and a central effect of oxytocin on pancreatic hormone release in rats. Neuroendocrinology 63: 377-383.

Blevins JE, Graham JL, Morton GJ, et al. (2015). Chronic oxytocin administration inhibits food intake, increases energy expenditure, and produces weight loss in fructose-fed obese rhesus monkeys. Am J Physiol Regul Integr Comp Physiol 308: R431-438.

Blevins JE, Schwartz MW & Baskin DG (2004). Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size. Am J Physiol Regul Integr Comp Physiol 287: R87-96.

Bocarsly ME, Fasolino M, Kane GA, et al. (2015). Obesity diminishes synaptic markers, alters microglial morphology, and impairs cognitive function. Proc Natl Acad Sci U S A 112: 15731-15736.

Bochukova EG, Lawler K, Croizier S, et al. (2018). A Transcriptomic Signature of the Hypothalamic Response to Fasting and BDNF Deficiency in Prader-Willi Syndrome. Cell Rep 22: 3401-3408.

Boyar RM, Wu RH, Roffwarg H, et al. (1976). Human puberty: 24-hour estradiol in pubertal girls. J Clin Endocrinol Metab 43: 1418-1421.

Boyers D, Retat L, Jacobsen E, et al. (2021). Cost-effectiveness of bariatric surgery and non-surgical weight management programmes for adults with severe obesity: a decision analysis model. Int J Obes (Lond) 45: 2179-2190.

Brancaccio M, Edwards MD, Patton AP, et al. (2019). Cell-autonomous clock of astrocytes drives circadian behavior in mammals. Science 363: 187-192.

Brancaccio M, Patton AP, Chesham JE, et al. (2017). Astrocytes Control Circadian Timekeeping in the Suprachiasmatic Nucleus via Glutamatergic Signaling. Neuron 93: 1420-1435 e1425.

Brede S, Fehr S, Dalla-Man C, et al. (2019). Intranasal oxytocin fails to acutely improve glucose metabolism in obese men. Diabetes Obes Metab 21: 424-428.

Buijs RM, la Fleur SE, Wortel J, et al. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. J Comp Neurol 464: 36-48.

Burmester V, Higgs S & Terry P (2018). Rapid-onset anorectic effects of intranasal oxytocin in young men. Appetite 130: 104-109.

Busnardo C, Tavares RF, Resstel LB, et al. (2010). Paraventricular nucleus modulates autonomic and neuroendocrine responses to acute restraint stress in rats. Auton Neurosci 158: 51-57.

Butler JV, Whittington JE, Holland AJ, et al. (2002). Prevalence of, and risk factors for, physical ill-health in people with Prader-Willi syndrome: a population-based study. Dev Med Child Neurol 44: 248-255.

Butler MG, Bittel DC, Kibiryeva N, et al. (2004). Behavioral differences among subjects with Prader-Willi syndrome and type I or type II deletion and maternal disomy. Pediatrics 113: 565-573.

Camerino C (2009). Low sympathetic tone and obese phenotype in oxytocin-deficient mice. Obesity (Silver Spring) 17: 980-984.

Campbell JN, Macosko EZ, Fenselau H, et al. (2017). A molecular census of arcuate hypothalamus and median eminence cell types. Nat Neurosci 20: 484-496.

Cao Y, AlHumaidi SS, Faqeih EA, et al. (2017). A novel deletion of SNURF/SNRPN exon 1 in a patient with Prader-Willi-like phenotype. Eur J Med Genet 60: 416-420.

Carulli D, Pizzorusso T, Kwok JC, et al. (2010). Animals lacking link protein have attenuated perineuronal nets and persistent plasticity. Brain 133: 2331-2347.

Cassidy SB, Schwartz S, Miller JL & Driscoll DJ (2012). Prader-Willi syndrome. Genet Med 14: 10-26.

Celio MR, Spreafico R, De Biasi S & Vitellaro-Zuccarello L (1998). Perineuronal nets: past and present. Trends Neurosci 21: 510-515.

Challis BG, Coll AP, Yeo GS, et al. (2004). Mice lacking pro-opiomelanocortin are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3-36). Proc Natl Acad Sci U S A 101: 4695-4700.

Chen H, Victor AK, Klein J, et al. (2020). Loss of MAGEL2 in Prader-Willi syndrome leads to decreased secretory granule and neuropeptide production. JCI Insight 5.

Chen R, Wu X, Jiang L & Zhang Y (2017). Single-Cell RNA-Seq Reveals Hypothalamic Cell Diversity. Cell Rep 18: 3227-3241.

Chew NWS, Ng CH, Tan DJH, et al. (2023). The global burden of metabolic disease: Data from 2000 to 2019. Cell Metab 35: 414-428 e413.

Chiodera P, Coiro V, Camellini L, et al. (1984). Effect of pharmacological doses of oxytocin on insulin response to glucose in normal man. Horm Res 20: 150-154.

Clement K, Vaisse C, Lahlou N, et al. (1998). A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392: 398-401.

Collet TH, Dubern B, Mokrosinski J, et al. (2017). Evaluation of a melanocortin-4 receptor (MC4R) agonist (Setmelanotide) in MC4R deficiency. Mol Metab 6: 1321-1329.

Correa-da-Silva F, Fliers E, Swaab DF & Yi CX (2021). Hypothalamic neuropeptides and neurocircuitries in Prader Willi syndrome. J Neuroendocrinol 33: e12994.

Correa-da-Silva F, Kalsbeek MJ, Gadella FS, et al. (2023). Reduction of oxytocin-containing neurons and enhanced glymphatic activity in the hypothalamic paraventricular nucleus of patients with type 2 diabetes mellitus. Acta Neuropathol Commun 11: 107.

Coskun T, Urva S, Roell WC, et al. (2022). LY3437943, a novel triple glucagon, GIP, and GLP-1 receptor agonist for glycemic control and weight loss: From discovery to clinical proof of concept. Cell Metab 34: 1234-1247 e1239.

Cui H, Lopez M & Rahmouni K (2017). The cellular and molecular bases of leptin and ghrelin resistance in obesity. Nat Rev Endocrinol 13: 338-351.

Cummings JL (1994). Vascular subcortical dementias: clinical aspects. Dementia 5: 177-180.

De Pablo-Fernandez E, Courtney R, Warner TT & Holton JL (2018). A Histologic Study of the Circadian System in Parkinson Disease, Multiple System Atrophy, and Progressive Supranuclear Palsy. JAMA Neurol 75: 1008-1012.

De Rubeis S, Pasciuto E, Li KW, et al. (2013). CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation. Neuron 79: 1169-1182.

De Souza CT, Araujo EP, Bordin S, et al. (2005). Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. Endocrinology 146: 4192-4199.

Delle C, Cankar N, Digebjerg Holgersson C, et al. (2023). Long-term high-fat diet increases glymphatic activity in the hypothalamus in mice. Sci Rep 13: 4137.

Di Dalmazi G, Pagotto U, Pasquali R & Vicennati V (2012). Glucocorticoids and type 2 diabetes: from physiology to pathology. J Nutr Metab 2012: 525093.

Djogo T, Robins SC, Schneider S, et al. (2016). Adult NG2-Glia Are Required for Median Eminence-Mediated Leptin Sensing and Body Weight Control. Cell Metab 23: 797-810.

Dominguez-Iturza N, Lo AC, Shah D, et al. (2019). The autism- and schizophrenia-associated protein CYFIP1 regulates bilateral brain connectivity and behaviour. Nat Commun 10: 3454.

Douglass JD, Ness KM, Valdearcos M, et al. (2023). Obesity-associated microglial inflammatory activation paradoxically improves glucose tolerance. Cell Metab 35: 1613-1629 e1618.

Duquenne M, Folgueira C, Bourouh C, et al. (2021). Leptin brain entry via a tanycytic LepR-EGFR shuttle controls lipid metabolism and pancreas function. Nat Metab 3: 1071-1090.

Eide PK, Valnes LM, Lindstrom EK, et al. (2021). Direction and magnitude of cerebrospinal fluid flow vary substantially across central nervous system diseases. Fluids Barriers CNS 18: 16.

Einfeld SL, Smith E, McGregor IS, et al. (2014). A double-blind randomized controlled trial of oxytocin nasal spray in Prader Willi syndrome. Am J Med Genet A 164A: 2232-2239.

El-Haschimi K, Pierroz DD, Hileman SM, et al. (2000). Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. J Clin Invest 105: 1827-1832.

Endo F, Kasai A, Soto JS, et al. (2022). Molecular basis of astrocyte diversity and morphology across the CNS in health and disease. Science 378: eadc9020.

Everett JW & Sawyer CH (1950). A 24-hour periodicity in the "LH-release apparatus" of female rats, disclosed by barbiturate sedation. Endocrinology 47: 198-218.

Farooqi IS, Drop S, Clements A, et al. (2006). Heterozygosity for a POMC-null mutation and increased obesity risk in humans. Diabetes 55: 2549-2553.

Fawcett JW, Oohashi T & Pizzorusso T (2019). The roles of perineuronal nets and the perinodal extracellular matrix in neuronal function. Nat Rev Neurosci 20: 451-465.

Figueiro MG, Plitnick BA, Lok A, et al. (2014). Tailored lighting intervention improves measures of sleep, depression, and agitation in persons with Alzheimer's disease and related dementia living in long-term care facilities. Clin Interv Aging 9: 1527-1537.

Freeman R (2014). Diabetic autonomic neuropathy. Handb Clin Neurol 126: 63-79.

Fricker M, Oliva-Martin MJ & Brown GC (2012). Primary phagocytosis of viable neurons by microglia activated with LPS or Abeta is dependent on calreticulin/LRP phagocytic signalling. J Neuroinflammation 9: 196.

Fu MX, Requena JR, Jenkins AJ, et al. (1996). The advanced glycation end product, Nepsilon-(carboxymethyl)lysine, is a product of both lipid peroxidation and glycoxidation reactions. J Biol Chem 271: 9982-9986.

Fuente-Martin E, Garcia-Caceres C, Granado M, et al. (2012). Leptin regulates glutamate and glucose transporters in hypothalamic astrocytes. J Clin Invest 122: 3900-3913.

Gao R, Wang M, Lin J, et al. (2018a). Spatiotemporal expression patterns of chondroitin sulfate proteoglycan mRNAs in the developing rat brain. Neuroreport 29: 517-523.

Gao Y, Bielohuby M, Fleming T, et al. (2017a). Dietary sugars, not lipids, drive hypothalamic inflammation. Mol Metab 6: 897-908.

Gao Y, Ottaway N, Schriever SC, et al. (2014). Hormones and diet, but not body weight, control hypothalamic microglial activity. Glia 62: 17-25.

Gao Y, Vidal-Itriago A, Kalsbeek MJ, et al. (2017b). Lipoprotein Lipase Maintains Microglial Innate Immunity in Obesity. Cell Rep 20: 3034-3042.

Gao Y, Vidal-Itriago A, Milanova I, et al. (2018b). Deficiency of leptin receptor in myeloid cells disrupts hypothalamic metabolic circuits and causes body weight increase. Mol Metab 7: 155-160.

Garcia-Caceres C, Quarta C, Varela L, et al. (2016). Astrocytic Insulin Signaling Couples Brain Glucose Uptake with Nutrient Availability. Cell 166: 867-880.

Goldstone AP, Unmehopa UA, Bloom SR & Swaab DF (2002). Hypothalamic NPY and agouti-related protein are increased in human illness but not in Prader-Willi syndrome and other obese subjects. J Clin Endocrinol Metab 87: 927-937.

Gropp E, Shanabrough M, Borok E, et al. (2005). Agouti-related peptide-expressing neurons are mandatory for feeding. Nat Neurosci 8: 1289-1291.

Gruber T, Pan C, Contreras RE, et al. (2021). Obesity-associated hyperleptinemia alters the gliovascular interface of the hypothalamus to promote hypertension. Cell Metab 33: 1155-1170 e1110.

Grugni G, Beccaria L, Corrias A, et al. (2013). Central adrenal insufficiency in young adults with Prader-Willi syndrome. Clin Endocrinol (Oxf) 79: 371-378.

Gudbjornsdottir S, Friberg P, Elam M, et al. (1994). The effect of metformin and insulin on sympathetic nerve activity, norepinephrine spillover and blood pressure in obese, insulin resistant, normoglycemic, hypertensive men. Blood Press 3: 394-403.

Guo L, Qi YJ, Tan H, et al. (2022). Different oxytocin and corticotropin-releasing hormone system changes in bipolar disorder and major depressive disorder patients. EBioMedicine 84: 104266.

Göbel J, Engelhardt E, Pelzer P, et al. (2020). Mitochondria-Endoplasmic Reticulum Contacts in Reactive Astrocytes Promote Vascular Remodeling. Cell Metab 31: 791-808 e798.

Hartley SL, Maclean WE, Jr., Butler MG, et al. (2005). Maladaptive behaviors and risk factors among the genetic subtypes of Prader-Willi syndrome. Am J Med Genet A 136: 140-145.

Healy LM, Zia S & Plemel JR (2022). Towards a definition of microglia heterogeneity. Commun Biol 5: 1114.

Herrera Moro Chao D, Kirchner MK, Pham C, et al. (2022). Hypothalamic astrocytes control systemic glucose metabolism and energy balance. Cell Metab 34: 1532-1547 e1536.

Hirsch HJ, Eldar-Geva T, Bennaroch F, et al. (2015). Sexual dichotomy of gonadal function in Prader-Willi syndrome from early infancy through the fourth decade. Hum Reprod 30: 2587-2596.

Hogenboom R, Kalsbeek MJ, Korpel NL, et al. (2019). Loss of arginine vasopressin- and vasoactive intestinal polypeptide-containing neurons and glial cells in the suprachiasmatic nucleus of individuals with type 2 diabetes. Diabetologia 62: 2088-2093.

Holm VA, Cassidy SB, Butler MG, et al. (1993). Prader-Willi syndrome: consensus diagnostic criteria. Pediatrics 91: 398-402.

Horvath TL, Naftolin F, Kalra SP & Leranth C (1992). Neuropeptide-Y innervation of beta-endorphin-containing cells in the rat mediobasal hypothalamus: a light and electron microscopic double immunostaining analysis. Endocrinology 131: 2461-2467.

Horvath TL, Sarman B, Garcia-Caceres C, et al. (2010). Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. Proc Natl Acad Sci U S A 107: 14875-14880.

Hsu EA, Miller JL, Perez FA & Roth CL (2018). Oxytocin and Naltrexone Successfully Treat Hypothalamic Obesity in a Boy Post-Craniopharyngioma Resection. J Clin Endocrinol Metab 103: 370-375.

Huggett RJ, Scott EM, Gilbey SG, et al. (2003). Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. Circulation 108: 3097-3101.

Hurd MW & Ralph MR (1998). The significance of circadian organization for longevity in the golden hamster. J Biol Rhythms 13: 430-436.

Iepsen EW, Zhang J, Thomsen HS, et al. (2018). Patients with Obesity Caused by Melanocortin-4 Receptor Mutations Can Be Treated with a Glucagon-like Peptide-1 Receptor Agonist. Cell Metab 28: 23-32 e23.

Iliff JJ, Wang M, Liao Y, et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. Sci Transl Med 4: 147ra111.

Ionescu E, Coimbra CC, Walker CD & Jeanrenaud B (1989). Paraventricular nucleus modulation of glycemia and insulinemia in freely moving lean rats. Am J Physiol 257: R1370-1376.

Jansen K, Van der Zee EA & Gerkema MP (1999). Organotypic suprachiasmatic nuclei cultures of adult voles reflect locomotor behavior: differences in number of vasopressin cells. Chronobiol Int 16: 745-750.

Jastreboff AM, Kaplan LM, Frias JP, et al. (2023). Triple-Hormone-Receptor Agonist Retatrutide for Obesity - A Phase 2 Trial. N Engl J Med 389: 514-526.

Johnson L, Manzardo AM, Miller JL, et al. (2016). Elevated plasma oxytocin levels in children with Prader-Willi syndrome compared with healthy unrelated siblings. Am J Med Genet A 170: 594-601.

Kalin S, Heppner FL, Bechmann I, et al. (2015). Hypothalamic innate immune reaction in obesity. Nat Rev Endocrinol 11: 339-351.

Kalsbeek A, van Heerikhuize JJ, Wortel J & Buijs RM (1996). A diurnal rhythm of stimulatory input to the hypothalamo-pituitary-adrenal system as revealed by timed intrahypothalamic administration of the vasopressin V1 antagonist. J Neurosci 16: 5555-5565.

Kalsbeek MJ, Wolff SE, Korpel NL, et al. (2020). The impact of antidiabetic treatment on human hypothalamic infundibular neurons and microglia. JCI Insight 5.

Khakh BS & Sofroniew MV (2015). Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 18: 942-952.

Khaodhiar L, McCowen KC & Blackburn GL (1999). Obesity and its comorbid conditions. Clin Cornerstone 2: 17-31.

Khatua B, El-Kurdi B, Patel K, et al. (2021). Adipose saturation reduces lipotoxic systemic inflammation and explains the obesity paradox. Sci Adv 7.

Kierdorf K & Prinz M (2017). Microglia in steady state. J Clin Invest 127: 3201-3209.

Klement J, Ott V, Rapp K, et al. (2017). Oxytocin Improves beta-Cell Responsivity and Glucose Tolerance in Healthy Men. Diabetes 66: 264-271.

Kobessho H, Oishi K, Hamaguchi H & Kanda F (2008). Elevation of cerebrospinal fluid protein in patients with diabetes mellitus is associated with duration of diabetes. Eur Neurol 60: 132-136.

Kochlamazashvili G, Henneberger C, Bukalo O, et al. (2010). The extracellular matrix molecule hyaluronic acid regulates hippocampal synaptic plasticity by modulating postsynaptic L-type Ca(2+) channels. Neuron 67: 116-128.

Konopka RJ & Benzer S (1971). Clock mutants of Drosophila melanogaster. Proc Natl Acad Sci U S A 68: 2112-2116.

Kozlov SV, Bogenpohl JW, Howell MP, et al. (2007). The imprinted gene Magel2 regulates normal circadian output. Nat Genet 39: 1266-1272.

Krude H, Biebermann H, Luck W, et al. (1998). Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. Nat Genet 19: 155-157.

Kuppens RJ, Donze SH & Hokken-Koelega AC (2016). Promising effects of oxytocin on social and food-related behaviour in young children with Prader-Willi syndrome: a randomized, double-blind, controlled crossover trial. Clin Endocrinol (Oxf) 85: 979-987.

Lang RE, Heil JW, Ganten D, et al. (1983). Oxytocin unlike vasopressin is a stress hormone in the rat. Neuroendocrinology 37: 314-316.

Larsen PJ, Tang-Christensen M, Stidsen CE, et al. (1999). Activation of central neuropeptide Y Y1 receptors potently stimulates food intake in male rhesus monkeys. J Clin Endocrinol Metab 84: 3781-3791.

Lassi G, Priano L, Maggi S, et al. (2016). Deletion of the Snord116/SNORD116 Alters Sleep in Mice and Patients with Prader-Willi Syndrome. Sleep 39: 637-644.

Lawson EA, Marengi DA, DeSanti RL, et al. (2015). Oxytocin reduces caloric intake in men. Obesity (Silver Spring) 23: 950-956.

Laxton AW, Tang-Wai DF, McAndrews MP, et al. (2010). A phase I trial of deep brain stimulation of memory circuits in Alzheimer's disease. Ann Neurol 68: 521-534.

Lechner L, Opitz R, Silver MJ, et al. (2023). Early-set POMC methylation variability is accompanied by increased risk for obesity and is addressable by MC4R agonist treatment. Sci Transl Med 15: eadg1659.

Lechuga-Sancho AM, Arroba AI, Frago LM, et al. (2006). Reduction in the number of astrocytes and their projections is associated with increased synaptic protein density in the hypothalamus of poorly controlled diabetic rats. Endocrinology 147: 5314-5324.

Lee GH, Proenca R, Montez JM, et al. (1996). Abnormal splicing of the leptin receptor in diabetic mice. Nature 379: 632-635.

Lee J, Dimitry JM, Song JH, et al. (2023). Microglial REV-ERBalpha regulates inflammation and lipid droplet formation to drive tauopathy in male mice. Nat Commun 14: 5197.

Lee J, Liu J, Feng X, et al. (2016). Withaferin A is a leptin sensitizer with strong antidiabetic properties in mice. Nat Med 22: 1023-1032.

Legros JJ (1992). Neurohypophyseal peptides and psychopathology. Horm Res 37 Suppl 3: 16-21.

Lehman MN, LeSauter J, Kim C, et al. (1995). How do fetal grafts of the suprachiasmatic nucleus communicate with the host brain? Cell Transplant 4: 75-81.

Lembo G, Napoli R, Capaldo B, et al. (1992). Abnormal sympathetic overactivity evoked by insulin in the skeletal muscle of patients with essential hypertension. J Clin Invest 90: 24-29.

Lensjo KK, Lepperod ME, Dick G, et al. (2017). Removal of Perineuronal Nets Unlocks Juvenile Plasticity Through Network Mechanisms of Decreased Inhibition and Increased Gamma Activity. J Neurosci 37: 1269-1283.

Leoutsakos JS, Yan H, Anderson WS, et al. (2018). Deep Brain Stimulation Targeting the Fornix for Mild Alzheimer Dementia (the ADvance Trial): A Two Year Follow-up Including Results of Delayed Activation. J Alzheimers Dis 64: 597-606.

Lewis DA (2002). The human brain revisited: opportunities and challenges in postmortem studies of psychiatric disorders. Neuropsychopharmacology 26: 143-154.

Li N, Baldermann JC, Kibleur A, et al. (2020). A unified connectomic target for deep brain stimulation in obsessive-compulsive disorder. Nat Commun 11: 3364.

Liharska LE, Park YJ, Ziafat K, et al. (2023). A study of gene expression in the living human brain. medRxiv.

Liu RY, Zhou JN, Hoogendijk WJ, et al. (2000). Decreased vasopressin gene expression in the biological clock of Alzheimer disease patients with and without depression. J Neuropathol Exp Neurol 59: 314-322.

Locke AE, Kahali B, Berndt SI, et al. (2015). Genetic studies of body mass index yield new insights for obesity biology. Nature 518: 197-206.

Logan RW & McClung CA (2019). Rhythms of life: circadian disruption and brain disorders across the lifespan. Nat Rev Neurosci 20: 49-65.

Lu R, Dong Y & Li JD (2020). Necdin regulates BMAL1 stability and circadian clock through SGT1-HSP90 chaperone machinery. Nucleic Acids Res 48: 7944-7957.

Lucassen EA & Cizza G (2012). The Hypothalamic-Pituitary-Adrenal Axis, Obesity, and Chronic Stress Exposure: Sleep and the HPA Axis in Obesity. Curr Obes Rep 1: 208-215.

Lupori L, Totaro V, Cornuti S, et al. (2023). A comprehensive atlas of perineuronal net distribution and colocalization with parvalbumin in the adult mouse brain. Cell Rep 42: 112788.

Lutomska LM, Miok V, Krahmer N, et al. (2022). Diet triggers specific responses of hypothalamic astrocytes in time and region dependent manner. Glia 70: 2062-2078.

Maffei M, Halaas J, Ravussin E, et al. (1995). Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1: 1155-1161.

Manni R, Politini L, Nobili L, et al. (2001). Hypersomnia in the Prader Willi syndrome: clinical-electrophysiological features and underlying factors. Clin Neurophysiol 112: 800-805.

Marchant EG & Mistlberger RE (1997). Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6j mice. Brain Res 765: 273-282.

McCarthy J, Lupo PJ, Kovar E, et al. (2018). Schaaf-Yang syndrome overview: Report of 78 individuals. Am J Med Genet A 176: 2564-2574.

McCormack SE, Blevins JE & Lawson EA (2020). Metabolic Effects of Oxytocin. Endocr Rev 41: 121-145.

Medetgul-Ernar K & Davis MM (2022). Standing on the shoulders of mice. Immunity 55: 1343-1353.

Melo HM, Seixas da Silva GDS, Sant'Ana MR, et al. (2020). Palmitate Is Increased in the Cerebrospinal Fluid of Humans with Obesity and Induces Memory Impairment in Mice via Pro-inflammatory TNF-alpha. Cell Rep 30: 2180-2194 e2188.

Menzies FM, Fleming A, Caricasole A, et al. (2017). Autophagy and Neurodegeneration: Pathogenic Mechanisms and Therapeutic Opportunities. Neuron 93: 1015-1034.

Mercer RE, Michaelson SD, Chee MJ, et al. (2013). Magel2 is required for leptin-mediated depolarization of POMC neurons in the hypothalamic arcuate nucleus in mice. PLoS Genet 9: e1003207.

Mestre H, Hablitz LM, Xavier AL, et al. (2018). Aquaporin-4-dependent glymphatic solute transport in the rodent brain. Elife 7.

Meziane H, Schaller F, Bauer S, et al. (2015). An Early Postnatal Oxytocin Treatment Prevents Social and Learning Deficits in Adult Mice Deficient for Magel2, a Gene Involved in Prader-Willi Syndrome and Autism. Biol Psychiatry 78: 85-94.

Mieda M, Ono D, Hasegawa E, et al. (2015). Cellular clocks in AVP neurons of the SCN are critical for interneuronal coupling regulating circadian behavior rhythm. Neuron 85: 1103-1116.

Milanova IV, Korpel NL, Correa-da-Silva F, et al. (2022). Loss of Microglial Insulin Receptor Leads to Sex-Dependent Metabolic Disorders in Obese Mice. Int J Mol Sci 23.

Miller BH, Olson SL, Levine JE, et al. (2006). Vasopressin regulation of the proestrous luteinizing hormone surge in wild-type and Clock mutant mice. Biol Reprod 75: 778-784.

Miller JL, Lynn CH, Driscoll DC, et al. (2011). Nutritional phases in Prader-Willi syndrome. Am J Med Genet A 155A: 1040-1049.

Miller JL, Tamura R, Butler MG, et al. (2017). Oxytocin treatment in children with Prader-Willi syndrome: A double-blind, placebo-controlled, crossover study. Am J Med Genet A 173: 1243-1250.

Milner KM, Craig EE, Thompson RJ, et al. (2005). Prader-Willi syndrome: intellectual abilities and behavioural features by genetic subtype. J Child Psychol Psychiatry 46: 1089-1096.

Mirzadeh Z, Alonge KM, Cabrales E, et al. (2019). Perineuronal Net Formation during the Critical Period for Neuronal Maturation in the Hypothalamic Arcuate Nucleus. Nat Metab 1: 212-221.

Moraes JC, Coope A, Morari J, et al. (2009). High-fat diet induces apoptosis of hypothalamic neurons. PLoS One 4: e5045.

Muller-Fielitz H, Stahr M, Bernau M, et al. (2017). Tanycytes control the hormonal output of the hypothalamic-pituitary-thyroid axis. Nat Commun 8: 484.

Muller J (1997). Hypogonadism and endocrine metabolic disorders in Prader-Willi syndrome. Acta Paediatr Suppl 423: 58-59.

Muller TD, Finan B, Bloom SR, et al. (2019). Glucagon-like peptide 1 (GLP-1). Mol Metab 30: 72-130.

Neumann P, Lenz DE, Streit WJ & Bechmann I (2023). Is microglial dystrophy a form of cellular senescence? An analysis of senescence markers in the aged human brain. Glia 71: 377-390.

Ohlsson B, Truedsson M, Djerf P & Sundler F (2006). Oxytocin is expressed throughout the human gastrointestinal tract. Regul Pept 135: 7-11.

Ott V, Finlayson G, Lehnert H, et al. (2013). Oxytocin reduces reward-driven food intake in humans. Diabetes 62: 3418-3425.

Ou YN, Ge YJ, Wu BS, et al. (2023). The genetic architecture of fornix white matter microstructure and their involvement in neuropsychiatric disorders. Transl Psychiatry 13: 180.

Ou Z, Ma Y, Sun Y, et al. (2019). A GPR17-cAMP-Lactate Signaling Axis in Oligodendrocytes Regulates Whole-Body Metabolism. Cell Rep 26: 2984-2997 e2984.

Ouyang Y, Andersson CR, Kondo T, et al. (1998). Resonating circadian clocks enhance fitness in cyanobacteria. Proc Natl Acad Sci U S A 95: 8660-8664.

Palm IF, Van Der Beek EM, Wiegant VM, et al. (1999). Vasopressin induces a luteinizing hormone surge in ovariectomized, estradiol-treated rats with lesions of the suprachiasmatic nucleus. Neuroscience 93: 659-666.

Paolisso G, Sgambato S, Passariello N, et al. (1988). Pharmacological doses of oxytocin affect plasma hormone levels modulating glucose homeostasis in normal man. Horm Res 30: 10-16.

Papazoglou I, Lee JH, Cui Z, et al. (2022). A distinct hypothalamus-to-beta cell circuit modulates insulin secretion. Cell Metab 34: 285-298 e287.

Patel P & Abate N (2013). Body fat distribution and insulin resistance. Nutrients 5: 2019-2027.

Peng S, Liu J, Liang C, et al. (2023). Aquaporin-4 in glymphatic system, and its implication for central nervous system disorders. Neurobiol Dis 179: 106035.

Pizzorusso T, Medini P, Berardi N, et al. (2002). Reactivation of ocular dominance plasticity in the adult visual cortex. Science 298: 1248-1251.

Plant TM (1981). Time courses of concentrations of circulating gonadotropin, prolactin, testosterone, and cortisol in adult male rhesus monkeys (Macaca mulatta) throughout the 24 h light-dark cycle. Biol Reprod 25: 244-252.

Plessow F, Marengi DA, Perry SK, et al. (2018). Effects of Intranasal Oxytocin on the Blood Oxygenation Level-Dependent Signal in Food Motivation and Cognitive Control Pathways in Overweight and Obese Men. Neuropsychopharmacology 43: 638-645.

Podlesny-Drabiniok A, Marcora E & Goate AM (2020). Microglial Phagocytosis: A Disease-Associated Process Emerging from Alzheimer's Disease Genetics. Trends Neurosci 43: 965-979.

Polex-Wolf J, Lam BY, Larder R, et al. (2018). Hypothalamic loss of Snord116 recapitulates the hyperphagia of Prader-Willi syndrome. J Clin Invest 128: 960-969.

Qian W, Zhu T, Tang B, et al. (2014). Decreased circulating levels of oxytocin in obesity and newly diagnosed type 2 diabetic patients. J Clin Endocrinol Metab 99: 4683-4689.

Qualls-Creekmore E & Munzberg H (2018). Modulation of Feeding and Associated Behaviors by Lateral Hypothalamic Circuits. Endocrinology 159: 3631-3642.

Reddy S, Bichler J, Wells-Knecht KJ, et al. (1995). N epsilon-(carboxymethyl)lysine is a dominant advanced glycation end product (AGE) antigen in tissue proteins. Biochemistry 34: 10872-10878.

Reis WL, Yi CX, Gao Y, et al. (2015). Brain innate immunity regulates hypothalamic arcuate neuronal activity and feeding behavior. Endocrinology 156: 1303-1315.

Reppert SM & Weaver DR (2002). Coordination of circadian timing in mammals. Nature 418: 935-941.

Rice LJ, Einfeld SL, Hu N & Carter CS (2018). A review of clinical trials of oxytocin in Prader-Willi syndrome. Curr Opin Psychiatry 31: 123-127.

Riemersma-van der Lek RF, Swaab DF, Twisk J, et al. (2008). Effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities: a randomized controlled trial. JAMA 299: 2642-2655.

Roberto CA, Swinburn B, Hawkes C, et al. (2015). Patchy progress on obesity prevention: emerging examples, entrenched barriers, and new thinking. Lancet 385: 2400-2409.

Rogers SL, Rankin-Gee E, Risbud RM, et al. (2018). Normal Development of the Perineuronal Net in Humans; In Patients with and without Epilepsy. Neuroscience 384: 350-360.

Rosenbaum JL, Melhorn SJ, Schoen S, et al. (2022). Evidence That Hypothalamic Gliosis Is Related to Impaired Glucose Homeostasis in Adults With Obesity. Diabetes Care 45: 416-424.

Rowe JW, Young JB, Minaker KL, et al. (1981). Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. Diabetes 30: 219-225.

Ryder RE, Owens DR, Hayes TM, et al. (1990). Unawareness of hypoglycaemia and inadequate hypoglycaemic counterregulation: no causal relation with diabetic autonomic neuropathy. BMJ 301: 783-787.

Sa M, Yoo ES, Koh W, et al. (2023). Hypothalamic GABRA5-positive neurons control obesity via astrocytic GABA. Nat Metab.

Saltiel AR & Kahn CR (2001). Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414: 799-806.

Sanjabi B, Dashty M, Ozcan B, et al. (2015). Lipid droplets hypertrophy: a crucial determining factor in insulin regulation by adipocytes. Sci Rep 5: 8816.

Schaller F, Watrin F, Sturny R, et al. (2010). A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. Hum Mol Genet 19: 4895-4905.

Schmitt U, Tanimoto N, Seeliger M, et al. (2009). Detection of behavioral alterations and learning deficits in mice lacking synaptophysin. Neuroscience 162: 234-243.

Schulze A, Hansen C, Skakkebaek NE, et al. (1996). Exclusion of SNRPN as a major determinant of Prader-Willi syndrome by a translocation breakpoint. Nat Genet 12: 452-454.

Shen L, Tso P, Woods SC, et al. (2008). Brain apolipoprotein E: an important regulator of food intake in rats. Diabetes 57: 2092-2098.

Sikich L, Kolevzon A, King BH, et al. (2021). Intranasal Oxytocin in Children and Adolescents with Autism Spectrum Disorder. N Engl J Med 385: 1462-1473.

Silva AI, Haddon JE, Ahmed Syed Y, et al. (2019). Cyfip1 haploinsufficient rats show white matter changes, myelin thinning, abnormal oligodendrocytes and behavioural inflexibility. Nat Commun 10: 3455.

Slomp M, Koekkoek LL, Mutersbaugh M, et al. (2023). Free-choice high-fat diet consumption reduces lateral hypothalamic GABAergic activity, without disturbing neural response to sucrose drinking in mice. Front Neurosci 17: 1219569.

Smith AJ, Duan T & Verkman AS (2019). Aquaporin-4 reduces neuropathology in a mouse model of Alzheimer's disease by remodeling peri-plaque astrocyte structure. Acta Neuropathol Commun 7: 74.

Soeda S, Ito D, Ogushi T, et al. (2023). Defects in early synaptic formation and neuronal function in Prader-Willi syndrome. Sci Rep 13: 12053.

Speakman JR (2019). Use of high-fat diets to study rodent obesity as a model of human obesity. Int J Obes (Lond) 43: 1491-1492.

Srinivasan J, Schachner M & Catterall WA (1998). Interaction of voltage-gated sodium channels with the extracellular matrix molecules tenascin-C and tenascin-R. Proc Natl Acad Sci U S A 95: 15753-15757.

Stanley S, Pinto S, Segal J, et al. (2010). Identification of neuronal subpopulations that project from hypothalamus to both liver and adipose tissue polysynaptically. Proc Natl Acad Sci U S A 107: 7024-7029.

Sternson SM (2013). Hypothalamic survival circuits: blueprints for purposive behaviors. Neuron 77: 810-824.

Stopa EG, Volicer L, Kuo-Leblanc V, et al. (1999). Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. J Neuropathol Exp Neurol 58: 29-39.

Streit WJ, Braak H, Xue QS & Bechmann I (2009). Dystrophic (senescent) rather than activated microglial cells are associated with tau pathology and likely precede neurodegeneration in Alzheimer's disease. Acta Neuropathol 118: 475-485.

Streit WJ, Sammons NW, Kuhns AJ & Sparks DL (2004). Dystrophic microglia in the aging human brain. Glia 45: 208-212.

Swaab DF, Purba JS & Hofman MA (1995). Alterations in the hypothalamic paraventricular nucleus and its oxytocin neurons (putative satiety cells) in Prader-Willi syndrome: a study of five cases. J Clin Endocrinol Metab 80: 573-579.

Takaya K, Ogawa Y, Isse N, et al. (1996). Molecular cloning of rat leptin receptor isoform complementary DNAs--identification of a missense mutation in Zucker fatty (fa/fa) rats. Biochem Biophys Res Commun 225: 75-83.

Tarpey PS, Smith R, Pleasance E, et al. (2009). A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. Nat Genet 41: 535-543.

Tauber M, Boulanouar K, Diene G, et al. (2017). The Use of Oxytocin to Improve Feeding and Social Skills in Infants With Prader-Willi Syndrome. Pediatrics 139.

Tauber M, Mantoulan C, Copet P, et al. (2011). Oxytocin may be useful to increase trust in others and decrease disruptive behaviours in patients with Prader-Willi syndrome: a randomised placebo-controlled trial in 24 patients. Orphanet J Rare Dis 6: 47.

Taylor R (2012). Insulin resistance and type 2 diabetes. Diabetes 61: 778-779.

Thaler JP, Yi CX, Schur EA, et al. (2012). Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 122: 153-162.

Thienel M, Fritsche A, Heinrichs M, et al. (2016). Oxytocin's inhibitory effect on food intake is stronger in obese than normal-weight men. Int J Obes (Lond) 40: 1707-1714.

Timper K, Paeger L, Sanchez-Lasheras C, et al. (2018). Mild Impairment of Mitochondrial OXPHOS Promotes Fatty Acid Utilization in POMC Neurons and Improves Glucose Homeostasis in Obesity. Cell Rep 25: 383-397 e310.

Tso CF, Simon T, Greenlaw AC, et al. (2017). Astrocytes Regulate Daily Rhythms in the Suprachiasmatic Nucleus and Behavior. Curr Biol 27: 1055-1061.

Tsuno Y, Peng Y, Horike SI, et al. (2023). In vivo recording of suprachiasmatic nucleus dynamics reveals a dominant role of arginine vasopressin neurons in circadian pacesetting. PLoS Biol 21: e3002281.

Valdearcos M, Robblee MM, Benjamin DI, et al. (2014). Microglia dictate the impact of saturated fat consumption on hypothalamic inflammation and neuronal function. Cell Rep 9: 2124-2138.

Vale W, Spiess J, Rivier C & Rivier J (1981). Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213: 1394-1397.

van den Beld AW, Kaufman JM, Zillikens MC, et al. (2018). The physiology of endocrine systems with ageing. Lancet Diabetes Endocrinol 6: 647-658.

van der Beek EM, Wiegant VM, van der Donk HA, et al. (1993). Lesions of the suprachiasmatic nucleus indicate the presence of a direct vasoactive intestinal polypeptide-containing projection to gonadotrophin-releasing hormone neurons in the female rat. J Neuroendocrinol 5: 137-144.

van Dierendonck X, Vrieling F, Smeehuijzen L, et al. (2022). Triglyceride breakdown from lipid droplets regulates the inflammatory response in macrophages. Proc Natl Acad Sci U S A 119: e2114739119.

van Wamelen DJ, Aziz NA, Anink JJ, et al. (2013). Suprachiasmatic nucleus neuropeptide expression in patients with Huntington's Disease. Sleep 36: 117-125.

Varela L, Kim JG, Fernandez-Tussy P, et al. (2021). Astrocytic lipid metabolism determines susceptibility to diet-induced obesity. Sci Adv 7: eabj2814.

Vgontzas AN, Bixler EO, Kales A, et al. (1996). Daytime sleepiness and REM abnormalities in Prader-Willi syndrome: evidence of generalized hypoarousal. Int J Neurosci 87: 127-139.

Viggiano D, Ibrahim M & Celio MR (2000). Relationship between glia and the perineuronal nets of extracellular matrix in the rat cerebral cortex: importance for volume transmission in the brain. Prog Brain Res 125: 193-198.

Vijayan V, Pradhan P, Braud L, et al. (2019). Human and murine macrophages exhibit differential metabolic responses to lipopolysaccharide – A divergent role for glycolysis. Redox Biol 22: 101147.

Vo T, Carulli D, Ehlert EM, et al. (2013). The chemorepulsive axon guidance protein semaphorin3A is a constituent of perineuronal nets in the adult rodent brain. Mol Cell Neurosci 56: 186-200.

Vollenweider P, Tappy L, Randin D, et al. (1993). Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest 92: 147-154.

Wang XL, Kooijman S, Gao Y, et al. (2021). Microglia-specific knock-down of Bmal1 improves memory and protects mice from high fat diet-induced obesity. Mol Psychiatry 26: 6336-6349.

Wilding JPH, Batterham RL, Calanna S, et al. (2021). Once-Weekly Semaglutide in Adults with Overweight or Obesity. N Engl J Med 384: 989-1002.

Winkler Z, Kuti D, Polyak A, et al. (2019). Hypoglycemia-activated Hypothalamic Microglia Impairs Glucose Counterregulatory Responses. Sci Rep 9: 6224.

Xu D, Hopf C, Reddy R, et al. (2003). Narp and NP1 form heterocomplexes that function in developmental and activity-dependent synaptic plasticity. Neuron 39: 513-528.

Yang J, Lunde LK, Nuntagij P, et al. (2011). Loss of astrocyte polarization in the tg-ArcSwe mouse model of Alzheimer's disease. J Alzheimers Dis 27: 711-722.

Yeo GS, Farooqi IS, Aminian S, et al. (1998). A frameshift mutation in MC4R associated with dominantly inherited human obesity. Nat Genet 20: 111-112.

Yi CX, Gericke M, Kruger M, et al. (2012). High calorie diet triggers hypothalamic angiopathy. Mol Metab 1: 95-100.

Yi CX, Walter M, Gao Y, et al. (2017). TNFalpha drives mitochondrial stress in POMC neurons in obesity. Nat Commun 8: 15143.

Yoo Y, Neumayer G, Shibuya Y, et al. (2023). A cell therapy approach to restore microglial Trem2 function in a mouse model of Alzheimer's disease. Cell Stem Cell 30: 1043-1053 e1046.

Zahova S & Isles AR (2021). Animal models for Prader-Willi syndrome. Handb Clin Neurol 181: 391-404.

Zhang G & Cai D (2011). Circadian intervention of obesity development via resting-stage feeding manipulation or oxytocin treatment. Am J Physiol Endocrinol Metab 301: E1004-1012.

Zhang H, Wu C, Chen Q, et al. (2013). Treatment of obesity and diabetes using oxytocin or analogs in patients and mouse models. PLoS One 8: e61477.

Zhang N, Yan Z, Liu H, et al. (2021). Hypothalamic Perineuronal Nets Are Regulated by Sex and Dietary Interventions. Front Physiol 12: 714104.

Zhou JN, Riemersma RF, Unmehopa UA, et al. (2001). Alterations in arginine vasopressin neurons in the suprachiasmatic nucleus in depression. Arch Gen Psychiatry 58: 655-662.

Zhuang L, Sachdev PS, Trollor JN, et al. (2013). Microstructural white matter changes, not hippocampal atrophy, detect early amnestic mild cognitive impairment. PLoS One 8: e58887.