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# Insulin Action in the Brain: Cell Types, Circuits, and Diseases

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## Abstract

Since its discovery over 100 years ago, insulin has been recognized as a key hormone in control of glucose homeostasis. Deficiencies of insulin signaling are central to diabetes and many other disorders. The brain is among the targets of insulin action, and insulin resistance is a major contributor to many diseases including brain disorders. Here, we summarize key roles of insulin action in the brain and how this involves different brain cell types. Disordered brain insulin signaling can also contribute to neuropsychiatric diseases, affecting brain circuits involved in mood and cognition. Understanding of insulin signaling in different brain cell types/circuits and how these are altered in disease may lead to the development of new therapeutic approaches to these challenging disorders.

### Keywords

Diabetes; Insulin resistance; Neurons; Astrocytes; Depression; Alzheimer’s Diseases

## The Brain is Insulin-Sensitive

**Type 2 Diabetes** (T2D, see Glossary), obesity and metabolic syndrome are increasing worldwide at epidemic rates. T2D affects over 450 million people worldwide, and over 2 billion people are overweight or obese [1]. In these disorders, impaired insulin signaling, i.e., **insulin resistance**, is a defining feature [2, 3]. While metabolic changes associated with insulin resistance have been largely related to alterations in so-called “classical” insulin-sensitive tissues: liver, skeletal muscle, and fat [2, 4], it is now known that most,

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if not all, tissues of the body, including the brain, express **insulin receptors** (InsR) and are insulin sensitive (Figure 1) [4]. Accumulating evidence suggests that brain insulin signaling not only plays a key role in regulation of metabolism [5], but also in regulation of mood, behavior, and cognition. Dysregulated insulin signaling has also been implicated in a range of brain disorders (Figure 1). Over the past few decades, epidemiological studies have demonstrated a high rate of **comorbidity** between T2D and brain disorders such as depression and Alzheimer's Disease (AD) [6, 7], leading to recommendations that the evaluation of neuropsychiatric comorbidities should be part of the initial assessment of T2D patients [8]. Understanding the role of brain insulin signaling in these disorders may also provide new therapeutics targeting the comorbidity between T2D and brain disorders.

In this review, we summarize the clinical evidence connecting insulin resistance/T2D and brain dysfunction, and explore this link at a mechanistic level with a particular focus on cell type-specific regulation in the brain mediated by insulin signaling. We also connect these cellular insights to brain-wide changes in circuits and to the pathogenesis of brain disorders.

## Comorbidity of T2D and Brain Disorders

Growing evidence has shown that T2D is closed associated with mood disorders and neurodegenerative diseases independent of dysglycemia. Thus, patients with **type 1 diabetes** have similar rates of depression as the general population [9], while the prevalence of depression in T2D is about twice the population average [10] and increases with insulin resistance [11]. Prospective studies have pointed to a bidirectional relationship: the incidence of depression is increased [12], and depressive symptoms are more persistent and remission less stable in patients with T2D [13]. Likewise, a previous diagnosis of depression is associated with increased incidence of T2D by 60% [14] and with more severe long-term diabetic complications [15]. The effect of depression on T2D is partially, but not completely, explained by the increased risk of T2D with antidepressant use, especially noradrenergic and tricyclic antidepressants [16, 17]. Conversely, diabetic patients treated with selective serotonin reuptake inhibitors experience improvements in glycemic control [18] and increased insulin sensitivity [19], although this has not been observed in all studies [20]. Understanding the molecular mechanisms underlying these interactions between diabetes and psychiatric medications should provide new insights into the pathogenesis of depression as well as diabetes, and how to improve treatment of both (see Outstanding questions).

Investigations of the relationship between T2D and anxiety have uncovered similar connections. The prevalence of clinically significant anxiety in patients with T2D is ~20% higher than the population average [21]. People with T2D also have increased prevalence of generalized anxiety disorder (GAD), panic disorder, post-traumatic stress disorder, and agoraphobia [22]. Attempts to establish causality, however, yielded mixed results. A meta-analysis demonstrated that patients with higher baseline levels of anxiety have an increased incidence of T2D [23]. In patients with GAD and T2D, treatment with alprazolam has been noted to improve glycemic control in a manner unrelated to the level of improvement in anxiety, suggesting these are occurring through independent mechanisms [24].

It has long been known that schizophrenia is associated with increased levels of hyperglycemia [25], and the prevalence of T2D in patients with schizophrenia is 2–3 times the population norm [26]. Part of this is attributable to medication side effects. All atypical antipsychotics, the first-line treatment for schizophrenia since the 1990s, increase the incidence of diabetes [27]. The co-existence of diabetes, however, is not fully explained by medications. Indeed, prior to the advent of antipsychotics, hospitalized patients with psychosis exhibited abnormal glucose tolerance [28] and hepatic insulin resistance [29]. Intriguingly, the efficacy of current antipsychotics tends to positively correlate with the severity of their metabolic side effects, suggesting that disruptions in insulin signaling and/or metabolism is linked to the therapeutic mechanism [30].

A growing body of evidence indicates that neurodegenerative diseases, such as AD, also have strong epidemiological connections with T2D and that T2D is a major risk factor for AD and other dementias [31]. Patients with T2D have a ~50% increased risk of developing mild cognitive impairment [32, 33], and a meta-analysis of prospective studies including more than 1.7 million patients has demonstrated a similar increase in AD risk for patients with T2D [34]. This robust epidemiological connection is supported by analysis of postmortem brain tissue of AD patients. Defects in insulin and IGF-1 signaling or associated signaling proteins have been shown to co-occur with Aβ plaques in the temporal lobe, hippocampus, and cerebellum [35, 36]. A decrease in the primary substrate of these receptors, IRS-1, appears to be a more consistent marker of insulin resistance in the brain [36, 37]. Interestingly, hyperactivation of the downstream targets of IRS-1, including phosphoinositide 3-kinase (PI3K), Akt, and glycogen synthase kinase 3β (GSK-3β), have also been observed in both early- and late-stage AD [38]. Furthermore, patients with AD have increased plasma insulin levels and decreased cerebrospinal fluid insulin levels [39, 40].

## Cell Type-Specific Regulation of Insulin Receptor Signaling in the Brain

To decipher the comorbidity of T2D and brain disorders, it is key to understand insulin signaling in the brain. Both the InsR and the closely-related **insulin-like growth factor 1 receptor** (IGF-1) receptor are highly expressed in the brain [41]. The first direct evidence for a role of the InsR in brain came with the generation of a brain InsR knockout (NIRKO) mouse, produced using the **Cre/LoxP system** with Cre driven by the Nestin promoter [42]. Nestin targets both neuronal and glial cell precursors in the brain and also to some extent the peripheral nervous system (Table 1 summarizes the use of the Cre/loxP system for probing InsR signaling in different cell types). NIRKO mice exhibited a complete loss of insulin-stimulated signaling in the brain at a molecular level [42]. This was accompanied by slightly increased food intake, mild obesity and systemic insulin resistance [5]. When NIRKO mice were challenged with hypoglycemia, the expected sympathoadrenal response with elevation in epinephrine and norepinephrine levels was significantly blunted, indicating that insulin signaling in the brain is required for a normal counterregulatory response to hypoglycemia [43, 44] (for more information on brain insulin action in regulation of peripheral metabolism, see reference [45]). In addition, NIRKO mice also exhibited increased anxiety- and depression-like behavioral changes [6] and demonstrated some biochemical hallmarks of AD, such as increased Tau phosphorylation [42]. These data

indicate the interplay between insulin signaling in brain function and its control of peripheral metabolism, behavior and brain disorders.

Using mice which express cell type specific Cre driven by promoters for unique neuropeptides or their receptors, it has been possible to explore the role of insulin signaling within specific neuronal subtypes (Table 2). Among these, the role of insulin signaling in hypothalamic proopiomelanocortin (POMC) and agouti-related neuropeptide/neuropeptide Y (AgRP/NPY) expressing neurons in terms of food intake and energy expenditure are the best characterized (Figure 2). Although the InsR is expressed in both of these neuronal types [46], insulin signaling in AgRP/NPY neurons acts to suppress hepatic glucose production and decrease energy expenditure [47, 48], whereas in POMC neurons, insulin acts to promote hepatic glucose production and increase energy expenditure, at least in part by activating TRPC5 channels and PI3K [49–51] and regulating neuronal plasticity [47, 51, 52]. These divergent effects occur as the result of signaling within competitive circuits (Figure 2, and see also discussion below and references [47, 48]).

Insulin signaling is also critical in other neuronal cell types. While mice with whole-body knockout of melanin-concentrating hormone (MCH) exhibit hypophagia and reduced body weight [53], deletion of InsR from MCH-expressing neurons produces no metabolic phenotype in lean mice but increases locomotor activity and improves insulin sensitivity in obese mice [54]. Orexin is another peptide that plays a key role in the CNS regulation of glucose and energy homeostasis [55], and deletion of orexin-producing neurons or manipulation of orexin-dependent neuronal activity leads to hepatic insulin resistance [56] and alters feeding behavior and metabolism [57]. For additional information on insulin signaling in neuronal subpopulations, see Table 2 and other excellent reviews [58–60].

Investigations into the roles that insulin signaling plays in non-neuronal cell types in the brain are relatively recent. Astrocytes play an important role in regulating neuronal functions, such as **synaptic plasticity** and neurotransmission [61]. InsR KO in astrocytes guided by the glial fibrillary acidic protein$^{+}$ (GFAP$^{+}$) Cre has been achieved using both constitutive and inducible Cre/LoxP-mediated recombination (GIRKO/iGIRKO mice, Table 1). These studies have revealed that in astrocytes, insulin stimulates tyrosine phosphorylation of Munc18c which regulates SNARE complex formation, leading to exocytosis of ATP, which in turn modulates synaptic plasticity at dopaminergic axonal terminals by acting through P2Y receptor (Figure 3) [7]. Strikingly, loss of insulin signaling in astrocytes reduces evoked dopamine (DA) release in dorsal striatum and nucleus accumbens (NAc) by almost 50%, leading to anxiety- and depression-like behaviors in GIRKO mice [7].

Astrocytic insulin signaling also regulates hypothalamic glucose sensing. In GIRKO mice, there is a reduction of glucose-induced activation of POMC neurons and an impairment of the physiological response to altered glucose availability, indicating that astrocytic insulin signaling plays an important role in systemic **glucose homeostasis** [62]. However, GFAP$^{+}$ astrocytes represent only one subpopulation of astrocytes in brain. Thus, going forward, it will be important to utilize other astrocyte markers, such as GLAST [62] or ALDH1L1

[61, 63] or to target disease-associated astrocytic subpopulations [64] (see Outstanding questions).

Studies on the role of insulin signaling in microglia, the resident immune cells in the brain, are still at a nascent stage. In obesity and metabolic syndrome, increases in inflammatory cells are observed in many organs, including adipose tissue, liver and even brain [65, 66], therefore it seems plausible that microglial responses could be altered during states of insulin resistance. In microglial cell lines, insulin inhibits $A\beta_{42}$ clearance induced by isoproterenol (Figure 3) [67]. This seems contradictory to the finding that intranasal insulin treatment has been shown to reduce Aβ levels and microglia activation and restore impaired insulin signaling in an AD mouse model [68]. It is possible that the beneficial effects of intranasal insulin are related to an impact on inflammation gene expression in the hippocampus [69, 70], suggesting these as potential targets of insulin in the AD brain. Microglial overactivation had also been observed in rats made insulin-resistant by high fructose diet, and this effect can be reduced with the antidiabetic PPARγ-agonist pioglitazone or the GLP-1 analogue exenatide associated with enhanced cognition [71]. These studies, together with others, suggest that microglia activation and inflammation occur in the brain in both AD and states of insulin resistance, and that insulin and other antidiabetic drugs might prevent microglia activation via reduction of inflammation.

Compared to astrocytes and microglia, little is known about insulin action on oligodendrocytes, but clinical clues are pointing to their potential involvement. In patients with multiple system atrophy (MSA), a neurodegenerative disorder characterized by α-synuclein aggregation in oligodendrocytes, there is increased phosphorylation of IRS-1 at serine 312, a site associated with insulin resistance [72]. Studies also suggest that both neurons and oligodendrocytes, but not microglia and astrocytes, are insulin resistant in MSA, implicating the potential for differentially regulated insulin signaling in different brain cell types [73]. Further studies are needed to determine the role of insulin signaling in oligodendrocyte function and survival.

Brain vascular endothelial cells (BVECs) also express InsR (Figure 3). Conditional InsR KO in vascular endothelium delays the onset of insulin signaling in brain areas such as the hippocampus, certain areas of the hypothalamus, and prefrontal cortex (Figure 4) [74]. In addition, loss of endothelial InsR impairs blood-brain barrier (BBB) function and causes systemic insulin resistance and mild obesity [74]. While early studies showed that insulin enters the brain via InsR-mediated transcytosis across BVECs [75], recent studies suggest that insulin can also be transported across BVECs via InsR-independent routes [76, 77]. While these alternative routes remain to be revealed, identification of insulin transport-related protein(s) [77] may provide crucial insights into understanding how brain insulin signaling contributes to the pathogenesis of brain disorders.

Recently, it has been shown that tanycytes, a form of non-neuronal cells that share some features with astrocytes and have processes extending into the hypothalamus, may play an important role in both transcytosis of peripheral hormones to the arcuate nucleus (ARC) and in nutrient sensing and regulation of energy homeostasis (Figure 4) [78, 79]. Comparison of mice with InsR deletion in endothelial cells versus tanycytes showed that InsR in tanycytes

maybe rate limiting in regulation of insulin transport into the ARC [79]. This study also suggested that insulin action in tanycytes has an important role in gating systemic insulin sensitivity, ghrelin action and AgRP neuronal activity [79].

In addition to insulin's actions in the periphery and in the brain, it remains debated whether some cells in the brain may actually produce insulin, at least under some conditions. Insulin-like peptides are produced in *Drosophila* brain [80], and comparison of mice with conditional targeting of *Ins1* versus *Ins2* gene have suggested that *Ins2* (which is expressed in the brain during development) may have a unique role in brain [81–83]. A recent study showed that insulin can also be released from the choroid plexus (ChP), and this release is modulated by serotonin, but not glucose [84]. This has been further supported by single-cell RNA-sequencing (scRNA-seq) showing expression of insulin in the ChP [85]. Future studies should further investigate whether serotonergic antidepressants modulate insulin release from the ChP (see Outstanding questions). For further reading on insulin signaling in BVECs, we refer readers to references [86, 87].

## Insulin Action in Brain Circuits

The mesolimbic dopaminergic pathway projects from the ventral tegmental area (VTA) to the NAc and plays a key role in circuits involving (among other brain regions) the hippocampus and amygdala, and that are critical to the regulation of mood, cognition, and reward-based behaviors. Insulin can modulate mesolimbic circuitry to alter feeding and reward behaviors by acting at the NAc and VTA, key brain regions in regulation of **motivational salience** and **emotional valence**. Direct infusion of insulin into the VTA decreases food ingestion [88], whereas loss of InsR in VTA dopaminergic neurons leads to hyperphagia and obesity [89], consistent with a role for insulin in satiety. Insulin modulates the excitability of VTA dopaminergic neurons by two mechanisms. First, insulin elicits long-term depression (LTD) of the presynaptic glutamatergic inputs to VTA dopaminergic neurons [90]. Second, insulin enhances spontaneous tonic activity of dopaminergic neurons in a cell-autonomous fashion [89]. The net effect of insulin on dopaminergic neuronal activity represents an integration of the negative presynaptic and positive cell-autonomous effects (Figure 5). Complicating the picture, insulin, acting via the PI3K-Akt pathway, also promotes the surface expression of the DA transporter [91], which counterbalances the increased DA release.

Insulin modulates reward behaviors by acting on the mesolimbic dopaminergic circuitry in the NAc (Figure 5), a primary target of VTA dopaminergic neurons. Insulin indirectly enhances evoked DA release in both dorsal striatum and the NAc by increasing the excitability of the cholinergic interneurons and enhancing astrocyte-derived ATP release [7, 92]. Thus, in NAc, insulin indirectly potentiates DA release through astrocyte-neuron and interneuron-neuron communication. Functionally, the potentiation of DA release in NAc by insulin is important for reward valuation, and ablating insulin action in NAc leads to impairment of food preference and development of anhedonic behavior [7]. In line with this, insulin resistant obese animals lose insulin-enhanced DA release in NAc [92], which contributes to the anhedonic behavior partly resulting from deficient reward processing. The mechanism by which impairment of insulin action in NAc contributes to depressive

symptoms, however, has yet to be fully elucidated (see below discussion and Outstanding questions).

Insulin, in addition to leptin and ghrelin, is a primary modulator of an important hypothalamic circuit projecting from the ARC to the paraventricular nucleus (PVN) involved in regulation of food intake and satiety. As noted above, insulin binds to its receptors on both POMC and AgRP neurons in the ARC. These cells project to PVN neurons that express melanocortin 4 receptors ($MC4R^+$). POMC and AgRP are released and competitively bind to MC4R with POMC potentiating downstream firing of these neurons, while AgRP inhibits firing. This competitive action results in POMC neurons suppressing appetite while AgRP neurons promote hunger and eating behavior (Figure 2). Insulin inhibits GABA release to AgRP neurons and hence alleviates the inhibitory constraint on POMC neurons [52, 93–95], resulting in reduced food intake. Electrophysiological recording of glucose-inhibited hypothalamic neurons from brain slices of NIRKO mice show a significant reduction in membrane potential and input resistance in response to a decrease of glucose concentration [43], i.e., impaired glucose sensing. The exact links between these changes in glucose sensing and altered InsR signaling remain to be determined.

The roles of gut microbiota in modulating mood and behavior, as well as brain insulin sensitivity [66], have been receiving growing attention, and represent one of the rapidly advancing areas in the field of brain-metabolism interactions. Modification of gut microbiota, due to high-fat diet feeding, is associated with altered insulin signaling and inflammation in key brain reward processing centers such as the NAc and amygdala [66]. Concordantly, gut microbiome transfer between mice on high-fat diet and high-fat diet plus antibiotics results in changes in many basic behaviors in recipient germ-free mice [66]. Further investigations should help reveal key metabolites or other mediators involved in modulating insulin sensitivity through the gut-brain axis.

## Insulin Action on Synaptic Transmission

Beyond modulation of distinct circuits, insulin can modulate global brain processes. In mice, increases of circulating insulin levels within a physiological range under conditions when glucose levels are clamped elicits robust transcriptional regulation of genes in brain, especially in hypothalamus, where the number of transcriptional changes exceeds those in classic insulin target tissues, such as liver and muscle [96]. Many of the regulated genes are involved in neurotransmission (Figure 5). Insulin regulates synaptic plasticity by upregulating $GABA_A$ receptor subunits and SNARE proteins responsible for neurotransmitter release, while altering expression of different glutamate receptor subunits (Figure 5). In addition, in hippocampus, insulin not only enhances phosphorylation of NMDA receptor subunits and promotes NMDA receptor membrane localization [97, 98], but also stimulates GluR1-containing AMPA receptor surface expression [99]. With chronic loss of insulin/IGF-1 signaling in hippocampus, a dramatic reduction of GluR1 expression is observed [100]. Insulin also stimulates endocytosis of GluR2-containing AMPA receptors [101] and triggers internalization of AMPA receptors in hippocampus [102], contributing to induction of LTD. Thus, through transcriptional and post-translational regulation affecting NMDA and AMPA receptors, insulin modulates hippocampal long-term plasticity, a cellular

component of learning and memory (Figure 5). Consistent with this, hippocampal deletion of InsR results in impairment of spatial and contextual learning [100, 103]. To add to this picture, a recent study demonstrated that insulin produces bidirectional effects on synaptic transmission in NAc involving a disinhibition mechanism mediated by opioid receptor [104]. These studies open an avenue for future studies of brain insulin dysfunction in cognitive deficits and mood disturbance. In fact, restoration of brain insulin signaling via intranasal insulin treatment has been suggested as a potential treatment for cognitive decline [105] and mood disorders [106] (see discussion below).

## Insulin Action on Global Brain Metabolism

The brain has high energy demands and relies on glucose as its main fuel. In addition, the brain is the most cholesterol-rich organ in the body, containing approximately 25% of total body cholesterol. In brain, cholesterol is important not only in myelin formation, but in neuronal membrane function. Since cholesterol-containing lipoproteins in the blood cannot cross the BBB, the brain relies on local production of cholesterol for myelination and synaptic formation and remodeling. Peripheral infusion of insulin under euglycemic conditions in mice induces expression of genes involved in cholesterol and long-chain fatty acid biosynthesis while suppressing expression of many genes involved in glycolysis and the pentose phosphate pathway [96]. The overall effect is to reroute carbon substrates from glucose metabolism to lipid metabolism. Thus, although insulin does not regulate brain glucose uptake, insulin can regulate substrate flux and brain metabolism, which affects many neural functions including cognition (Figure 5). Consistent with this, in streptozotocin-induced diabetic mice with low circulating insulin levels, genes involved in cholesterol biosynthesis in brain are downregulated, and brain cholesterol synthesis is low [107]. Likewise, in the hypothalamus of NIRKO mice, there is downregulation of SREBP2, the master regulator of cholesterol metabolism [107]. Reduced brain cholesterol synthesis impairs LTP and contextual learning in mice [108], which in the context of the other findings discussed earlier indicates that the anabolic effect of insulin on brain cholesterol synthesis is necessary for normal memory formation.

Mitochondria are central regulators of cellular metabolism and energetics. Mitochondrial dysfunction in multiple peripheral tissues has been associated with diabetes and insulin resistance [109]. It is therefore not surprising that, in brain, insulin can regulate mitochondrial biogenesis, morphology, and function, which in turn can modulate metabolism and higher-order brain functions and cognition (Figure 5). Insulin also mediates the innate stress-response machinery in mitochondria by regulating chaperone proteins, including Hsp60 and Hsp10 [89, 90]. Thus, in NIRKO mice, there are fewer and smaller mitochondria in brain and decreased expression of mitochondrial oxidative phosphorylation complexes [6]. In addition, normal insulin signaling prevents mitochondrial swelling and accumulation of reactive oxygen species in hippocampus [110]. Intranasal insulin treatment increases mitochondrial ATP production in heathy mouse brain, demonstrating that insulin direct regulating brain mitochondrial function [111]. However, increased mitochondrial ATP release could also increase oxidative stress, which might contribute to AD (recently reviewed in [112]). Given the vulnerability of neurons to oxidative stress, a question that

requires further investigation is how mitochondrial dysfunction related to abnormal insulin signaling contributes to neurodegenerative disorders.

## Cell Type-Specific Regulation of IGF-1 Receptor Signaling in the Brain

Insulin-like growth factors (IGFs) bind with high affinity to the IGF1 receptor (IGF1R) on target cells and modulate multiple processes, including cell growth and differentiation [113]. Insulin and IGF-1 receptors are expressed in overlapping neuroanatomical patterns, but at different levels in different regions [45]. Despite many similarities in signaling, these two receptors have preferential pathways of signaling and exert distinct downstream biological effects [114]. Both ligands can also cross-react with each other's receptors, albeit with lower affinity.

The neuroprotective effects of IGF-1 signaling have been studied for decades [115]. In mouse models of neurodegeneration, IGF-1 administration has been shown to improve the neurologic phenotype [116–118], suggesting that IGF-1 might serve as a treatment for these disorders. Conversely, dysfunction of IGF-1 signaling leads to poor outcomes after traumatic brain injury [119, 120]. Not all effects of IGF-1, however, are positive. For instance, a recent study found that microglia-produced IGF-1 may serve as a tumor-supporting factor [121]. Thus, a balance of homeostasis of IGF-1 signaling is essential.

In contrast to insulin which is almost exclusively produced in pancreatic β-cells, IGF-1 is produced by many cell types. scRNA-seq has revealed that IGF-1 expression is enriched in specific subsets of inhibitory neurons [122], and this might form a bridge between neuronal activity and IGF-1-related cognitive function [123]. While IGF1Rs are present on both neurons and glial cells, IGF-1 signaling in astrocytes appears largely responsible for its neuroprotective role. Consistent with this, mice with an astrocyte-specific IGF1R KO have impaired hippocampal-dependent learning [124]. In addition, studies have shown that loss of IGF-1 signaling in astrocytes impairs the astrocytic protection of neurons during oxidative stress in a human astrocyte-neuron co-culture system [125] and in mice subjected to ischemic brain injury [126].

Microglia-derived IGF-1 also plays a role in protection of neurons from damage and apoptosis. In contrast to astrocytes, microglial IGF-1 signaling mainly protects from focal ischemic injury [127, 128]. Following ischemic brain injury, *Igf1* expression is increased in reactive microglia, but not in astrocytes [127], indicating a cell-type-specific response. Thus, insulin/IGF-1 action in different glia cell types play distinct roles in the CNS response to injury.

## New Technologies to Probe Insulin and IGF-1 Signaling in the Brain

New technologies are enabling a better characterization of individual brain cell types and may help elucidate the roles of insulin/IGF-1 signaling in the brain. As discussed above, distinct VTA dopaminergic subpopulations exhibit different responses to insulin. Thus, insulin increases the spontaneous firing rates of only ~50% of midbrain dopaminergic neurons [89]. Going forward, technologies such as scRNA-seq should help identify these phenotypically distinct neuronal subsets and help better understand the CNS response to

insulin and IGF-1. Additionally, *in vivo* $Ca^{2+}$ imaging via a fiber or lens implanted in the brain enables simultaneous recording of activity of hundreds of brain cells while animals are engaged in behavioral tasks [129]. A recent study has applied a fiber photometry-based $Ca^{2+}$ imaging in freely moving mice with tanycytic InsR deletion and discovered a role of insulin signaling in tanycytes in regulation of AgRP neuronal activity [79]. Although it is clear that fiber photometry lacks single-cell resolution, this has already offered significant insights into tanycyte's regulatory effects upon application of stimuli such as gut-secreted hormones and feeding. Future studies should also investigate the dynamic neuronal response in real-time to different metabolic states, such as insulin resistance and diabetes (see Outstanding Questions).

## A Translational View

While translational studies are limited, preliminary clinical trials in healthy subjects [130] and small trials[i,ii] in AD patients [131–133] suggested that intranasal insulin therapy might produce modest cognitive improvement. In AD, patients without the *Apoe4* risk allele showed a more profound response to intranasal insulin than patients carrying *Apoe4* [134]. However, results from the larger multicenter trial have been mixed. While one clinical trial[iii] found no cognitive benefits of 12-month intranasal insulin treatment [135], a recent follow-up analysis of brain imaging of the study participants demonstrated a reduction in white matter hyperintensity with insulin therapy [136]. Intranasal insulin treatment of patients with mood disorders has also shown mixed results. An early clinical trial[iv] found a significant improvement of neurocognitive performance in bipolar patients [106], however, the same research group failed to demonstrate beneficial effects of intranasal insulin on overall mood or neurocognition in patients with major depressive disorders[v] [137]. Interestingly, animal studies and pilot clinical trials of other antidiabetic medications, including liraglutide (a GLP-1 analogue)[vi] and pioglitazone[vii,viii], have shown promising effects in AD [138, 139], but this effect was not observed with rosiglitazone[ix,x], another thiazolidinedione [140].

Investigations of the basic mechanisms of insulin action in the brain have also raised the potential of identifying new therapeutic targets. Cholesterol synthesis provides a promising example. Thus, acute insulin signaling regulates *de novo* cholesterol synthesis in neurons and glia, whereas chronic hyperinsulinemia in diabetes and insulin resistance leads to brain insulin resistance and a loss of the normal dynamic regulation of brain cholesterol production. This could contribute to the acceleration of AD progression, possibly through altered apoE metabolism, which is strongly genetically linked to development of AD [141]. Further advances to our understanding of brain insulin and IGF-1 signaling should help pinpoint the precise site where insulin acts on different subtypes of astrocytes and neurons in regulating cholesterol and long-chain fatty acid biosynthesis and understand how these might contribute to the pathogenesis of brain disorders such as depression and AD (see Outstanding Questions). These results raise hope for new therapeutic approaches to brain disorders centered around insulin signaling and metabolism, but also underscore the need for better understanding of the multiple roles of these pathways in brain.

## Concluding Remarks

Insulin signaling regulates a broad range of physiological processes at the systemic level by modulating substrate uptake and metabolism in classic target organs - liver, fat and muscle. Insulin also plays important roles in brain, modulating both neurons and glial cells through changes in gene expression and cell function at multiple levels. Accumulating evidence demonstrates that dysregulated insulin signaling plays a role in psychiatric disorders and neurodegenerative diseases. In animal models, manipulation of brain InsR or IGF1R using transgenic approaches, as well as regional manipulation, has increased our understanding of insulin action and its role in brain functions. However, the brain has an incomparable level of cellular heterogeneity, and a deep cell-type-specific understanding of insulin action remains an important goal for future research. New tools, such as scRNA-seq, optogenetics, chemogenetics, and neuronal tracing techniques, as well as ways to modify insulin action in specific cell populations, should provide deeper insights and better understanding of insulin signaling in the brain and hopefully provide new targets for the treatment of both metabolic and brain disorders.

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## Glossary

**Comorbidity**
the existence of two or more medical conditions in an individual, which is often associated with worse outcomes

**Cre/LoxP system**
a technique enabling precise genetic modification based on the recognition of Cre recombinase of the 34 bp LoxP sites inserted around a gene or exon

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a technique enabling precise genetic modification based on the recognition of Cre recombinase of the 34 bp LoxP sites inserted around a gene or exo

**Emotional valence**
a term broadly defining emotional state as positive or negative

**Glucose homeostasis**
the tightly regulated process to maintain the blood glucose within a narrow range

**Insulin-like growth factor 1 receptor (IGF1R)**
a closely related tyrosine kinase receptor activated primarily by IGF-1 and IGF-2, and to a lesser extent by insulin

**Insulin receptor (InsR)**

a member of the class of tyrosine kinase receptors activated primarily by insulin, and to a lesser extent by IGF-1 and IGF-2. The InsR plays a key role in maintaining glucose homeostasis

**Insulin resistance**
a state when cells become unresponsive to insulin. In individuals in which hyperinsulinemia cannot overcome the insulin resistance, the result is elevated level of blood glucose

**Motivational salience**
a cognitive process regulating behavior to pursue a reward (incentive salience) or to avoid a punishment (aversive salience)

**Synaptic plasticity**
the ability of synapses to modify the strength, efficacy, or target of synaptic transmission

**Type 1 diabetes**
a chronic autoimmune condition in which the β cells in the pancreas are destroyed resulting in low insulin levels. While historically this was viewed as a form of diabetes which appeared in childhood, it is now clear that it can develop at any age

**Type 2 diabetes (T2D)**
a chronic condition in which body cells do not respond appropriately to insulin; as a result, glucose remains in the blood and cannot be utilized as fuel. Circulating insulin levels may be high, low, or normal

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## Resources

i. https://clinicaltrials.gov/ct2/show/NCT00438568
ii. https://clinicaltrials.gov/ct2/show/NCT01595646
iii. https://clinicaltrials.gov/ct2/show/NCT01767909
iv. https://clinicaltrials.gov/ct2/show/NCT00314314
v. https://clinicaltrials.gov/ct2/show/NCT00570050
vi. https://clinicaltrials.gov/ct2/show/NCT01469351
vii. https://clinicaltrials.gov/ct2/show/NCT00982202
viii. https://clinicaltrials.gov/ct2/show/NCT02284906
ix. https://clinicaltrials.gov/ct2/show/NCT00428090
x. https://clinicaltrials.gov/ct2/show/NCT00550420

## Outstanding questions

- Does insulin signaling in the mesolimbic dopamine reward pathway directly modulate this pathway's function, and could this play a role in the manifestation of mood and anxiety symptoms in disorders of insulin signaling?
- Given the marked heterogeneity of brain cell types, including neurons, astrocytes and microglia, how does InsR or IGF1R signaling in these subtypes change with normal brain development and during disease progression? Are some subtypes more vulnerable than others to systemic metabolic dysfunction? How does insulin resistance in one subtype affect function in other subtypes?
- Would targeting dopaminergic or serotonergic neurons modulate brain insulin release and alleviate the comorbidity of T2D and psychiatric disorders? How do antidepressants or cognition-enhancing drugs modify systemic metabolic status?
- Are there InsR-independent mechanisms or actions of insulin on other cells which can modulate how insulin enters the brain, and if so, how do they change in disease?
- What are the best preclinical animal models to study comorbidity of T2D and brain disorders and the most efficient way to achieve clinical translation of these studies?

## Highlights

- Insulin is a key hormone in regulation of energy metabolism. Insulin resistance, a state when cells become unresponsive to insulin, is a key feature of type 2 diabetes (T2D), obesity, and many other metabolic disorders.
- The brain is an insulin sensitive tissue. Insulin signaling plays a key role in different brain cell types.
- Insulin modulates neuronal and glial function, resulting in changes in mood, cognition, and behavior.
- Dysfunction in brain insulin signaling underlies comorbidity of T2D and disorders such as depression and Alzheimer's disease.
- Better understanding of brain insulin actions may lead to new therapeutic targets for the treatment of brain disorders.

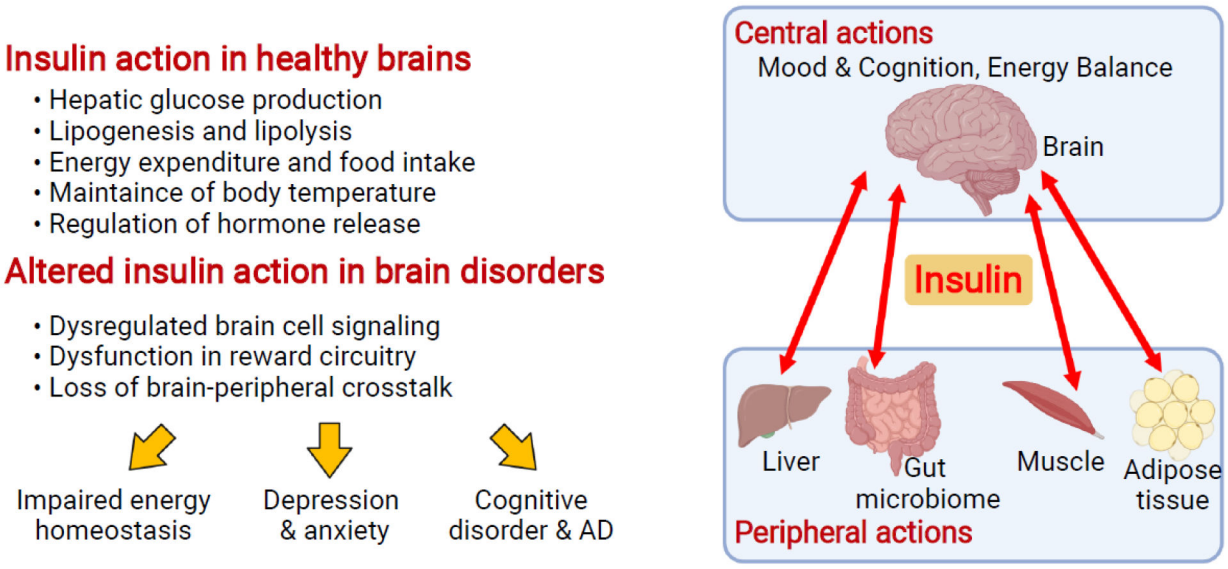


**Figure 1.**

Brain insulin signaling in healthy and disordered brains. Insulin not only serves as a master regulator of systemic metabolism and normal functions within organs such as liver, fat, and muscle, but also regulates key brain functions such as mood and cognition. As a result, altered brain insulin signaling leads to brain disorders such as emotional and cognitive disorders. Created with BioRender.com.

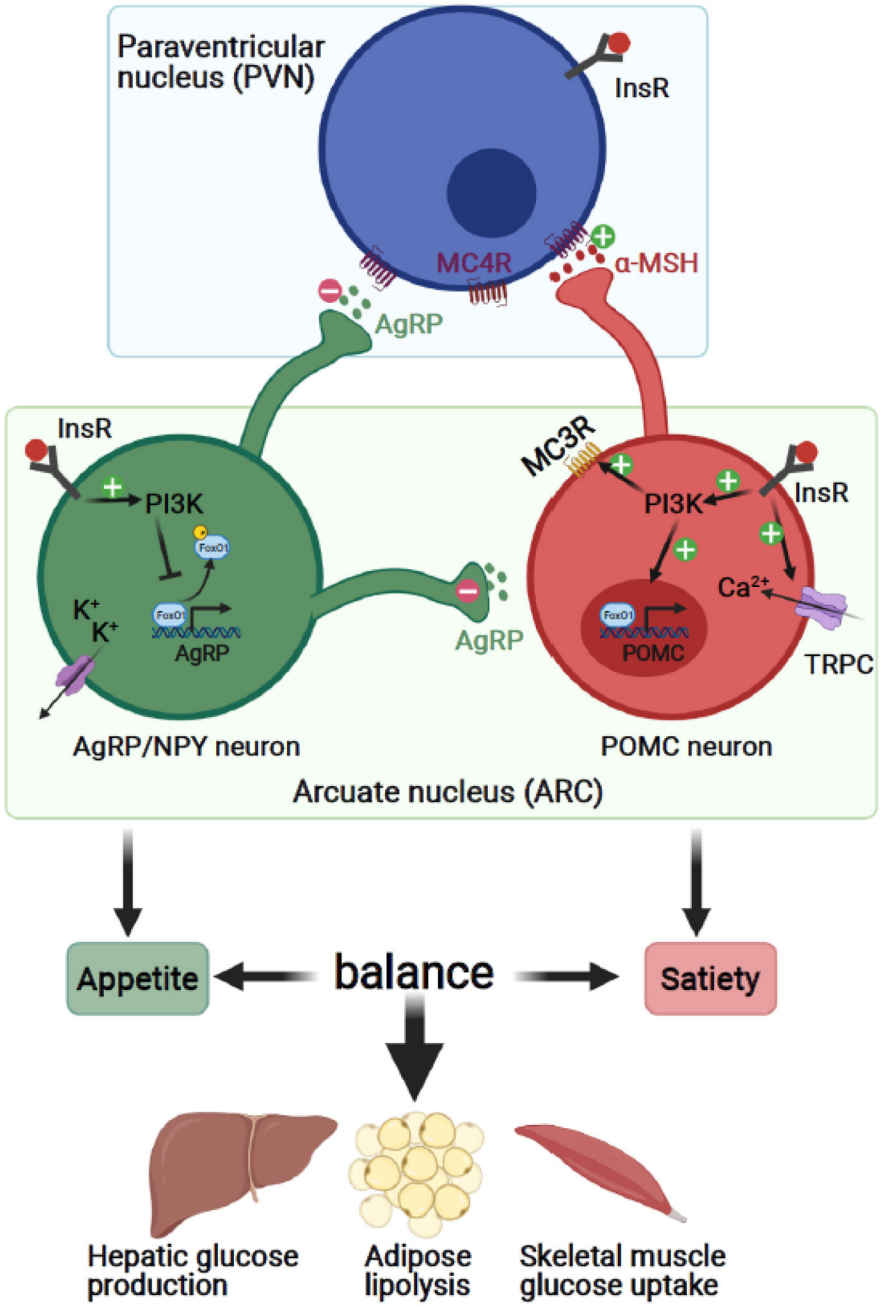


**Figure 2.**

Insulin regulates food intake and energy expenditure through hypothalamic neurons. A prominent role of insulin signaling in the brain is to regulate food intake and energy metabolism through hypothalamic POMC and AgRP/NYP neurons. The interplay across these cell types contributes to the maintenance of the balance between appetite and satiety. Created with BioRender.com.

Abbreviations: AgRP/NPY, agouti-related neuropeptide/neuropeptide Y; α-MSH, alpha-Melanocyte-stimulating hormone; InsR, insulin receptor; MC3R, melanocortin 3

receptor; MC4R, melanocortin 4 receptor; PI3K, Phosphoinositide 3-kinase; POMC, proopiomelanocortin; TRPC, transient receptor potential canonical channels.

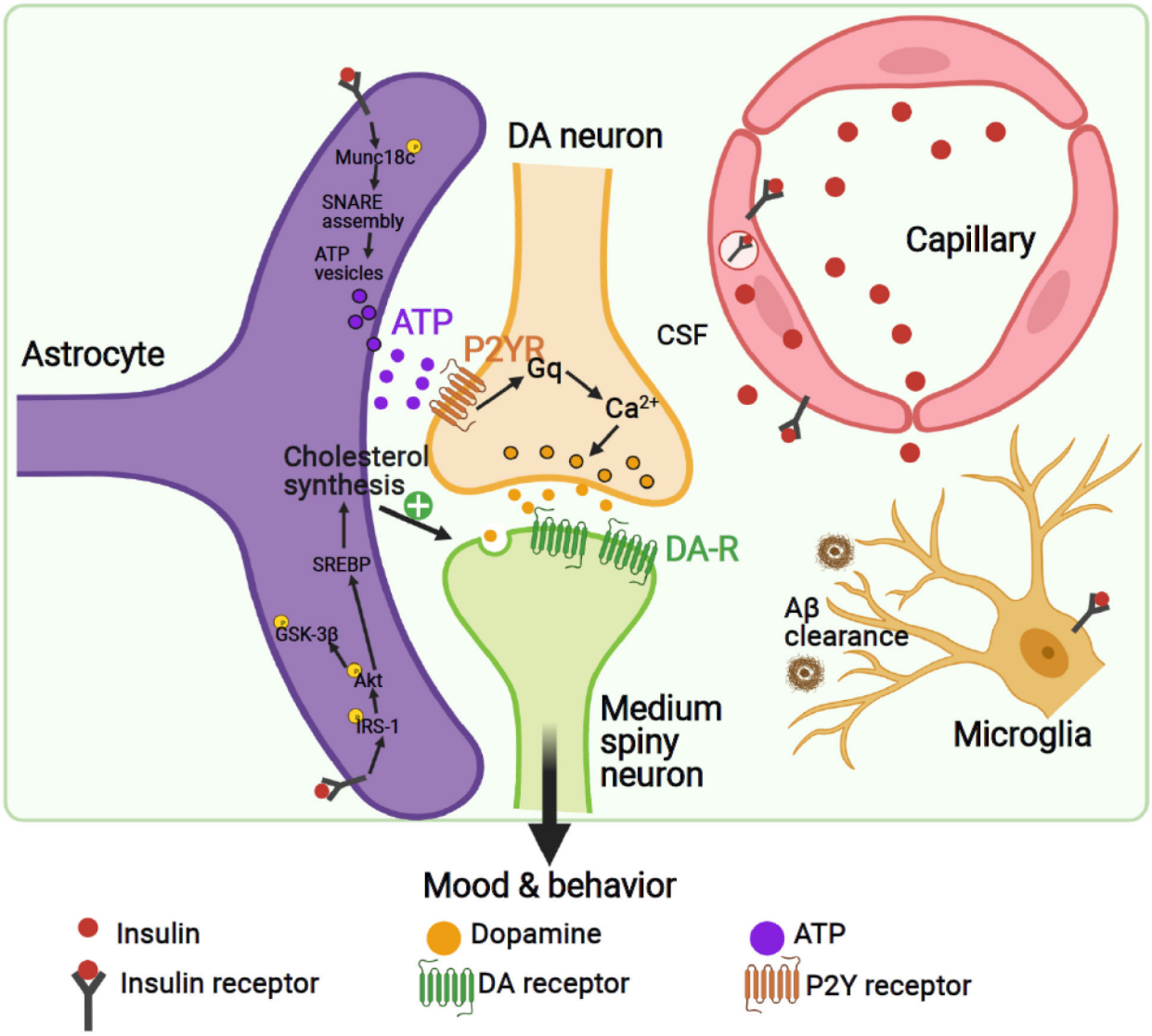


**Figure 3.**

Cell type-specific regulation of insulin signaling in the brain. Insulin regulates a wide range of brain cell types, including neurons, astrocytes, endothelial cells, and microglia. Created with BioRender.com.

Abbreviations: ATP, adenosine triphosphate; CSF, cerebrospinal fluid; DA, dopamine; DA-R, dopamine receptors; P2YR, P2Y receptors; SREBP, sterol regulatory element-binding protein.

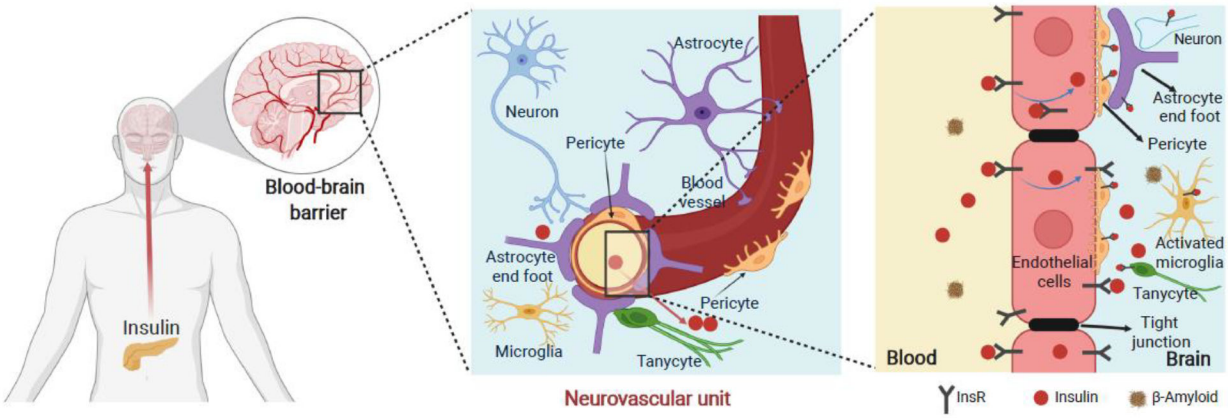


**Figure 4.**

Insulin signaling in brain vascular endothelial system. The blood-brain barrier (BBB) is a highly selective barrier that dynamically maintains a stable milieu for the brain. Endothelial InsR regulates transendothelial insulin delivery thus differentially modulating the kinetics of insulin signaling across brain regions. Altered BBB function plays a role in brain insulin resistance and contributes to the comorbidity of T2D and AD. Blue lines within the endothelial cells (in the right panel) denote the direction of insulin transport across the brain capillary. Created with BioRender.com.

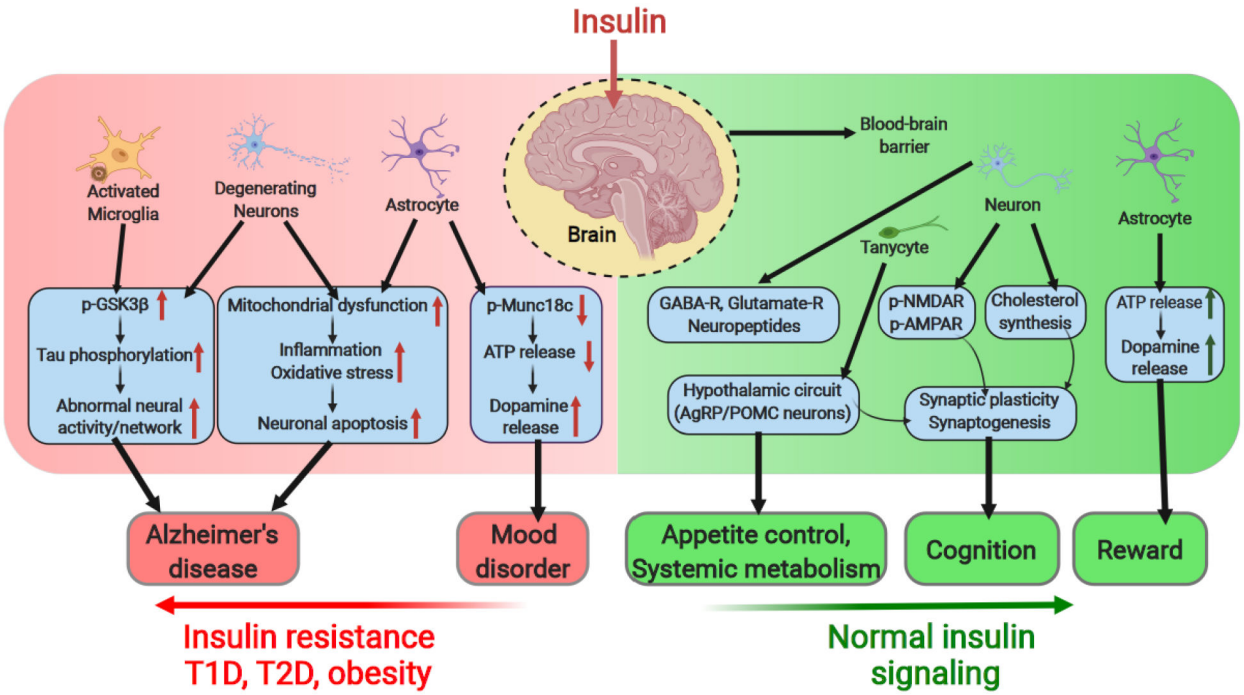


**Figure 5.**

Insulin regulates key brain functions through multiple mechanisms involving different cell types and neural circuits. Insulin not only regulates functions of various brain cell types, including neurons, astrocytes, microglia, and tanycytes, but also modulates key brain pathways such as the mesolimbic dopaminergic pathway and hypothalamic circuits. In addition, insulin modulates global brain processes such as synaptic transmission and brain metabolism. Dysregulated insulin signaling in the brain contributes to brain disorders such as depression and AD. Created with BioRender.com.

Abbreviations: AgRP/NPY, agouti-related neuropeptide/neuropeptide Y; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; ATP, adenosine triphosphate; GABA, γ-aminobutyric acid; GSK-3β, glycogen synthase kinase 3β; NMDA, N-Methyl-d-aspartic acid; POMC, proopiomelanocortin.

**Table 1.**

A summary of studies using Cre/loxP system to probe physiological roles of InsR signaling in different brain cell types

| Name | Cell-type of interests | Cre-driver | Main findings of the InsR KO phenotypes | Physiological importance | Refs |
|---|---|---|---|---|---|
| NIRKO | brain-specific | *Nestin-Cre* | • increased food intake in females;<br>• mild insulin resistance and dyslipidemia;<br>• hypothalamic hypogonadism;<br>• loss of insulin stimulated PIP3 signaling<br>• Tau hyperphosphorylation;<br>• attenuated phosphorylated hepatic STAT3;<br>• impaired GLUT4 translocation;<br>• brain mitochondrial dysfunction;<br>• dopaminergic dysfunction;<br>• late-age onset-related anxiety- and depression-like behaviors | Regulation of energy disposal, fuel metabolism, reproduction, brain-liver crosstalk, glucose sensing, brain cholesterol metabolism, mitochondrial function, and dopamine signaling | [5, 6, 42–44, 107, 142] |
| GIRKO | Astrocyte-specific | GFAP-Cre, GFAP-Cre$^{ERT2}$, GLAST-Cre$^{ERT2}$ | • impaired responses to changes in glucose availability;<br>• decreased ATP release from astrocytes;<br>• anxiety- and depression-like behaviors;<br>• longer and irregular estrus cycles;<br>• decreased pregnancy rates and reduced litter sizes;<br>• dysregulation of the HPG axis | hypothalamic glucose sensing, regulation of systemic glucose metabolism, dopaminergic signaling, ATP exocytosis, HPG axis | [7, 62, 143] |
| EndoIRKO | Vascular endothelial cells-specific | Cdh5-Cre Slcolc1-Cre$^{ERT2}$ | • delayed onset of insulin signaling in the brain;<br>• decreased levels of hypothalamic POMC, increased food intake and obesity;<br>• delayed acute hypoglycemic effect of systemic insulin administration and impaired glucose tolerance;<br>• Not necessary to maintain insulin sensitivity | Transendothelial insulin delivery; kinetics of insulin signaling | [74, 77, 79] |

**Table 2.**

A summary of InsR signaling in different neuronal subtypes

| Neuronal subtypes | Transgenic approaches | Main findings | Physiological role | Ref. |
|---|---|---|---|---|
| ARC POMC neurons | POMC InsR KO<br>POMC InsR KI mice<br>POMC-eGFP<br>POMC InsR/LepR double KO<br>IRS2 KO mice | • insulin depolarizes POMC neurons via activating TRPC5 channels and inhibits POMC neurons via PI3K activation<br>• IRS2 KO mice show lower POMC mRNA levels and higher levels of inflammation<br>• POMC InsR KO increased hepatic triglyceride levels in HFD mice<br>• overexpression of InsR in POMC neurons leads to increased energy expenditure and locomotor activity | Regulation of adipose lipolysis, neuronal plasticity, energy expenditure, glucose homeostasis and fertility | [47, 48, 50–52, 144–146] |
| ARC AgRP neurons | AgRP InsR KO<br>AgRP InsR KI mice | • increase in appetite, decrease in metabolism/energy expenditure<br>• restrained hepatic glucose production | Regulation of glucose metabolism | [47, 48] |
| LH MCH neurons | MCH InsR KO<br>MCH-GFP<br>$MCH^{-/-}$ mice | • insulin increases excitability of MCH neurons<br>• MCH InsR KO mice showed no alterations in energy homeostasis or glucose metabolism in lean mice<br>• Increased hepatic insulin sensitivity in obese MCH InsR KO mice<br>• MCH ablation mice showed increased body weight and insulin resistance | Regulation of body weight, insulin sensitivity, and energy homeostasis | [53, 54, 147] |
| Orexin-A neurons | $Orexin^{-/-}$ mice<br>Orexin-cre (DREADDs, viral ablation) | • Orexin global KO leads to impaired hepatic insulin action<br>• manipulation of orexin neurons shows phenotypes in food intake and metabolism<br>• insulin i.c.v. injection activates orexin neurons<br>• Orexin A regulates plasma insulin levels | Prevention of hepatic insulin resistance Regulation of feeding behavior, metabolism | [56, 57, 148–150] |
| HPA axis | $InsR^{Nkx2.1}$ KO (hypothalamus) mice,<br>$InsR^{Sim1}$ KO (PVN), mice<br>$InsR^{AgRP}$ KO (ARH) mice | This study used separate Cre lines to achieve disruption of InsR signals in selective hypothalamic areas and found:<br>• $InsR^{Nkx2.1}$ KO leads to heightened HPA axis response to stress and anxiety-like behaviors in males<br>• $InsR^{AgRP}$ KO show reduced AVP release in median eminence | Regulation of HPA axis and stress response;<br>Regulation of mood | [151] |
| Sensory neurons | Advillin-cre mice (SNIRKO) | • no behavioral, physiological, or morphological signs of peripheral diabetic neuropathy<br>• elevated serum insulin levels, glucose intolerance, increased insulin in islets of pancreas | Regulation of islet function | [152] |