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Insulin on the Brain: The Role of Central Insulin Signalling in Energy and Glucose Homeostasis

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1. Abstract:

Insulin signals to the brain where it coordinates multiple physiological processes underlying energy and glucose homeostasis. This review explores where, and how insulin, interacts within the brain parenchyma; how brain insulin signalling functions to coordinate energy and glucose homeostasis; and how this contributes to the pathogenesis of metabolic disease.

2. Introduction:

Insulin is a peptide hormone that is synthesised and secreted by beta cells of the pancreas (1). Following secretion into the blood, insulin influences a constellation of cells expressed throughout the body (1). The physiological role of insulin is best described in the context of glucose homeostasis where it plays a critical role in maintaining blood glucose levels within an "optimum range". In this context, insulin targets peripheral tissues including the skeletal muscle and adipose tissue to promote glucose uptake from the circulation, and the liver to represses gluconeogenesis and glycogenolysis (1). Alongside insulin's "traditional" peripheral targets, insulin also signals to the brain (2-4). Here, insulin signalling within the brain's parenchyma plays a vital role in how the brain controls whole-body glucose and energy homeostasis. Considering the epidemic proportions and growing incidence of metabolic diseases such as obesity and Type-2 diabetes (T2D), there is an imperative need to understand the neuroendocrine mechanisms coordinating metabolism and how these go awry during the development of metabolic disease. A cornerstone of metabolic disease is the development of insulin resistance, whereby insulin's target tissues in the periphery become insensitive to the action of insulin (1). Exciting evidence suggests that the brain also becomes insulin resistant during the development of metabolic disease (5), yet the relative contributions of insulin resistance within the brain to the development of metabolic disease remains unclear. With the recent advent of novel transgenic and pharmacological technologies to explore the brain evidence is now emerging that brain insulin signalling plays a causative role in the pathogenesis of metabolic disease and represents an exciting and relatively unexplored therapeutic avenue.

This review discusses the role of insulin signalling within the brain and its contribution to energy and glucose homeostasis. We will explore the current understanding of how insulin interacts with the brain, how this influences energy and glucose homeostasis, and how dysfunctions in central insulin signalling contribute to the development of metabolic disease.

3. The History of Insulin and the Brain

The discovery of insulin in 1921 by Frederick Banting, Charles Best, James Collip and John Macleod marks a historic milestone in both the therapy and prognosis of diabetes. Whilst this landmark discovery, now a century ago, has revolutionised our understanding of glycaemic control, the stage was set for the role of the brain in glycaemia many years prior. In 1855, the French physiologist, Claude Bernard was the first to identify the role of the brain in glucose metabolism after he discovered that puncturing the fourth ventricle in dogs resulted in glycosuria (6). This was then followed up by two German physiologists, Oskar Minkowski and Joseph von Mering, who in 1889 observed that pancreatectomy in dogs also resulted in glycosuria, implicating an integral role of

pancreatic hormone secretion in glycaemic control (7). Critically, Minkowski and von Mering also discovered that implanting a small piece of the pancreas subcutaneously following pancreatectomy in dogs prevented hyperglycaemia until the implant was removed or had degenerated (7). These early insights provided the bedrock for establishing the vital nexus between the pancreas, the brain and glycaemic control. The missing link was then instilled in 1921 when Banting and Best extracted and purified insletin, later called insulin, from the pancreas of dogs (8). They later went on to demonstrate that intravenously administered insulin to de-pancreatised dogs resulted in a marked drop in blood sugar and herein demonstrated the pancreatic origin and functional role of insulin (8). In 1922, insulin was administered for the first time to a diabetic human (9), marking a defining moment in history for the treatment of diabetes and the recognition of insulin as a critical metabolic hormone.

Since its discovery, insulin has long been pigeonholed as a "peripheral" hormone, exerting its effects on glycaemia primarily through peripheral tissues of the body. This "peripheral-centric" view was further engrained by early observations reporting that glucose transport into the brain occurred independently of insulin and as such the brain was long considered to be an "insulin insensitive" organ (10, 11). The importance of the brain in glucose metabolism re-emerged in the 1950s when Jean Mayer postulated that hypothalamic 'glucoreceptors' could sense changes in circulating glucose levels (12). Electrophysical patch-clamp recordings in the 1960s revealing the presence of glucose-sensing neurons in the hypothalamus (13, 14) rekindled interest in the role of the CNS in glycaemic control. Since then, the peripheral-centric view of insulin has now been demystified by a significant body of evidence supporting a neuroregulatory role of insulin within the brain.

4. Insulin Signalling in the Brain

4.1. The Insulin Receptor

Central and peripheral insulin signalling occurs through binding and phosphorylation/activation of the insulin receptor (INSR, **Fig 1**). The INSR is a receptor tyrosine kinase with two disulphide-linked extracellular α-subunits encompassing ligand-binding domains, and two transmembrane β-subunits containing the cytoplasmic protein tyrosine kinase (PTK) (3). In humans, the *INSR* gene is comprised of 22 exons and encoded on chromosome 19 (15). Based on the alternative splicing of exon 11, two INSR isoforms exist due to the exclusion (INSR-A) or inclusion (INSR-B) of a 12-amino-acid sequence encoded by exon 11 (16). While INSR-A and INSR-B are both expressed in all tissues in the human body, the ratio of INSR-A:INSR-B favours INSR-B in the liver, skeletal muscle, adipose tissue, and

kidney; and favours INSR-A in the brain (17-19). Insulin can also bind to insulin-like growth factor (IGF)-1 receptors (IGF-1R), which are also ubiquitously expressed within the brain (20, 21). IGF-1R and INSR can form both hetero- and homodimers which exhibit different affinities towards insulin and IGF-1 (22). Homodimers of IGF-1R and INSR demonstrate increased affinity for their endogenous ligands, whereas heterodimers show higher affinity towards IGF-1 than insulin (23). The INSR has been found to exist predominantly as heterodimers within the rabbit brain (24), however, the regional abundance of INSR heterodimers and homodimers and their relative contribution to the metabolic actions of insulin are unknown.

4.2. Insulin Receptor Expression

In situ hybridisation and immunohistochemistry studies have identified INSR distribution throughout the CNS (2, 25-29). In rodents, INSR expression is highest in the olfactory bulb, cortex, hippocampus, hypothalamus, and cerebellum, with low levels also observed in the midbrain, striatum, and brainstem (25-29). Similarly, IGF-1R expression is highest in the cortex, hypothalamus, and cerebellum (25). Kleinridders *et al* compared the expression of known insulin-responsive receptors across brain regions using quantitative real-time PCR and found that *Igf1r* mRNA levels were almost double the level of *Insr,* with the highest expression in the cerebellum (30). Interestingly, the INSR substrate proteins (IRS), IRS-1 and IRS-2 were expressed far more abundantly throughout the brain than the INSR or IGF-1R (30). Exactly how these transcript levels reflect translated protein or influence functional signalling among the different cell types/regions of the brain remains to be determined. The subcellular expression of the INSR within the CNS has begun to emerge. Within hippocampal neurons, the INSR is localised to the synaptic membrane and within synaptosomes (31), whilst in astrocytes, the INSR is expressed within end-feet (32).

4.3. Insulin Receptor Signal Cascade

Circulating insulin binds to the extracellular α-subunits of the membrane-bound INSR ectodomain (33) (**Fig. 1**). Ligand activation of the INSR then promotes receptor conformational changes to induce intracellular autophosphorylation (34). A comprehensive review of INSR binding properties or signalling is beyond the scope of this review but can be found elsewhere (15, 16, 22, 34). In short, insulin activation of the INSR induces intracellular autophosphorylation of residues Y1150/Y1151 (35) (Y1162/Y1163 on INSR isoform B) (35, 36) leading to the activation of the cytoplasmic INSR protein tyrosine kinase domains (35). This tyrosine kinase activity phosphorylates additional INSR

sites (including Y960, Y1146, Y1316 and Y1322) as well as several downstream cellular INSR substrates including the IRS proteins 1-4 or Shc (named because it has an SH2 domain and a collagen homologous region) (37). From this vantage point, INSR signalling can activate two distinct intracellular signalling networks; 1) the canonical phosphoinositide 3-kinase (PI3K) and protein kinase B (AKT) pathway, and/or 2) the mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) pathway (1). The IRS activated PI3K/AKT pathway recruits downstream signalling pathways involving glycogen synthase kinase 3β, mammalian target of rapamycin complex 1 and forkhead box O (FoxO) signalling, to regulate synaptic plasticity, gene transcription and neuronal excitability (**Fig. 1**) (38-40). The insulin-MAPK/ERK pathway initiates the Ras-mediated phosphorylation of MAPK and ERK to regulate cellular proliferation/differentiation and astrocytic glucose transport (41-44). Whilst this evidence supports a differential role of AKT and MAPK/ERK signal transduction networks in insulin actions, it is important to note that significant functional crosstalk and feedback exist (45).

4.4. Negative Regulators of Insulin Receptor Signalling

Just as INSR signalling is propagated by protein kinase mediated phosphorylation, it is also negatively regulated by protein tyrosine phosphatases. Protein tyrosine phosphatases function to de-phosphorylate the INSR and/or its downstream components. One of the most well-characterised protein tyrosine phosphatases is protein tyrosine phosphatase 1B (PTP1B). PTP1B dephosphorylates Y1162/Y1163 on the INSR (46, 47) and possibly IRS-1 (48, 49) to attenuate INSR signalling (**Fig. 1**). Single nucleotide polymorphisms in the *PTPN1* gene, which encodes PTP1B, are linked to insulin resistance and diabetes in humans (50), and the expression of PTP1B is elevated in the hypothalamus of diet-induced obese mice (51, 52). Whole-body-, muscle- or liver-specific PTP1B knockout (KO) mice display enhanced INSR phosphorylation and enhanced whole-body glycaemic control (53-56). PTP1B knockdown in the brain enhances PI3K/AKT insulin signalling, protects against diet-induced obesity, and improves whole-body glucose homeostasis (53, 56-58). Deletion of PTP1B in proopiomelanocortin (POMC) neurons enhanced leptin but not insulin signalling (59), whereas PTP1B deletion in agouti-related peptide neurons (AgRP) enhanced insulin signalling to repress food intake and improve glycaemic control (60). PTP1B deletion specifically within steroidogenic factor-1 (SF-1) neurons in the ventromedial hypothalamus (VMH) promoted insulin signalling yet enhanced adiposity and weight gain (61).

Another key INSR phosphatase is T-cell like protein tyrosine phosphatase (TCPTP). TCPTP is expressed within the brain and functions to dephosphorylate the INSR at the Y1162/Y1163 sites to

attenuate insulin signalling (**Fig. 1**) (62). TCPTP deletion in both POMC or AgRP neurons robustly enhances insulin-induced AKT Ser-473 phosphorylation and improves whole-body glucose metabolism (5, 51, 63-65). TCPTP levels within the mediobasal hypothalamus (MBH) are markedly elevated in response to fasting (51, 64, 65), (an effect driven by fasting-induced glucocorticoid levels) and actively degraded in response to re-feeding (51), suggesting that TCPTP may functionally coordinate hypothalamic INSR signalling in response to nutritional status (51, 64, 65).

Suppressor of cytokine signalling 3 (SOCS3) also negatively regulates INSR signalling (**Fig. 1**). The role of SOCS3 in the brain has been mostly studied in the context of leptin and its signalling through the leptin receptor long isoform (LepRb). Leptin signalling via the LepRb activates the Janus-activated kinase-2 (JAK-2)/signal transducer and activator of transcription 3 (STAT-3) which in turn drives SOCS3 dependant expression (66). SOCS3 then negatively regulates JAK-STAT by inhibiting JAK kinase activity which in turn creates a negative feedback loop (67). Obesity is associated with elevated leptin levels (hyperleptinemia) which are known to promote basal LepRb JAK/STAT-3 signalling (60) resulting in chronic SOCS3 expression (68-72). From here, SOCS3 then impinges on INSR signalling by binding to the INSR or IRS to signal protein ubiquitination (73). Neuron/glial specific SOCS3 KO mice exhibit weight loss and protection from diet-induced insulin resistance (74). Single nucleotide polymorphisms around the *SOCS3* locus are associated with human obesity (75), and obese rodents exhibit elevated SOCS3 expression within hypothalamic neurons (51, 76). The hyperleptinemia and neuronal inflammation associated with obesity have also been shown to promote PTP1B and TCPTP expression which in turn can dephosphorylate JAK2/STAT3 to further ensue brain insulin resistance (52, 77-79).

5. Insulin Transport Into the CNS

Insulin is secreted into the circulation by the pancreas in response to rises in postprandial blood glucose. In turn, this affords insulin access to numerous tissues around the body including the brain. Insulin must navigate the blood-brain barrier (BBB) to access the brain (**Fig. 1**), however, as insulin is a 51-amino acid peptide, paracellular diffusion across the BBB is limited (80, 81). In pathophysiological states of brain insulin resistance, the ratio of insulin levels in cerebrospinal fluid (CSF) is significantly lower than that in plasma (82, 83). Impaired insulin transportation or appearance within the brain may therefore mechanistically contribute to the development of central insulin resistance.

5.1. Insulin Transport Across the BBB

Peripheral circulating insulin reaches the brain parenchyma via a saturable transport system that is considered concentration-dependent (81). Early studies in the 1960s and 1970s defined a correlation between plasma insulin level and insulin levels within the CSF (84-86). This correlation between insulin plasma and CSF insulin concentration was only observed when insulin was within a physiologically relevant range, suggesting that insulin transport into the brain is saturable (80, 81, 84-86). Several barriers exist to regulate cellular exchanges between the periphery and the CNS (see reviews (87)), of which the BBB is thought to have the largest influence on the microenvironment of cells within the CNS (88). The BBB is a cellular portmanteau, a component of both the periphery and the CNS, acting as the interface mediating cellular transport and uptake from circulation into the brain (89). The BBB is formed by brain capillaries consisting of endothelial cells which form tight junctions to restrict paracellular movement (90). Brain endothelial cells are encompassed by pericytes embedded in a vascular basement membrane, which are covered by astrocytic perivascular end-feet that also connect with neurons (**Fig. 1**) (90). A synergistic relationship exists between endothelial BBB cell types to regulate intracellular transport and communication (91).

Circulating insulin is transported across the BBB by receptor-mediated transcytosis (**Fig. 1**) (91, 92). Once bound to the INSR on endothelial cells, insulin is then internalised into vesicles (92-94), transported across the endothelial cells and presented by receptor-mediated exocytosis to the abluminal side of the cell and into the brain (**Fig. 1**) (92, 93). The process of receptor-mediated transcytosis has been widely assumed to be undertaken by the INSR, as endothelial cell-specific INSR KO mice show altered BBB permeability and impaired insulin signalling within the hypothalamus, hippocampus and prefrontal cortex (95). Interestingly, a loss of endothelial INSRs attenuates hypothalamic *Pomc* expression and promotes hyperphagia, hyperinsulinemia and accelerated development of systemic insulin resistance (95). Notably, insulin transport across the BBB has also been shown to occur independently of endothelial INSR activity as both endothelial-specific INSR KO mice and mice receiving a pharmacological INSR inhibition still show functional insulin transport into the brain (96, 97). Although insulin transport into the brain is attenuated without the presence of INSRs it does however imply the presence of a possible unidentified insulin transport-related protein (98).

The transcytosis of insulin is attenuated in metabolic disease, however, the pathophysiological mechanisms underlying this phenomenon are unclear (91, 99). Obesity driven nuclear factor kappa-B expression within brain endothelial cells reduces cellular insulin uptake *in vitro* (98). INSRs have been detected in astrocytic end-feet which ensheath the BBB endothelial cells, and ablation of INSRs in

astrocytes limits the uptake of circulating insulin (32). The development of astrocyte INSR KO mice has consistently demonstrated the vital role of the INSR in astrocytes in the maintenance of the BBB structure and permeability (44, 100, 101), as well as the neural network response to circulating peripheral hormones (101). Pericytes also act to modulate the BBB by downregulating *trans*-endothelial permeability and astrocyte coupling (102). Increased BBB permeability, pathophysiology associated with insulin resistance and metabolic disease, is observed in pericyte KO mice (102) and in streptozotocin-induced diabetic mice which correspondingly exhibit a loss of pericytes as well as a retraction of pericytes from the BBB (100). The symbiotic relationship between astrocytes, pericytes and endothelial cells highlights the integrity of multiple cell types in insulin journey into the brain.

5.2. Insulin Transport Through the Median Eminence

The brain is not entirely protected by the BBB. Specialised areas of the brain termed circumventricular organs are characterised by highly permeable capillaries devoid of a BBB and allow for the passive extravasation of bloodborne factors such as insulin into the brain (**Fig. 1**) (103). The peripheral administration of [^{125}]-Insulin shows that insulin rapidly penetrates an area of the brain termed the median eminence (ME) (81, 104, 105). The ME is a circumventricular organ that is situated below the MBH, ventral to the third ventricle and adjacent to the arcuate nucleus of the hypothalamus (ARC) (**Fig. 1**). Neurons in the MBH such as AgRP, POMC and SF-1 neurons have privileged access to detect and signal insulin levels in the blood (**Fig. 1**). Microdialysis studies show that postprandial insulin appears within the interstitial fluid of the medial hypothalamus within 30mins of feeding and returns to basal levels after 1h (106, 107), rapid effects that are consistent with entry through a circumventricular organ. The transport of insulin via the ME is however not unregulated. The ME contains specialized ependymal cells, termed tanycytes, which line the floor of the third ventricle (**Fig. 1**) (108). The presence of tight junctions between adjacent tanycytes functions as physical barriers controlling the extravasation of insulin from the portal capillary spaces and CSF into the hypothalamus (**Fig. 1**) (109, 110). When blood glucose levels are low, such as during fasting, the blood-hypothalamus barrier is remodelled so that the extravasation of blood-borne hormones into the ARC is augmented, which in turn enhances hormonal signalling (110). The structural organization of the blood-hypothalamus barrier in the ME during periods of nutritional change provides an elegant mechanism by which INSR signalling may be regulated with metabolic demand (110).

6. Insulin Targets in the Brain

The molecular machinery to transport, transduce and integrate insulin signalling from the blood to the brain parenchyma is present throughout the CNS. Whilst abundant, diverse heterogeneity exists in how insulin is functionally integrated by different regions and cells of the brain. Understanding the precise cellular populations and anatomical distribution of these correlates is fundamental to understanding the vital role of insulin signalling in the brain.

6.1. Hypothalamic Targets

A plethora of studies in both human and animals provide unequivocal evidence that insulin signals to cells within the hypothalamus (51, 81, 105, 111). Suppression of INSR translation within the hypothalamus using an antisense oligodeoxynucleotide leads to hyperphagia and insulin resistance in rodents (112). Functional immunohistochemical studies in rodents have revealed several hypothalamic regions show enhanced functional c-Fos immunoreactivity in response to insulin administration, including the ARC, VMH, paraventricular hypothalamus (PVH) and dorsomedial hypothalamus (DMH) (113, 114).

The proximity of ARC neurons to the ME affords them a prime position to detect and respond to peripheral metabolic hormones and nutrients in systemic circulation. Neurons within the ARC have a manifold of bi-directional connections to regions such as the DMH, PVH, lateral hypothalamus, dorsal vagal complex (DVC), and the nucleus tractus solitarius (115-117), which affords insulin signalling access to a diverse set of multifunctioning neurocircuits (**Fig. 2**). The ARC is composed of several metabolically relevant neuronal populations (118), the most notable of which are AgRP (that co-express neuropeptide Y, NPY) and POMC neurons (**Fig. 2**). AgRP and POMC neurons are critical regulators of both energy and glucose homeostasis and are one of the first-order cells through which insulin signals to the brain. INSRs are expressed on AgRP and POMC neurons and the delivery of systemic insulin administration promotes a robust increase in Ser-473 phosphorylation of AKT in the ARC, particularly within AgRP and POMC neurons (119, 120) (**Fig. 1 and 4a**). Chemogenic inhibition or genetic ablation of AgRP neurons attenuates food intake, enhances energy expenditure and decreases body weight (121-123). Within AgRP neurons, INSR signalling inhibits both *Agrp* and *NPY* transcription and activates adenosine triphosphate (ATP)-sensitive potassium channels, resulting in AgRP membrane hyperpolarisation (63, 119, 124). In contrast to AgRP neurons, which are orexigenic and promote positive energy balance (increased food intake, increased body weight, decreased energy expenditure); POMC are anorexigenic and promote negative energy balance (decreased food intake, decreased body weight, increased energy expenditure) (124-126). As such, chemogenic activation of POMC neurons decreases feeding behaviour and bodyweight and enhances energy expenditure (64). INSR activation on POMC neurons promotes α-melanocyte-stimulating hormone

to activate melanocortin-4 receptors on second-order neurons in other brain regions, including the PVH, VMH and DMH (64, 125). The upregulation of α-melanocyte-stimulating hormone, and activation of melanocortin-4 receptors, activate catabolic pathways causing a reduction in food intake and an increase in energy expenditure (64, 125, 127). Understanding the functionality of insulin signalling within POMC neurons has been thwarted with contradictory studies reporting that insulin can both activate and inhibit POMC neurons (113, 128-133). Investigating the insulin responsivity of POMC neurons throughout the entire rostro-caudal extent of the ARC demonstrated that functional heterogeneity exists in how POMC neurons respond to insulin (64), with POMC neurons displaying either excitation (11%), inhibition (51%) or no-responsivity (38%). Interestingly, this functional heterogeneity is highly dynamic and is shifted in POMC neurons in diet-induced obese mice (3% excitation, 65% inhibition, 32% no-response), effects that have been directly attributed to the expression of TCPTP (64).

Beyond the ARC, melanin-concentrating-hormone neurons in the lateral hypothalamus also exhibit divergent insulin-sensitive subsets that exhibit insulin-dependent excitability, inhibition, or non-responsivity (134). Mice with INSR deletion in melanin-concentrating-hormone neurons exhibit enhanced locomotor activity and insulin sensitivity during the development of diet-induced obesity (134). Insulin also targets neurons within the VMH which play pivotal roles in glucose metabolism and energy homeostasis (135, 136). INSR activation hyperpolarises SF-1 positive VMH neurons via PI3K-dependent activation of K_{ATP} channels (136, 137). Conditional INSR deletion in SF-1 positive VMH neurons protects against diet-induced obesity and improves glucose metabolism through enhanced glutamatergic innervation of POMC neurons (136). Specific deletion of FoxO, a downstream target of PI3K, in SF-1 neurons of mice promotes energy expenditure and improves glucose tolerance (138).

Another mechanism by which insulin exerts its effects on metabolic homeostasis is through its interaction with adenosine monophosphate (AMP)-activated protein kinase (AMPK), a proposed energy sensor and regulator (139). AMPK responds to peripheral nutritional and hormonal signals within the hypothalamus to regulate metabolic homeostasis through modulation of feeding behaviour (140, 141), glucose homeostasis (142, 143) and energy expenditure (144, 145). Pharmacological activation of AMPK within the hypothalamus increases the transcriptional levels of *NPY* and *AgRP* leading to increased food intake (141). POMC and AgRP neurons lacking AMPKα2 respond to insulin but are insensitive to glucose (140), suggesting there may be distinct pathways for glucose and insulin signalling with the MBH.

In the hypothalamus, insulin infusion reduces AMPKα2 expression by 25-40% (141) and attenuates its activity through Ser385 and Ser491 phosphorylation (146). The state of equipoise between AMPK and insulin within the CNS appears to be integral to metabolic health as streptozotocin-induced diabetic mice demonstrate heightened hypothalamic AMPK phosphorylation but chronic insulin treatment suppresses AMPK activity and abolishes diabetes-induced increases in food intake (147). Genetic inhibition of AMPK in SF-1 neurons of the VMH protects against diet-induced obesity by enhancing brown adipose (BAT) thermogenesis and improving glucose and lipid metabolism (148). Given insulin is a potent inhibitor of AMPK throughout the hypothalamus (141) these findings are somewhat counterintuitive to the lean phenotype observed in mice with impaired insulin signalling in SF-1 neurons (136, 138).

Whilst insulin signalling in hypothalamic neurons is established, emerging evidence suggests important functions are governed by insulin activity in non-neuronal cells. Insulin signalling in astrocytes controls glucose transport from the blood into the CNS and regulates glucose-induced activation in POMC neurons (43, 101). Furthermore, hypothalamic-specific astrocyte INSR ablation impairs CNS glucose sensing and metabolism resulting in systemic insulin resistance (43). These effects may be due to astrocyte insulin signalling mediating transport into the brain, synaptic reorganisation resulting in reduced activation of POMC neurons, or enhanced AgRP activation (101, 149, 150). Pericytes, a cell comprising the BBB, have also been found to express INSRs (151) and reside within the hypothalamus (152). Intriguingly, hypothalamic neurons show increased sensitivity to insulin and enhanced AKT phosphorylation in response to pericyte-conditioned media, an effect not seen in response to astrocyte or aortic smooth muscle cell-conditioned media (152). Thus, the functionality and importance of insulin signalling within diverse cell types and sub-populations are beginning to emerge.

6.2. Extrahypothalamic Targets

Two distinct neurocircuitries that have been implicated in brain insulin action are the dopaminergic reward circuitry, comprised of interconnected structures such as the ventral tegmental area (VTA), nucleus accumbens (NAc) and striatum (153); and the hypothalamic-pituitary-adrenal (HPA) axis, a network spanning the PVH, the anterior lobe of the pituitary gland and the adrenal glands (154). The dopaminergic reward neurocircuitry is associated with hedonic food seeking, and dysregulation within the dopamine system is associated with obesity and insulin resistance (155-159). Insulin has been found to act upon dopaminergic neurons in the VTA and NAc to suppress excitatory synaptic transmission resulting in decreased food seeking behaviour and food salience (156, 160-162).

Inhibition of insulin signalling or conditional deletion of the INSR in the VTA/NAc results in increased adiposity and hyperphagia (44, 163). Regulation of food consumption also involves the higher-order circuitry and the cognitive processing of food cues (164). In humans, activation of the pre-frontal cortex in response to food pictures is suppressed by insulin administration, an effect not observed in obese individuals (165, 166). The link between hippocampal insulin signalling and food salience has also been endorsed by human magnetic resonance imaging (MRI) studies which demonstrate significant positive correlations between fasting insulin plasma levels, waist circumference and hippocampal activity following exposure to high-caloric food images (167).

The HPA axis is responsible for the body's reaction to stress (168). Elevated stress levels are associated with increased consumption of palatable food, high in fat or sugar, in both animals and humans (169, 170). Critically, animals and humans with diabetes frequently exhibit impaired functioning in the HPA axis (154, 171). Glucocorticoids are key regulators of energy metabolism in response to stress-induced energy demands. Glucocorticoids increase the levels of lipids in systemic circulation (172), stimulate pre-adipocyte differentiation (173), and promote lipolysis (174). As such, insulin and glucocorticoids have opposing effects on metabolic homeostasis. Glucocorticoids attenuate IRS-1 expression and phosphorylation resulting in the suppression of PI3K/AKT activity (175). One mechanism by which glucocorticoids promote food consumption and energy homeostasis is through stimulation of NPY (176, 177). Critically, glucocorticoid response elements are located within the promoter region for the NPY gene (177) and the administration of dexamethasone, a glucocorticoid receptor agonist, promotes *Npy* an effect that is attenuated in response to insulin administration (178). Interestingly, stress combined with a high-fat diet has been found to increase *Npy* expression in the amygdala and ARC, resulting in increased palatable food intake and decreased energy expenditure likely due to a loss of insulin responsiveness (179). Critically, on a chow diet, chemogenetic activation of NPY neurons in the amygdala led to increased food intake and reduced energy expenditure, similar to effects observed in response to hypothalamic NPY activation (179). This aligns with previous studies that have reported enhanced NPY expression in the ARC is associated with increased food intake and adiposity, and a decrease in energy expenditure and cold-induced thermogenesis (51, 180-182). Insulin's regulation of the HPA axis is therefore a vital component in the neuroendocrine control of food intake and energy expenditure.

7. The Functional Role of Insulin Signalling within the Brain

Insulin signalling within the CNS is integral to a diverse range of physiological and behavioural functions and plays a governing role in metabolism. Here, we will discuss the integral role of central

insulin signalling on glucose and energy homeostasis through its regulation of feeding behaviour and the orchestration of systemic metabolic processes (**Fig. 2**).

7.1. Insulin Signalling in the Brain Regulates Feeding Behaviour

Central insulin's effect on food intake was first demonstrated by Woods *et al* in the late 1970s, whereby they showed insulin infusion into the brain reduced food intake in baboons (85). Since then, studies delivering insulin or insulin mimetics to the brain by ICV or intranasal administration (to target the brain) have shown significant effects on food intake and body weight (183-186). In line with this, genetic ablation of insulin signalling in the brain or pharmacological blockage of insulin signalling promotes food intake, increases body weight and reduces energy metabolism (187, 188).

Neurons within the hypothalamus, and specifically within the ARC, are well-established as critical regulators of energy intake (51, 123, 125, 127, 182). Studies attempting to define the neuronal population underlying insulin's actions on food intake have largely explored the role of AgRP and POMC neurons (described in section 6.1). Exogenous insulin signals via the PI3K/AKT pathway in both AgRP and POMC neurons which is coordinated by TCPTP, PTP1B and SOCS (**Fig. 1 and 2**). The expression of TCPTP but not PTP1B is known to be driven by nutritional status which actively regulates INSR signalling and AgRP neurons (51). ICV infusion or peripheral administration of insulin has been shown to inhibit *Npy* expression and promote *Pomc* expression in the ARC (64, 127, 181, 189). Genetic studies employing Cre-lox technology to conditionally delete INSRs within AgRP, POMC or more broadly within most ARC/VMH neurons (using NKx2.1 linage marker) generally show limited effects on feeding behaviour (129, 180). One possible explanation is that INSR deletion during development is likely compensated resulting in functional redundancy of the ISNR and the maintenance of the feeding response. As adult ablation of AgRP causes rapid starvation, yet neonatal ablation has minimal effect, it's clear that compensatory mechanisms exist (122). This is further illustrated by the delivery of antisense oligonucleotides delivered into the adult ARC, which attenuates INSR expression and evokes a rapid feeding response and increased adiposity (188). Furthermore, inducible deletion of the INSR in NPY neurons in adulthood increases food intake (182), whereas combined ablation of *Ptpn1* and *Ptpn2,* but not *Ptpn2* alone, in the MBH of diet-induced obese mice represses food intake (5). Interestingly, mice with ablated islet insulin secretion (streptozotocin-treatment), show no alterations in cumulative daily food intake but exhibit an attenuated microstructure of feeding behaviour (190).

Whilst many diverse neuronal populations likely functionally integrate insulin signalling in the brain to regulate feeding behaviour, it is only AgRP neurons that have been conclusively shown to respond to endogenous postprandial insulin secretion (51). Exactly how AgRP neurons functionally integrate this information to the rest of the brain remains unclear. Circuit mapping studies show that optogenetic and pharmacogenetic stimulation of AgRP projections to the PVH and ventrolateral part of the aBNST are associated with activation of feeding, whereas AgRP projections to the lateral hypothalamus, dorsomedial part of the aBNST, dorsal raphe or DVC are not (116, 191). These findings suggest a functional distinction of AgRP projections in how hormonal information is integrated into the brain.

In addition to ARC neurons, insulin signals to astrocytes (101, 192). INSR ablation in glial fibrillary acidic protein (GFAP) or glutamate aspartate transporter expressing cells (markers of astrocytes) attenuated astrocytic glucose uptake through the regulation of mitochondrial dynamics (101). Within the forebrain, INSR astrocytic signalling has also been shown to act in concert with IGF-1 signalling to regulate insulin-stimulated glucose uptake (43). The ablation of astrocytic INSR signalling impacted upon the functional responses and synaptic profile of POMC neurons, attenuating feeding behaviour and glycaemic control (101). Specific deletion of the INSR in astrocytes within the hypothalamus (predominantly the MBH) impaired glucose availability in the brain resulting in attenuated feeding responses and glycaemic adaptions to glucoprivation (101). Although an emerging role of astrocytes within the hypothalamus exists (150, 193), the specific contribution of astrocytic INSR signalling to the functionality of other hypothalamic cells types or its contribution to the pathogenesis of metabolic disease remains unclear.

The sensory detection and perception of food play an intrinsic role in feeding behaviour (194). The integration of this information is highly dependent upon the nutritional and motivational state, information that is encoded in part by POMC and AgRP neurons (195, 196). ARC neurons receive direct input from olfactory sensing neurons in the olfactory bulb which serves to prime ARC neurons to the olfactory perception of foods. Nutritional status directly regulates olfactory sensitivity (197), thus the nutritional-state dependent modulation of POMC and AgRP neuron excitation may be mediated by olfactory stimulation levels. Mice with hyposmia exhibit increased energy expenditure due to enhanced sympathetic nervous system activity innervating of BAT and white adipose tissue (WAT). Insulin has been found to directly enhance olfactory performance in humans (198, 199), effects that are attenuated in obesity and hyperinsulinemia (200, 201). Insulin signalling in the olfactory bulb may therefore impinge information relating to the sensory properties of food onto the hypothalamic tuning of energy and glucose homeostasis.

Beyond the hypothalamus, INSR signalling has been implicated in the regulation of dopaminergic neurocircuitry associated with hedonic and motivational components of feeding (see section 6.2). Given food seeking behaviour requires memory and learning functions, cortical and hippocampal circuits are also implicit (167, 202). Dysregulation of both homeostatic and dopaminergic systems are associated with obesity and insulin resistance (155), and conjunctively overnutrition impairs dopamine and insulin function within the brain (157, 158). INSRs are expressed on dopaminergic neurons in the VTA (161, 203) and inhibition of INSR signalling in the VTA promotes food intake and weight gain (163). Furthermore, insulin administration directly into the VTA reduced the consumption of sweetened high-fat food in satiated mice (160) and suppressed synaptic tone onto VTA neurons diminishing food seeking behaviour (204). Importantly, the capacity for insulin to regulate VTA dopaminergic neurons is attenuated in the context of hyperinsulinemia (161).

Central insulin signalling also mediates dopamine signalling to control feeding via its regulation of tyrosine hydroxylase (TH), a rate-limiting enzyme for dopamine synthesis (205). Mice lacking INSRs in TH-neurons exhibit diet-induced obesity and hyperphagia (163). Astrocyte INSR signalling modulates dopamine signalling and the secretion of neurotransmitters, such as glutamate (44, 206). Mice lacking astrocyte INSRs exhibit impaired dopamine release from NAc neurons resulting in increased anxiety- and depressive-like behaviours (44). The development of obesity has been associated with impaired insulin action in the dopaminergic system (156, 159, 162). Obese individuals demonstrate potentiated reward circuitry and exhibit increased activation of the dopamine system in response to images of high-calorie foods (207). Rodents in hyperinsulinemic states exhibit an impairment in insulin mediation of food seeking behaviour (160, 161), and attenuated insulin action is observed in the striatum of overweight but not lean humans (208).

7.2. Insulin Signalling in the Brain Regulates Energy Expenditure

Energy expenditure functions to coordinate energy homeostasis and is made up from several components, including basal metabolic rate (~60%), physical activity (~25%) and adaptive thermogenesis (~15%) (see review (209)). Of relevance to central INSR signalling is adaptive thermogenesis (**Fig. 2 and 3**) which has received significant attention in the context of metabolic disease due to its profound action on body weight and glucose metabolism. Adaptive thermogenesis is defined as the concerted production of heat energy in response to changes in ambient temperature and nutritional status (diet/feeding-induced thermogenesis) (210). Adaptive thermogenesis occurs through the activation of brown adipocytes (located within the interscapular region of rodents/infant humans and the supraclavicular region in adult humans (211-214)), and the

activation and recruitment of beige adipocytes, located within WAT depots (a process referred to as WAT browning (**Fig. 2 and 3**)) (211-214). Whilst white adipocytes primarily function to store energy, brown and beige adipocytes function to dissipate energy in the form of heat (215). In response to caloric intake or cold exposure, sympathetically derived norepinephrine activates β3-adrenergic receptors on brown and beige adipocytes to promote the release of free fatty acids (FFA) and the expression of uncoupling protein-1 (UCP-1) (**Fig. 3**)(216). UCP-1 uncouples fatty acid and glucose oxidation from ATP production to expend energy and generate heat (**Fig. 3**) (217). Notably, UCP-1 independent mechanisms of thermogenesis also exist (see review (218)). In humans, BAT levels are directly correlated with body mass index, and BAT thermogenesis is impaired in obese individuals (219, 220). In healthy humans, BAT activation through cold exposure triggers a substantial increase in BAT glucose disposal which is not observed in other organs (219, 221).

The role of central insulin signalling in thermogenesis was deduced as early as the 1980s, when plasma insulin rises in response to refeeding were associated with enhanced thermogenic responses, an effect attenuated by chemical blockade of β-cell insulin secretion (222). Postprandial plasma insulin elevation is associated with increased glucose uptake into BAT compared to physiological states of low insulin levels (i.e., fasting) or impaired insulin action (obesity) (221, 223, 224). Insulin administration dramatically enhances BAT glucose uptake in lean humans (221, 223), whereas obese insulin resistant individuals exhibit impaired thermogenesis (220). The actions of insulin to regulate thermogenesis likely involves the brain. ICV administration of insulin promotes sympathetic outflow to peripheral tissue (225, 226), and intranasal administration of insulin promotes hypothalamic functional activity to promote postprandial thermogenesis (183, 227).

The neurocircuitry underpinning central insulin's action upon thermogenesis involves neurons of the ARC. Deletion of the INSR phosphatase, TCPTP, in AgRP neurons promotes BAT and beige *Ucp-1* expression, thermogenesis and glucose uptake (51, 64). These effects are directly attributed to INSR signalling in AgRP neurons and protect against diet-induced obesity (51). INSR signalling in AgRP neurons has further been shown to coordinate feeding induced thermogenesis, through the expression of TCPTP within the hypothalamus (51, 64). Feeding-induced activation of beige adipocytes is attenuated in obesity which has been attributed to insulin resistance within the ARC (51). TCPTP deletion in the ARC of obese mice promotes neuronal insulin signalling which in turn reinstates adipose tissue thermogenesis resulting in weight loss and improved glycaemic control (5, 51). Furthermore, pharmacological inhibition of TCPTP, or TCPTP and PTP1B, within the MBH robustly promotes adipose tissue thermogenesis and weight loss in diet-induced obese mice (5). INSR signalling in POMC neurons also regulates energy expenditure (63). Mice lacking TCPTP and PTP1B in POMC neurons exhibit enhanced brown and beige adipocyte activation, energy

expenditure and protection against diet-induced obesity (63, 65). Furthermore, re-expression of the INSR in POMC neurons in INSR deficient mice promotes energy expenditure and locomotor activity (228).

Whilst there is a growing recognition of the role of insulin signalling within CNS astrocytes in the regulation of food intake, there has been little examination of a role in adipose tissue thermogenesis. Recently, Manaserh *et al* showed INSR deletion in astrocytes reduced BAT thermogenesis and BAT β3-adrenergic receptor expression (229). As these mice could not engage a thermogenic response to cold exposure this implies a functional role of astrocytic INSR signalling in thermogenesis. Given INSR deletion in astrocytes alters astrocyte morphology, and neurocircuitry structure and connectivity (101), understanding how regional neuronal circuits and their cellular components integrate insulin signalling to regulate adipose tissue thermogenesis is still to be determined.

7.3. Central Insulin Regulation of Glucose Metabolism

Although Claude Bernard first demonstrated the role of the brain in glycaemic control in 1855 (6), the actions of insulin to stimulate glucose uptake in peripheral tissues has long overshadowed potential contributions by the brain. Insulin signalling in peripheral tissues such as skeletal muscle and adipose tissue facilitates glucose transport from the blood via the translocation of the insulin-dependent glucose transporter (GLUT4) (230). A subset of hypothalamic neurons express GLUT4 (231), however, glucose transport into neurons is generally accepted to occur independently of insulin (10, 11). Although insulin's effects on glucose uptake into the brain parenchyma is untenable, it does play a significant neuromodulatory role in how the brain regulates glycaemic control.

Central insulin action has been implicated in the regulation of hepatic glucose production (HGP, **Fig. 2 and 3a**). HGP refers to the appearance of *de novo* glucose generated by the liver through gluconeogenesis and glycogenolysis in the blood (1). In the postabsorptive or fasted state, HGP is upregulated in order to maintain glucose homeostasis (1). In the absorptive state (after feeding) when plasma glucose and insulin levels are elevated, HGP is shutoff to facilitate liver glucose storage via glycolysis and glycogenesis (1). The orchestration of HGP to maintain glucose homeostasis becomes dysregulated in diabetic and obese individuals and significantly contributes to hyperglycaemia (232). Mice with brain INSR deletion exhibit an inability for insulin to attenuate HGP (233), and ICV infusion of insulin suppresses HGP and lowers blood glucose levels even in the context of peripheral insulin resistance (112, 188, 234). Consistent with this, the attenuation of INSR

signalling using INSR antibodies or antisense oligonucleotides vitiates insulin's ability to suppress HGP (112). Insulin infusion into the MBH activates hypothalamic ATP-sensitive potassium channels which attenuates HGP via vagal efferent neurons innervating the liver (188, 235). INSR signalling in the brain is known to suppress hepatic vagal activation which, via α7-nicotinic acetylcholine receptors, promotes interleukin-6 (IL-6) secretion from hepatic Kupffer cells (**Fig. 3**) (236). IL-6, in turn, acts upon IL-6 receptors expressed on hepatocytes to repress HGP via a STAT3 mediated repression of gluconeogenic enzymes glucose-6-phosphatase (G6pc) and phosphoenolpyruvate-carboxykinase (Pck-1) (**Fig. 3**) (233, 236). The ability of central insulin to act on HGP is lost following hepatic vagotomy and sympathectomy implying that intact innervation from the brain to the liver is necessary (237, 238).

In humans, intranasal insulin administration promotes hypothalamic activity and improves insulin sensitivity, implicating the hypothalamus as a key site coordinating central insulin's actions on glycaemic control (183). The ability of insulin to suppress HGP is lost in mice lacking INSRs in AgRP neurons of the hypothalamus (163), and chemogenetic excitation of AgRP neurons impairs whole-body insulin sensitivity by selectively repressing BAT glucose uptake (191). Consistent with this, enhanced insulin signalling in AgRP neurons promotes whole-body insulin sensitivity via BAT/WAT glucose uptake and enhanced insulin-induced suppression of HGP (65). Chemogenetic inhibition and excitation of POMC neurons elicit elevated and attenuated HGP respectively (64), illustrating the capacity of POMC neurons to influence HGP. Whilst the deletion of INSRs specifically in POMC neurons has little effect on HGP (129), enhancing insulin signalling within POMC neurons promotes insulin-mediated attenuation of HGP and is capable of suppressing HGP even in the context of whole-body insulin resistance (65). Combined deletion of the INSR and LepR in POMC neurons has also been shown to regulate glucose metabolism (239). Infusion of insulin into the brain represses liver glucose metabolism in a fed but not the fasted state (64), an effect that is coordinated by the fed/fast fluctuations in TCPTP expression in both AgRP and POMC neurons (64, 65). Central insulin signalling in AgRP and POMC neurons may therefore function to coordinate food intake with glucose homeostasis via the regulation of HGP.

Whilst it seems implicit that central insulin signalling regulates HGP directly via vagal efferent neurons, it is important to note that studies in both humans and dogs have shown limited impairment in glucose metabolism following liver denervation (235, 240, 241). To account for the actions of central insulin signalling in the control of hepatic glucose metabolism it is likely that other "indirect" mechanisms exist. One such mechanism may be through the central regulation of adipose tissue lipolysis (**Fig. 2**). Lipolysis occurs within adipocytes and functions to supply energy through the hydrolysis of triglycerides into FAAs and glycerol (242). Lipolysis is regulated by the sympathetic

nervous system and functions to maintain energy homeostasis during periods of negative energy balance, such as fasting, or to produce substrates required for thermogenesis or gluconeogenesis (242). A key role of central insulin signalling in the control of WAT lipolysis was demonstrated by unrestrained lipolysis and decreased *de novo* lipogenesis in mice lacking INSRs specifically in the brain (243). Insulin action in the hypothalamus appears to be important in WAT lipolytic activity, as insulin infusion into the MBH of rodents increases WAT lipogenic gene expression and suppresses lipolysis (243). Critically, a high-fat diet has been shown to abolish the ability of the MBH to suppress WAT lipolysis and HGP, consistent with the development of central insulin resistance (244). Notably, insulin signalling in the brain inhibits lipolysis independently of changes in peripheral insulin levels, suggesting that these actions must occur through the inhibition of sympathetic outflow to WAT (243). Retrograde tracing studies have identified the neural circuitry innervating WAT and BAT, implicating areas of the brainstem, midbrain, and hypothalamus (245). The hypothalamic melanocortin system, a direct target of insulin, has been shown to have significant effects on WAT lipolytic and lipogenic activity. Chronic blockade of hypothalamic melanocortin receptor 3 and 4 promoted WAT lipogenic activity (246), and INSR deletion in POMC but not AgRP neurons impaired WAT lipolytic regulation (247). It is intriguing that insulin signalling in the hypothalamus can elicit both lipolysis and browning within WAT, considering these processes require a respective suppression and promotion of β-adrenergic receptor activation (51, 63, 243). It is tentative to think that heterogeneity in the functional innervation of the WAT may exist however this has yet to be elucidated.

8. Insulin Resistance and Metabolic Disease

A hallmark of metabolic diseases such as obesity, pre-diabetes and T2D is impaired cellular insulin signalling (1). As the impaired insulin action occurs despite heightened insulin levels in the blood, this phenomenon is termed “insulin resistance” (**Fig. 4**). Insulin resistance within the brain, particularly the hypothalamus, has been demonstrated in both obese rodents and humans. The mechanisms underlying this pathophysiology are complex and not fully understood, however, a significant contribution is through defects in insulin signalling occurring downstream of the INSR (1). In the context of neuronal insulin resistance, negative regulators of insulin signalling such as TCPTP, PTP1B and SOCS3 play a role (**Fig. 4**). The levels of INSR negative regulators are driven by the hyperleptinemia (248, 249), enhanced hypothalamic inflammation (226, 250), reactive gliosis (251) and enhanced circulating glucocorticoid levels (5, 51) associated with obesity. Exactly when neuronal insulin resistance occurs is unclear, however, reduced hypothalamic insulin sensitivity has been

reported after 72 hours of high-fat feeding (226). The expression of SOCS3 is elevated after 2 days of high-fat feeding in AgRP neurons and after 2 weeks in POMC neurons (76). PTP1B and TCPTP expression is elevated within the hypothalamus of mice after 6 and 9 weeks of high-fat feeding and these levels remain elevated beyond 12 weeks of high-caloric exposure (5, 51, 52). The consequence of elevated levels of TCPTP, PTP1B and SOCS3 is evident as their genetic ablation in cells of the MBH promotes hypothalamic insulin signalling and enhances whole-body glucose metabolism (5, 51, 63, 64, 72, 76, 252, 253). More specifically, the deletion of TCPTP and PTP1B in the MBH of diet-induced obese mice after 12 weeks of high-fat diet reinstated INSR mediated signalling, despite the mice continuing to eat a high-fat diet (5). Deletion of TCPTP with the MBH of diet-induced obese mice promoted adipose tissue thermogenesis and energy expenditure whereas PTP1B deletion repressed food intake (5). Combined deletion of TCPTP and PTP1B repressed both food intake and promoted adipose tissue thermogenesis resulting in a synergistic reduction in body weight (5).

Whilst it is apparent that the key neuronal populations within the brain governing energy and glucose homeostasis become insulin resistant, there are currently no effective therapeutic strategies targeting insulin resistance within the brain. Pharmacological inhibition of PTP1B through systemic or central administration of trodusquemine (MSI-1436) or its highly selective analogue, claramine, decreases food intake and promotes weight loss in lean and obese mice (51, 254, 255). Central TCPTP inhibition through ICV administration of Compound 8 enhanced energy expenditure (256), repressed food intake and body weight (52), and promoted hypothalamic *Pomc* expression (63). Hypothalamic TCPTP levels are attenuated by ICV administration of the glucocorticoid receptor antagonist, RU486 (5, 51). Central daily administration of RU486 over 10 days to diet-induced obese mice dose-dependently attenuated body weight and enhanced energy expenditure through the promotion of adipose tissue thermogenesis (5). Furthermore, the combined administration of RU486 and claramine to attenuate central TCPTP and PTP1B synergistically promoted weight loss in obese mice (5). Celastrol, a natural pentacyclic triterpene has recently been shown to noncompetitively inhibit PTP1B and TCPTP (257). Systemic daily administration of celastrol to diet-induced obese mice for 10 days promoted ~10% weight loss, actions that are mediated through the inhibition of PTP1B and TCPTP in the hypothalamus (257).

There is a clear emerging precedent set for therapeutically targeting central insulin resistance for the treatment of metabolic disease. Despite this, specifically targeting regions of the brain in a repeatable and viable way has long been a significant therapeutic roadblock. This has greatly limited the use of several efficacious pharmacological interventions delivered systemically due to confounding non-specific actions on peripheral tissues. For example, deletion of PTP1B within myeloid cells drives the development of leukaemia and shortened lifespan (258) whilst global TCPTP

deletion promotes systemic inflammation and mortality (259, 260). To potentially overcome these limitations the intranasal route of administration is a possible option for the viable delivery of agents into the brain. Drugs can be delivered into the brain via the intranasal route of administration by passing through the paracellular space across the nasal epithelium (261). Compounds then enter the CSF and directly interact with the hypothalamic parenchyma coordinating metabolism (5, 183, 262). In healthy humans, intranasal delivery of insulin regulates hypothalamic neuronal activity which in turn attenuates HGP and food intake with minimal spill over into the blood (183, 263). Intranasal insulin in humans has also been shown to promote adipocyte glucose metabolism to improve whole-body insulin sensitivity (183, 227). The efficacy of intranasal insulin to regulate hypothalamic activity and hepatic glucose metabolism is lost in obese men, consistent with the development of central insulin resistance (183). The lack of efficacy of intranasal insulin in the insulin resistant state limits its therapeutic potential. An emerging alternative approach to ameliorate insulin resistance within the brain may be to intranasally target TCPTP and PTP1B. Combined intranasal delivery of RU486 and claramine, to inhibit TCPTP and PTP1B respectively, promoted hypothalamic insulin-induced AKT Ser-473 phosphorylation and promoted weight loss in diet-induced obese mice (5). Collectively it seems apparent that the dysfunction of central insulin signalling plays a substantial role in the development of metabolic disease. Recent advances in our understanding of the molecular mechanisms underlying neuronal insulin resistance along with refinements in therapeutically targeting the brain have highlighted the potential of intranasally targeting INSR signalling in the treatment of metabolic disease.

9. Conclusion

Energy and glucose homeostasis function to deliver an adequate supply of nutrients to the body in order to maintain the *internal milieu*. To facilitate this, the brain has evolved a complex array of neuroendocrine mechanisms capable of integrating the body's nutritional status and transducing this information into physiological responses capable of fine-tuning metabolism. Insulin signalling in the brain plays a significant role in the coordination of energy and glucose homeostasis through the regulation of feeding behaviour (182), energy expenditure (51, 63), insulin sensitivity (64, 65, 191), glucose metabolism (5, 64, 65, 129, 182) and lipid metabolism (31, 243, 247) (**Fig. 2**). Many of these actions are mediated by insulin signalling in AgRP and POMC neurons in the ARC. Emerging evidence now substantiates novel insulin-responsive neuronal and non-neuronal subsets in the hypothalamus (44, 101, 149, 152, 229); however, the functional contribution of this signalling is still in its infancy. Beyond the hypothalamus, insulin is known to regulate cells within areas of the brain involved with

reward (160, 161, 163, 204), olfaction (198, 199) and stress (178). Further studies examining insulin's role in these areas will afford an invaluable insight into how the brain integrates external and hedonic cues with nutritional status to regulate energy and glucose homeostasis.

The brain becomes insulin resistant during the development of metabolic diseases, the functional significance of which is only now starting to emerge. Emerging evidence suggests that insulin resistance in the brain is responsible for the elevated food intake (5), HGP (64), lipolysis (244), and impaired adipose tissue thermogenesis (51, 220, 264) associated with diet-induced obesity. Exciting inroads have been made towards understanding the cellular mechanisms driving neuronal insulin resistance and a clear precedent has emerged for the delivery of agents intranasally to promote brain insulin sensitivity (5, 183, 262, 265). The therapeutic significance of targeting INSR signalling in the brain to treat metabolic disease is evident (**Fig 4**), and the stage may therefore be set for insulin signalling in the brain to take the therapeutic spotlight in the fight against metabolic diseases.

FIGURE LEGENDS

Figure 1: Insulin transport and receptor signalling within the brain. a) Insulin within the systemic circulation reaches its targets within the mediobasal hypothalamus via 1) receptor-mediated transcytosis across the blood-brain barrier (BBB), 2) passive extravasation through the fenestrated capillaries and ependymal cells of the median eminence (ME) or 3) through tanycyte mediated transport from the cerebral spinal fluid (CSF). 1) The BBB is formed by endothelial cells joined by tight junctions which regulate the paracellular diffusion of insulin into the brain (90). Circulating insulin binds to insulin receptors (INSR) expressed on endothelial cells (91, 92). Once bound to the INSR, insulin is internalised into vesicles and transported across the endothelial cells and presented by receptor-mediated exocytosis to the abluminal side of the endothelial cell where it enters the brain (92-94). Once in the brain insulin can interact with the surrounding neurons, pericytes and glia (including astrocytes). 2) Insulin can enter the brain via passive diffusion via the ME. The ME is a circumventricular organ composed of fenestrated capillaries that allows passive entry of insulin into the brain (81, 104, 105). 3) Insulin is also present within the cerebrospinal fluid (CSF) where it can be transported into the brain via tanycytes that line the floor of the third ventricle and project processes into the hypothalamus including the arcuate nucleus of the hypothalamus (ARC), ventromedial hypothalamus (VMH) and the ME (108-110). **b)** Insulin receptor signalling cascade within agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) neurons of the ARC. Insulin binds to the extracellular α-subunits of the INSR expressed on the cell surface of neurons in the ARC. INSR activation results in receptor autophosphorylation of the β-subunits (34) and a cascade of

phosphorylation events in either the Phosphoinositide 3-kinase (PI3K)/Protein kinase B (AKT) or Mitogen-activated protein kinase (MAPK)/Extracellular signal-regulated kinase (ERK) pathways (38-40). In the schematic, autophosphorylation of tyrosine residues initiates the phosphorylation of insulin receptor substrate (IRS) proteins which in turn activates PI3K leading to AKT phosphorylation. INSR activated AKT promotes the expression of *Pomc* and represses the expression of *Agrp* within the neuron (64, 127, 181, 189). INSR signalling is also negatively regulated by protein tyrosine phosphatases, such as protein tyrosine phosphatase 1B (PTP1B) (46-49) and T-cell like protein tyrosine phosphate (TCPTP)(62), and suppressor of cytokine signalling 3 (SOCS3) (73) which function to dephosphorylate the INSR and/or its downstream components.

Figure 2: Physiological effects of the insulin signalling in the brain. Insulin is secreted postprandially from the pancreas into the blood circulation. Insulin reaches the brain where it signals to regulate olfaction (olfactory bulb) (198, 199), hedonic aspects of feeding behaviour (pre-frontal cortex (111, 164-166), nucleus accumbens (NAc) (162), striatum (162), ventral tegmental area (VTA)(153, 156, 160, 161)) and memory/learning (hippocampus) (164, 167). Insulin signals to the cells in the hypothalamus, in particular to two discrete population of neurons expressing agouti-related peptide (AgRP) and pro-opiomelanocortin (POMC) (51, 63, 64, 129). The representative photomicrograph depicts immunofluorescent expression of AgRP (red) and POMC (green) within neurons of the arcuate nucleus of the hypothalamus (ARC) of an 8-week old male *Agrp*-Cre::lox-STOP-lox-tdTomato::*Pomc*-eGFP transgenic mouse. Insulin signalling in hypothalamic AgRP and POMC neurons coordinates food intake (182), peripheral insulin sensitivity (64, 65), hepatic glucose production (64, 65, 129, 247), lipolysis (247), brown adipose tissue (BAT) thermogenesis and white adipose tissue (WAT) browning (51, 63-65). Abbreviation: 3rd ventricle (3rd V), beige adipose tissue (BeigeAT), dorsal vagal complex (DVC), dorsomedial hypothalamus (DMH), free fatty acids (FFA), lateral hypothalamus (LH), paraventricular hypothalamus (PVH), ventromedial hypothalamus (VMH).

Figure 3: Central insulin signalling regulates hepatic glucose production and adipose tissue thermogenesis. Insulin signals to cells in the arcuate nucleus of the hypothalamus (ARC) (51, 65). ARC insulin signalling is functionally integrated into the brain and is propagated via the autonomic nervous systems to coordinate hepatic glucose production in the liver and adipose tissue thermogenesis in white adipose tissue (WAT) and brown adipose tissue (BAT). **a)** Insulin signalling in the ARC suppresses the activation of vagal cholinergic efferent neurons innervating the liver parenchyma (236). The attenuated release of acetylcholine (Ach) from vagal afferents stimulates

Kupffer cells to secrete interleukin-6 (IL-6) into the liver parenchyma, an effect that is mediated by the α7-nicotinic acetylcholine receptors (α7-nAchR) expressed on the Kupffer cell surface (236). IL-6 activates its receptor (IL-6R) expressed on hepatocytes, which in turn, signalling via Signal transducer and activator of transcription 3 (STAT3) represses the transcription of gluconeogenic enzymes phosphoenolpyruvate-carboxykinase (Pck-1) and glucose-6-phosphatase (G6pc) (236). Pck-1 and G6pc are enzymes essential for gluconeogenesis as they convert oxaloacetate into phosphoenolpyruvate and glucose 6-phosphate into glucose respectively (1). The STAT3 mediated suppression of Pck-1 and G6pc stops gluconeogenesis and the appearance of *de novo* glucose in the blood. **b)** Insulin signalling in the ARC promotes the release of norepinephrine (NE) from tyrosine-hydroxylase (TH) -expressing sympathetic efferent neurons (266). NE activates β-adrenoreceptors expressed upon the cell surface of beige and brown adipocytes (267). β-adrenoreceptor activation in turn stimulates uncoupling protein 1 (UCP1) located on the inner mitochondrial membrane to facilitate the transport of protons into the inner mitochondrial membrane (267). In a futile attempt to maintain the proton gradient across the inner mitochondrial membrane, the activity of the electron transport chain is upregulated which expends energy in the form of heat (267).

Figure 4: Insulin resistance in the arcuate nucleus of the hypothalamus (ARC). a) Insulin signals to neurons in the ARC via the insulin receptor (INSR) (51, 65). Insulin binding to the INSR initiates receptor autophosphorylation which in turn promotes the downstream phosphorylation of Protein kinase B (AKT) (1). The representative photomicrograph depicts immunoreactivity for insulin-stimulated Ser-473 AKT phosphorylation in the ARC of an 8-week-old C57BL/6J mouse. INSR signal transduction in ARC cells regulates energy and glucose homeostasis by attenuating food intake (182), lipolysis (247), hepatic glucose production (64, 65, 129, 247), and promoting brown adipose tissue (BAT) thermogenesis and white adipose tissue (WAT) browning (51, 63-65). **b)** In diet-induced obesity neurons of the ARC become insulin resistant (5). Despite the heightened levels of circulating insulin, the actions of insulin to induce INSR signalling are attenuated (1, 5). The representative photomicrograph depicts immunoreactivity for insulin-stimulated Ser-473 AKT phosphorylation in the ARC of a 12-week high-fat fed C57BL/6J mouse. During the development of diet-induced obesity, cells within the ARC exhibit heightened expression of INSR negative regulators such as T-cell protein tyrosine phosphatase (TCPTP), protein tyrosine phosphatase 1b (PTP1B) and suppressor of cytokine signalling 3 (SOCS3) (51, 52, 63, 64). TCPTP, PTP1B and SOCS3 functionally attenuate INSR signalling and contribute towards cellular insulin resistance. Insulin resistance within ARC promotes food intake (5), lipolysis (243, 244), hepatic glucose production (65, 183) and represses BAT and WAT thermogenesis (5, 51, 63) leading the development and maintenance of metabolic disease.

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References

1. Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. Physiological reviews. 2018;98(4):2133-223.

2. Hill JM, Lesniak MA, Pert CB, Roth J. Autoradiographic localization of insulin receptors in rat brain: Prominence in olfactory and limbic areas. Neuroscience. 1986;17(4):1127-38.

3. Dodd GT, Tiganis T. Insulin action in the brain: Roles in energy and glucose homeostasis. J Neuroendocrinol. 2017;29(10).

4. Werther GA, Hogg A, Oldfield BJ, McKinley MJ, Figdor R, Allen AM, et al. Localization and characterization of insulin receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. Endocrinology. 1987;121(4):1562-70.

5. Dodd GT, Xirouchaki CE, Eramo M, Mitchell CA, Andrews ZB, Henry BA, et al. Intranasal Targeting of Hypothalamic PTP1B and TCPTP Reinstates Leptin and Insulin Sensitivity and Promotes Weight Loss in Obesity. Cell Rep. 2019;28(11):2905-22.e5.

6. Bernard C. Leçons de physiologie expérimentale appliquée à la médecine, faites au Collège de France. Paris: J.B. Baillière et fils; [etc., etc.]; 1855.

7. v. Mering J, Minkowski O. Diabetes mellitus nach Pankreasexstirpation. Archiv für experimentelle Pathologie und Pharmakologie. 1890;26(5):371-87.

8. Best CH. The internal secretion of the pancreas. Can Med Assoc J. 1962;87(20):1046-51.

9. Karamanou M, Protogerou A, Tsoucalas G, Androutsos G, Poulakou-Rebelakou E. Milestones in the history of diabetes mellitus: The main contributors. World J Diabetes. 2016;7(1):1-7.

10. Hom FG, Goodner CJ, Berrie MA. A [3H]2-deoxyglucose method for comparing rates of glucose metabolism and insulin responses among rat tissues in vivo. Validation of the model and the absence of an insulin effect on brain. Diabetes. 1984;33(2):141-52.

11. Hasselbalch SG, Knudsen GM, Videbaek C, Pinborg LH, Schmidt JF, Holm S, et al. No effect of insulin on glucose blood-brain barrier transport and cerebral metabolism in humans. Diabetes. 1999;48(10):1915-21.

12. Mayer J. Decreased activity and energy balance in the hereditary obesity-diabetes syndrome of mice. Science. 1953;117(3045):504-5.

13. Oomura Y, Ono T, Ooyama H, Wayner MJ. Glucose and osmosensitive neurones of the rat hypothalamus. Nature. 1969;222(5190):282-4.

14. Anand BK, Chhina GS, Sharma KN, Dua S, Singh B. Activity of single cell neurons in the hypothalamic feeding centers: effect of glucose. Am J Physiol. 1964;207:1146-54.

15. Belfiore A, Malaguarnera R, Vella V, Lawrence MC, Sciacca L, Frasca F, et al. Insulin Receptor Isoforms in Physiology and Disease: An Updated View. Endocrine Reviews. 2017;38(5):379-431.

16. Siddle K. Signalling by insulin and IGF receptors: supporting acts and new players. J Mol Endocrinol. 2011;47(1):R1-10.

17. Mosthaf L, Grako K, Dull TJ, Coussens L, Ullrich A, McClain DA. Functionally distinct insulin receptors generated by tissue-specific alternative splicing. Embo j. 1990;9(8):2409-13.

18. Moller DE, Yokota A, Caro JF, Flier JS. Tissue-specific expression of two alternatively spliced insulin receptor mRNAs in man. Mol Endocrinol. 1989;3(8):1263-9.

19. Seino S, Bell GI. Alternative splicing of human insulin receptor messenger RNA. Biochem Biophys Res Commun. 1989;159(1):312-6.

20. Adem A, Jossan SS, d'Argy R, Gillberg PG, Nordberg A, Winblad B, et al. Insulin-like growth factor 1 (IGF-1) receptors in the human brain: quantitative autoradiographic localization. Brain Res. 1989;503(2):299-303.

21. De Keyser J, Wilczak N, De Backer JP, Herroelen L, Vauquelin G. Insulin-like growth factor-I receptors in human brain and pituitary gland: an autoradiographic study. Synapse. 1994;17(3):196-202.

22. Kleinridders A. Deciphering Brain Insulin Receptor and Insulin-Like Growth Factor 1 Receptor Signalling. Journal of Neuroendocrinology. 2016;28(11).

23. Slaaby R, Schäffer L, Lautrup-Larsen I, Andersen AS, Shaw AC, Mathiasen IS, et al. Hybrid receptors formed by insulin receptor (IR) and insulin-like growth factor I receptor (IGF-IR) have low insulin and high IGF-1 affinity irrespective of the IR splice variant. J Biol Chem. 2006;281(36):25869-74.

24. Bailyes EM, Navé BT, Soos MA, Orr SR, Hayward AC, Siddle K. Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. Biochem J. 1997;327 (Pt 1)(Pt 1):209-15.

25. Baron-Van Evercooren A, Olichon-Berthe C, Kowalski A, Visciano G, Van Obberghen E. Expression of IGF-I and insulin receptor genes in the rat central nervous system: A developmental, regional, and cellular analysis. Journal of Neuroscience Research. 1991;28(2):244-53.

26. Dou J-T, Chen M, Dufour F, Alkon DL, Zhao W-Q. Insulin receptor signaling in long-term memory consolidation following spatial learning. Learn Mem. 2005;12(6):646-55.

27. Havrankova J, Roth J, Brownstein M. Insulin receptors are widely distributed in the central nervous system of the rat. Nature. 1978;272(5656):827-9.

28. Unger J, McNeill TH, Moxley RT, 3rd, White M, Moss A, Livingston JN. Distribution of insulin receptor-like immunoreactivity in the rat forebrain. Neuroscience. 1989;31(1):143-57.

29. Zhao WQ, Chen H, Quon MJ, Alkon DL. Insulin and the insulin receptor in experimental models of learning and memory. Eur J Pharmacol. 2004;490(1-3):71-81.

30. Kleinridders A, Ferris HA, Cai W, Kahn CR. Insulin action in brain regulates systemic metabolism and brain function. Diabetes. 2014;63(7):2232-43.

31. Abbott MA, Wells DG, Fallon JR. The insulin receptor tyrosine kinase substrate p58/53 and the insulin receptor are components of CNS synapses. J Neurosci. 1999;19(17):7300-8.

32. Fernandez AM, Navarrete M, Davila JC, Garcia-Caceres C, Palenzuela R, de Martin Esteban SR, et al. The Insulin Receptor in Astrocytes is Involved in the Entrance of Circulating Insulin into the Brain. bioRxiv. 2019:720813.

33. LeRoith D, Shemer J, Adamo M, Raizada MK, Heffez D, Zick Y. Insulin and IGF-I stimulate phosphorylation of their respective receptors in intact neuronal and glial cells in primary culture. Journal of Molecular Neuroscience. 1989;1(1):3.

34. Jensen M, De Meyts P. Molecular mechanisms of differential intracellular signaling from the insulin receptor. Vitam Horm. 2009;80:51-75.

35. White MF, Takayama S, Kahn CR. Differences in the sites of phosphorylation of the insulin receptor in vivo and in vitro. J Biol Chem. 1985;260(16):9470-8.

36. Galic S, Hauser C, Kahn BB, Haj FG, Neel BG, Tonks NK, et al. Coordinated regulation of insulin signaling by the protein tyrosine phosphatases PTP1B and TCPTP. Mol Cell Biol. 2005;25(2):819-29.

37. Plum L, Schubert M, Brüning JC. The role of insulin receptor signaling in the brain. Trends Endocrinol Metab. 2005;16(2):59-65.

38. Stoica L, Zhu PJ, Huang W, Zhou H, Kozma SC, Costa-Mattioli M. Selective pharmacogenetic inhibition of mammalian target of Rapamycin complex I (mTORC1) blocks long-term synaptic plasticity and memory storage. Proc Natl Acad Sci U S A. 2011;108(9):3791-6.

39. Salcedo-Tello P, Ortiz-Matamoros A, Arias C. GSK3 Function in the Brain during Development, Neuronal Plasticity, and Neurodegeneration. Int J Alzheimers Dis. 2011;2011:189728-.

40. Kim M-S, Pak YK, Jang P-G, Namkoong C, Choi Y-S, Won J-C, et al. Role of hypothalamic Foxo1 in the regulation of food intake and energy homeostasis. Nat Neurosci. 2006;9(7):901-6.

41. Heffron DS, Landreth GE, Samuels IS, Mandell JW. Brain-specific deletion of extracellular signal-regulated kinase 2 mitogen-activated protein kinase leads to aberrant cortical collagen deposition. Am J Pathol. 2009;175(6):2586-99.

42. Satoh Y, Kobayashi Y, Takeuchi A, Pagès G, Pouysségur J, Kazama T. Deletion of ERK1 and ERK2 in the CNS causes cortical abnormalities and neonatal lethality: Erk1 deficiency enhances the impairment of neurogenesis in Erk2-deficient mice. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2011;31(3):1149-55.

43. Fernandez AM, Hernandez-Garzón E, Perez-Domper P, Perez-Alvarez A, Mederos S, Matsui T, et al. Insulin Regulates Astrocytic Glucose Handling Through Cooperation With IGF-I. Diabetes. 2017;66(1):64-74.

44. Cai W, Xue C, Sakaguchi M, Konishi M, Shirazian A, Ferris HA, et al. Insulin regulates astrocyte gliotransmission and modulates behavior. J Clin Invest. 2018;128(7):2914-26.

45. Arkun Y. Dynamic Modeling and Analysis of the Cross-Talk between Insulin/AKT and MAPK/ERK Signaling Pathways. PLoS One. 2016;11(3):e0149684-e.

46. Bandyopadhyay D, Kusari A, Kenner KA, Liu F, Chernoff J, Gustafson TA, et al. Protein-tyrosine phosphatase 1B complexes with the insulin receptor in vivo and is tyrosine-phosphorylated in the presence of insulin. J Biol Chem. 1997;272(3):1639-45.

47. Seely BL, Staubs PA, Reichart DR, Berhanu P, Milarski KL, Saltiel AR, et al. Protein tyrosine phosphatase 1B interacts with the activated insulin receptor. Diabetes. 1996;45(10):1379-85.

48. Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M. Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. Possible facilitation by the formation of a ternary complex with the Grb2 adaptor protein. J Biol Chem. 2000;275(6):4283-9.

49. Matsuo K, Bettaieb A, Nagata N, Matsuo I, Keilhack H, Haj FG. Regulation of brown fat adipogenesis by protein tyrosine phosphatase 1B. PLoS One. 2011;6(1):e16446.

50. Tsou RC, Bence KK. The Genetics of PTPN1 and Obesity: Insights from Mouse Models of Tissue-Specific PTP1B Deficiency. J Obes. 2012;2012:926857.

51. Dodd GT, Andrews ZB, Simonds SE, Michael NJ, DeVeer M, Bruning JC, et al. A Hypothalamic Phosphatase Switch Coordinates Energy Expenditure with Feeding. Cell Metab. 2017;26(2):375-93.e7.

52. Loh K, Fukushima A, Zhang X, Galic S, Briggs D, Enriori PJ, et al. Elevated hypothalamic TCPTP in obesity contributes to cellular leptin resistance. Cell metabolism. 2011;14(5):684-99.

53. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science. 1999;283(5407):1544-8.

54. Delibegovic M, Zimmer D, Kauffman C, Rak K, Hong EG, Cho YR, et al. Liver-specific deletion of protein-tyrosine phosphatase 1B (PTP1B) improves metabolic syndrome and attenuates diet-induced endoplasmic reticulum stress. Diabetes. 2009;58(3):590-9.

55. Bence KK, Delibegovic M, Xue B, Gorgun CZ, Hotamisligil GS, Neel BG, et al. Neuronal PTP1B regulates body weight, adiposity and leptin action. Nat Med. 2006;12(8):917-24.

56. Klaman LD, Boss O, Peroni OD, Kim JK, Martino JL, Zabolotny JM, et al. Increased energy expenditure, decreased adiposity, and tissue-specific insulin sensitivity in protein-tyrosine phosphatase 1B-deficient mice. Mol Cell Biol. 2000;20(15):5479-89.

57. Sugiyama M, Banno R, Mizoguchi A, Tominaga T, Tsunekawa T, Onoue T, et al. PTP1B deficiency improves hypothalamic insulin sensitivity resulting in the attenuation of AgRP mRNA expression under high-fat diet conditions. Biochem Biophys Res Commun. 2017;488(1):116-21.

58. Picardi PK, Calegari VC, Prada PO, Moraes JC, Araújo E, Marcondes MC, et al. Reduction of hypothalamic protein tyrosine phosphatase improves insulin and leptin resistance in diet-induced obese rats. Endocrinology. 2008;149(8):3870-80.

59. De Jonghe BC, Hayes MR, Banno R, Skibicka KP, Zimmer DJ, Bowen KA, et al. Deficiency of PTP1B in POMC neurons leads to alterations in energy balance and homeostatic response to cold exposure. Am J Physiol Endocrinol Metab. 2011;300(6):E1002-E11.

60. Balland E, Chen W, Dodd GT, Conductier G, Coppari R, Tiganis T, et al. Leptin Signaling in the Arcuate Nucleus Reduces Insulin's Capacity to Suppress Hepatic Glucose Production in Obese Mice. Cell Rep. 2019;26(2):346-55.e3.

61. Chiappini F, Catalano KJ, Lee J, Peroni OD, Lynch J, Dhaneshwar AS, et al. Ventromedial hypothalamus-specific Ptpn1 deletion exacerbates diet-induced obesity in female mice. J Clin Invest. 2014;124(9):3781-92.

62. Galic S, Klingler-Hoffmann M, Fodero-Tavoletti MT, Puryer MA, Meng T-C, Tonks NK, et al. Regulation of insulin receptor signaling by the protein tyrosine phosphatase TCPTP. Molecular and cellular biology. 2003;23(6):2096-108.

63. Dodd GT, Decherf S, Loh K, Simonds SE, Wiede F, Balland E, et al. Leptin and insulin act on POMC neurons to promote the browning of white fat. Cell. 2015;160(1-2):88-104.

64. Dodd GT, Michael NJ, Lee-Young RS, Mangiafico SP, Pryor JT, Munder AC, et al. Insulin regulates POMC neuronal plasticity to control glucose metabolism. Elife. 2018;7.

65. Dodd GT, Lee-Young RS, Brüning JC, Tiganis T. TCPTP Regulates Insulin Signaling in AgRP Neurons to Coordinate Glucose Metabolism With Feeding. Diabetes. 2018;67(7):1246-57.

66. Jiang L, Li Z, Rui L. Leptin stimulates both JAK2-dependent and JAK2-independent signaling pathways. The Journal of biological chemistry. 2008;283(42):28066-73.

67. Croker BA, Kiu H, Nicholson SE. SOCS regulation of the JAK/STAT signalling pathway. Semin Cell Dev Biol. 2008;19(4):414-22.

68. Bjørbaek C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS. Identification of SOCS-3 as a potential mediator of central leptin resistance. Mol Cell. 1998;1(4):619-25.

69. Bjorbak C, Lavery HJ, Bates SH, Olson RK, Davis SM, Flier JS, et al. SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. J Biol Chem. 2000;275(51):40649-57.

70. Howard JK, Cave BJ, Oksanen LJ, Tzameli I, Bjørbaek C, Flier JS. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. Nat Med. 2004;10(7):734-8.

71. Shi H, Cave B, Inouye K, Bjørbaek C, Flier JS. Overexpression of suppressor of cytokine signaling 3 in adipose tissue causes local but not systemic insulin resistance. Diabetes. 2006;55(3):699-707.

72. Reed AS, Unger EK, Olofsson LE, Piper ML, Myers MG, Jr., Xu AW. Functional role of suppressor of cytokine signaling 3 upregulation in hypothalamic leptin resistance and long-term energy homeostasis. Diabetes. 2010;59(4):894-906.

73. Shi H, Tzameli I, Bjørbaek C, Flier JS. Suppressor of cytokine signaling 3 is a physiological regulator of adipocyte insulin signaling. J Biol Chem. 2004;279(33):34733-40.

74. Mori H, Hanada R, Hanada T, Aki D, Mashima R, Nishinakamura H, et al. Socs3 deficiency in the brain elevates leptin sensitivity and confers resistance to diet-induced obesity. Nat Med. 2004;10(7):739-43.

75. Talbert ME, Langefeld CD, Ziegler J, Mychaleckyj JC, Haffner SM, Norris JM, et al. Polymorphisms near SOCS3 are associated with obesity and glucose homeostasis traits in Hispanic Americans from the Insulin Resistance Atherosclerosis Family Study. Hum Genet. 2009;125(2):153-62.

76. Olofsson LE, Unger EK, Cheung CC, Xu AW. Modulation of AgRP-neuronal function by SOCS3 as an initiating event in diet-induced hypothalamic leptin resistance. Proc Natl Acad Sci U S A. 2013;110(8):E697-706.

77. Williams KW, Liu T, Kong X, Fukuda M, Deng Y, Berglund ED, et al. Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis. Cell metabolism. 2014;20(3):471-82.

78. Gamber KM, Huo L, Ha S, Hairston JE, Greeley S, Bjørbæk C. Over-expression of leptin receptors in hypothalamic POMC neurons increases susceptibility to diet-induced obesity. PLoS One. 2012;7(1):e30485-e.

79. Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB. Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem. 2008;283(21):14230-41.

80. Baura GD, Foster DM, Porte D, Jr., Kahn SE, Bergman RN, Cobelli C, et al. Saturable transport of insulin from plasma into the central nervous system of dogs in vivo. A mechanism for regulated insulin delivery to the brain. J Clin Invest. 1993;92(4):1824-30.

81. Banks WA, Jaspan JB, Huang W, Kastin AJ. Transport of insulin across the blood-brain barrier: saturability at euglycemic doses of insulin. Peptides. 1997;18(9):1423-9.

82. Craft S, Peskind E, Schwartz MW, Schellenberg GD, Raskind M, Porte D, Jr. Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease: relationship to severity of dementia and apolipoprotein E genotype. Neurology. 1998;50(1):164-8.

83. Kern W, Benedict C, Schultes B, Plohr F, Moser A, Born J, et al. Low cerebrospinal fluid insulin levels in obese humans. Diabetologia. 2006;49(11):2790-2.

84. Margolis RU, Altszuler N. Insulin in the cerebrospinal fluid. Nature. 1967;215(5108):1375-6.

85. Woods SC, Porte D, Jr. Relationship between plasma and cerebrospinal fluid insulin levels of dogs. Am J Physiol. 1977;233(4):E331-4.

86. Greco AV, Ghirlanda G, Fedeli G, Gambassi G. Insulin in the cerebro spinal fluid of man. Eur Neurol. 1970;3(5):303-7.

87. Reinhold AK, Rittner HL. Barrier function in the peripheral and central nervous system-a review. Pflugers Arch. 2017;469(1):123-34.

88. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7(1):41-53.

89. Hawkins BT, Davis TP. The blood-brain barrier/neurovascular unit in health and disease. Pharmacol Rev. 2005;57(2):173-85.

90. Ballabh P, Braun A, Nedergaard M. The blood–brain barrier: an overview: Structure, regulation, and clinical implications. Neurobiology of Disease. 2004;16(1):1-13.

91. Rhea EM, Banks WA. Role of the Blood-Brain Barrier in Central Nervous System Insulin Resistance. Frontiers in neuroscience. 2019;13:521-.

92. Duffy KR, Pardridge WM. Blood-brain barrier transcytosis of insulin in developing rabbits. Brain Res. 1987;420(1):32-8.

93. King GL, Johnson SM. Receptor-mediated transport of insulin across endothelial cells. Science. 1985;227(4694):1583-6.

94. Bar RS, DeRose A, Sandra A, Peacock ML, Owen WG. Insulin binding to microvascular endothelium of intact heart: a kinetic and morphometric analysis. Am J Physiol. 1983;244(5):E447-52.

95. Konishi M, Sakaguchi M, Lockhart SM, Cai W, Li ME, Homan EP, et al. Endothelial insulin receptors differentially control insulin signaling kinetics in peripheral tissues and brain of mice. Proceedings of the National Academy of Sciences. 2017;114(40):E8478-E87.

96. Rhea EM, Rask-Madsen C, Banks WA. Insulin transport across the blood-brain barrier can occur independently of the insulin receptor. J Physiol. 2018;596(19):4753-65.

97. Hersom M, Helms HC, Schmalz C, Pedersen T, Buckley ST, Brodin B. The insulin receptor is expressed and functional in cultured blood-brain barrier endothelial cells but does not mediate insulin entry from blood to brain. Am J Physiol Endocrinol Metab. 2018;315(4):E531-e42.

98. Gray SM, Aylor KW, Barrett EJ. Unravelling the regulation of insulin transport across the brain endothelial cell. Diabetologia. 2017;60(8):1512-21.

99. Takechi R, Lam V, Brook E, Giles C, Fimognari N, Mooranian A, et al. Blood-Brain Barrier Dysfunction Precedes Cognitive Decline and Neurodegeneration in Diabetic Insulin Resistant Mouse Model: An Implication for Causal Link. Frontiers in Aging Neuroscience. 2017;9(399).

100. Salameh TS, Shah GN, Price TO, Hayden MR, Banks WA. Blood-Brain Barrier Disruption and Neurovascular Unit Dysfunction in Diabetic Mice: Protection with the Mitochondrial Carbonic Anhydrase Inhibitor Topiramate. J Pharmacol Exp Ther. 2016;359(3):452-9.

101. Garcia-Caceres C, Quarta C, Varela L, Gao Y, Gruber T, Legutko B, et al. Astrocytic Insulin Signaling Couples Brain Glucose Uptake with Nutrient Availability. Cell. 2016;166(4):867-80.

102. Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, et al. Pericytes regulate the blood–brain barrier. Nature. 2010;468(7323):557-61.

103. McKinley MJ, Denton DA, Ryan PJ, Yao ST, Stefanidis A, Oldfield BJ. From sensory circumventricular organs to cerebral cortex: Neural pathways controlling thirst and hunger. J Neuroendocrinol. 2019;31(3):e12689.

104. Blasberg RG, Patlak CS, Fenstermacher JD. Selection of experimental conditions for the accurate determination of blood--brain transfer constants from single-time experiments: a theoretical analysis. J Cereb Blood Flow Metab. 1983;3(2):215-25.

105. van Houten M, Posner B, Kopriwa B, Brawer. Insulin binding sites localized to nerve terminals in rat median eminence and arcuate nucleus. Science. 1980;207(4435):1081-3.

106. Orosco M, Gerozissis K, Rouch C, Nicolaïdis S. Feeding-related immunoreactive insulin changes in the PVN-VMH revealed by microdialysis. Brain Res. 1995;671(1):149-58.

107. Gerozissis K, Orosco M, Rouch C, Nicolaidis S. Insulin responses to a fat meal in hypothalamic microdialysates and plasma. Physiol Behav. 1997;62(4):767-72.

108. Mullier A, Bouret SG, Prevot V, Dehouck B. Differential distribution of tight junction proteins suggests a role for tanycytes in blood-hypothalamus barrier regulation in the adult mouse brain. J Comp Neurol. 2010;518(7):943-62.

109. Prevot V, Langlet F, Dehouck B. Flipping the tanycyte switch: how circulating signals gain direct access to the metabolic brain. Aging (Albany NY). 2013;5(5):332-4.

110. Langlet F, Levin BE, Luquet S, Mazzone M, Messina A, Dunn-Meynell AA, et al. Tanycytic VEGF-A boosts blood-hypothalamus barrier plasticity and access of metabolic signals to the arcuate nucleus in response to fasting. Cell Metab. 2013;17(4):607-17.

111. Kullmann S, Heni M, Fritsche A, Preissl H. Insulin action in the human brain: evidence from neuroimaging studies. J Neuroendocrinol. 2015;27(6):419-23.

112. Obici S, Feng Z, Karkanias G, Baskin DG, Rossetti L. Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. Nat Neurosci. 2002;5(6):566-72.

113. Qiu J, Zhang C, Borgquist A, Nestor CC, Smith AW, Bosch MA, et al. Insulin excites anorexigenic proopiomelanocortin neurons via activation of canonical transient receptor potential channels. Cell Metab. 2014;19(4):682-93.

114. Porter JP, Bokil HS. Effect of intracerebroventricular and intravenous insulin on Fos-immunoreactivity in the rat brain. Neurosci Lett. 1997;224(3):161-4.

115. Wang D, He X, Zhao Z, Feng Q, Lin R, Sun Y, et al. Whole-brain mapping of the direct inputs and axonal projections of POMC and AgRP neurons. Front Neuroanat. 2015;9:40-.

116. Atasoy D, Betley JN, Su HH, Sternson SM. Deconstruction of a neural circuit for hunger. Nature. 2012;488(7410):172-7.

117. Betley JN, Cao ZFH, Ritola KD, Sternson SM. Parallel, redundant circuit organization for homeostatic control of feeding behavior. Cell. 2013;155(6):1337-50.

118. Waterson Michael J, Horvath Tamas L. Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding. Cell Metabolism. 2015;22(6):962-70.

119. Varela L, Horvath TL. Leptin and insulin pathways in POMC and AgRP neurons that modulate energy balance and glucose homeostasis. EMBO Rep. 2012;13(12):1079-86.

120. Yang L, McKnight GS. Hypothalamic PKA regulates leptin sensitivity and adiposity. Nat Commun. 2015;6:8237.

121. Krashes MJ, Koda S, Ye C, Rogan SC, Adams AC, Cusher DS, et al. Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. J Clin Invest. 2011;121(4):1424-8.

122. Luquet S, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science. 2005;310(5748):683-5.

123. Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, et al. Agouti-related peptide-expressing neurons are mandatory for feeding. Nat Neurosci. 2005;8(10):1289-91.

124. Zhang ZY, Dodd GT, Tiganis T. Protein Tyrosine Phosphatases in Hypothalamic Insulin and Leptin Signaling. Trends Pharmacol Sci. 2015;36(10):661-74.

125. Millington GW. The role of proopiomelanocortin (POMC) neurones in feeding behaviour. Nutr Metab (Lond). 2007;4:18.

126. Xu B, Xie X. Neurotrophic factor control of satiety and body weight. Nat Rev Neurosci. 2016;17(5):282-92.

127. Benoit SC, Air EL, Coolen LM, Strauss R, Jackman A, Clegg DJ, et al. The Catabolic Action of Insulin in the Brain Is Mediated by Melanocortins. The Journal of Neuroscience. 2002;22(20):9048-52.

128. Hill JW, Williams KW, Ye C, Luo J, Balthasar N, Coppari R, et al. Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice. J Clin Invest. 2008;118(5):1796-805.

129. Konner AC, Janoschek R, Plum L, Jordan SD, Rother E, Ma X, et al. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. Cell Metab. 2007;5(6):438-49.

130. Qiu J, Wagner EJ, Rønnekleiv OK, Kelly MJ. Insulin and leptin excite anorexigenic pro-opiomelanocortin neurones via activation of TRPC5 channels. J Neuroendocrinol. 2018;30(2).

131. Plum L, Ma X, Hampel B, Balthasar N, Coppari R, Münzberg H, et al. Enhanced PIP3 signaling in POMC neurons causes KATP channel activation and leads to diet-sensitive obesity. J Clin Invest. 2006;116(7):1886-901.

132. Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford ML. Insulin activates ATP-sensitive K+ channels in hypothalamic neurons of lean, but not obese rats. Nat Neurosci. 2000;3(8):757-8.

133. Williams KW, Margatho LO, Lee CE, Choi M, Lee S, Scott MM, et al. Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. J Neurosci. 2010;30(7):2472-9.

134. Hausen AC, Ruud J, Jiang H, Hess S, Varbanov H, Kloppenburg P, et al. Insulin-Dependent Activation of MCH Neurons Impairs Locomotor Activity and Insulin Sensitivity in Obesity. Cell Reports. 2016;17(10):2512-21.

135. Choi Y-H, Fujikawa T, Lee J, Reuter A, Kim KW. Revisiting the Ventral Medial Nucleus of the Hypothalamus: The Roles of SF-1 Neurons in Energy Homeostasis. Frontiers in neuroscience. 2013;7:71-.

136. Klöckener T, Hess S, Belgardt BF, Paeger L, Verhagen LA, Husch A, et al. High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. Nat Neurosci. 2011;14(7):911-8.

137. Sohn JW, Oh Y, Kim KW, Lee S, Williams KW, Elmquist JK. Leptin and insulin engage specific PI3K subunits in hypothalamic SF1 neurons. Mol Metab. 2016;5(8):669-79.

138. Kim KW, Donato J, Jr., Berglund ED, Choi YH, Kohno D, Elias CF, et al. FOXO1 in the ventromedial hypothalamus regulates energy balance. J Clin Invest. 2012;122(7):2578-89.

139. Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 2005;1(1):15-25.

140. Claret M, Smith MA, Batterham RL, Selman C, Choudhury AI, Fryer LGD, et al. AMPK is essential for energy homeostasis regulation and glucose sensing by POMC and AgRP neurons. J Clin Invest. 2007;117(8):2325-36.

141. Minokoshi Y, Alquier T, Furukawa N, Kim Y-B, Lee A, Xue B, et al. AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus. Nature. 2004;428(6982):569-74.

142. Canabal DD, Song Z, Potian JG, Beuve A, McArdle JJ, Routh VH. Glucose, insulin, and leptin signaling pathways modulate nitric oxide synthesis in glucose-inhibited neurons in the ventromedial hypothalamus. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2007;292(4):R1418-R28.

143. Han SM, Namkoong C, Jang PG, Park IS, Hong SW, Katakami H, et al. Hypothalamic AMP-activated protein kinase mediates counter-regulatory responses to hypoglycaemia in rats. Diabetologia. 2005;48(10):2170-8.

144. Martínez de Morentin Pablo B, González-García I, Martins L, Lage R, Fernández-Mallo D, Martínez-Sánchez N, et al. Estradiol Regulates Brown Adipose Tissue Thermogenesis via Hypothalamic AMPK. Cell Metabolism. 2014;20(1):41-53.

145. Beiroa D, Imbernon M, Gallego R, Senra A, Herranz D, Villarroya F, et al. GLP-1 Agonism Stimulates Brown Adipose Tissue Thermogenesis and Browning Through Hypothalamic AMPK. Diabetes. 2014;63(10):3346-58.

146. Valentine RJ, Coughlan KA, Ruderman NB, Saha AK. Insulin inhibits AMPK activity and phosphorylates AMPK Ser^{485}/^{491} through Akt in hepatocytes, myotubes and incubated rat skeletal muscle. Arch Biochem Biophys. 2014;562:62-9.

147. Namkoong C, Kim MS, Jang PG, Han SM, Park HS, Koh EH, et al. Enhanced Hypothalamic AMP-Activated Protein Kinase Activity Contributes to Hyperphagia in Diabetic Rats. Diabetes. 2005;54(1):63-8.

148. Seoane-Collazo P, Roa J, Rial-Pensado E, Liñares-Pose L, Beiroa D, Ruíz-Pino F, et al. SF1-Specific AMPKα1 Deletion Protects Against Diet-Induced Obesity. Diabetes. 2018;67(11):2213-26.

149. Yang L, Qi Y, Yang Y. Astrocytes Control Food Intake by Inhibiting AGRP Neuron Activity via Adenosine A1 Receptors. Cell Reports. 2015;11(5):798-807.

150. Yang Y. Astrocytes: targets in obesity. Oncotarget. 2015;6(15):12835-6.

151. James CR, Cotlier E. Fate of insulin in the retina: an autoradiographic study. Br J Ophthalmol. 1983;67(2):80-8.

152. Takahashi H, Takata F, Matsumoto J, Machida T, Yamauchi A, Dohgu S, et al. Brain pericyte-derived soluble factors enhance insulin sensitivity in GT1-7 hypothalamic neurons. Biochem Biophys Res Commun. 2015;457(4):532-7.

153. Fiory F, Perruolo G, Cimmino I, Cabaro S, Pignalosa FC, Miele C, et al. The Relevance of Insulin Action in the Dopaminergic System. Frontiers in Neuroscience. 2019;13(868).

154. Yolanda D-C, Manuel G-L, Laura T, Juan F, Hugo O, Lucas CG-M, et al. Stressing diabetes? The hidden links between insulinotropic peptides and the HPA axis. Journal of Endocrinology. 2016;230(2):R77-R94.

155. Volkow ND, Wang G-J, Baler RD. Reward, dopamine and the control of food intake: implications for obesity. Trends in Cognitive Sciences. 2011;15(1):37-46.

156. Fordahl SC, Jones SR. High-Fat-Diet-Induced Deficits in Dopamine Terminal Function Are Reversed by Restoring Insulin Signaling. ACS Chem Neurosci. 2017;8(2):290-9.

157. Barry RL, Byun NE, Williams JM, Siuta MA, Tantawy MN, Speed NK, et al. Brief exposure to obesogenic diet disrupts brain dopamine networks. PLoS One. 2018;13(4):e0191299.

158. Cone JJ, Chartoff EH, Potter DN, Ebner SR, Roitman MF. Prolonged High Fat Diet Reduces Dopamine Reuptake without Altering DAT Gene Expression. PLoS One. 2013;8(3):e58251.

159. Oginsky MF, Ferrario CR. Eating "junk food" has opposite effects on intrinsic excitability of nucleus accumbens core neurons in obesity-susceptible versus -resistant rats. J Neurophysiol. 2019;122(3):1264-73.

160. Mebel DM, Wong JCY, Dong YJ, Borgland SL. Insulin in the ventral tegmental area reduces hedonic feeding and suppresses dopamine concentration via increased reuptake. Eur J Neurosci. 2012;36(3):2336-46.

161. Liu S, Labouèbe G, Karunakaran S, Clee SM, Borgland SL. Effect of insulin on excitatory synaptic transmission onto dopamine neurons of the ventral tegmental area in a mouse model of hyperinsulinemia. Nutr Diabetes. 2013;3(12):e97-e.

162. Stouffer MA, Woods CA, Patel JC, Lee CR, Witkovsky P, Bao L, et al. Insulin enhances striatal dopamine release by activating cholinergic interneurons and thereby signals reward. Nature Communications. 2015;6(1):8543.

163. Könner AC, Hess S, Tovar S, Mesaros A, Sánchez-Lasheras C, Evers N, et al. Role for Insulin Signaling in Catecholaminergic Neurons in Control of Energy Homeostasis. Cell Metabolism. 2011;13(6):720-8.

164. Rangel A. Regulation of dietary choice by the decision-making circuitry. Nat Neurosci. 2013;16(12):1717-24.

165. Guthoff M, Stingl KT, Tschritter O, Rogic M, Heni M, Stingl K, et al. The Insulin-Mediated Modulation of Visually Evoked Magnetic Fields Is Reduced in Obese Subjects. PLoS One. 2011;6(5):e19482.

166. Guthoff M, Grichisch Y, Canova C, Tschritter O, Veit R, Hallschmid M, et al. Insulin Modulates Food-Related Activity in the Central Nervous System. The Journal of Clinical Endocrinology & Metabolism. 2010;95(2):748-55.

167. Wallner-Liebmann S, Koschutnig K, Reishofer G, Sorantin E, Blaschitz B, Kruschitz R, et al. Insulin and Hippocampus Activation in Response to Images of High-Calorie Food in Normal Weight and Obese Adolescents. Obesity. 2010;18(8):1552-7.

168. Smith SM, Vale WW. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. Dialogues Clin Neurosci. 2006;8(4):383-95.

169. Stone AA, Brownell KD. The stress-eating paradox: Multiple daily measurements in adult males and females. Psychology & Health. 1994;9(6):425-36.

170. Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, et al. Chronic stress and obesity: a new view of "comfort food". Proc Natl Acad Sci U S A. 2003;100(20):11696-701.

171. Chan O, Inouye K, Riddell MC, Vranic M, Matthews SG. Diabetes and the hypothalamo-pituitary-adrenal (HPA) axis. Minerva Endocrinol. 2003;28(2):87-102.

172. Miell JP, Engloro P, Blum WF. Dexamethasone Induces an Acute and Sustained Rise in Circulating Leptin Levels in Normal Human Subjects. Horm Metab Res. 1996;28(12):704-7.

173. Tomlinson JJ, Boudreau Al, Wu D, Atlas E, Haché RJG. Modulation of Early Human Preadipocyte Differentiation by Glucocorticoids. Endocrinology. 2006;147(11):5284-93.

174. Xu C, He J, Jiang H, Zu L, Zhai W, Pu S, et al. Direct effect of glucocorticoids on lipolysis in adipocytes. Mol Endocrinol. 2009;23(8):1161-70.

175. Sakoda H, Ogihara T, Anai M, Funaki M, Inukai K, Katagiri H, et al. Dexamethasone-induced insulin resistance in 3T3-L1 adipocytes is due to inhibition of glucose transport rather than insulin signal transduction. Diabetes. 2000;49(10):1700-8.

176. Heinrichs SC, Menzaghi F, Pich EM, Hauger RL, Koob GF. Corticotropin-releasing factor in the paraventricular nucleus modulates feeding induced by neuropeptide Y. Brain Research. 1993;611(1):18-24.

177. Cavagnini F, Croci M, Putignano P, Petroni ML, Invitti C. Glucocorticoids and neuroendocrine function. International Journal of Obesity. 2000;24(2):S77-S9.

178. Wilding JP, Gilbey SG, Lambert PD, Ghatei MA, Bloom SR. Increases in neuropeptide Y content and gene expression in the hypothalamus of rats treated with dexamethasone are prevented by insulin. Neuroendocrinology. 1993;57(4):581-7.

179. Ip CK, Zhang L, Farzi A, Qi Y, Clarke I, Reed F, et al. Amygdala NPY Circuits Promote the Development of Accelerated Obesity under Chronic Stress Conditions. Cell Metabolism. 2019;30(1):111-28.e6.

180. Chong AC, Vogt MC, Hill AS, Brüning JC, Zeltser LM. Central insulin signaling modulates hypothalamus-pituitary-adrenal axis responsiveness. Mol Metab. 2015;4(2):83-92.

181. Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurink A, et al. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology. 1992;130(6):3608-16.

182. Loh K, Zhang L, Brandon A, Wang Q, Begg D, Qi Y, et al. Insulin controls food intake and energy balance via NPY neurons. Mol Metab. 2017;6(6):574-84.

183. Heni M, Wagner R, Kullmann S, Veit R, Mat Husin H, Linder K, et al. Central insulin administration improves whole-body insulin sensitivity via hypothalamus and parasympathetic outputs in men. Diabetes. 2014;63(12):4083-8.

184. Woods SC, Seeley RJ, Porte D, Jr., Schwartz MW. Signals that regulate food intake and energy homeostasis. Science. 1998;280(5368):1378-83.

185. Air EL, Strowski MZ, Benoit SC, Conarello SL, Salituro GM, Guan XM, et al. Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. Nat Med. 2002;8(2):179-83.

186. McGowan MK, Andrews KM, Grossman SP. Chronic intrahypothalamic infusions of insulin or insulin antibodies alter body weight and food intake in the rat. Physiol Behav. 1992;51(4):753-66.

187. Bruning JC, Gautam D, Burks DJ, Gillette J, Schubert M, Orban PC, et al. Role of brain insulin receptor in control of body weight and reproduction. Science. 2000;289(5487):2122-5.

188. Obici S, Zhang BB, Karkanias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. Nat Med. 2002;8(12):1376-82.

189. Sipols AJ, Baskin DG, Schwartz MW. Effect of Intracerebroventricular Insulin Infusion on Diabetic Hyperphagia and Hypothalamic Neuropeptide Gene Expression. Diabetes. 1995;44(2):147-51.

190. Davidson AJ, Stokkan KA, Yamazaki S, Menaker M. Food-anticipatory activity and liver per1-luc activity in diabetic transgenic rats. Physiol Behav. 2002;76(1):21-6.

191. Steculorum SM, Ruud J, Karakasilioti I, Backes H, Engström Ruud L, Timper K, et al. AgRP Neurons Control Systemic Insulin Sensitivity via Myostatin Expression in Brown Adipose Tissue. Cell. 2016;165(1):125-38.

192. Garwood CJ, Ratcliffe LE, Morgan SV, Simpson JE, Owens H, Vazquez-Villaseñor I, et al. Insulin and IGF1 signalling pathways in human astrocytes in vitro and in vivo; characterisation, subcellular localisation and modulation of the receptors. Molecular Brain. 2015;8(1):51.

193. Chen N, Sugihara H, Kim J, Fu Z, Barak B, Sur M, et al. Direct modulation of GFAP-expressing glia in the arcuate nucleus bi-directionally regulates feeding. eLife. 2016;5:e18716.

194. Chen Y, Lin Y-C, Zimmerman CA, Essner RA, Knight ZA. Hunger neurons drive feeding through a sustained, positive reinforcement signal. eLife. 2016;5:e18640.

195. Chen Y, Lin Y-C, Kuo T-W, Knight ZA. Sensory detection of food rapidly modulates arcuate feeding circuits. Cell. 2015;160(5):829-41.

196. Betley JN, Xu S, Cao ZFH, Gong R, Magnus CJ, Yu Y, et al. Neurons for hunger and thirst transmit a negative-valence teaching signal. Nature. 2015;521(7551):180-5.

197. Critchley HD, Rolls ET. Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. J Neurophysiol. 1996;75(4):1673-86.

198. Edwin Thanarajah S, Hoffstall V, Rigoux L, Hanssen R, Brüning JC, Tittgemeyer M. The role of insulin sensitivity and intranasally applied insulin on olfactory perception. Scientific Reports. 2019;9(1):7222.

199. Schopf V, Kollndorfer K, Pollak M, Mueller C, Freiherr J. Intranasal insulin influences the olfactory performance of patients with smell loss, dependent on the body mass index: A pilot study. Rhinology. 2015;53(4):371-8.

200. Van Regemorter V, Hummel T, Rosenzweig F, Mouraux A, Rombaux P, Huart C. Mechanisms Linking Olfactory Impairment and Risk of Mortality. Frontiers in Neuroscience. 2020;14(140).

201. Ketterer C, Heni M, Thamer C, Herzberg-Schäfer SA, Häring HU, Fritsche A. Acute, short-term hyperinsulinemia increases olfactory threshold in healthy subjects. Int J Obes (Lond). 2011;35(8):1135-8.

202. Serrenho D, Santos SD, Carvalho AL. The Role of Ghrelin in Regulating Synaptic Function and Plasticity of Feeding-Associated Circuits. Frontiers in cellular neuroscience. 2019;13:205-.

203. Figlewicz DP, Evans SB, Murphy J, Hoen M, Baskin DG. Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. Brain Res. 2003;964(1):107-15.

204. Liu S, Globa AK, Mills F, Naef L, Qiao M, Bamji SX, et al. Consumption of palatable food primes food approach behavior by rapidly increasing synaptic density in the VTA. Proceedings of the National Academy of Sciences. 2016;113(9):2520-5.

205. Kleinridders A, Pothos EN. Impact of Brain Insulin Signaling on Dopamine Function, Food Intake, Reward, and Emotional Behavior. Curr Nutr Rep. 2019;8(2):83-91.

206. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. Physiol Rev. 2006;86(3):1009-31.

207. Stoeckel LE, Weller RE, Cook EW, 3rd, Twieg DB, Knowlton RC, Cox JE. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. Neuroimage. 2008;41(2):636-47.

208. Heni M, Wagner R, Kullmann S, Gancheva S, Roden M, Peter A, et al. Hypothalamic and Striatal Insulin Action Suppresses Endogenous Glucose Production and May Stimulate Glucose Uptake During Hyperinsulinemia in Lean but Not in Overweight Men. Diabetes. 2017;66(7):1797-806.

209. Westerterp KR. Control of energy expenditure in humans. Eur J Clin Nutr. 2017;71(3):340-4.

210. Wu J, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: is beige the new brown? Genes Dev. 2013;27(3):234-50.

211. Betz MJ, Enerbäck S. Human Brown Adipose Tissue: What We Have Learned So Far. Diabetes. 2015;64(7):2352-60.

212. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and importance of brown adipose tissue in adult humans. The New England journal of medicine. 2009;360(15):1509-17.

213. Kajimura S, Spiegelman BM, Seale P. Brown and Beige Fat: Physiological Roles beyond Heat Generation. Cell Metab. 2015;22(4):546-59.

214. Rosen Evan D, Spiegelman Bruce M. What We Talk About When We Talk About Fat. Cell. 2014;156(1):20-44.

215. Ikeda K, Maretich P, Kajimura S. The Common and Distinct Features of Brown and Beige Adipocytes. Trends in Endocrinology & Metabolism. 2018;29(3):191-200.

216. Saito M, Matsushita M, Yoneshiro T, Okamatsu-Ogura Y. Brown Adipose Tissue, Diet-Induced Thermogenesis, and Thermogenic Food Ingredients: From Mice to Men. Front Endocrinol (Lausanne). 2020;11:222-.

217. Tews D, Pula T, Funcke JB, Jastroch M, Keuper M, Debatin KM, et al. Elevated UCP1 levels are sufficient to improve glucose uptake in human white adipocytes. Redox Biol. 2019;26:101286-.

218. Ikeda K, Yamada T. UCP1 Dependent and Independent Thermogenesis in Brown and Beige Adipocytes. Front Endocrinol (Lausanne). 2020;11(498).

219. Ouellet V, Labbé SM, Blondin DP, Phoenix S, Guérin B, Haman F, et al. Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. J Clin Invest. 2012;122(2):545-52.

220. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-activated brown adipose tissue in healthy men. N Engl J Med. 2009;360(15):1500-8.

221. Orava J, Nuutila P, Lidell ME, Oikonen V, Noponen T, Viljanen T, et al. Different metabolic responses of human brown adipose tissue to activation by cold and insulin. Cell Metab. 2011;14(2):272-9.

222. Rothwell NJ, Saville ME, Stock MJ. Role of insulin in thermogenic responses to refeeding in 3-day-fasted rats. Am J Physiol. 1983;245(2):E160-5.

223. M UD, Saari T, Raiko J, Kudomi N, Maurer SF, Lahesmaa M, et al. Postprandial Oxidative Metabolism of Human Brown Fat Indicates Thermogenesis. Cell Metab. 2018;28(2):207-16.e3.

224. Saari TJ, Raiko J, U-Din M, Niemi T, Taittonen M, Laine J, et al. Basal and cold-induced fatty acid uptake of human brown adipose tissue is impaired in obesity. Scientific Reports. 2020;10(1):14373.

225. Morgan DA, Rahmouni K. Differential effects of insulin on sympathetic nerve activity in agouti obese mice. J Hypertens. 2010;28(9):1913-9.

226. Clegg DJ, Gotoh K, Kemp C, Wortman MD, Benoit SC, Brown LM, et al. Consumption of a high-fat diet induces central insulin resistance independent of adiposity. Physiol Behav. 2011;103(1):10-6.

227. Benedict C, Brede S, Schiöth HB, Lehnert H, Schultes B, Born J, et al. Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes. 2011;60(1):114-8.

228. Lin HV, Plum L, Ono H, Gutiérrez-Juárez R, Shanabrough M, Borok E, et al. Divergent Regulation of Energy Expenditure and Hepatic Glucose Production by Insulin Receptor in Agouti-Related Protein and POMC Neurons. Diabetes. 2010;59(2):337-46.

229. Iyad HM, Emily M, Marziyeh J, Lakshmikanth C, Joshua P, Jennifer H. Insulin sensing by astrocytes is critical for normal thermogenesis and body temperature regulation. Journal of Endocrinology. 2020;247(1):39-52.

230. Sophie EL, Jeremy MT. The molecular basis of insulin-stimulated glucose uptake: signalling, trafficking and potential drug targets. Journal of Endocrinology. 2009;203(1):1-18.

231. Ren H, Yan S, Zhang B, Lu TY, Arancio O, Accili D. Glut4 expression defines an insulin-sensitive hypothalamic neuronal population. Molecular metabolism. 2014;3(4):452-9.

232. Sharabi K, Tavares CD, Rines AK, Puigserver P. Molecular pathophysiology of hepatic glucose production. Mol Aspects Med. 2015;46:21-33.

233. Inoue H, Ogawa W, Asakawa A, Okamoto Y, Nishizawa A, Matsumoto M, et al. Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. Cell Metab. 2006;3(4):267-75.

234. Gelling RW, Morton GJ, Morrison CD, Niswender KD, Myers MG, Jr., Rhodes CJ, et al. Insulin action in the brain contributes to glucose lowering during insulin treatment of diabetes. Cell Metab. 2006;3(1):67-73.

235. Yi CX, la Fleur SE, Fliers E, Kalsbeek A. The role of the autonomic nervous liver innervation in the control of energy metabolism. Biochim Biophys Acta. 2010;1802(4):416-31.

236. Kimura K, Tanida M, Nagata N, Inaba Y, Watanabe H, Nagashimada M, et al. Central Insulin Action Activates Kupffer Cells by Suppressing Hepatic Vagal Activation via the Nicotinic Alpha 7 Acetylcholine Receptor. Cell Rep. 2016;14(10):2362-74.

237. Pocai A, Lam TK, Gutierrez-Juarez R, Obici S, Schwartz GJ, Bryan J, et al. Hypothalamic K(ATP) channels control hepatic glucose production. Nature. 2005;434(7036):1026-31.

238. van den Hoek AM, van Heijningen C, Schröder-van der Elst JP, Ouwens DM, Havekes LM, Romijn JA, et al. Intracerebroventricular administration of neuropeptide Y induces hepatic insulin resistance via sympathetic innervation. Diabetes. 2008;57(9):2304-10.

239. Hill JW, Elias CF, Fukuda M, Williams KW, Berglund ED, Holland WL, et al. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. Cell metabolism. 2010;11(4):286-97.

240. Perseghin G, Regalia E, Battezzati A, Vergani S, Pulvirenti A, Terruzzi I, et al. Regulation of glucose homeostasis in humans with denervated livers. J Clin Invest. 1997;100(4):931-41.

241. Wada M, Connolly CC, Tarumi C, Neal DW, Cherrington AD. Hepatic denervation does not significantly change the response of the liver to glucagon in conscious dogs. American Journal of Physiology-Endocrinology and Metabolism. 1995;268(2):E194-E203.

242. Scherer PE. The many secret lives of adipocytes: implications for diabetes. Diabetologia. 2019;62(2):223-32.

243. Scherer T, O'Hare J, Diggs-Andrews K, Schweiger M, Cheng B, Lindtner C, et al. Brain insulin controls adipose tissue lipolysis and lipogenesis. Cell metabolism. 2011;13(2):183-94.

244. Scherer T, Lindtner C, Zielinski E, O'Hare J, Filatova N, Buettner C. Short term voluntary overfeeding disrupts brain insulin control of adipose tissue lipolysis. The Journal of biological chemistry. 2012;287(39):33061-9.

245. Bamshad M, Aoki VT, Adkison MG, Warren WS, Bartness TJ. Central nervous system origins of the sympathetic nervous system outflow to white adipose tissue. Am J Physiol. 1998;275(1):R291-9.

246. Wiedmer P, Chaudhary N, Rath M, Yi CX, Ananthakrishnan G, Nogueiras R, et al. The HPA axis modulates the CNS melanocortin control of liver triacylglyceride metabolism. Physiol Behav. 2012;105(3):791-9.

247. Shin AC, Filatova N, Lindtner C, Chi T, Degann S, Oberlin D, et al. Insulin Receptor Signaling in POMC, but Not AgRP, Neurons Controls Adipose Tissue Insulin Action. Diabetes. 2017;66(6):1560-71.

248. Leon-Cabrera S, Solís-Lozano L, Suárez-Álvarez K, González-Chávez A, Béjar YL, Robles-Díaz G, et al. Hyperleptinemia is associated with parameters of low-grade systemic inflammation and metabolic dysfunction in obese human beings. Front Integr Neurosci. 2013;7:62-.

249. Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. Int J Obes Relat Metab Disord. 2000;24(5):639-46.

250. Ono H, Pocai A, Wang Y, Sakoda H, Asano T, Backer JM, et al. Activation of hypothalamic S6 kinase mediates diet-induced hepatic insulin resistance in rats. J Clin Invest. 2008;118(8):2959-68.

251. de Aquino CC, Leitão RA, Oliveira Alves LA, Coelho-Santos V, Guerrant RL, Ribeiro CF, et al. Effect of Hypoproteic and High-Fat Diets on Hippocampal Blood-Brain Barrier Permeability and Oxidative Stress. Front Nutr. 2019;5:131-.

252. Banno R, Zimmer D, De Jonghe BC, Atienza M, Rak K, Yang W, et al. PTP1B and SHP2 in POMC neurons reciprocally regulate energy balance in mice. J Clin Invest. 2010;120(3):720-34.

253. Kievit P, Howard JK, Badman MK, Balthasar N, Coppari R, Mori H, et al. Enhanced leptin sensitivity and improved glucose homeostasis in mice lacking suppressor of cytokine signaling-3 in POMC-expressing cells. Cell Metab. 2006;4(2):123-32.

254. Qin Z, Pandey NR, Zhou X, Stewart CA, Hari A, Huang H, et al. Functional properties of Claramine: a novel PTP1B inhibitor and insulin-mimetic compound. Biochem Biophys Res Commun. 2015;458(1):21-7.

255. Lantz KA, Hart SGE, Planey SL, Roitman MF, Ruiz-White IA, Wolfe HR, et al. Inhibition of PTP1B by Trodusquemine (MSI-1436) Causes Fat-specific Weight Loss in Diet-induced Obese Mice. Obesity. 2010;18(8):1516-23.

256. Zhang S, Chen L, Luo Y, Gunawan A, Lawrence DS, Zhang ZY. Acquisition of a potent and selective TC-PTP inhibitor via a stepwise fluorophore-tagged combinatorial synthesis and screening strategy. Journal of the American Chemical Society. 2009;131(36):13072-9.

257. Kyriakou E, Schmidt S, Dodd GT, Pfuhlmann K, Simonds SE, Lenhart D, et al. Celastrol Promotes Weight Loss in Diet-Induced Obesity by Inhibiting the Protein Tyrosine Phosphatases PTP1B and TCPTP in the Hypothalamus. Journal of Medicinal Chemistry. 2018;61(24):11144-57.

258. Le Sommer S, Morrice N, Pesaresi M, Thompson D, Vickers MA, Murray GI, et al. Deficiency in Protein Tyrosine Phosphatase PTP1B Shortens Lifespan and Leads to Development of Acute Leukemia. Cancer Research. 2018;78(1):75-87.

259. Wiede F, Chew SH, van Vliet C, Poulton IJ, Kyparissoudis K, Sasmono T, et al. Strain-Dependent Differences in Bone Development, Myeloid Hyperplasia, Morbidity and Mortality in Ptpn2-Deficient Mice. PLoS One. 2012;7(5):e36703.

260. You-Ten KE, Muise ES, Itié A, Michaliszyn E, Wagner J, Jothy S, et al. Impaired bone marrow microenvironment and immune function in T cell protein tyrosine phosphatase-deficient mice. J Exp Med. 1997;186(5):683-93.

261. Liu Q, Zhang Q. 10 - Nanoparticle systems for nose-to-brain delivery. In: Gao H, Gao X, editors. Brain Targeted Drug Delivery System: Academic Press; 2019. p. 219-39.

262. Opstal AMv, Akintola AA, Elst Mvd, Westendorp RG, Pijl H, Heemst Dv, et al. Effects of intranasal insulin application on the hypothalamic BOLD response to glucose ingestion. Scientific reports. 2017;7(1):13327-.

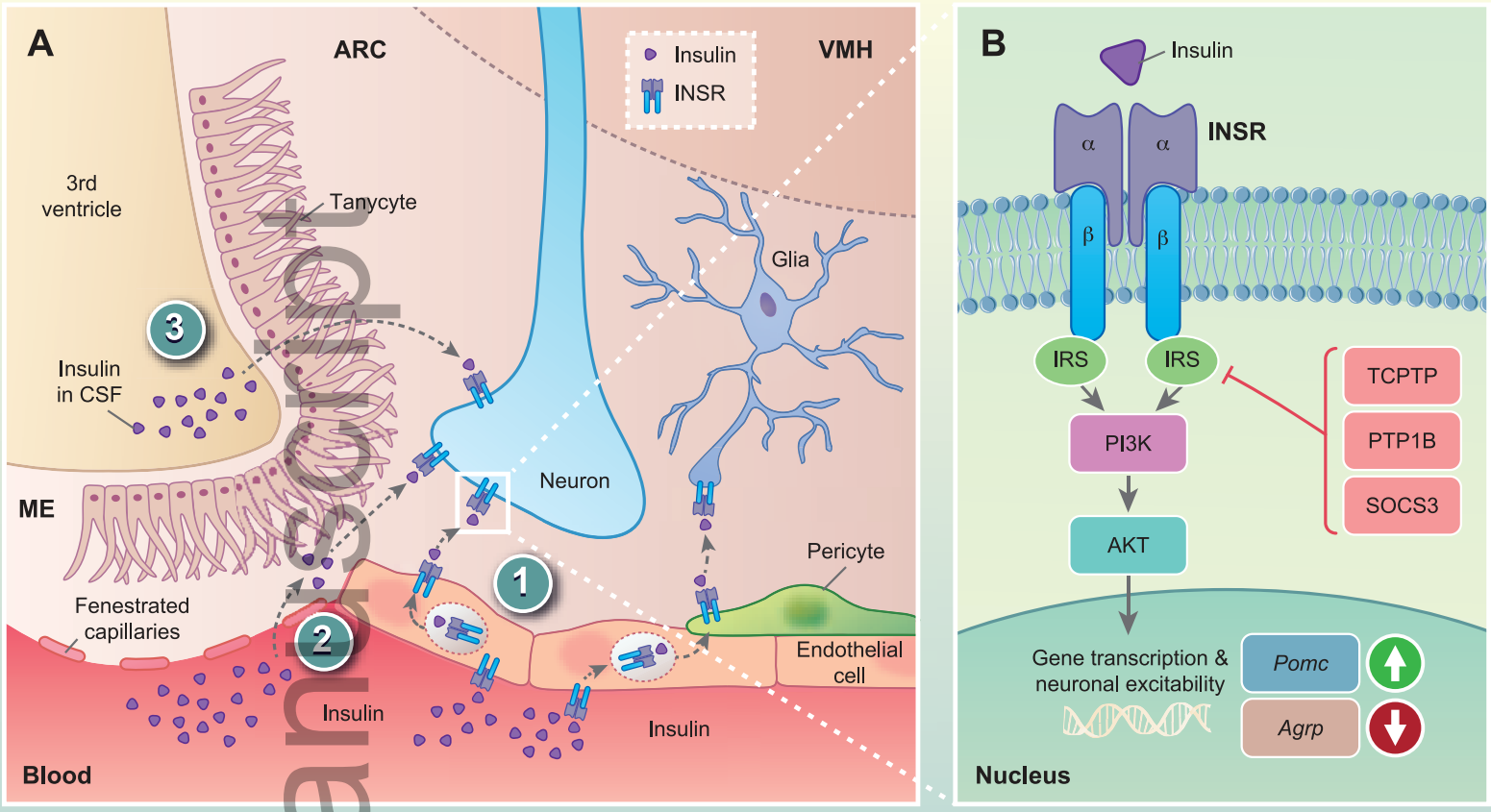
263. Jauch-Chara K, Friedrich A, Rezmer M, Melchert UH, H GS-E, Hallschmid M, et al. Intranasal insulin suppresses food intake via enhancement of brain energy levels in humans. Diabetes. 2012;61(9):2261-8.

264. Poher A-L, Altirriba J, Veyrat-Durebex C, Rohner-Jeanrenaud F. Brown adipose tissue activity as a target for the treatment of obesity/insulin resistance. Front Physiol. 2015;6:4-.

265. Claxton A, Baker LD, Hanson A, Trittschuh EH, Cholerton B, Morgan A, et al. Long-acting intranasal insulin detemir improves cognition for adults with mild cognitive impairment or early-stage Alzheimer's disease dementia. J Alzheimers Dis. 2015;44(3):897-906.

266. Ruan H-B, Dietrich MO, Liu Z-W, Zimmer MR, Li M-D, Singh JP, et al. O-GlcNAc transferase enables AgRP neurons to suppress browning of white fat. Cell. 2014;159(2):306-17.

267. Evans BA, Merlin J, Bengtsson T, Hutchinson DS. Adrenoceptors in white, brown, and brite adipocytes. British Journal of Pharmacology. 2019;176(14):2416-32.



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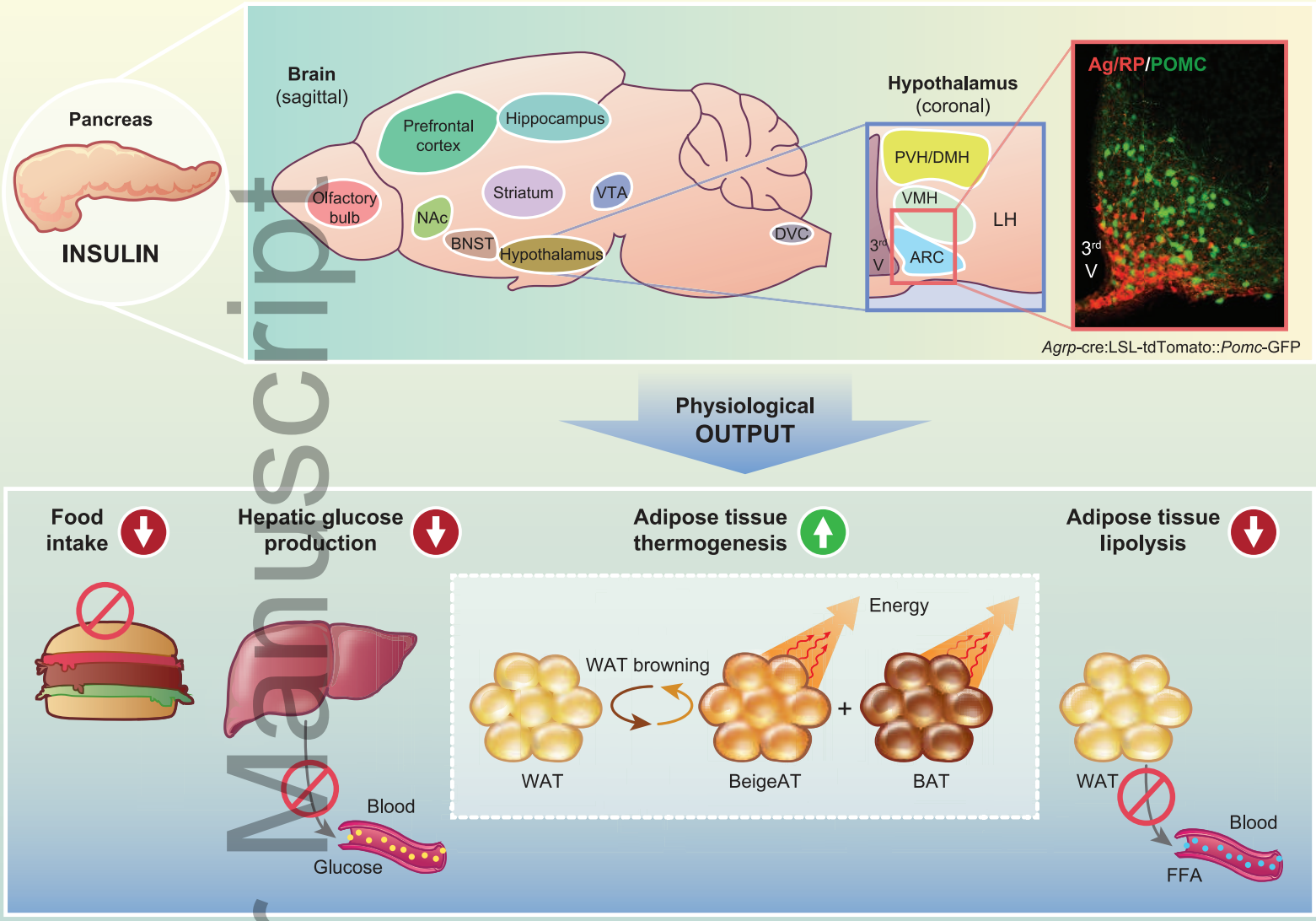

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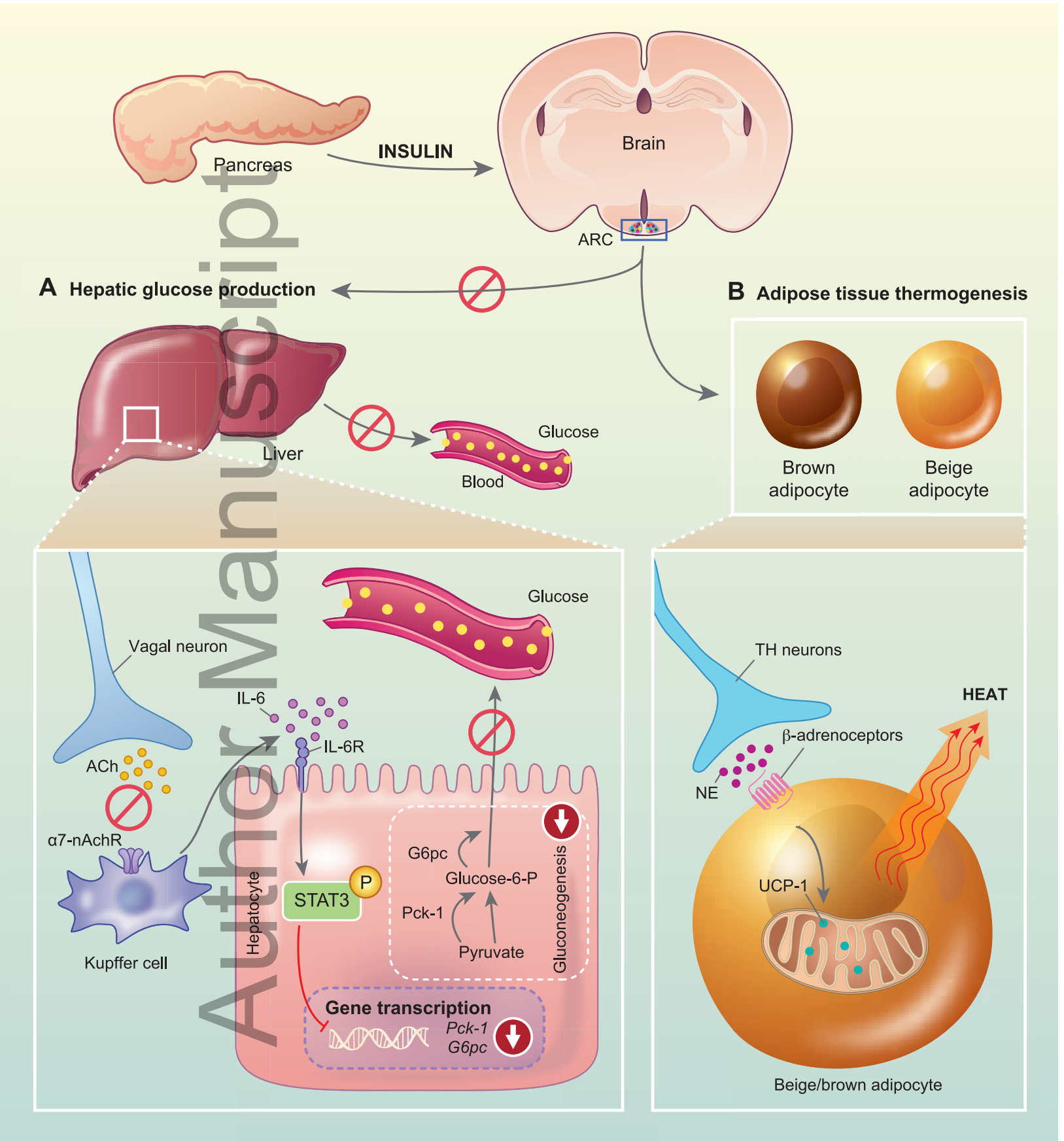

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