Brain Insulin Resistance at the Crossroads of Metabolic and Cognitive Disorders in Humans

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Outline

Abstract

Ever since the brain was identified as an insulin-sensitive organ, evidence has rapidly accumulated that insulin action in the brain produces multiple behavioral and metabolic effects, influencing eating behavior, peripheral metabolism, and cognition.

Disturbances in brain insulin action can be observed in obesity and type 2 diabetes (T2D), as well as in aging and dementia. Decreases in insulin sensitivity of central nervous pathways, i.e., brain insulin resistance, may therefore constitute a joint pathological feature of metabolic and cognitive dysfunctions.

Modern neuroimaging methods have provided new means of probing brain insulin action, revealing the influence of insulin on both global and regional brain function. In this review, we highlight recent findings on brain insulin action in humans and its impact on metabolism and cognition. Furthermore, we elaborate on the most prominent factors associated with brain insulin resistance, i.e., obesity, T2D, genes, maternal metabolism, normal aging, inflammation, and dementia, which could be the cause or consequence of brain insulin resistance. We also describe the beneficial effects of enhanced brain insulin signaling on human eating behavior and cognition, and discuss potential applications in the treatment of metabolic and cognitive disorders.

I. Introduction

Insulin resistance is a shared hallmark feature of obesity, type 2 diabetes (T2D) and neuropathological processes underlying cognitive aging and dementia. As the population gets older, age-related chronic diseases become more prevalent and are of increasing public concern. The number of individuals diagnosed with T2D is expected to reach 592 million by the year 2035 (91). By this time, the number of people with dementia will have doubled, reaching 75.6 million (388). In parallel, global obesity rates are on the rise, increasing the risk of T2D, hypertension, coronary heart disease and certain forms of cancer (111, 112). Furthermore, the influence of obesity and T2D on brain structure and function has been well established and shows a higher risk of cognitive decline and even dementia (30, 137, 212), particularly in the elderly population (30, 242). The mechanisms underlying cognitive decline and brain structure changes in obesity and T2D are, however, still unclear. Since chronic and acute dysregulations of blood glucose concentrations have both been linked to compromised neurocognitive functions (96), the majority of prediabetes and T2D research has focused on the effects of glycemia extremes (317). Due to its importance in brain functioning, insulin signaling within the brain has been receiving more attention recently. One reason for this is because significant disturbances in brain insulin action are not only observed in obesity and T2D, but also in brain aging and dementia. It has therefore been proposed that decreases in the sensitivity of central nervous pathways to insulin, i.e., brain insulin resistance, constitute a potential link between metabolic and cognitive dysfunctions (56, 377).

1. The role of insulin in the brain: a historical perspective

Following its discovery in 1921, the vital role of insulin in the periphery was quickly recognized and extensively studied before scientific interest turned to the role of the brain in insulin signaling and vice versa. Peripheral tissue depends on insulin to translocate and activate glucose transporters on cell membranes which, in turn, trigger glucose uptake from the circulation. However, the central nervous system (CNS) can utilize glucose independently

of insulin-mediated processes inasmuch as glucose can enter the brain by diffusing across the blood-brain barrier. It is then absorbed by brain cells via a range of insulin-insensitive glucose transporters. However, insulin, being a large peptide hormone, does not passively cross the blood-brain barrier. For a long time, brain function was therefore considered to be insulin-independent. However, studies in the 1970s and early 1980s, in particular by Jesse Roth and colleagues, demonstrated that insulin receptors are abundantly distributed throughout the brain (15, 16, 72, 157, 158, 328, 375) (Figure 1). The seminal work of Stephen Woods and colleagues highlighted the pivotal role of the brain in insulin action, showing that intracerebroventricular infusion of insulin decreases food intake and body weight in baboons (397). Although mostly performed in rats (1, 2, 187, 238), this central catabolic action has been replicated across a number of species including mice (37), chickens (89, 179), sheep (113) and marmots (64). Despite this diversity, not all results are confirmatory (193, 253). Some investigators failed to observe a reduction in food intake after insulin administration. A recent study by McAllister et al. (234) systematically evaluated potential confounds by performing several cross-over designs with insulin versus placebo in mice. The authors proposed that recent experience with intracereboventricular administration can contribute to the variability in the effect of insulin. When insulin and placebo trials were spaced only two days apart, order-dependent effects were indeed identified. Virtually no significant insulin effect on food intake was observed when insulin was delivered on the first and placebo on the second trial. Thus, environmental cues can alter eating-related responses to insulin, probably due to an associative learning process.

Besides its effects on food intake, evidence has been rapidly accumulating that brain insulin action produces multiple behavioral and metabolic effects, influencing eating behavior, peripheral metabolism (166), and cognition, in particular memory formation (266), in animals and humans.

Until the 1990's, the mechanisms responsible for the transport of insulin to the central nervous system had not been identified (273). Controversy over the source of central

nervous insulin led to the hypothesis that insulin is produced within the central nervous system (159, 243). While local insulin release in the CNS seems to be important in lower organisms (133), its quantitative relevance in comparison to pancreas-derived insulin in higher animals is still under debate (133). However, two recent studies suggest that local production of small quantities of insulin might exist in the CNS (239, 243). With regard to pancreas-derived insulin that enters the brain via the bloodstream, a saturable, insulin receptor-mediated pathway was observed to transport insulin into the brain (12, 17, 18, 208). Once released into the blood stream by pancreatic beta cells, circulating insulin binds to receptors on endothelial cells of the blood-brain barrier, where it is further transported into the brain's interstitial fluid by receptor-mediated transcytosis (18). Here it binds to numerous insulin receptors distributed throughout the olfactory bulb, cerebral cortex, hippocampus, hypothalamus, amygdala and septum (16, 157, 328, 365, 375) (Figure 1). It then induces a number of central nervous and peripheral effects (as discussed in section III). More specifically, as soon as the insulin receptor binds the hormone insulin, it becomes active as a tyrosine kinase. This activation causes autophosphorylation of the receptor as well as phosphorylation of the tyrosine residues of the docking protein known as insulin receptor substrate (IRS). This subsequently activates the downstream signaling cascade (386). Of the six IRS family members identified to date, IRS-1 and IRS-2 are responsible for most of the abundant effects of insulin associated with the activation of two main signaling pathways, namely the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) pathway and the Ras-mitogen-activated protein kinase (MAPK) pathways (224, 385). The former is responsible for most of the metabolic actions of insulin (344). With regard to the IRS family expression in the brain, although IRS-1 was identified in parts of the brain, the ventral hypothalamus showed no expression of IRS-1 and knockout mice revealed normal energy homeostasis (278). IRS-2 was abundantly found in the arcuate nucleus of the ventral hypothalamus (354) and knockout mice exemplified an obese phenotype (227). Hence, IRS-2 would appear to play an important role in brain insulin signaling. The specific roles of IRS-1 and IRS-2 in cognitive brain functions are not completely understood. Several studies

indicate memory-enhancing effects of insulin in IRS pathways (81). IRS-1 seems to positively regulate memory and is inhibited in Alzheimer's disease brains and animal models (34, 244, 342). Reducing IRS-1 inhibition improved cognition in mice (34). IRS-2, on the other hand, acts as a negative modulator of memory impairing for example dendritic spine formation (188). Beneficial effects of IRS-2 deletion are seen on Alzheimer's disease pathology reducing cognitive deficits in transgenic mouse model of AD (34, 206).

2. Evidence for brain insulin resistance

Insulin resistance refers to the reduced ability of insulin to exert its action on target tissues. In the periphery, this has long been considered a hallmark feature of obesity and T2D. Once the central effects of insulin on food intake and body weight in animals and humans were identified, it transpired that the brain is a further important site of insulin resistance. In a pioneering study, the selective disruption of neuronal insulin receptors in mice induced a diet-induced obese phenotype with increased body fat and peripheral insulin resistance (39). Parts of this effect were later attributed to insulin receptors in specific hypothalamic subnuclei (246, 379). The restoration of insulin receptor function in non-canonical insulin target tissue such as the brain prevents diabetes and maintains homeostasis (262). The significance of brain insulin action for peripheral metabolism was first revealed in rodent models, in which an alteration of the CNS-liver circuit was shown to contribute to hyperglycemia. More specifically, insulin signaling in the hypothalamus was discovered to be necessary for controlling hepatic glucose production (258, 259) and the surgical resection of the hepatic branch of the vagus nerve negated brain insulin action (277). Like insulin, numerous other hormonal signals from the periphery, such as GLP-1, CCK or ghrelin, influence the brain by exerting their effects on food intake, thereby establishing a multifactorial signal crosstalk between the periphery and brain (393).

In humans, Tschritter and colleagues (362) were the first to show brain unresponsiveness to exogenous insulin in obese adults. This opened up the new field of the study of brain insulin

resistance in-vivo in humans using neuroimaging techniques (as discussed under sections III and IV).

Interestingly, many of the metabolic disturbances found in prediabetes and T2D can also be observed in Alzheimer's disease (AD). Patients with AD display reduced peripheral insulin sensitivity and are typically hyperinsulinemic in both a state of fasting and in response to an oral glucose tolerance test, (75). Furthermore, prolonged peripheral hyperinsulinemia can decrease insulin receptors at the blood-brain barrier, thereby reducing insulin transport into the brain (315). Notably, in patients with AD and mild-cognitive impairments as well as in patients with whole-body insulin resistance, T2D and obesity, plasma insulin levels are high, whereas cerebrospinal fluid (CSF) insulin levels are decreased (76, 169, 199, 306). Reduced brain insulin uptake has therefore been postulated to lead to impaired brain insulin action. Underlying mechanisms might include insulin resistance at the blood-brain-barrier (377). The number of insulin receptors in the brain decreases with age, particularly in AD (123). This suggests that, in this condition, the development of brain insulin resistance is independent of diabetes. More importantly, recent evidence suggests that insulin directly influences neuropathology and behavioral characteristics of AD by influencing beta-amyloid load and synaptic plasticity that underlie memory formation (56, 377).

This review focuses on brain insulin resistance as a shared pathological feature of metabolic and cognitive disturbances in obesity, T2D and dementia patients (for a schematic overview please see Figure 2). We will first provide a brief introduction into cognitive dysfunctions and underlying brain alterations in obesity, T2D and dementia. In detail, we will discuss recent findings on brain insulin action/resistance in humans as assessed with neuroimaging techniques. Moreover, we will illustrate beneficial effects of brain insulin on human eating behavior and cognition and consider potential applications in the treatment of metabolic and cognitive disorders. For an introduction into the methods applied to study brain insulin action, please see supplementary text as well as Tables 1 and 2.

II. Neurocognitive dysfunction in obesity, T2D and dementia

Longitudinal studies in mostly middle-aged to older adults (for review, please see (212, 242)) have reported a modest cognitive decrement in T2D in comparison to matched control groups over a period of less than 6 years (155, 254, 265, 370). Patients with T2D displayed impaired performance in almost all neuropsychological tests. The greatest decrements were found in memory, information-processing speed and executive function (242). Cross-sectional studies likewise indicate that T2D patients perform worse in several cognitive domains. These include executive function, information-processing speed, memory, psychomotor efficiency, verbal fluency and learning (242). Such signs of cognitive decline are associated with duration of illness, glycemic control and hypoglycemic episodes. Thus, women who suffered from diabetes for more than 15 years showed a 57% to 114% greater risk of cognitive decline (134). They also had a fourfold increased risk of cognitive decline in verbal fluency, which correlated negatively with glycohemoglobin (195). Furthermore, a history of severe hypoglycemic episodes in older type 2 diabetes patients was associated with a greater risk of dementia (387). Obesity has a general negative effect on cognitive function, even when controlling for T2D, hypertension, smoking, and other confounding factors (27), the strongest effect being in early midlife. Midlife obesity was negatively associated with visuospatial performance and executive function over 12 years (396). However, at a later age, an increased body mass index (BMI) can have positive effects on cognitive functions (30). Such a non-linear relationship might suggest that the maintenance of skeletal mass or lean body mass via increased BMI protects cognitive functions, while T2D independently (396) or additively (327) mediates obesity-related cognitive dysfunctions.

A) Structural brain alterations in obesity and T2D

T2D and obesity, alongside their accompanying risk factors such as dyslipidemia and hypertension, have adverse effects on brain function and structure. Obesity itself is associated with brain atrophy, i.e., a loss of gray matter and reduced integrity of white fiber tracts (222). T2D is also related to vascular damage, which results in white matter hyperintensities, infarcts and microbleeds (38). Brain atrophy can be global as well as region-specific, including loss of neurons, axodendritic pruning and reduced synaptic plasticity, such as is also observed in normal aging and in dementia (309). Using advanced magnetic resonance imaging (MRI), a reduction in gray matter volume and cortical thickness as well as a loss of white matter integrity has been observed to be associated with obesity-related factors and T2D (30, 38, 222). A longitudinal study over 6 years in older adults identified BMI as the strongest predictor of declining gray matter volume, particularly in the frontal lobe and subcortical regions such as the hippocampus (30, 284). The medial temporal lobe, including the hippocampus, seems to be particularly affected by diabetes. Hippocampal atrophy, a marker of neurodegeneration, was identified in individuals with impaired glucose tolerance and insulin resistance (67, 366). Phylogenetically and ontogenetically younger regions, such as the temporal and frontal lobe, are therefore more sensitive to obesity/T2D as well as aging. This may lead to accelerated aging in obese and T2D subjects. More importantly, brain atrophy is associated with behavioral cognitive deficits (212).

B) Brain alterations in dementia

Since it is a memory disorder without impairments in other cognitive domains, mild-cognitive impairment (MCI) is related to abnormalities in cognitive test performance without qualifying for dementia. However, patients diagnosed with MCI run a higher risk of developing dementia, which is diagnosed when multiple cognitive deficits disrupt social or occupational functioning (212). Several underlying diseases can cause dementia, of which AD is the most common sub-type. The greatest risk factors for AD are advanced age, carrier of the apolipoprotein (APOE) ε4 allele and a family history of the disease (320). Hallmark characteristics include an accumulation of extracellular neuritic plaques and fibrils, i.e.

aggregated amyloid-beta (Aβ) peptides, intracellular neurofibrillary tangles, accumulation of hyperphoshorylated tau, followed by a widespread loss of neurons and changes in neurotransmitter systems (7, 65, 281). The deposition of Aβ plaques is considered as a central event in AD pathogenesis. Failure to clear this peptide or overproduction leads to amyloid deposition. This, in turn, triggers a plethora of events such as the production of neurofibrillary tangles, cell death and, ultimately, cognitive dysfunctions (150, 151). Imaging biomarkers associated with cognitive decline and dementia are temporal lobe atrophy, mainly in the hippocampus (14), but also in the prefrontal and parietal cortices. In addition to structural markers, positron emission tomography (PET) tracers facilitate the detection of amyloid deposition, using Pittsburgh compound B (PiB), and glucose hypometabolism (^{18}F fluorodeoxyglucose (FDG)) of AD-vulnerable regions as early pathological features (for reviews see (65, 236)). Amyloid PET has a prognostic role in MCI inasmuch as longitudinal studies have shown that high PiB retention in MCI patients makes a conversion to AD more likely (65). Glucose hypometabolism of AD-vulnerable regions such as the lateral parietal cortex, frontal cortex and precuenus/posterior cingulate cortex are well established in AD patients. In individuals at risk for AD due to genetic predisposition (i.e., carriers of the ApoE4 allele and subjects with a family history) hypometabolism of these regions has been identified by FDG-PET. Furthermore, hypometabolism has been associated with cognitive decline in otherwise healthy elderly and in type 2 diabetes patients (65). AD-vulnerable brain regions exhibiting hypometabolism overlap with the regional distribution of amyloid deposition. These regions are also termed “default mode network” (DMN), which is a network of interacting brain regions highly active during rest as a person is not focused on a particular task. The DMN is essential for higher cognitive processes like memory and executive function. Patients with dementia show a loss in functional connectivity in the default mode network comprising the precuneus/posterior cingulate, prefrontal cortex, lateral temporal cortex and the hippocampus (324). Interestingly, T2D patients (181, 252) and obese individuals (219) also show diminished functional connectivity within this network. This means that there is an

extensive overlap between brain regions affected by AD as well as by T2D and obesity, particularly in regions belonging to the default mode network (Figure 3).

T2D and obesity have adverse effects on brain structure and function, affecting several cognitive domains. Midlife obesity in particular is negatively associated with memory and executive function, whereas patients with T2D display impaired performance in almost all neuropsychological tests. At the same time, a reduction in gray matter volume, cortical thickness as well as a loss of white matter integrity are all associated with obesity and T2D. The frontal lobe and subcortical regions such as the hippocampus are particularly susceptible to such a decline. Similarly, imaging biomarkers of dementia include atrophy, amyloid deposition and hypometabolism of the temporal lobe, mainly in the hippocampus, but also in the prefrontal and parietal cortices. These are considered to be early pathological features of AD and overlap with cognitive decline in otherwise healthy obese and T2D patients. Hence, a network of brain regions, termed the “default-mode” network, seems to be compromised in function and structure, which is associated with decreased cerebral blood flow and altered connectivity (Figure 3).

III. Brain insulin action in healthy humans

a) Insulin action on global brain function

Influence of insulin-mediated cortical electrical activity on higher cognitive processes

In accordance with behavioral studies, imaging studies in healthy normal-weight individuals have ascertained that insulin plays a prominent role in brain functions that regulate metabolism and cognition. Since electroencephalography (EEG) is highly sensitive to hypoglycemic states (32, 356), some of the earliest studies on brain insulin action evaluated event-related potentials using both memory tasks and visual and auditory-evoked potentials as indicators of cortical responses to insulin. When euglygemic hyperinsulinemic clamps were used, no effect on visual-evoked potentials was observed (26, 318). This indicates that

insulin has no effect on low-level sensory processes. For auditory-evoked responses and event-related potentials during a memory task, a reduction in the amplitude of N100 and P300 component was found in response to intranasal and intravenous insulin (clamp technique) (26, 200). Concomitantly, magnetoencephalography (MEG) studies revealed enhanced amplitudes for auditory and visual evoked responses during a hyperinsulinemic euglycemic clamp, as well as after intranasal insulin in healthy lean individuals (141, 362). The rapid changes in event-related potentials indicated that insulin may be a rapidly acting feedback signal that contributes to the sensation of satiety. Furthermore, these studies provided the first indication that higher cortical areas are particularly reactive to insulin. This is also reflected by the increased amplitude of auditory mismatch responses to increased exogenous insulin (362), the pronounced P300 amplitude reduction over frontal areas to auditory stimuli (200), and the negative shift in frontal direct current potentials after intravenous insulin injection (147). Whereas the latter experiment showed that a surprisingly rapid central nervous effect of increases in circulating insulin manifested itself within 7 min, effects on oscillatory EEG activity were found only when hypoglycemia was not prevented by glucose infusion after insulin administration. In this case, an increase in theta frequency activity accompanied the nadir in blood glucose concentrations (147). Theta rhythms are usually generated within the hippocampus (43, 154), a region with high densities of insulin receptors that plays a prominent role in memory formation. In rodent studies, insulin signaling in the hippocampus has been shown to promote cell survival and synaptic plasticity (13). Insulin binding may, in turn, influence EEG theta activity during complex task paradigms. The strong modulatory effect of insulin on (frontocortical) EEG activity paved the way for research on the effects of insulin on higher cognitive processes. One disadvantage of both EEG and MEG, however, is their limited spatial resolution. For deeper brain structures in particular, they provide only limited insight into the specific target regions involved in healthy insulin brain signaling. Recent advances in high field MRI have given us the opportunity to fill this knowledge gap by using blood-oxygen-level dependent contrast (BOLD) and cerebral blood

341 flow (by either MRI or PET) to indirectly measure neural activity with high spatial resolution
342 (for details, see supplementary text and Table 2).

343 ***Postprandial changes influence cerebral blood flow particularly in subcortical regions***

344 Early neuroimaging studies used PET to measure cerebral blood flow in response to hunger
345 and satiation when evaluating brain activity after a meal (86). These studies indicated that,
346 after administration of a meal to hungry subjects, neural activity decreases within the
347 limbic/paralimbic areas including the fusiform gyrus, striatum, thalamus and hypothalamus,
348 but increases in the prefrontal cortex (86). These in-vivo studies provided pivotal evidence for
349 the concept that, when controlling eating behavior, the homeostatic system of the human
350 brain does not act independently, but works in tandem with regions that belong to the
351 decision and reward circuitry for food intake control (for review see (28)). However, a
352 plethora of hormonal postprandial changes and increases in the availability of macronutrients
353 could be the underlying cause of these activation patterns. This is plausible, since the
354 reactivity of these regions correlated with changes in different hormones and metabolites
355 including insulin (86, 127, 345).

356 It is crucial that the role of insulin in the CNS regulation of eating behavior and cognition is
357 selectively studied. For this reason, functional magnetic resonance imaging (fMRI) studies
358 usually investigate brain insulin action by oral glucose ingestion, the clamp technique or
359 intranasal insulin application. To differentiate between metabolic and cognitive/task-specific
360 effects, insulin-stimulated brain activity is evaluated under spontaneous (resting-state)
361 conditions or in response to a particular task, thus recruiting different cognitive domains such
362 as memory. For further methodological details, please see supplementary section or Tables
363 1 and 2.

364 Studies in which global brain function was investigated using EEG and MEG showed that
365 higher cortical areas are particularly responsive to insulin. Moreover, PET studies revealed
366 that hormonal changes after a meal induce a specific activation pattern of the reward and

homeostatic system. The next section introduces the functions of target brain regions of insulin action, their involvement in healthy insulin signaling and their relationship to metabolic and cognitive functions (for overview, please see Figure 4).

b) ***Brain target regions of insulin action***

1) Hypothalamus

The hypothalamus has been extensively studied on account of its fundamental role in the physiological processes essential for survival and for controlling vital bodily functions. The latter include sleep, thermoregulation, food and fluid homeostasis, sexual behavior, stress, immune responses, autonomic and various endocrine functions (45, 304). Rodent models have provided us with a particularly detailed blueprint of the hypothalamic insulin-signaling pathway, revealing a profound regulatory influence of hypothalamic subregions in energy intake and feeding behavior (379, 393).

Hypothalamic responsiveness to peripheral signals, especially to glucose

Glucose-sensing neurons in the hypothalamus, which are usually found in the ventromedial hypothalamic nucleus, respond to local glycopenia by stimulating the release counterregulatory hormones such as growth hormone from the pituitary gland, glucagon from pancreatic islets, as well as epinephrine, and cortisol from the adrenal glands (35, 231, 297). Accordingly, rising glucose levels suppress the release of some of these hormones and parts of this effect are mediated through the hypothalamus. Although the small size and central position of the hypothalamus within the walls of the floor of the third ventricle has proved challenging for in-vivo studies in humans. Recently, fMRI has shed some light on the hypothalamic responsiveness to peripheral signals. As shown by a recent ultra-high field MRS study (241), the hypothalamus, particularly in the hypoglycemic state, responds with a persistent increase in cerebral blood flow (8, 268). This is potentially modulated by a local γ-Aminobutyric acid (GABA) drop (241). An increase in glucose levels during either an oral glucose load or after intravenous glucose administration results in a decrease in the

hypothalamic fMRI signal (164, 269, 330, 331). It is important to note that oral glucose ingestion lowers hypothalamic activity more effectively than glucose infusion, suggesting that other hormonal signals like incretins, are involved in this response (331).

Hypothalamic responsiveness to insulin

The hypothalamic response to insulin has been studied less extensively. For the intranasal route, a hypothalamic decrease of the BOLD signal and cerebral blood flow was observed 15 and 30 minutes after insulin application (165, 171, 216, 220). This was associated with whole-body insulin sensitivity (165, 171) and unfavorable fat distribution (220). Although animal models have yielded promising clues, the specific role of different hypothalamic nuclei in insulin signaling in humans remains obscure. Whereas the resolution of anatomical MRI is sufficient to distinguish lateral and medial subregions of the hypothalamus, their functional dissection is much more challenging. Recent efforts to investigate functional connections of the hypothalamus with fMRI suggest that the medial and lateral hypothalamus tap into different parts of the dopaminergic fronto-striatal circuitry of the brain, including projections to and from the striatal regions (218) (Figure 5). Furthermore, Page and coworkers (269) showed that glucose ingestion increases functional connectivity between the hypothalamus and the striatum, presumably via insulin.

2) Frontal cortex

The functional role of the frontal cortex in eating behavior

The prefrontal cortex can be divided into three subregions: lateral, orbital and medial/cingulate (124). The function of each prefrontal cortex subregion strongly depends on its connections since these are highly interwined with the brainstem, hypothalamus, thalamus, striatum and limbic system as well as with each other. By way of its afferent connections, particularly from the hypothalamus and amygdala, the prefrontal cortex receives

information about the internal state and the motivational significance of a stimulus, and plays an important role in the enactment of a certain kind of behavior (124). The lateral prefrontal cortex is of the utmost importance in cognitive function, including the inhibitory control of eating behavior (152). The orbitofrontal cortex and anterior cingulate cortex are essential for reward-based decision-making. The orbitofrontal cortex is a critical convergence zone for sensory information containing the secondary gustatory cortex and encoding the value, probability and magnitude of, for example, taste reward (296). The anterior cingulate cortex is involved in the motivational aspect of reward processing, reduces or increases the motivation to obtain rewarding stimuli such as palatable food. Studies investigating dieting revealed lateral prefrontal cortex activation to be a significant predictor for successful weight loss (87, 130, 160, 235). The orbitofrontal cortex and anterior cingulate cortex are particularly sensitive to an individual's internal state (i.e. hunger versus satiated) and the rewarding content of a food stimulus, and therefore respond more strongly to palatable food under fasting conditions (84).

Frontal cortex response to increasing insulin levels

Using the glucose clamp technique, glucose ingestion, and intranasal insulin administration, all prefrontal regions were shown to be significantly responsive to insulin across all modalities (140, 164, 165, 197, 213, 216, 217, 269, 270). Following oral glucose ingestion, the subject's endogenous serum insulin levels determined the reactivity of the prefrontal cortex and the anterior cingulate cortex to food cues. Individuals with a higher postprandial elevation in insulin showed a more pronounced frontal decrease (164, 213, 269). Similarly, exogenous intranasal insulin induced a decrease in the response of the prefrontal cortex to food cues (140) and a decrease in orbitofrontal cortex resting-state activity (216). This correlated significantly with whole-body insulin sensitivity (165). Insulin also plays an important role in the metabolism of the prefrontal cortex. In a euglycemic-hyperinsulinemic clamp study, neurometabolites were assessed by proton magnetic resonance spectroscopy in healthy young men (197). Interestingly, subjects with a high whole-body insulin sensitivity

showed improved neural metabolism in the frontal cortex after insulin stimulation. In particular, the ratio of N-acetylaspartate, a marker of neuronal density and integrity (301), increased after insulin infusion and significantly correlated with whole-body insulin sensitivity. This indicates that individuals with low insulin sensitivity have an impaired neuronal metabolism (197). Moreover, intranasal insulin increased brain energy levels (i.e. ATP) assessed by magnetic resonance spectroscopy to a degree that correlated with the subsequent reduction in food intake (191).

These recent findings indicate that the prefrontal cortex is particularly sensitive to increasing peripheral and central insulin levels. Based on the function and connections of the prefrontal cortex, it is tempting to speculate that healthy brain insulin signaling leads to an inhibition of food intake by reducing the rewarding properties of food and motivation for consumption via striato-prefrontal pathways (Figure 5). ***3) Striatum***

The functional role of the striatum in reward-mediated behavior

The striatum is generally associated with reward-motivated behavior, including the drive for food intake as promoted by the neurotransmitter dopamine. Impaired dopamine signaling in the striato-cortico pathways has been postulated to be the greatest overlap between obesity and addiction (352). The cortico-ventral striatal circuit, which includes the orbitofrontal cortex, the anterior cingulate cortex, the ventral striatum and parts of the midbrain, is at the center of the reward network. The cortico-dorsal striatal circuit, on the other hand, is associated with executive function and motor control and includes the dorsal striatum, temporal and prefrontal regions (142). Initially, reward processes in the ventral striatum prompt the motivation to repeat a certain behavior such as drug or food intake (352). The dorsal striatum is of special importance for the actual consumption of the reward (e.g. food) since its output to other cortical areas couples motivation with the motor responses required for goal-directed behavior (352, 381). These circuits work in concert to reach appropriate decisions, and to decide upon goal-directed actions such as, for example, the initiation and termination of a

meal. The hypothalamus is embedded in these dopamine-modulated cortico-striatal circuitries (218) that facilitate food reward (Figure 5).

The role of insulin in the reward circuitry of the brain

Hormones can directly influence dopaminergic striatal activity to stimulate or inhibit feeding. Insulin suppresses dopamine release by clearing the synapses of dopamine, thus reducing the rewarding properties of food (107, 108). Concomitantly, imaging studies revealed that striatal regions are responsive to changes in endogenous insulin (164, 213, 269), induced by both oral glucose ingestion and exogenous insulin (165, 312). Following glucose ingestion, the striatum showed a reduction in spontaneous neural activity and in response to food cue stimulation (213, 269), whereas intranasal insulin increased striatal cerebral blood flow (312). Furthermore, the reward circuitry may well act as a link to peripheral metabolism, as activity in the putamen, orbitofrontal cortex and insula correlated positively with enhanced peripheral insulin sensitivity two hours after intranasal insulin application (165). It is important to note, however, that the rewarding properties of the sweet glucose taste and ingestion itself could also be key players responsible for limbic activation, since the main cortical sensory input to the ventral striatum is via the orbitofrontal cortex and adjacent insula (142). The anterior part of the insula in particular is regarded as the primary taste cortex of the brain, contributing to the gustatory perception represented by taste, smell and the visual input of food. Higher insula activity is observed when more rewarding food items are perceived by an individual (for review see (114)). A number of studies probing insulin action identified the insula cortex as insulin-reactive, eliciting a decrease after glucose ingestion (213) and an increase after nasally applied exogenous insulin (165, 312), as well as during a hypoglycemic condition (270).

4) ***Hippocampus and neighboring gyri***

The hippocampus and its neighboring gyri, i.e parahippocampal and fusiform gyrus, are regions within the temporal lobe which are particularly important for memory formation.

497 Furthermore both the parahippocampal and the fusiform gyrus are linked to neural pathways
498 of recognition for visual scenes.

499 ***Insulin-mediated activity of the temporal/occipital cortex in response to visual cues***

500 By probing working memory using food cues, insulin modulates regions within the temporal
501 and occipital brain regions in particular. Studies with fMRI showed that the hippocampus and
502 its neighboring gyri respond to food cues by reducing activity after oral glucose ingestion
503 (164, 213) and intranasal insulin application (140). In line with the findings on increases in
504 EEG theta activity during insulin-induced hypoglycemia, there is also growing evidence that
505 insulin mediates hippocampal activity. Thus possibly affecting memory formation. Studies
506 investigating memory processes in obesity have further confirmed the importance of these
507 findings. Reduced memory performance in obese individuals is associated with neural
508 activity in temporal brain regions including the hippocampus (141, 160, 337). (Please see
509 section on brain insulin effects on cognition for more details on insulin's memory improving
510 properties). Furthermore, the fusiform gyrus is the most concurrently activated brain region
511 elicited by visual food cues (371), responding with increased activity to high as opposed to
512 low caloric food (221). Moreover, event-related potentials recorded by MEG in a visual
513 working memory task containing food and non-food images showed a clear categorization
514 effect in primary visual areas (338). Hence, the insulin-mediated effect in the visual system
515 could be specific to visual food-cue elicited brain response, which may in turn lead to
516 reduced visual attention to food cues in the postprandial state when insulin levels are high.
517 Further effects of insulin on visual memory tasks are discussed in the next section on brain
518 insulin resistance in obesity.

519 Neuroimaging studies investigating target brain regions of insulin action in healthy normal-
520 weight individuals identified the hypothalamus as well as the frontal and striatal regions as
521 particularly insulin-sensitive (see Figure 4). In response to complex tasks, mainly probing
522 memory, hippocampal and visual brain regions are additionally modulated by insulin, which
523 could contribute to a reduced attention to food cues. Healthy insulin signaling modulates

brain networks involved in homeostatic control, reward processing and cognitive control functions, thus influencing different aspects of human eating behavior. The majority of these studies investigated brain insulin action in healthy young men (approx. 25-30 years old). Possible sex and age effects on brain insulin action therefore still require investigation.

c) ***Effect of brain insulin action on eating behavior***

Central nervous insulin administration inhibits food intake and reduces body weight in humans

In accordance with animal experiments (226, 246, 278), central nervous effects of insulin on energy homeostasis partly oppose the peripheral impact of the peptide. Following intravenous or subcutaneous administration, insulin promotes gain of body weight in the form of muscle and fat (237, 300), i.e., it has anabolic properties. However, when administered to the brain via the intranasal or intracerebroventricular pathway in humans and animals, respectively, insulin acts in an anorexigenic fashion. At the same time, brain insulin might also promote anabolic processes in peripheral tissues (see next section). The effect on eating behavior in humans is, however, clearly hypophagic. Healthy young men were observed to consume fewer calories when they acutely received 160 U of regular human insulin via the intranasal pathway (25, 145). The same dose, when administered daily over a period of eight weeks, reduced body weight by 1.3 kg and body fat content by 1.4 kg, while

549 also decreasing waist circumference and leptin concentrations, in normal-weight male
550 participants (144) (Figure 6). These findings in humans corroborated respective results in
551 animals shaping the concept that central nervous insulin is a pivotal negative feedback signal
552 in the control of ingestive behavior (37, 63, 179).

553 ***Indicators of sex-specific insulin effects on eating behavior***

554 The anorexigenic effects of intranasal insulin administration were considerably more salient
555 in male in comparison to female subjects (25, 144). Accordingly, in animal experiments, male
556 rats decreased their food intake after intracerebroventricular insulin infusion and lost weight
557 after 24 h of treatment, whereas age- and weight-matched female rats remained unaffected
558 (63). Leptin administration yielded a reverse pattern, i.e. it exerted a stronger impact in
559 female rats (63). These sex differences might be related to respective differences in body fat
560 storage. The amount of visceral fat, which is correlated with whole-body insulin resistance, is
561 by average proportionally higher in men than in women. On the other hand, women have
562 more of the metabolically favorable subcutaneous fat than men (382). However, it remains
563 unclear as to what extent this differential pattern contributes to brain insulin sensitivity and
564 resistance in humans. Animal data also suggest that estrogen signaling modulates the
565 brain's sensitivity to the impact of insulin on food intake (62). However post-menopausal and
566 young women basically show comparable responses to acute intranasal insulin (214).
567 Interestingly, when administered after lunch intake, intranasal insulin intensifies satiety and
568 reduces rated palatability and consumption of chocolate cookies in healthy women (145)
569 (Figure 7). This suggests that (in women) prandial insulin secretion acts as a satiety signal
570 that contributes above all to the regulation of the reward-related ('hedonic') aspect of food
571 intake. It is still unclear as to whether this also holds true for insulin effects in men.
572 Nevertheless, this conclusion is supported by observations that intranasal insulin
573 administration changes activity of reward-processing brain circuitries (assessed in the fasted
574 state) in normal-weight women (216) (for further evidence from neuroimaging studies, see
575 above).

Insulin and olfactory function

Central nervous insulin effects on eating behavior might also be mediated via changes in olfactory function (185). Both hyperinsulinemia in the presence of fasting glucose levels (204) and intranasal insulin administration (40) impair the performance in a standardized test of olfactory function ("Sniffin' Sticks" task) in healthy men and women. However, in both studies, the odors presented were not related to foods. It therefore remains to be established whether the compromising effect of insulin on olfactory functions affects ongoing calorie intake.

In sum, the preclinical data available on the contribution of brain insulin signaling to eating behavior in humans suggest that intranasal insulin induces a reduction in energy intake and, therefore, a catabolic net effect.

d) ***Brain insulin effects on cognition***

Insulin administration improves declarative memory function

In parallel to the discovery that central nervous insulin administration has an impact on metabolic control in humans, a number of experimental studies provided evidence that the peptide moreover contributes to memory function. Particularly the formation of hippocampus-dependent memory contents is affected (131). (For more details on probing cognitive functions in humans, see supplementary material). The hippocampal formation is essential for the formation and storage of declarative memory traces, i.e. memory for facts and events that are accessible to conscious recollection (for review see (92)). Beneficial effects of (intranasal) insulin administration to the brain on memory in healthy subjects have been repeatedly reported (23-25, 202). In one of the first experiments, Kern and colleagues (202) found that infusing healthy men with a higher (15 mU/kg*min) rather than a lower dose of

602 insulin (1.5 mU/kg*min) in an euglycemic clamp lasting for 360 min induced a relative
603 improvement in their ability to remember word lists, especially food-related and emotional
604 words. In addition to metabolic parameters, the above-mentioned study on the subchronic
605 effects of intranasal insulin also tested declarative memory in 38 normal-weight, young men
606 and women before and after eight weeks of insulin treatment (160 U/d). Lists of 30 nouns
607 (e.g., tree, father, chocolate) were presented aurally and had to be recalled immediately
608 afterwards and again one week later (23). This delayed recall of words was enhanced by
609 insulin (words recalled, placebo 2.92 ± 1.00, insulin 6.20 ± 1.03, Figure 7) whereas
610 immediate recall, non-declarative memory (assessed by a wordstem-priming task) and
611 selective attention (assessed by the Stroop task) were not altered. Respective improving
612 effects on delayed word list recall, albeit at a generally lower performance level, were found
613 in obese men who were also treated with intranasal insulin over a period of eight weeks
614 (143) (Figure 8).

615 When the fast-acting insulin analog insulin aspart was intranasally administered to a group of
616 lean participants for eight weeks, they displayed even an enhanced improvement in delayed
617 word recall in comparison to the group who had received regular insulin (24). This superior
618 effect of insulin aspart might be attributed to the faster dissociation of its molecules from
619 hexamers into monomers and dimers (36). This process accelerates absorption of the
620 compound after subcutaneous delivery (196) and might also bring about improved and/or
621 increased permeation into the central nervous compartment after intranasal uptake.

622 ***Immediate insulin effects on memory function***

623 Intranasal insulin delivery can also induce acute improvements in memory function as
624 illustrated by the observation that healthy young women perform tasks on verbal working
625 memory (digit span) and hippocampus-dependent visuospatial memory (2-D object location)
626 better after receiving 160 U of intranasal insulin than after placebo (25). This improvement in
627 verbal working memory, a capacity mediated by frontal cortical areas (240), was likewise
628 observed in postmenopausal women (214) after the same insulin dose. Acute memory-

improving effects of intranasal insulin were also observed in young men who memorized associations between odors and object locations (41). Olfactory pathways directly project to cortical areas and influence emotional processing and memory formation via amygdala and hippocampus, respectively.

Potential mediators of insulin's memory-improving properties

The observation that both the olfactory bulb and the hippocampal formation express high amounts of insulin receptors (365, 402) provided first clues as to the mechanisms behind insulin's beneficial effect on declarative memory function. Experiments in differentiated cultures of hippocampal neurons harvested in rats indicate a punctuate pattern of the dendritic distribution of insulin receptors that is in accordance with synaptic localization (82, 403). Accordingly, insulin modulates synaptic plasticity in the hippocampus by stimulating processes of long-term depression and potentiation, both of which are assumed to confer the strength of a memory representation (103, 184) (e.g., ref. (223); for review see ref. (248)). Moreover, insulin signaling enhances synaptic plasticity by increasing synapse density in those brain regions that process visual input (55). Since sleep plays a crucial role in the formation of memory traces, particularly in the declarative memory domain (286), the role of central nervous insulin in the sleep-related formation of memory contents was investigated in healthy men and women (102). In that study, insulin did not alter the retrieval of memories learned before insulin administration and subsequent sleep, but impaired the acquisition of new contents in both the declarative and procedural memory systems on the evening of the subsequent day. This outcome suggests that sleep-associated memory consolidation is not a primary mediator of insulin's acute memory-improving effect, but that the peptide acts on mechanisms, which diminish the subsequent encoding of novel contents. Thus, insulin might benefit memory function in healthy humans by reducing the interfering influence of newly encoded information.

In its capacity as a growth factor, the peptide might also support neuronal survival (225) and trigger the release of neurotrophic factors from glial cells (106, 399). Neurotransmitter

656 systems with relevance for memory function, including norepinephrine and acetylcholine,
657 also receive insulinergic input (128). Although glucose supply of the CNS is generally
658 considered to be insulin-independent (101, 153, 319), insulin may also promote glucose
659 utilization in neuronal networks (e.g., (29)). In rodent experiments, hyperinsulinemia has
660 been observed to yield effects on glucose metabolism in structures such as the anterior
661 hypothalamus and the basolateral amygdala (95). Finally, intranasal insulin attenuates
662 cortisol secretion in normal-weight and obese participants (23, 143) and reduces the
663 response of the hypothalamic-pituitary-adrenal axis to stress (33), the overactivation of which
664 is known to compromise hippocampal function (for review see (261)).

665 ***Central nervous insulin and emotional regulation***

666 Evidence exists that central nervous insulin signaling affects not only cognitive function but
667 also emotional regulation. Lentivirus-mediated downregulation of hypothalamic insulin
668 receptors triggers depression- and anxiety-like behaviors in rats (135). Intranasal insulin
669 administration in mice not only enhances object-memory but also yields anxiolytic effects on
670 behavior (230). Interestingly, animals with diet-induced obesity and impaired glucose
671 tolerance did not show the respective effects (230), thus supporting the assumption that
672 obesity is associated with central nervous insulin resistance (see section VI). In experiments
673 in humans, 8-week intranasal insulin administration improved well-being and self-confidence
674 as rated on an adjective check list in normal-weight participants (23), whereas respective
675 effects in obese men were restricted to a slight reduction in self-rated feelings of anger (143).

676 Behavioral studies on the role of brain insulin action in cognitive function in humans indicate
677 an improving effect on short-term memory as well as on the long-term formation of memory
678 contents. These findings suggest that hippocampus-dependent memory processes benefit
679 particularly from insulin. Preliminary evidence moreover indicates that the peptide modulates
680 central nervous signaling pathways underlying emotional regulation.

681

682 **e) Effect of brain insulin action on peripheral metabolism**

683 The first animal models with genetically disturbed brain insulin signaling revealed that insulin
684 action in the brain has an influence on peripheral metabolism. Neuron-specific knockout of
685 the insulin receptor in mice not only caused obesity but also introduced whole-body insulin
686 resistance and hypertriglyceridemia (39). Over the following years, animal research
687 characterized both the involved neuronal structures and the peripheral tissues that are
688 regulated by insulin action in the brain (210). These include liver, skeletal muscle, and
689 adipose tissue. Insulin action in specific hypothalamic neurons suppresses endogenous
690 glucose production in the liver and leads to lower blood glucose (109, 259, 277). In skeletal
691 muscle, it promotes glucose uptake and storage as glycogen (69, 211, 275), which again
692 reduces blood glucose levels. In adipose tissue, brain insulin action inactivates hormone-
693 sensitive lipase, induces expression of lipogenic proteins, and suppresses lipolysis thereby
694 promoting energy storage in adipocytes (70, 211, 311). However, particularly for the liver, the
695 relevance of these findings for larger organisms has been questioned. In studies in dogs,
696 some of the results obtained in rodents could not be replicated (129, 285).

697 ***Effect of brain insulin action on peripheral insulin sensitivity as assessed in studies***
698 ***applying intranasal insulin***

699 Up to now, brain insulin signaling has not been characterized as thoroughly in humans as in
700 animals. However, accumulating evidence suggests that brain insulin action contributes to
701 the modulation of peripheral metabolism in humans. One study used intranasal insulin to
702 investigate the postprandial situation in 19 healthy young men. When insulin spray was
703 administered before a mixed meal, postprandial blood insulin levels were, despite
704 comparable plasma glucose concentrations, significantly lower after insulin than after
705 placebo spray administration (19). One possible explanation for this unusual observation is
706 that brain insulin action improved peripheral insulin sensitivity. In this case, a smaller amount
707 of circulating insulin would be needed to control blood glucose.

The contribution of brain insulin action to peripheral insulin sensitivity was first evaluated in a study with over 100 participants that used the homeostasis model assessment of insulin resistance (HOMA-IR) following intranasal insulin administration (165). HOMA-IR as an estimation of whole-body insulin sensitivity derived from fasting blood glucose and insulin concentrations (233). It thereby depends on plasma insulin concentrations. HOMA-IR is therefore difficult to interpret when measured directly after intranasal insulin administration, since small amounts of intranasally administered insulin are absorbed into the systemic bloodstream (19, 23, 125, 140, 165, 171). However, since insulin has a short biological half-life of less than 10 minutes (353), this index is useful with some delay after nasal insulin administration. At later points of time after nasal insulin application, HOMA-IR indeed indicated improved whole-body insulin sensitivity (165).

Moreover, Heni and colleagues (171) used the hyperinsulinemic-euglycemic glucose clamp (85) to assess peripheral insulin sensitivity more precisely. To maintain euglycemia in ten lean males, significantly more glucose had to be infused after intranasal insulin than after placebo spray (171). In this type of clamp experiment, higher glucose infusion rate indicates higher peripheral insulin sensitivity (85). Of note, the insulin-sensitizing effect observed after intranasal insulin administration persisted until the end of the experiment after 2 hours. However, when the experiment was repeated in overweight males, no effect of intranasal insulin was detected (171). This result may indicate that the relative brain insulin resistance in obesity (see section below) disrupts the modulating effects on peripheral insulin sensitivity and might contribute to whole-body insulin resistance such as is often found in obese humans. To address the underlying mechanisms, this study also quantified both brain insulin effects by fMRI and autonomous nervous system activity using heart rate variability. The effect of nasal insulin on the peripheral metabolism was correlated with both hypothalamic insulin effects and parasympathetic nervous system activity (171) (Figure 9). A spillover of small amounts of nasal insulin into the circulation was also reported in this study. However, the results did not change when the measured plasma insulin levels were taken into account by calculating insulin sensitivity indices. Nevertheless, further studies are required to

736 experimentally clarify the contribution of spillover insulin when it acts directly in the body
737 periphery.

738 ***Effects of brain insulin action on glucose metabolism in the fasting state***

739 While the above-mentioned study experimentally increased systemic insulin concentrations
740 to assess whole-body insulin sensitivity, three further studies on this topic were conducted
741 with nasal insulin under fasting insulin concentrations (78, 125, 267). Systemic
742 hyperinsulinemia, which is physiologically present after food intake, was absent in these
743 studies and one study even blocked portal insulin and glucagon by somatostatin infusion
744 (78). To further investigate whether intranasal insulin impacts systemic glucose metabolism,
745 Ott and colleagues studied lean men who underwent three experiments with repeated
746 intranasal spray application (every 15 minutes) for around six hours (267). In one session,
747 participants received placebo spray, whereas in the other sessions they repeatedly received
748 10U or 20U of the insulin analog aspart every 15 minutes, resulting in a total dose of 210U
749 and 420U, respectively. This paradigm of repeated intranasal administration of insulin aspart
750 caused a decline in blood glucose, a decrease in the circulating levels of endogenous insulin,
751 and an elevation of the counter-regulatory hormones cortisol and growth hormone.
752 Exogenous insulin (i.e., insulin aspart) also permeated into the circulation after repeated
753 intranasal administration. When this spillover of intranasal insulin was mimicked by iv
754 administration of insulin aspart, a comparable reduction in blood glucose was observed. This
755 indicated that intranasal insulin delivery to the CNS had no net impact on basal glucose
756 levels in this study (267). Nevertheless, since no clamp was performed in that particular
757 study, subtle alterations in insulin or glucagon secretion might have emerged and
758 superimposed regulatory effects of the brain (97). Dash and coworkers (78) applied what is
759 known as a “pancreatic clamp”, in which both portal insulin and glucagon are blocked by
760 somatostatin and systemically replaced at fasting concentrations (83). This study (78)
761 quantified endogenous glucose production by tracer dilution technique after nasal application
762 of the insulin analog lispro versus placebo in eight lean men. To mimic spillover, small

amounts of insulin lispro were administered intravenously concurrently with the placebo spray. Three hours after nasal insulin lispro application, endogenous glucose production was seen to be markedly suppressed and more glucose had to be infused to maintain euglycemia. This reaction was not observed after placebo spray (78). These results show that brain insulin action contributes to the regulation of endogenous glucose production, also in a later postprandial state (79). The interpretation of these two clinical studies is somewhat complicated by the fact that both used insulin analogs. While this approach facilitates the differentiation between exogenous and endogenous insulin in the circulation, insulin analogs might not necessarily induce the same brain responses as human insulin. In fact, previous experiments in humans have yielded evidence that insulin analogs, unlike human insulin, can induce stronger brain effects (for insulin aspart, see (24), for insulin detemir, see (146, 359)).

Effects of brain insulin action on the liver

Gancheva et al. (125) also assessed endogenous glucose production by tracer dilution technique in ten lean persons and ten overweight patients with type 2 diabetes. This study differed from that of Dash and coworkers (78) in that it did not infuse somatostatin. Endogenous insulin and glucose were therefore not blocked. Furthermore, instead of an insulin analog, Gancheva et al. (125) used human insulin as a nasal spray. The experiments ended three hours after spray administration, which might explain why this work failed to replicate the suppression of endogenous glucose production reported by Dash et al (78). However, the study used MRI techniques to investigate liver metabolism and liver fat content. In the group of lean subjects, intranasal insulin lowered liver fat content significantly, while a bolus of iv insulin to mimic insulin spillover actually increased liver fat. This suggests that peripheral and central insulin effects on liver fat might oppose each other. Further studies are required to clarify which effect predominates under physiological conditions. Furthermore, the study detected an increase in hepatic ATP synthesis after nasal insulin, indicating that higher mitochondrial activity is a possible mechanism underlying the liver fat findings. The effect was found on neither liver fat nor ATP in the group of obese type 2 diabetes patients.

790 ***Effects of brain insulin action on lipolysis***

791 Besides postprandial glucose control, animal research has also identified adipose tissue
792 metabolism as a further target of brain insulin action (70, 211, 311). The peripherally and
793 centrally mediated effects of insulin on adipose tissue appear to converge to play a joint
794 anabolic role. Peripheral insulin enhances fat storage by inducing de novo lipogenesis and
795 by inhibiting lipolysis in white adipose tissue (100, 339). Central nervous insulin similarly
796 reduces lipolysis and increases lipogenesis in animals (100, 211, 310). Accordingly, the
797 intranasal administration of 160 U insulin to healthy young men acutely suppressed the
798 circulating concentrations of free fatty acids and the rate of appearance of deuterated
799 glycerol (an estimate of lipolysis), without altering lipolytic protein expression in
800 subcutaneous adipose tissue (190). The observed anti-lipolytic effect of intranasal insulin
801 was confirmed in an independent sample of subjects, yielding a cumulative group size of 41
802 participants. Although adipose tissue is highly sensitive to even small alterations in insulin
803 levels (192), the slight spillover of intranasal insulin into the bloodstream was not
804 experimentally controlled for in that study. However, the detected reaction was statistically
805 independent of the circulating insulin levels. If and how obesity interacts with effects of insulin
806 on adipocyte metabolism has not yet been studied in humans. Moreover, since neither of the
807 recent studies that mimicked insulin spillover by an iv insulin bolus (78, 125) nor a study
808 under systemic hyperinsulinemia (170) has detected effects of nasal insulin on lipolysis, the
809 physiological contribution of brain insulin action to adipose tissue function requires further
810 investigation.

811 **Effect of brain insulin action on thermogenesis, blood pressure and locomotor activity**

812 Studies in mice have shown that central nervous insulin signaling increases sympathetic
813 nervous system outflow to brown adipose tissue (283) and inhibits warm-sensitive neurons
814 (303). Intranasal insulin, on the other hand, acutely enhances postprandial thermogenesis in
815 healthy men (19). Related studies suggest that insulin-induced sympathoexcitation (80, 251)
816 may also trigger increases in blood pressure (5, 201). Fittingly, acute intranasal

administration of 240 U insulin over a period of 120 min to healthy, normal-weight men slightly increased diastolic and mean arterial blood pressure compared to placebo, suggesting transient changes in the baroreflex set point. By contrast, eight weeks of intranasal insulin administration, as described above, had no effect on blood pressure (21). This suggests that potential clinical applications of long-term intranasal insulin delivery are not associated with (adverse) effects on blood preasure. Moreover, murine studies indicate that intracerebroventricular insulin administration enhances locomotor activity (172). Although this effect has not yet been investigated in humans, it might add to the catabolic impact of intranasal/central nervous insulin administration. Interestingly, obese mice did not increase their physical activity after brain administration of insulin whereas their normal-weight counterparts did (172). This is in accordance with the concept that central nervous insulin resistance is a pathophysiological trait in metabolic disorders.

Studies on the effect of brain insulin action on peripheral metabolism support the hypothesis that following food intake, insulin from the pancreas reaches the brain via the bloodstream. It then activates specific brain regions including frontal areas and the hypothalamus. By turn, brain-derived signals may use autonomic outflows to improve peripheral insulin sensitivity and to alter metabolic function in peripheral tissues. The latter promotes the postprandial storage of nutrients, suppresses endogenous glucose production, and regulates hepatic energy metabolism. Moreover, postprandial energy expenditure via thermogenesis is increased by insulin. While the brain-derived effects on peripheral insulin sensitivity under systemic hyperinsulinemia (171) and on liver fat (125) might be more rapid, the effect on endogenous glucose production under fasting insulinemia might be considerably delayed (79). Further research is required to account for these differences and to determine the importance of each of these findings for human physiology. Furthermore, animal research postulates that, in addition to peripheral insulin sensitivity, the brain modulates two further crucial mechanisms in the regulation of blood glucose. These are insulin secretion from pancreatic beta cells (49, 52, 272, 293, 398) and glucose effectiveness, i.e. the insulin-independent uptake of glucose into tissues (316). Since neither of these two mechanisms

has been investigated in humans to date, it should be addressed in further studies. These physiological effects of insulin action on metabolism, eating behavior and cognition are summarized in Figure 10.

IV. Brain insulin resistance

a) Brain insulin resistance in obesity

Due to the strong link between obesity and peripheral insulin resistance, the contribution of obesity-associated factors to brain insulin resistance is particularly worthy of investigation. To this end, neural activity in response to insulin is compared between normal-weight and overweight and obese persons. ***Diminished brain insulin action in higher cognitive brain regions in obesity***

In addition to alertness and attentiveness, behavioral paradigms capturing the neural signature of memory-related processes revealed enhanced cortical activity in response to increasing insulin levels, with an attenuated or even diminished response in overweight and obese individuals. More specifically, the first study to evaluate brain insulin resistance in obese adults via MEG showed that spontaneous and stimulated cortical activity within the beta and theta frequency band increased during a hyperinsulinemic euglycemic clamp in normal-weight but not in obese participants (362). Increased theta activity was associated with enhanced memory and improved cognitive performance (308), which might partly explain memory-enhancing effects of insulin. Furthermore, when compared to normal-weight individuals, obese patients demonstrated lower memory performance along with enhanced prefrontal cortex activity to achieve a simple one-back memory task (160, 337). Failure to modulate theta activity, together with the increased cognitive effort to perform memory tasks, could be a predictor for cognitive dysfunction in obesity as age increases (138). Again using MEG, increased evoked potentials were observed to food stimuli in higher visual brain areas after intranasal insulin in the fusiform gyrus. Here again, this applied to normal-weight but not overweight individuals (141). Apart from MEG studies, insulin-mediated changes within the

visual system were reported using fMRI, indicating that the fusiform gyrus in particular is insulin-resistant in obese individuals (164). On the basis of the intrinsic state of an individual (i.e. hunger versus satiated), a dissociable activity pattern emerges in the fusiform gyrus of normal-weight insulin-sensitive individuals. This pattern showed reduced attention to high caloric foods in the postprandial state with increased insulin levels (213). Similarly, in the fasting state, the fusiform gyrus and surrounding regions are known to track the energy value of food by responding to high caloric foods with an increase in activity and functional connectivity within visual networks (115, 164, 205, 221, 326, 351). The relationship between peripheral and central insulin resistance is such that subjects can also be stratified according to their peripheral insulin sensitivity instead of using a continuous correlative measure. By means of FDG-PET, peripherally insulin-sensitive men showed an increase in prefrontal cortex and striatum glucose metabolism during a hypinsulinemic euglycemic clamp, while the insulin-resistant men displayed a reduced response (6). The same applied to women suffering from polycystic ovary syndrome (PCO), a disease accompanied by peripheral insulin resistance. Only the insulin-sensitive PCOS patients showed a significant prefrontal cortex and striatal inhibition after glucose ingestion in response to food pictures (376). This was also observed for normal-weight as opposed to obese individuals in response to glucose (164) ingestion and intranasal insulin (220).

Brain insulin resistance is associated with success of life-style intervention

Working memory-related activity of the fusiform gyrus and prefrontal cortex were predictive for the outcome of a lifestyle intervention study (160). Here, individuals who reduced their BMI by approximately 7% after a 6-month dietary intervention showed increased activity in the fusiform gyrus during a working memory paradigm using food stimuli. Non-responders, by contrast, showed an increase in prefrontal cortex activity. In agreement with these results, our group (361) identified a significant relationship between insulin-stimulated theta activity using MEG and the amount of weight lost, as well as a reduction in the metabolically unhealthy visceral adipose tissue (VAT) during lifestyle intervention. The greater the brain

insulin response prior to the lifestyle intervention, the more weight and VAT was lost by an individual for up to 2 years after the intervention (Figure 11D). Interestingly, the insulin-stimulated hypothalamic response was also compromised in obese individuals (232), with partial reversibility after massive reduction of body weight (369). Depending on the amount of VAT, intranasal insulin strongly reduced the hypothalamic cerebral blood flow as measured by fMRI (220) (Figure 11E). However, overweight and obese subjects with high VAT failed to show this reduction in neural activity (220), indicating a relationship between brain insulin resistance and metabolically unfavorable abdominal adiposity (336) (Figure 11). It is worth bearing in mind that, independent of visceral and liver fat, high levels of circulating saturated nonesterified free fatty acid were associated with diminished insulin effects on theta band brain activity, suggesting that nonesterified free fatty acids are independent predictors of brain insulin resistance (360). Hence, soluble factors such as fatty acids derived from visceral fat could be one cause of cerebral insulin resistance, which may then aggravate cerebral dysfunction.

Insulin-mediated brain function in morbidly obese individuals and after bariatric surgery

In morbidly obese individuals, the dorsal striatal regions in particular react strongly to insulin that has been elevated by a hyperinsulinemic euglygemic clamp (257). This insulin-stimulated increase in glucose metabolism, measured by FDG-PET, is reversed after bariatric surgery (364). Functional connections of the cortico-striatal network, which are important for food reward (369) also showed a normalization of insulin-mediated brain function after bariatric surgery. Failure to activate the lateral prefrontal cortex in response to high caloric foods correlated significantly with the insulin-mediated response of the dorsal striatum, giving the cortico-striatal brain network a prominent role in morbid obesity (257). The prefrontal cortex, the striatum and the hypothalamus are key players in this network. Besides the hypothalamus, the prefrontal cortex seems to be particularly prone to insulin resistance. The insulin-stimulated prefrontal cortex response correlates significantly with

peripheral insulin sensitivity assessed by an oral glucose tolerance test (oGTT). A negative correlation between prefrontal cortex activity and insulin levels was identified in insulin-sensitive individuals (220). Furthermore, the insulin-induced activation pattern correlated positively with measures of cognition related to eating behavior. Hence, individuals susceptible to uncontrolled eating, together with a craving for food, showed insulin resistance in the prefrontal cortex. Similarly, as assessed by a hyperinsulinemic euglycemic clamp (362) insulin-stimulated theta activity was positively correlated with peripheral insulin sensitivity.

Studies investigating brain insulin action in obesity revealed an attenuated or even diminished response in overweight and obese individuals to both endogenous and exogenous insulin stimulation. The hypothalamus, fusiform gyrus, striatal regions and prefrontal cortex seem to be particularly vulnerable to obesity-associated insulin resistance (see Figure 4 for details on insulin-sensitive regions). At present it is unclear as to whether brain insulin resistance is a cause or consequence of obesity. Nonetheless, these studies show that brain insulin resistance is highly relevant for peripheral metabolism and eating behavior.

b) Brain insulin resistance and the influence of obesity- and diabetes-related risk genes

Insulin receptor substrate (IRS-1)

The first common single nucleotide polymorphism (SNP) found to be associated with the brain responds to insulin is located in the *IRS-1* (insulin receptor substrate-1) locus. Together with its isoforms, this adapter protein couples the insulin receptor to its signaling cascade and is therefore crucial for molecular signal transduction when the insulin receptor is activated (344). One polymorphism in this locus, SNP rs1801278, introduces a Gly927Arg amino-acid exchange, thereby impairing the insulin signaling cascade (4). Initially identified as a diabetes-risk polymorphism (321), this SNP also determines the brain's response to the hyperinsulinemic euglycemic glucose clamp as assessed by MEG (as described in section III

951 on insulin effects on global brain function). While beta-activity in non-risk allele carriers
952 responded to the increased insulin levels, a diminished response was observed in risk-allele
953 carriers. This is indicative of brain insulin resistance (362).

954 ***Fat-mass and obesity-associated gene (FTO)***

955 Common variation in the *FTO* gene is the strongest genetic determinant for an increased
956 BMI, explaining differences of up to 3 kg body weight (118). By predisposition to obesity and
957 therefore to peripheral insulin resistance, variation in the gene region also increases the risk
958 for type 2 diabetes (93, 118). In humans, the increased body weight is driven by an increase
959 in food intake rather than in energy expenditure (48, 156, 250).

960 On the cellular level, recent research proposed that *FTO* obesity-risk variation is associated
961 with altered mitochondrial function and thereby with thermogenesis in adipose tissue (59).
962 This seems to be regulated via the functional connection with the distant genes *IRX3/5*. The
963 latter might be responsible for the weight effect rather than the *FTO* gene product itself (59,
964 282, 332). It is still not completely clear how these experimental findings relate to the
965 observations of food intake effects in multiple clinical studies (174, 334). It is possible that
966 adipose-tissue derived signals reach further organs to modulate function there. Specialized
967 neuronal subpopulations might also be affected by alterations of *IRX3/5* in *FTO* risk allele
968 carriers. Indeed, obesity-risk polymorphisms in *FTO* are associated with expression levels of
969 *IRX3* in the human brain; the gene transcript potential responsible for *FTO* associations
970 (332). However, such mechanisms have not yet been conclusively tested beyond the
971 hypothalamus.

972 In humans, carriers of the *FTO* obesity risk allele show an attenuated satiation response after
973 a meal, increased food intake and impulsivity (57) and cognitive restraint (71), indicating
974 distinct differences in eating behavior. *FTO* is highly expressed within the brain, where
975 expression levels are regulated by food intake (119). In terms of anatomy, healthy elderly risk
976 allele carriers show reduced frontal (57) and occipital brain volume (177). Also functionally,

977 the FTO risk allele affects brain areas important for reward processing and food-cue
978 reactivity (215, 263, 389). Moreover, brain insulin reactivity is strongly attenuated in *FTO* risk
979 allele carriers (167, 198). In the postprandial state, neural food-cue reactivity showed a
980 pronounced reduction in prefrontal regions (167) and reward-associated brain regions such
981 as the striatum in *FTO* carriers using fMRI (198). While our group (167) found differences in
982 the postprandial state only, Karra et al. (198) also identified changes within the reward
983 system in response to food cues in the fasted and fed state. This indicates that the nutritional
984 status plays an important role in *FTO*-associated brain insulin resistance. The importance of
985 insulin sensitivity in the reward system was underscored by a recent study investigating an
986 interaction between the *FTO* gene and the dopamine D2 receptor gene *ANKK1*. A common
987 polymorphism in this locus determines the D2 receptor density (194). In rodent models, the
988 variation of the *FTO* gene has been shown to influence dopamine signaling such that a loss
989 of *FTO* selectively influences reward sensitivity and food intake in dopamine neurons (175).
990 Risk allele carries of the *ANKK1* gene polymorphism show reduced D2 receptor density,
991 have an increased risk for substance abuse (66), attenuated neural response to palatable
992 food (104), and difficulties in losing and maintaining body weight (297, 395). Furthermore, the
993 *FTO* risk allele influenced D2 receptor dependent behavior and brain reward responses in
994 interaction with the *ANKK1* variation (322). Regarding brain insulin action, the association of
995 the *FTO* SNP rs8050136 in the striatum depends on dopamine D2 receptor density as
996 determined by the *ANKK1* polymorphism rs1800497. Carriers of both risk alleles have an
997 exaggerated striatal response to intranasal insulin, as well as increased body fat and
998 reduced peripheral insulin sensitivity (168). All in all, this suggests that carriers of both risk
999 alleles have an increased risk for obesity and type 2 diabetes. Moreover, insulin-stimulated
1000 beta activity as assessed by MEG was reduced in *FTO* allele carriers during a
1001 hyperinsulinemic clamp (362, 363). The *FTO* and *IRS1* polymorphisms affected the beta
1002 frequency in particular. Until recently, this was regarded as being related mainly to motor-
1003 related processes. However, several studies have shown that beta band activity is largely
1004 involved in attentional processing (99). Although the reduced insulin induced change for the

risk carriers may point to specific changes in the attentional control system, this assumption is open to further investigation.

Melanocortin receptor 4 (MC4R)

One important receptor for cell-to-cell signaling in specific hypothalamic neurons is the melanocortin receptor 4 (MC4R). Following activation of the insulin receptor, the anorexigenic POMC-neurons release α-MSH, a peptide that activates the MC4R in secondary neurons. Genome-wide association studies have confirmed that polymorphisms in the locus encoding for MC4R are associated with increased BMI, affecting energy homeostasis and peripheral insulin sensitivity (186, 249). One study in humans suggests that the MC4R polymorphism rs17782313 associates with impaired insulin action on theta brain activity (357). Notably, the long-term (6-week) intranasal administration of MSH/$ACTH_{4-10}$ reduced body weight and fat mass in normal-weight but not in overweight subjects (148). This indicates that overweight, in combination with brain insulin resistance, is also associated with reduced sensitivity to relevant central nervous downstream signals of insulin.

Cannabinoid receptor 2 (CNR2)

The endocannabinoid system is a regulatory network that contributes to the control of body weight, food intake, and whole-body energy metabolism. This system is also known to mediate some of insulin's metabolic effects (90). The endocanabinoids are a group of specialized lipids that usually transmit via two receptors (CNR1 and CNR2). Whereas the role of CNR1 in the brain was established some time ago (394), the expression of *CNR2* was only recently identified in different brain cells and regions (264). However, carriers of SNP rs3123554 in the *CNR2* gene showed reduced brain insulin sensitivity, which was indicated by attenuated insulin stimulated theta activity (203).

The aforementioned obesity and diabetes risk gene carriers showed an attenuated insulin-mediated response in beta and theta band brain activity as assessed by MEG and attenuated food-cue reactivity in the postprandial state in prefrontal and reward associated

brain regions. This indicates that each genetic determinant for brain insulin resistance encompasses different neuronal systems. Since all these studies were carefully matched for BMI, sex and age, a genetically determined brain insulin resistance may be proposed. None of these genetic associations has yet been replicated in another cohort, presumably due to the complex techniques involved to quantify brain insulin action in humans. Such a replication is particularly necessary to verify results from hypothesis-free approaches.

c) Brain insulin resistance in type 2 diabetes

Hypothalamic insulin resistance in T2D patients is normalized after dietary restrictions

So far, only very few studies have investigated the effect of insulin resistance in T2D on the homeostatic system of the brain controlling metabolism. By means of oral glucose ingestion, endogenous stimulated insulin typically induces a profound decrease in the hypothalamus (232, 330, 331). However, T2D patients fail to show this inhibitory hypothalamic response. In view of the fundamental role of the hypothalamus in energy homeostasis (378), this may contribute to the metabolic imbalance in these patients. Nonetheless, a very low caloric diet over a period of four days normalized the hypothalamic responsiveness to glucose ingestion in T2D patients (162, 346). This shows that short-term caloric restriction can be beneficial. The mechanism responsible for this hypothalamic normalization could be based on both glucose and insulin sensing neurons. Teeuwisse et al. (346) observed no significant correlation between the peripheral increase in insulin and the hypothalamic response to glucose ingestion. This could be because the strong reaction of the glucose-sensing neurons masks the insulin-mediated effects. However, studies using the intranasal approach, which results in higher cerebral insulin concentrations, showed an insulin-mediated attenuation in hypothalamic activity in non-diabetic individuals (165, 216, 220). Caloric restriction could therefore lead to an increased sensitivity to hypothalamic glucose or insulin sensitive neurons. However, whether or not this normalization can be maintained in T2D patients has not yet been investigated. T2D patients who manage to adhere to their dietary restrictions on a more long-term scale show an enhanced response to food cues in reward associated brain

regions. They therefore tend to be more successful in resisting the temptation to eat foods that are high in carbohydrates and fat (51).

Brain insulin resistance in higher cognitive functional networks in T2D

Functional connectivity is used for the characterization of brain networks in health and disease. Regions in the brain in which a consistent pattern of synchronous activity is found reflect a functional network (31, 42). In T2D patients, the disruption of functional connectivity is related to the severity of peripheral insulin resistance as well as of cognitive performance (54, 252, 400). Functional connectivity between precuneus/posterior cingulate and frontal regions (Figure 3) was negatively associated with HOMA-IR (54, 252) and positively associated with verbal fluency performance (54). The latter reflects semantic memory performance, which is a prominent characteristic in T2D cognitive dysfunctions (9, 10). Moreover, in T2D patients, less efficient executive functions, as assessed by the trail-making tests, and the degree of insulin resistance are related to reduced interhemispheric functional connectivity in the temporal cortex (400). Interestingly, increasing CSF insulin by the administration of intranasal insulin normalizes these functional connectivity alterations. A single dose of 40U of insulin in older T2D patients acutely increased functional connectivity between the hippocampus and frontal regions, restoring complex neural networking that are important for higher cognitive functions (132, 401). In addition, verbal fluency and visual spatial memory tended to be higher after intranasal insulin application (256). In T2D subjects, who had received intranasal insulin, better cognitive performance correlated with the enhanced functional connectivity between the hippocampus and frontal regions (401). Intranasal insulin may therefore restore functional connectivity in higher cognitive regions, thereby improving memory and executive function.

Effect of intranasal insulin on cerebral blood flow in T2D

Intranasal insulin also enhanced regional perfusion in the insular cortex (256, 312). Similar to dementia patients, T2D individuals show reduced cerebral blood flow and cerebrovascular reactivity, particularly with advanced complications (38, 255). However, since the effects do not remain significant after correction for atrophy, the cerebral blood flow reduction could be related to brain atrophy (38, 302). Likewise, Rusinek et al. (298) found reduced cerebral blood flow in insulin resistant subjects but not in treated T2D individuals. This could be related to the efficient antidiabetic drug treatment. Nonetheless, intranasal insulin was able to increase cerebral blood flow (256, 312) and to vasodilate the middle cerebral artery, which is in accordance with the enhanced cognitive performance in T2D subjects (256).

So far, only a small number of studies have investigated brain insulin action in T2D. These have mainly identified brain insulin resistance in those brain regions that are relevant for cognitive function. Since communication within and between brain hemispheres is essential for intact cognitive functions, measures of functional connectivity in T2D are of particular interest. Indeed, exogenous application of insulin to the brain is able to restore functional connectivity between the hippocampus and frontal regions. However, further studies are necessary to comprehend the development of brain insulin resistance in T2D. Currently, little is known as to whether other target regions of insulin action are prone to insulin–resistance in T2D and whether obesity-associated brain insulin resistance can be differentiated from T2D-related brain insulin resistance.

d) ***Brain insulin resistance and gestational diabetes***

The developmental aspects of brain insulin resistance are largely unknown. However, it is well-established that the metabolic and psychological status of the pregnant mother can strongly influence the metabolic and cognitive development of the offspring. Gestational diabetes is therefore an interesting model for the study of brain insulin resistance. Typically, in insulin resistance, mothers with gestational diabetes expose the fetus to a hyperglycemic state, since glucose is transported to the fetus through the placenta. It is important to note, however, that maternal insulin does not cross the placenta. Nonetheless, the fetus itself

reacts to the increased glucose levels with increased insulin release. Due to the maternal hyperglycemic condition, this puts the fetus into a hyperinsulinemic and hyperglycemic state. This was already proposed by Pedersen (in 1952) to cause fetal macrosomia (274). Furthermore, the offspring of mothers with gestational diabetes have higher amniotic fluid insulin levels in utero as well as higher insulin and C-peptide levels in umbilical cord blood at birth (384). Since increased insulin resistance is present at birth, fetuses probably develop impaired insulin action while still *in utero* (46).

Gestational diabetes and its long-term risks for mother and child

Although gestational diabetes usually disappears immediately after birth, it is nevertheless associated with several adverse effects for both mother and child. A mother suffering from gestational diabetes has a higher risk of developing type 2 diabetes in later life (207). For the newborn offspring, there are immediate risks such as macrosomia, large for gestational age, perinatal mortality and cesarean delivery (136). Importantly, the maternal environment during gestational diabetes also increases the risk of the offspring for obesity and type 2 diabetes in later life (333). This increased risk is independent of genetic or environmental background (77). The detailed molecular mechanisms of such metabolic changes in the offspring of mothers with gestational diabetes have still not been well investigated. However, recent epidemiological and experimental data suggest that elevated insulin levels during perinatal life directly program the development of obesity and diabetes (117). Epigenetic mechanisms, which are labeled as a phenomenon of fetal programming (276) are therefore liable to play a very important role. As shown in animal studies, the change of insulin action in fetuses of diabetic mothers affects not only the peripheral tissues, but also the development of the fetal central nervous system (139, 380). These studies mainly investigated hypothalamic changes and pointed out the adverse anatomical and functional effects of the hyperinsulinemic state.

Investigating the effect of maternal metabolism on the fetal brain using MEG

Fetal magnetoencephalography (fMEG) and magnetic resonance imaging are the two methods by which the development of the human fetal brain can currently be assessed. As already mentioned, MEG is a non-invasive method by which biomagnetic fields can be assessed in the framework of studies on brain activity. Since the magnetic signals generated in the fetal brain can be recorded at the abdomen of the mother, this technique can also be used to record fetal neuronal signals directly (279). In the last trimester of gestation, the fetal brain is mature enough to respond to external stimuli and show distinctive spontaneous activity. Auditory stimulation is well suited to the investigation of functional fetal brain development. From around the 20th week of gestation, the fetus reacts to external sounds. It is therefore possible to record auditory evoked fields from this point onwards. Since the latency of the evoked fields decreases over gestation, it is used to assess the functional maturation (178). In a group of healthy pregnant women, the latency of the fetal auditory response decreases after a glucose challenge to the mother using an oGTT. In this study, the fetal auditory responses were recorded before, and 60 and 120 Minutes after glucose ingestion. A significant latency decrease was observed 60 minutes post ingestion, whereas baseline levels were reached again after 120 minutes. The group of mothers was split according to their maternal insulin sensitivity (median split of HOMA-IR) for further analysis. Interestingly, the insulin-sensitive group showed a stronger decrease in latency (228) (Figure 12). In a follow-up study, fetal responses in pregnant woman with gestational diabetes were investigated. One hour after a glucose challenge, a decrease in latency was observed in the fetuses of non-diabetic mothers. However, under baseline conditions, there was no difference in latency between fetuses of diabetic and non-diabetic mothers (229) (Figure 12). If the mother had gestational diabetes, no endogenous insulin-induced decrease in latency was observed in her fetus. This indicates that the metabolic status of the mother interacts with the functional organization of the fetal brain. Under normal metabolic conditions, endogenous increase in glucose and insulin induces a latency decrease, which does not occur in mothers with a hyperinsulinemic and hyperglycemic state. Interestingly, no

difference was observed in the baseline between the two groups until a challenge corresponding to a postprandial state was performed.

The metabolic and psychological condition of the pregnant mother can strongly influence the development of the offspring. Mothers with gestational diabetes expose the fetus to a hyperinsulinemic and hyperglycemic state. This also increases the offspring's risk of developing obesity and type 2 diabetes in later life. By virtue of non-invasive imaging, and using latency of auditory evoked responses as a measure of functional maturation, fetal neural signals can be recorded directly to investigate functional fetal brain development. Indeed, the latency of the fetal auditory response decreases after glucose challenge in healthy mothers, while insulin resistance and gestational diabetes diminishes such an insulin-induced response in the fetus. However, further studies are required to determine the functional significance of this effect and its possible outcome on long-term brain development.

e) Brain insulin resistance in normal aging

Aging has been associated with peripheral insulin resistance and the maintenance of insulin sensitivity has been observed in familial human longevity and centenarians (for review see (3)). As indicated in post-mortem brain studies, both cortical insulin levels as well as insulin receptor binding decrease as age increases (123). In comparison to healthy controls, AD brains displayed lower insulin signaling and insulin receptors (294, 335). Besides the direct effect on brain insulin signaling and receptor expression, the transport of insulin from the periphery to the brain might be responsible for brain insulin resistance in the elderly. Altered blood-brain barrier function for carrier-mediated transport systems have been observed in aging animals and humans (323). The ratio CSF/serum insulin is markedly related to whole-body sensitivity. A reduced ratio in insulin resistant individuals provides evidence of an altered transport of insulin across the blood-brain barrier (169, 306). Furthermore, by

administering insulin directly into the ventricles, bypassing the blood-brain barrier, it was possible to improve its action in aging mice (306).

Brain insulin resistance with increased age in response to food-cues

In middle-aged adults, food-cue elicited brain activity in response to a meal diminishes with increasing age (50). This may reduce the satiety effect while increasing the risk for obesity in middle-aged adults. Concomitantly, increasing insulin levels by means of a hyperinsulinemic clamp failed to modulate beta activity evaluated by MEG with increased age, possibly resulting in increased attention towards food cues in the postprandial state (358).

AD-like brain alterations in aging individuals with insulin resistance

Even in older individuals without dementia, insulin resistant individuals and T2D patients showed more medial temporal lobe atrophy particularly in the hippocampus and amygdala (88). Moreover, the Leiden Longevity study reports microstructural brain changes with insulin resistance as assessed by oGTT. This is indicative of a loss of homogeneity of brain tissue with increasing age in insulin resistant individuals. Following glucose ingestion, increasing plasma glucose and insulin levels appear in an AD-like pattern in cognitively healthy adults (11, 189). In insulin sensitive participants, a reduction in FDG uptake and cerebral blood flow (hypometabolism) was observed in AD-vulnerable regions. These regions include the frontal cortex, lateral parietal cortex and precuneus (189) (Figure 3). Similarly, in older patients with prediabetes and diabetes, peripheral insulin resistance correlated with the AD like pattern of reduced FDG uptake in the above-mentioned brain regions (11). Moreover, the Baltimore Longitudinal Study of Aging revealed that, in impaired glucose tolerance individuals, the cerebral blood flow declined more quickly with age (349). The population-based Mayo Clinic Study of Aging showed that an FDG-hypometabolism in AD brain regions was more common in older diabetes patients (295). These results suggest that high circulating insulin and insulin resistance are important contributors for neurodegenerative disease.

Disrupted functional connectivity in AD-vulnerable regions

A loss in functional connectivity within the default-mode network has been observed in patients suffering from dementia, T2D and obesity (Figure 3). Furthermore, as discussed in the sections above, evidence has accumulated that insulin resistance also affects these regions. This network is involved in higher cognitive functions including the hippocampus, posterior cingulate cortex/precuneus and prefrontal regions. A loss in connectivity within the default mode network could hence explain cognitive dysfunctions in obesity, T2D and dementia. The posterior cingulate cortex, a central hub of the default mode network, was also recently shown to be functionally closely connected to both the lateral as well as medial hypothalamus (218). This hub may therefore constitute a link between the networks controlling metabolism and those responsible for cognition. Whether the default mode network can be modulated by centrally administrated insulin on account of its hypothalamic connections remains to be investigated. Studies in healthy young adults have shown that particularly higher cognitive brain regions are affected by obesity-associated brain insulin resistance, as discussed in section IV. Hence these regions constitute a potential overlap between AD-vulnerable and cerebral insulin resistant brain areas (Figure 13).

f) Brain insulin resistance in dementia and AD

1) The role of insulin in Aβ metabolism

Human and animal cell culture and in-vivo animal studies indicate that insulin and insulin resistance play a prominent role in Aβ metabolism and, conversely, that Aβ affects brain insulin signaling (for review (56, 377)). Insulin regulates beta amyloid by reducing the phosphorylation of the amyloid precursor protein. It also increases anti-amyloidogenic

proteins, such as the insulin degrading enzyme, a metalloprotease that catabolizes insulin. In addition to regulating peripheral insulin levels, insulin degrading enzyme is highly expressed in the brain and fosters Aβ clearance and intracellular degradation (56, 377). Reduced insulin degrading enzyme activity levels have been reported in AD patients as well as in the postmortem brain tissue of deceased AD patients (68). During the progression from MCI to AD, levels of insulin degrading enzyme continue to decrease, correlating inversely with Aβ levels. This endorses the notion that insulin degrading enzyme dysfunction is a prodromal phenomenon of AD (404). Low CSF insulin levels and high peripheral insulin levels in AD (76) result in reduced Aβ clearance in the brain and the periphery. High insulin levels in the periphery may result in competitive inhibition of insulin degrading enzyme, thus preventing Aβ degradation. As a result, Aβ accumulated in the periphery would cross the blood-brain barrier and access the brain (126, 377).

Insulin counteracts the reduction in insulin receptors on dendritic surfaces (82) as well as on the serine phosphorylation of insulin receptor substrate-1 as induced by Aβ oligomers (34). The latter is known to inhibit downstream insulin signaling and induces peripheral insulin resistance (305, 403). Moreover, the peptide protects synapses against Aβ oligomers by decreasing respective binding sites (82), thereby attenuating the detrimental effect of Aβ oligomers on neuronal survival (355). It is not surprising that in turn, insulin resistance accelerates Aβ production and facilitates its accumulation (for review see (81, 377)). Vice versa, Aβ oligomers impair insulin action by binding to insulin receptors, thereby disrupting the signaling capacity and down-regulating insulin receptors in the hippocampus (355, 403).

2) Association between peripheral insulin resistance and AD pathology

Only a small number of studies have used modern imaging techniques to investigate the relationship between peripheral insulin resistance and amyloid load in humans. In healthy late middle-aged adults with normoglycemia, higher peripheral insulin resistance correlated with higher PiB uptake. This is indicative of increased amyloid deposition (390). However, in a longitudinal study, which included PiB-PET data, multiple oGTTs, and postmortem brain

slices, amyloid load was not found to be associated with either glucose intolerance or insulin resistance (350). Moreover, although hypometabolism of AD related regions was observed, there was no increase in amyloid load in T2D patients (295). This led to the conclusion that there is no coherent pattern between insulin resistance/T2D and amyloid load, which is supported by a cross-sectional study in subjects with AD or MCI (245). In these subjects T2D was associated with decreased frontal and parietal cortical thickness, but not with Aβ concentrations in CSF. Remarkably, however, T2D was linked to increases in total and phosphorylated microtubule-associated tau protein in CSF. As outlined above, hyperphosphorylation of tau, a hallmark and biomarker of AD pathology, leads to the accumulation of neurofibrillary tangles and, consequently, to neuronal demise (281). Notably, insulin administration appears to counteract taupathy in AD (see below).

In cognitively intact adults with pre-diabetes or T2D, peripheral insulin resistance is associated with reduced cerebral glucose metabolism in frontal, temporo-parietal, and cingulate areas (11) (brain regions displayed in Figure 3). Moreover, decreased peripheral insulin sensitivity has frequently been observed to be associated with MCI- and AD-related brain glucose hypometabolism, evaluated by FDG-PET (247, 260, 291, 349, 350, 377, 391). Interestingly, within the medial temporal lobe there appears to be a shift from hyper- to hypometabolism in MCI progressors. This is predicted by peripheral insulin resistance, while those patients who have a stable MCI show no such shift (391).

Evidence has accumulated that measures of peripheral insulin resistance correspond to lower hippocampal volume in middle-aged to elderly individuals who are healthy (20) or who suffer from T2D (343), as well as in individuals at risk for AD (287, 392), due to genetic predisposition or family history. Concomitantly, peripheral insulin resistance has been associated with compromised cognitive function in these studies, i.e. verbal fluency (20), verbal learning (392) and executive function (343).

3. Impact of central nervous insulin administration on AD pathology

Craft and colleagues (74) were the first to show that acute and chronic exposure of a low dose of intranasal insulin (20 and 40 U) provides promising results in reducing neuropathological changes in AD (further discussed in section V). These behavioral studies provide encouraging findings that increasing CSF insulin by intranasal application benefits cognitive function in MCI and early to moderate AD patients (60, 61, 74, 288-290). In an acute setting, verbal memory was tested 15 minutes after application of intranasal insulin. Insulin improved verbal memory in patients without the APOE ε4 mutation, with the most pronounced effect being observed for the relatively low dose of 20U, whereas 60U were not effective (288, 289). Notably, in carriers of the APOE ε4 allele insulin remained without effect or even impaired performance. It is not known if this difference is related to the stronger association between insulin resistance and AD observed in patients without risk allele in comparison to risk allele carriers (76). Insulin might even aggravate impairments in central nervous glucose metabolism, such as is the case in carriers of the APOE ε 4-positive genotype (292).

In a pilot study with 24 patients, 21 days of 20U insulin administration modulated Aβ levels in the CSF, and improved attention and verbal memory (290). For the latter measurement, participants were requested to recall a story containing 44 informational bits immediately and again after a 20-minute delay. In a follow-up study, 104 patients with MCI or moderate AD underwent 4 months of treatment with either 20 or 40U of intranasal insulin or placebo (74). Both insulin doses preserved caregiver-rated functional ability (such as orientation, judgement, social interaction, home activities etc.) and preserved general cognition as assessed by the Alzheimer's Assessment Scale. The improvement in episodic memory due to 20U-insulin treatment was still present 2 months after the intervention had been completed. Of note, changes in memory and functional ability were related to changes in Aβ and tau protein (74). Remarkably, 4 months treatment with intranasal insulin minimized the progression of reduced FDG-uptake in AD-vulnerable brain regions such as the precuneus and frontal and parietal areas (74). So far, this is the only study using brain-imaging techniques to evaluate brain insulin action in AD patients. Craft and colleagues further

analyzed the insulin-induced behavioral improvements of this cohort on the basis of sex and APOE ε4 genotype (61). Only men showed improvements in delayed story recall after being administered 20U of insulin for 4 months. This sex difference was most pronounced in the group of APOE ε4 non-carriers. In accordance with the effects of acute intranasal insulin administration (288, 289), performance of APOE ε4 female carriers who had received the 40U-insulin treatment even deteriorated (61). Interestingly, in APOE ε4 carriers, Claxton and coworkers found beneficial effects on verbal and spatial memory using 40U of insulin detemir (a long-acting human insulin analogue) but also an improvement in peripheral insulin sensitivity (see also below) (60).

Besides insulin's role in Aβ clearance and production, insulin resistance also exacerbates neurodegeneration by hyperphosphorylation of tau protein to form neurofibrillary tangles (245). Four months of intranasal insulin application changed the tau protein to Aβ ratio in CSF (74). Furthermore, when intranasally applied to mice, insulin attenuated hyperphosphorylation of tau-promoting brain insulin signaling (53). Such effects might be mediated by changes in glycogen-synthase kinase-3-beta, which inhibits tau phosphorylation (180).

g) Inflammation as a potential shared pathophysiology of metabolic and cognitive disorders

Role of inflammation in brain insulin resistance

Inflammation occurs in the brain and the periphery to defend the body against multiple threats. It involves soluble factors and specialized cells that are mobilized to restore normal body physiology. Chronic inflammation, however, is detrimental as it leads to tissue damage and degenerative diseases. In the brain, glial cells (including the insulin-responsive astrocytes (161) and microglia) become activated, thereby increasing the production of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin (IL) 6 and IL-1β. In AD patients, these inflammatory mediators are significantly higher in the blood and

CSF (105, 340). Hyperinsulinemia facilitates brain inflammatory responses and influences AD pathology by increasing Aβ oligomers (73, 110). These, in turn, inhibit insulin receptor substrate by activating TNF-α (105). Conversely, β oligomers can also activate microglia, secreting pro-inflammatory cytokines (377). Chronic low-grade inflammation of the periphery is also a key characteristic of obesity and T2D and originates from increased adipose tissue. Pro-inflammatory cytokines, such as TNF- α, are upregulated in adipose tissue in obese individuals and can cause peripheral insulin resistance (182, 183). Elevated TNF-α can interfere with insulin signaling by hindering intracellular actions of insulin. Blocking TNF-α in the obese mouse ameliorates insulin sensitivity and glucose homeostasis (for review see (105)). In the “obese brain”, hypothalamic inflammation and gliosis are particularly prevalent during a high-fat diet. While these salient findings were mainly revealed in diet-induced obesity rodent models, a few modern imaging studies also detected gliosis in humans (for review see (94)). With increasing BMI, gliosis was observed in the mediobasal hypothalamus (348). In response to intranasal insulin, hypothalamic insulin signaling was impaired in young adults with high amounts of visceral adipose tissue (220) (Figure 11E), which is considered a highly inflamed adipose tissue. Furthermore, measures of hypothalamic structural integrity were inversely correlated with systemic inflammation (47, 280). Even more importantly, increased damage was correlated with impaired cognitive performance (280). At the same time, chronic inflammation in AD and older patients with T2D is related to cognitive decline and brain atrophy and vasoregulation (44, 58, 271).

All in all, inflammation has the potential to be a shared mechanism between metabolic disorders and AD (for recent review see (105)).

With increasing age, both peripheral as well as central insulin sensitivity declines. Older individuals with peripheral insulin resistance are more prone to show an AD-like brain pattern (Figure 3). This is characterized by reduced cerebral blood flow (i.e. hypometabolism) and a reduction in brain tissue, mainly in the frontal, temporal, lateral parietal cortices and precuneus. In young adults, obesity-associated brain insulin resistance displays an overlap

with AD affected regions mainly in the prefrontal cortex and hippocampus (Figure 13). Higher circulating insulin levels and insulin resistance could therefore be a possible mediator of neurodegenerative ailments. Indeed, cell culture studies have shown that insulin plays a prominent role in beta amyloid metabolism. However, no consistent pattern was observed between amyloid load and peripheral insulin resistance. Other hallmarks of AD, such as glucose hypometabolism and a loss in brain tissue are nevertheless strongly associated with peripheral insulin resistance. Furthermore, first evidence exists that intranasal insulin can minimize the progression of glucose hypometabolism in AD-vulnerable regions.

V. Therapeutic advances in treating brain insulin resistance

a) Effect of lifestyle intervention on brain insulin sensitivity

There are no studies available on the effects of lifestyle intervention specifically on human brain insulin sensitivity. However, it can be assumed that interventions which improve peripheral insulin sensitivity of muscle and liver also enhance insulin action in the brain. Exercise and weight reduction are known to influence whole body insulin sensitivity. Thus, diabetes prevention studies, such as the Diabetes Prevention Program, have conclusively shown that an increase in the amount of physical activity and a reduction of body weight by caloric restriction improves whole body insulin sensitivity (209). However, it remains to be seen whether weight loss and increased physical activity by lifestyle intervention can also improve or even reverse brain insulin resistance. As outlined above, we propose that brain insulin resistance is imprinted on the fetal brain. Such brain insulin resistance, if developed in early life, might not be treatable by lifestyle intervention. By contrast, brain insulin sensitivity may determine the success of a lifestyle intervention. Indeed, individuals with high cerebral insulin sensitivity were able to reduce their body fat, in particular visceral fat, more effectively during a lifestyle intervention than individuals with a low cerebral insulin sensitivity (361). Cerebral insulin sensitivity is liable to influence cognitive control of food intake, i.e., the ability

to intentionally restrain eating behavior and successfully lose weight (160). In addition, cerebral insulin sensitivity might not only affect the behavioral aspects that determine the success of lifestyle intervention, but also directly modulate metabolic processes. In this respect, it is important to note that hypothalamic insulin sensitivity is associated with the brain-derived modulation of peripheral metabolism (171) as well as the amount of visceral fat (220), and may therefore directly influence visceral fat.

Since bariatric surgery has the most dramatic weight-lowering effect, it is interesting to study brain function in individuals before and after such interventions. Evidence exists that obesity-associated alterations in brain activity can be reversed by bariatric surgery (116, 314). However, at present there is no evidence to suggest that these beneficial effects lead to an increased central nervous insulin sensitivity. So far, only one study has shown that massive weight reduction in obese humans at least partially corrects the dysfunctional activity in response to glucose in specific brain areas such as the hypothalamus (369). One might speculate that this outcome is related to improved insulin sensitivity in the respective central nervous areas after weight loss.

***b)* Therapeutic advances in treating brain insulin resistance**

Brain effects of anti-diabetic drugs in T2D

Whole body insulin resistance is improved by insulin sensitizers such as metformin or PPAR gamma agonists. Hyperglycemia is also known to induce insulin resistance. Agents such as sulfonlyureas, GLP-1 agonists, DPP-4 inhibitors and SGLT2 inhibitors therefore improve whole body insulin sensitivity by lowering glycemia. One might speculate that agents that improve whole body insulin resistance also improve brain insulin resistance thereby secondarily further improving systemic glucose metabolism. Specific brain imaging studies to assess the direct impact of anti-diabetic drugs on the brain will be necessary to confirm this assumption. However, the influence of antihyperglycemic agents specifically on brain insulin sensitivity has not yet been investigated, and only very of the few human studies available

show that antidiabetic drugs have a direct influence on brain function. Several of these studies have examined GLP-1 agonists. GLP-1 receptors in the brain, in particular in the arcuate nucleus, are known from animal studies to be a prerequisite for the anorectic effect of GLP-1 agonist treatment (329). The influence of peripherally injected GLP-1 agonist on brain function in humans, in particular on brain areas involved in the regulation of hunger and satiety, has been demonstrated using fMRI and PET brain imaging techniques (368). GLP-1 agonist exenatide decreased food intake and food-related brain responses in T2D patients and obese subjects in the insula, amygdala, putamen, and orbitofrontal cortex. Interestingly, these effects could be blocked by exenidin 9-39, a pharmacological GLP-1 receptor antagonist (367). In addition, in patients with type 2 diabetes, CNS activation in response to food pictures was reduced after meal intake in patients with type 2 diabetes (347). This postprandial reduction in brain activation was prevented by exenidin 9-39 infusion. In accordance with these findings, our group recently showed that postprandially elevated endogenous GLP-1 levels are associated with a suppression of activity in the orbitofrontal cortex, a brain area that regulates hunger and satiety. Notably, some of these endogenous GLP-1 effects appear to be independent of brain insulin action (163).

Brain effects of insulin-sensitizing diabetes drugs in AD

As discussed above, patients with AD show impaired insulin signaling in specific brain areas like the hippocampus (342). Since brain insulin resistance is believed to be an early and common feature of Alzheimer's disease, the treatment of AD-related brain insulin resistance with insulin sensitizers or with insulin itself seems to be a worthwhile approach. Small pilot studies in humans suggest that the insulin-sensitizing PPAR gamma agonists may preserve or improve cognitive function in Alzheimer's disease (307, 383), probably via improved insulin signalling in the brain. Animal studies have provided evidence that neuronal pathologies induced by impaired central nervous insulin signalling can be prevented by the use of the GLP-1 receptor agonist exendin-4 (34). Likewise, the GLP-1 receptor agonist liraglutide has been found to ameliorate neuropathology and improve cognitive function in an

AD mouse model (149). Its efficiency in patients with AD is currently under investigation (98). Against the background of these pilot studies, which indicate that activation of GLP-1 receptors enhances brain insulin signalling and counteract memory impairments, boosting GLP-1 signalling might be considered a helpful tool in the treatment pf AD (341).

Intranasal insulin as a treatment

Overcoming peripheral insulin resistance by intranasal insulin

In peripheral tissues, insulin itself can overcome insulin resistance when administered in appropriate doses. In the brain, proof-of-concept studies indicate that intranasal insulin might also hold some potential in the clinical setting. Eight weeks of intranasal insulin treatment reduced body weight and body fat content in healthy men but not in women (144), whereas obese men did not lose body fat during intranasal insulin treatment but still showed improved declarative memory performance (Figure 8) (143). Short-term studies show that a single dose of intranasal insulin reduces free fatty acid levels (190) and improves peripheral insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp technique (171). In addition, increases in hypothalamic and parasympathetic output in normal-weight but not obese men have been recorded (171). More specifically, intranasal insulin improved peripheral insulin sensitivity in this study. This effect was correlated with the high frequency band of heart rate variability, an estimate of the parasympathetic output, and insulin-stimulated hypothalamic activity (171). This provides further evidence that peripheral and central insulin sensitivity are highly linked processes. Notably, these effects were found in normal-weight participants whereas in accordance with the concept of central nervous insulin resistance, overweight and obese subjects did not show respective effects (143, 171). It is therefore unclear at present as to whether or not improved paradigms of intranasal insulin administration constitute a suitable means of overcoming central nervous insulin resistance in the obese or diabetic state.

Beneficial effects of intranasal insulin in developmental delayed children

The 22q13 deletion syndrome (Phelan-McDermid syndrome) is characterized by global developmental delay, absent or delayed speech, generalized hypotonia, autistic behavior and a characteristic phenotype. In an exploratory clinical trial (313), children suffering from this syndrome received intranasal insulin (maximal dose of 0.5-1.5 U/kg*day) for twelve months, which is, to our knowledge, the longest duration of intranasal insulin delivery tested up to now. These children appeared to benefit from insulin delivery in their parent-assessed motor development, cognitive function and spontaneous activity. The beneficial effect of intranasal insulin on cognitive function has been discussed in detail in a recent review (325). Results supporting the notion that intranasal insulin could become an effective means of treating Alzheimer's disease have been described above and moreover have been extensively reviewed elsewhere (120).

Effect of the insulin analogue detemir

Due to its pharmacokinetic properties, the long-acting insulin analogue detemir may have a more pronounced effect on the brain than on the rest of the body. Detemir has a weight-sparing role in the treatment of diabetes compared to other insulins (122). It has been proposed that this is the consequence of detemir action on the central nervous system, where it mediates reduced energy intake (299). Animal studies indicate that insulin detemir has a tissue-selective action, with a relative preference for brain (173). The time course and extent of insulin signaling in peripheral tissues were similar following the treatment with insulin detemir and with human insulin. However, insulin signaling in hypothalamic and cerebrocortical tissue occurred more quickly and was enhanced on account of a higher insulin detemir concentration in the brain. This was accompanied by an increased cortical activity, as measured by epidural EEG in mice with detemir treatment (173). In humans, acute euglycemic infusion with detemir rather than human insulin exerts a stronger EEG-assessed brain effect and reduces food intake while inducing similar systemic effects (146). Importantly, in obese humans, the somewhat weaker impact of systemic, euglycemic insulin infusion on the brain in comparison to lean individuals can be restored by insulin detemir

(359). In two studies in patients with type 1 diabetes, the effects of chronic detemir therapy on the brain were studied by means of PET and fMRI (372-374). In comparison to NPH insulin, detemir showed a weight-sparing effect (121) and increased activiation in appetite-regulating brain regions. One recent study investigated intranasal administration of insulin detemir over a period of 3 weeks in older subjects with cognitive impairment. It reported improved peripheral insulin resistance in subjects with one or two APOE-4 alleles, a genetic variant associated with Alzheimer's disease. Surprisingly, however, participants without the APOE-4 allele showed a deterioration in peripheral insulin sensitivity (60). Taken together, these results provide the first promising evidence that insulin analogues with brain-affine action profiles such as insulin detemir may be able to at least temporarily overcome insulin resistance associated with metabolic and cognitive impairments. In addition, when other insulin analogues such as insulin aspart were used, brain responses were different than for human insulin, which is reported earlier in this review (24).

In summary, treating brain insulin resistance with pharmacologic agents that – directly or indirectly - improve brain insulin signaling would seem to be a promising approach in the prevention and/or treatment of both metabolic diseases and cognitive impairments. Current evidence for respective effects in humans is strongest for insulin and insulin analogs when administered specifically to the brain via the intranasal pathway.

VI. Concluding remarks

As outlined in this review, there are strong indications that brain insulin resistance is a shared pathological feature of the metabolic and cognitive disturbances found in obesity, T2D and dementia (for overview see Figure 2). Recent neuroimaging studies using exogenous and endogenous insulin stimulation have probed the sensitivity of central nervous pathways to insulin and have revealed remarkable effects on hypothalamus, frontal cortex, limbic regions and the hippocampus including its surrounding gyri (Figure 4). Accordingly, brain insulin action can be assumed to influence homeostatic, reward-related and higher cognitive brain functions, as reflected by multiple behavioral and metabolic effects. Studies on eating

behavior show that central insulin administration inhibits food intake and reduces body weight particularly in men, which suggests a sex-specific component to insulin effects on eating behavior. Olfaction, emotional regulation and cognition are similarly affected by brain insulin action, with particularly relevant beneficial effects on memory function. Brain insulin action mediates whole-body metabolism with immediate effects on blood glucose and FFAs and long-term effects on energy storage in multiple organs presumably by altering the autonomic nervous system function (summarized in Figure 10) (for recent review see (166)).

Brain insulin resistance in humans was first described in obese individuals. Unlike normal-weight subjects, obese show a diminished neural response to insulin in higher cognitive brain regions, with the severity of bran insulin resistance determining the success of life-style intervention. Recent findings suggest that especially metabolically unfavorable abdominal adipose tissue is particularly associated with brain insulin resistance in the hypothalamus and higher cognitive brain regions (Figure 11). Similarly, T2D patients show brain insulin resistance in the above-mentioned brain regions, with normalization after dietary restriction. However, it is currently unclear whether brain insulin resistance is a cause or consequence of obesity/T2D. Some studies point to a genetic predisposition for brain insulin resistance, with the obesity-associated *FTO* variants being the most extensively studied genetic determinant. Other factors known to influence brain insulin sensitivity/resistance are age and inflammation. Considering that it is a key characteristic of both metabolic disorders and AD, chronic inflammation in particular might represent a mechanism shared by these two afflictions. Inflammatory mediators have adverse effects on beta amyloid metabolism and some studies in humans point to hypothalamic inflammation in obesity. With increasing age, insulin signaling in the periphery and the central nervous system decreases. Individuals with relatively diminished brain insulin sensitivity have a particularly high risk for an AD-like brain pattern (Figure 3), and so insulin resistance is more liable to be a mediator of neurodegeneration. In AD, insulin plays a prominent role in beta amyloid metabolism, albeit no coherent pattern has been observed between peripheral insulin resistance and amyloid load in the brain. Nonetheless, brain glucose hypometabolism and lower hippocampal

volume, hallmarks of AD, are strongly associated with peripheral insulin resistance. Moreover, first evidence of alterations in functional connectivity point to network-related rather than merely localized changes due to brain insulin resistance. In their capacity as a common neural signature linking metabolic and cognitive dysfunctions, connections between the default mode network and hypothalamus network should receive more attention in future studies.

Increasing insulin signaling in the brain by intranasal administration of the hormone reduces neuropathological changes in AD and induces beneficial effects on cognitive function in patients with MCI and early to moderate AD, with the APOE ε4 mutation as a potential genetic determinant of cognitive improvements. Hence, counteracting brain insulin resistance by improving insulin signaling could be promising in treating or preventing cognitive dysfunctions. Moreover, intranasal administration of insulin and insulin analogues might be an efficient means of overcoming brain insulin resistance in the obese or diabetic state. Short-term studies have shown an improvement in peripheral insulin sensitivity and a reduction in FFAs. Up to now, only a small number of studies have investigated the effects of long-term intranasal insulin administration. These indicated weight-loss in normal-weight, but not obese men. Longitudinal studies on brain insulin action in humans are therefore urgently required. There are, notably, already first indications that maternal metabolism significantly influences brain insulin signaling in the fetus, suggesting that the metabolic status of the mother interacts with the functional organization of the fetal brain. These results show that central nervous insulin signaling is relevant – and malleable – even at very early stages of development. They also suggest that brain insulin signaling is a promising target for interventions aiming at the prevention and treatment of metabolic and cognitive disorders.

VII. Future perspectives

While brain insulin resistance can certainly be considered a common trait or potential link between obesity, T2D, and dementia, there is still no evidence on the trajectory of brain insulin resistance. Whereas dementia usually affects cognitive target regions of insulin

action, obesity-associated brain insulin resistance has been predominantly traced in homeostatic and reward-processing areas. Hypothalamic insulin resistance may therefore be a precursor of brain insulin resistance in regions relevant for cognitive function. Alternatively, obesity and dementia/aging-associated brain insulin resistance may be independent processes. In the light of the plethora of functional connections in the brain and the loss of functional connectivity in T2D and dementia, insulin resistance in one region possibly alters other target regions over time. To tie up such loose ends, studies investigating brain insulin action need to cover the patients' whole life span. Preliminary findings in the human fetus suggest that brain insulin resistance plays a role in prenatal development. Hence, it is conceivable that brain insulin resistance is a cause rather than a consequence of obesity/T2D and that it is perhaps even a precursor to dementia. Recent therapeutic progress in treating brain insulin resistance with intranasal insulin sparks hope that the related decline in cognitive functions can be halted. Whether metabolic consequences of insulin resistance can also be treated by boosting brain insulin action is currently unclear. However, the findings summarized in this review clearly call for a thorough investigation of this question in the near future.

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Figure legends

Figure 1. Insulin receptor distribution in the rat brain as assessed using ^{125}I-labeled insulin. Circulating insulin, released into the blood stream by pancreatic beta cells, is transported into the brain via a saturable receptor-mediated pathway. As seen on the color-coded reconstruction of the audioradiographs (left), insulin binds to its abundantly distributed receptors in the olfactory bulb, cerebral cortex, hippocampus, hypothalamus, amygdala and septum, thereby inducing a number of central nervous as well as peripheral effects (see section III). Abbreviations on line drawings of brain section (right): CA1, CA1 of the hippocampus; Cg, cingulate cortex; CG, central grey; DG, dentate gyrus; ENT, entorhinal cortex; SNR, substantia nigra; Str, striate cortex; SU, subiculum (adapted from (176)).

Figure 2. Overview of the central role of brain insulin resistance in cognitive and metabolic disturbances. Brain insulin resistance has been identified in obesity and T2D as well as in normal aging and dementia. Brain insulin resistance is associated with peripheral metabolic dysfunctions and with cognitive decline particularly with regard to memory function (as discussed in section IV). Factors such as adipose tissue, hormones, nutrition, certain genetic mutations, overnutrition and age are associated with brain insulin resistance.

Figure 3. **Overview of brain regions mainly affected by dementia.** Brain regions within the default mode network are particularly vulnerable to aging and dementia. They are the first to develop amyloid deposition, show decreased glucose metabolism and a loss in functional

connectivity. The disruption of functional connectivity and reduced cerebral glucose metabolism in these regions is related to the severity of peripheral insulin resistance and cognitive impairment. Hence, a potential overlap between brain regions associated with peripheral insulin resistance and AD has been proposed. Moreover, a loss in functional connectivity within this network has been observed in T2D and obesity. The default mode network is essential for higher cognitive functions like memory-related processes including the precuneus/posterior cingulate cortex, lateral temporal cortex, prefrontal regions and the hippocampus. The displayed brain regions associated with AD are a schematic overview of the default mode network on a standard anatomical image.

Figure 4. **Insulin-sensitive brain regions** and their functions displayed on a standard anatomical template. Modern neuroimaging studies have revealed a significant insulin-induced brain response, mainly in the fusiform gyrus (white), prefrontal cortex (green and cyan), hippocampus (red), striatum (yellow), insular cortex (blue) and the hypothalamus (violet). For this purpose, oral glucose ingestion, the clamp technique or intranasal insulin application were used. To distinguish task-specific effects, insulin-stimulated brain activity is evaluated under spontaneous (resting-state) conditions or in response to particular tasks recruiting different cognitive domains such as memory. The hypothalamus, fusiform gyrus, striatal regions and the prefrontal cortex appear to be particularly vulnerable to obesity-associated insulin resistance. General functions of the displayed areas include: hypothalamus (violet), control of vital bodily functions such as food and fluid homeostasis; striatum (yellow), reward-related behavior including food reward; insular cortex (blue), the anterior part of the insula is the primary taste cortex of the brain, contributing to the gustatory perception represented by taste, smell and the visual input of food; fusiform gyrus (blue), visual attention, recognition of visual stimuli including food cues; hippocampus (red), memory function, spatial navigation; lateral prefrontal cortex (bright green), cognitive function including inhibitory control of eating behavior; orbitofrontal cortex and anterior cingulate cortex (cyan), reward/motivation-based decision-making.

Figure 5. **Hypothalamus functional connectivity network.** The hypothalamus has been extensively studied on account of its its fundamental role in physiological processes essential for survival and the control of vital bodily functions. Rodent models have provided us with a particularly detailed blueprint of the hypothalamic insulin-signaling pathway, revealing a profound regulatory influence of hypothalamic subregions in energy intake and feeding behavior. However, in humans, the specific role of different hypothalamic nuclei in insulin signaling in humans so far has remained obscure. While the resolution of anatomical MRI is sufficient to distinguish lateral and medial subregions of the hypothalamus, their functional dissection is much more challenging. Recent efforts to investigate the functional connections of the hypothalamus suggest that the medial and lateral hypothalamus tap into different parts of the dopaminergic fronto-striatal circuitry. This figure shows that both the lateral and medial hypothalamus (LH and MH), in humans, are functionally connected with reward-processing regions as well as higher cognitive brain regions such as the posterior cingulate cortex/precuneus, prefrontal cortex and hippocampus. The hypothalamus network is overlaid on a standard anatomical template (adapted from (218)).

Figure 6. **Intranasal insulin administration reduces body weight and fat in healthy, normal-weight men.** Average body weight (±SEM) during 8 weeks of intranasal insulin or placebo administration and in a follow-up examination (**left panel**) and body fat (±SEM) after 8 weeks of treatment (**right panel**). One group of subjects received 160 U of insulin per day (black circles/bar), the other group received placebo (white circles/bar). Values are baseline-adjusted as derived from analyses of covariance. n=12 for each group, * $p \leq 0.05$. (adapted from (144)).

Figure 7. **Postprandial intranasal insulin administration reduces appetite and snack intake in healthy women. (A)** Mean (± SEM) appetite ratings on visual analog scales anchored at 0 and 100 given by female participants who received insulin (160 U; black dots and solid lines; n=15) at 1300 h (nose symbol) and control subjects (placebo administration; white dots and dashed lines; n=15). Standard lunch (~400 kcal) was consumed at 1230 h

and snacks were offered at 1505 h. **(B)** Mean (± SEM) snack intake (kcal) assessed at 1505 h under the pretext of a taste rating session in the placebo group (white bars) and the insulin group (black bars). Three different types of cookies were offered. **(C)** Mean (± SEM) snack palatability rated on visual analog scales anchored at 0 (not palatable) and 100 (highly palatable) during the snack test at 1505 h. * $p < 0.05$ for comparisons between groups (t-tests). Adapted from (145).

Figure 8. **Intranasal insulin improves declarative memory in normal-weight and obese subjects.** Mean sum scores in a delayed recall of words (e.g., car, tree, chocolate) learned one week earlier in **(A)** normal-weight (both groups, n = 19) and **(B)** obese subjects (both groups, n = 15) assessed after eight weeks of intranasal administration of regular human insulin (160 U/day, black bars) or placebo (white bars). Baseline-adjusted means ± SEM are indicated. * $P \leq 0.05$, for pairwise comparisons between groups. Adapted from (A) (22) and (B) (143).

Figure 9. **Intranasal insulin administration improves whole-body insulin sensitivity**. In this experiment, peripheral insulin sensitivity was assessed by hyperinsulinemic-euglycemic glucose clamp in combination with intranasal insulin administration. A) Change in peripheral insulin sensitivity after intranasal insulin application is associated with hypothalamic activity in response to intranasal insulin. The hypothalamus is marked in red on a standardized brain. The scatter plot shows the change in insulin sensitivity index in eight lean and three obese participants from before to after intranasal insulin application. This is plotted against hypothalamic activity after intranasal insulin adjusted for baseline. B) Change in peripheral insulin sensitivity after intranasal insulin application. In lean participants, insulin sensitivity improved more significantly after application of insulin than after placebo spray application. In obese participants, no difference was detected between insulin and placebo spray. Improvement in the insulin sensitivity index different significantly between lean and obese participants. Data are mean ±SEM. Adapted from (171)

Figure 10. **Summary of physiological and behavioral effects of brain insulin action.** Mainly by means of intranasal insulin, numerous advantages effects of brain insulin on metabolism and cognition have been identified. Most notably, brain insulin action inhibits food intake, reduces body weight, increases peripheral insulin sensitivity, decreases gluconeogenesis and lipolysis in the fasting state and increases postprandial thermogenesis. Furthermore, brain insulin action has been identified as a predictor for successful weight loss success. With respect to cognitive function, brain insulin action improves visual and spatial episodic memory, working memory as well as declarative memory. Brain insulin action has also been shown to improve mood and counteract cognitive dysfunction in dementia. More details on these effects can be found in section III.

Figure 11. **Brain insulin sensitivity is associated with visceral adipose tissue.** A) Segmentation of visceral (VAT) and subcutaneous fat using magnetic resonance imaging (MRI) by means of a T1-weighted contrast. B) Sketch of a magnetoencephalograph (MEG) depicting the superconducting sensors that measure the magnetic field generated by the electric activity of neurons. C) Hypothalamic brain activity in response to intranasal insulin compared to placebo in 25 lean and 23 overweight/obese adults overlayed on a standardized brain (adapted from (220)). D) High brain insulin sensitivity before lifestyle intervention is associated with loss in visceral adipose tissue after 9 months of lifestyle intervention. Scatter plot shows that changes in VAT in 28 participants correlated negatively with insulin-stimulated cortical theta activity at baseline, as measured by MEG during a hyperinsulinaemic-euglacaemic clamp (adapted from (361)). E) Scatter plot shows a significant positive correlation between the change in hypothalamic activity 15 min after insulin application and VAT adjusted for other fat compartments in 25 lean and 23 overweight/obese participants (adapted from (220)).

Figure 12. **Maternal insulin sensitivity is associated with oral glucose-induced changes in fetal brain activity**. A) Schematic of fetal biomagnetic field recording. Magentic fields generated by electrical currents in the fetus are recorded by highly sensitive magnetic

sensors (SQUID) (for further details please see supplements) B) Pregnant mother seated on the fetal MEG device. Auditory stimulation is delivered by an air-filled balloon positioned between mother and recording device, which is well suited to the investigation of fetal brain development. Since it decreases over gestation, the latency of the evoked fields is used to assess the functional maturation. C) Graphs show maternal glucose and insulin levels and fetal response latencies during an oral glucose tolerance test in insulin-sensitive and insulin-resistant mothers as well as in mothers with gestational diabetes (GDM). The latency of the fetal auditory response decreases after a glucose challenge to the healthy mother. The endogenous insulin-induced decrease in latency was not observed in the mothers of fetuses with gestational diabetes indicating that the metabolic status of the mother interacts with the functional organization of the fetal brain. Data are shown as mean±SEM (adapted from (228, 229)).

Figure 13. An overview of brain regions particularly affected by cerebral insulin resistance in response to endogenous or exogenous insulin stimulation (in blue). These regions include the prefrontal cortex, fusiform gyrus, hippocampus, striatum, insular cortex and the hypothalamus. The functional details of these regions are explained in figure 4. There is an overlap between regions affected by cerebral insulin resistance and AD especially in the prefrontal cortex and hippocampus (indicated by dashed circles). The displayed brain regions associated with cerebral insulin resistance are a schematic overview on a standard anatomical image.

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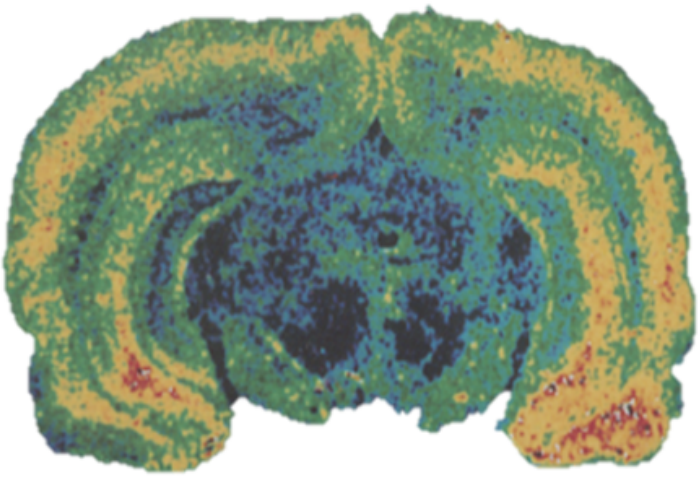
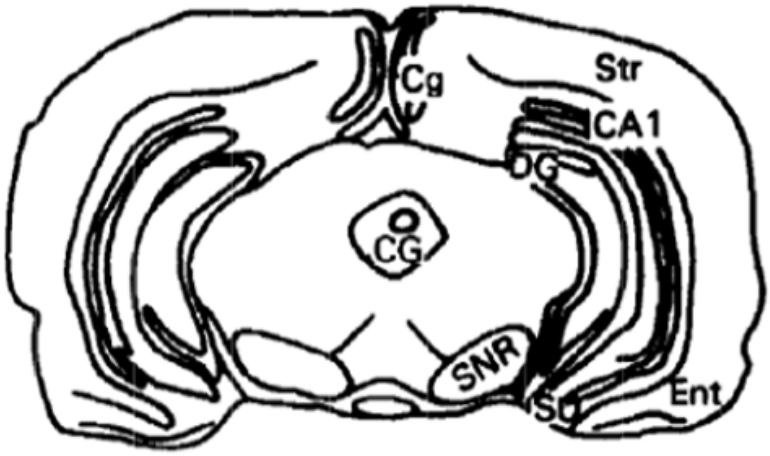
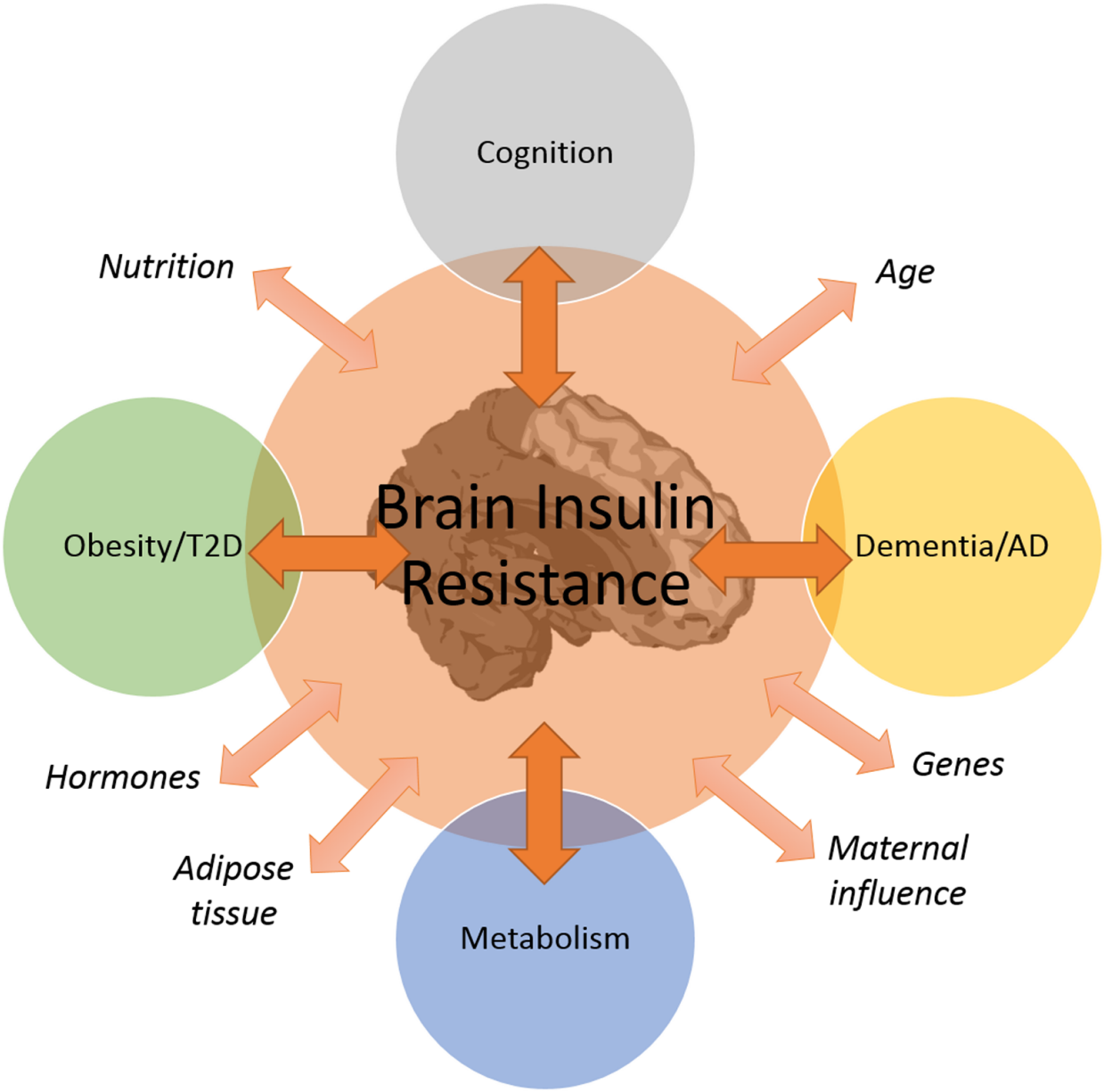
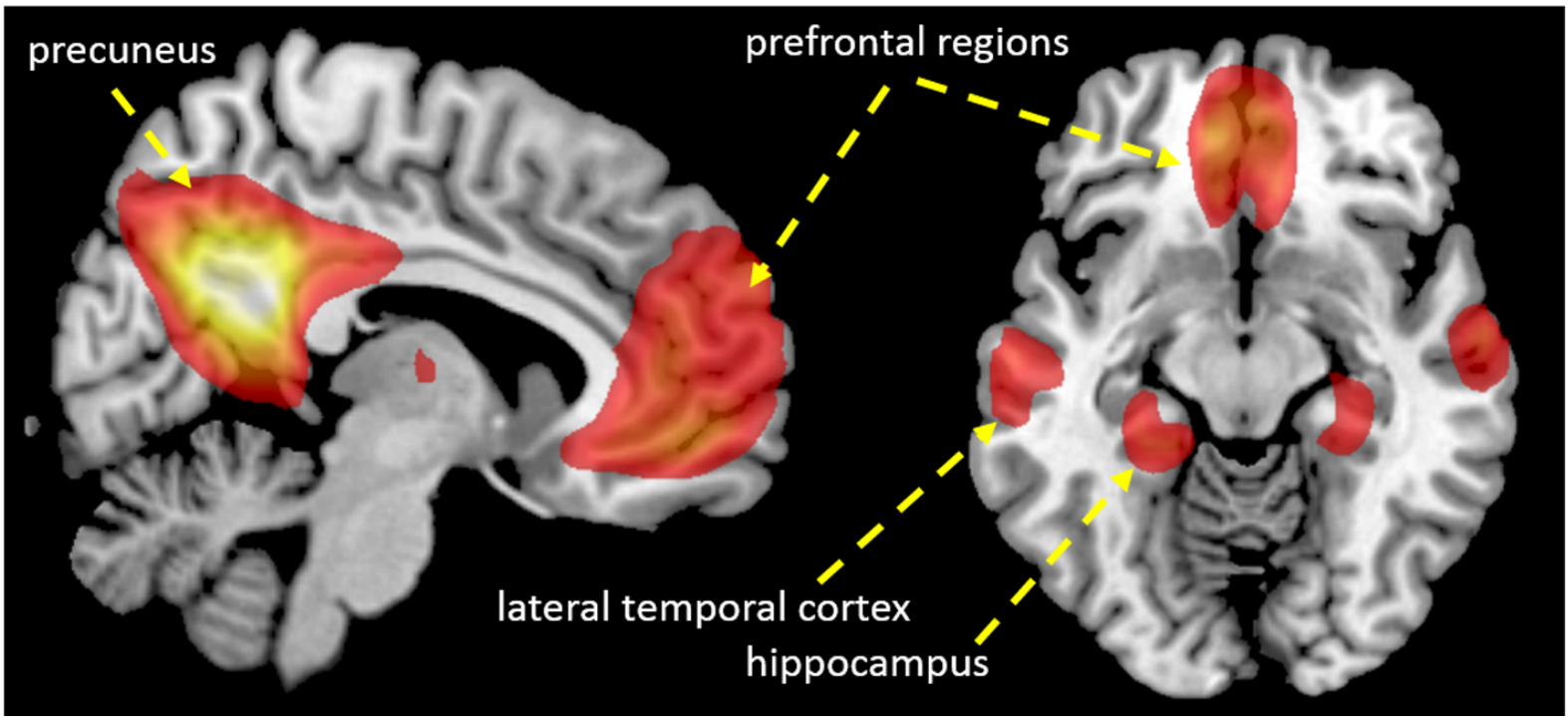
^{125}I-labeled insulin receptor distribution of rat brain

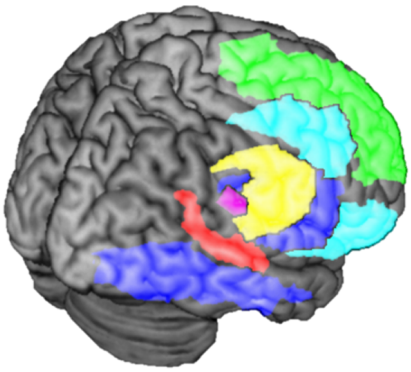




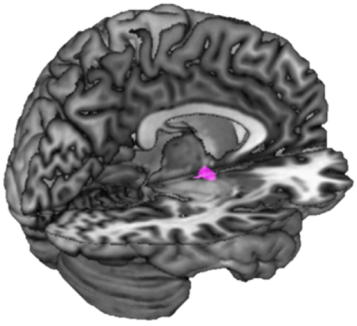

Cognition
Nutrition
Age
Brain Insulin Resistance
Obesity/T2D
Dementia/AD
Hormones
Genes
Adipose tissue
Maternal influence
Metabolism

Brain regions associated with Alzheimers Disease

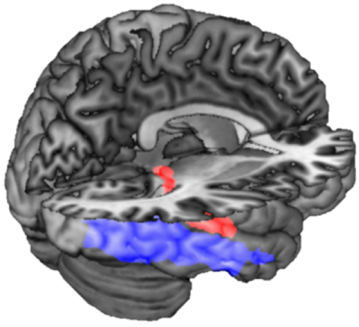




Insulin sensitive brain regions

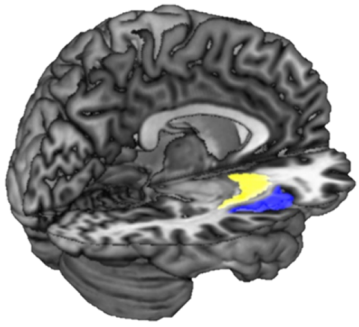


Homeostasis
Hypothalamus



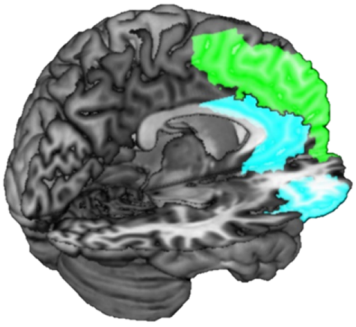
Memory
Hippocampus

Attention
Fusiform gyrus



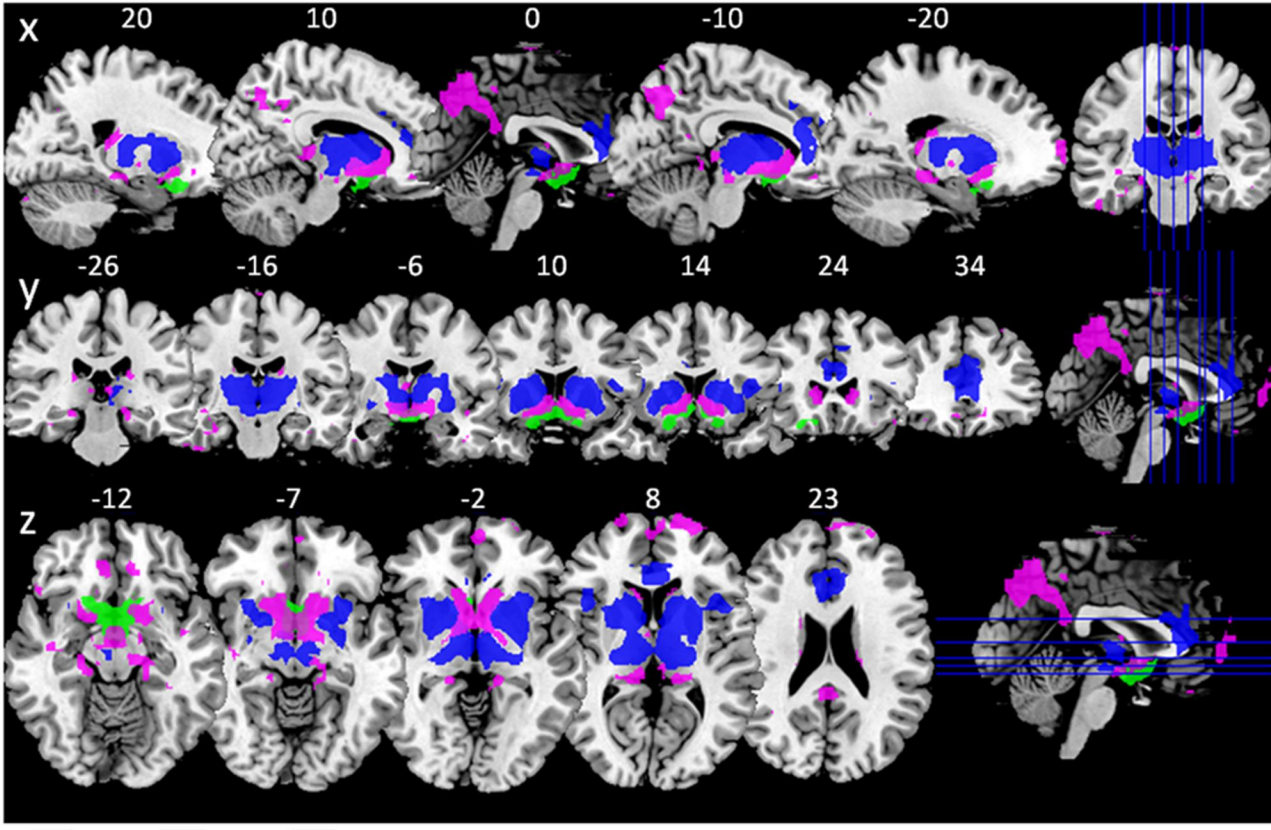
Reward
Striatum

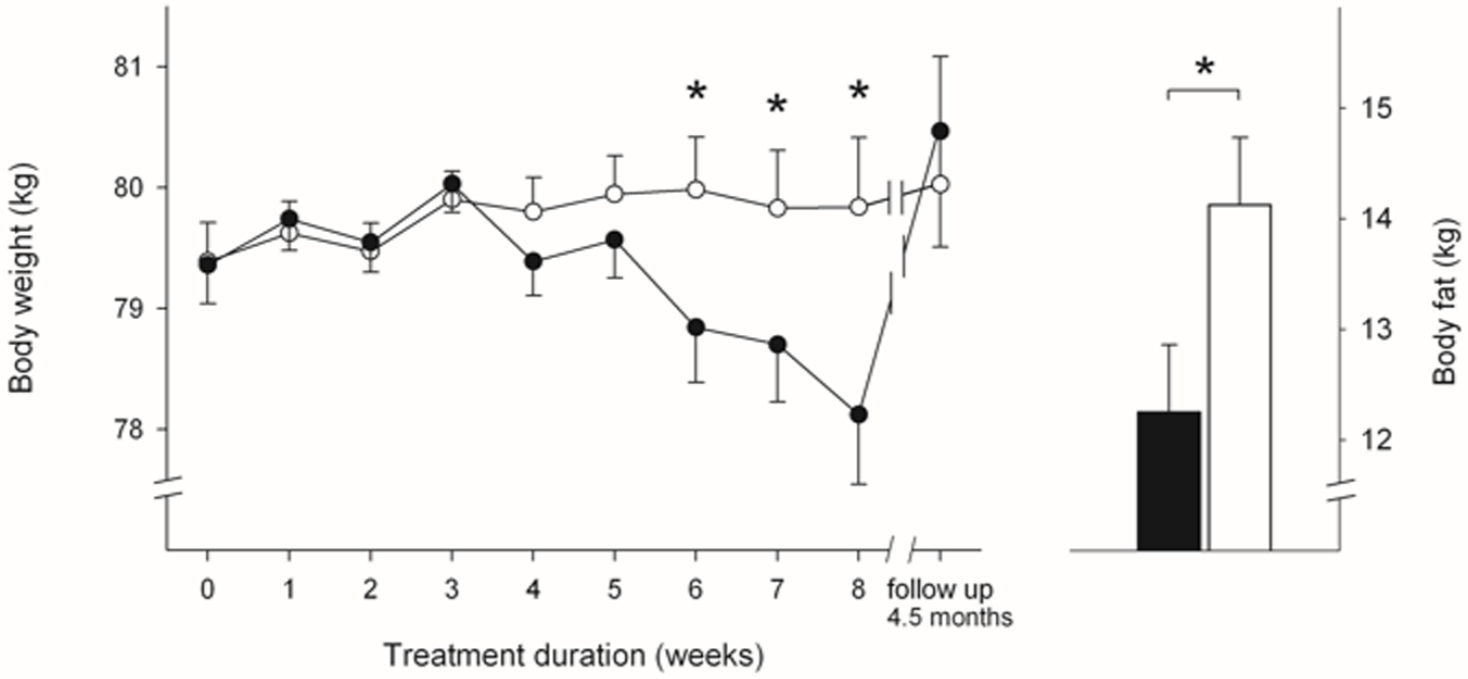
Sensory perception
Insular cortex

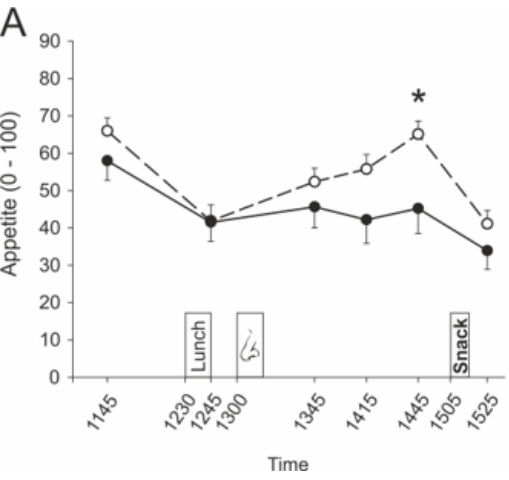


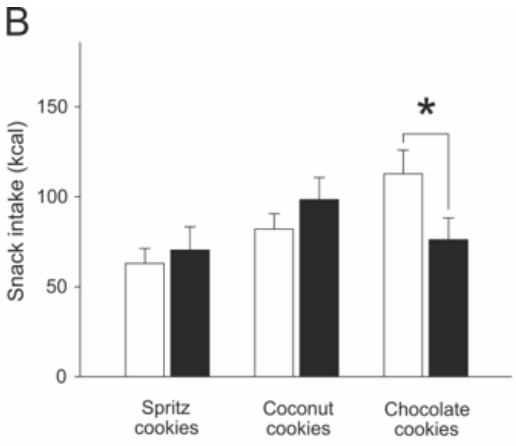
Inhibition
Lateral prefrontal cortex

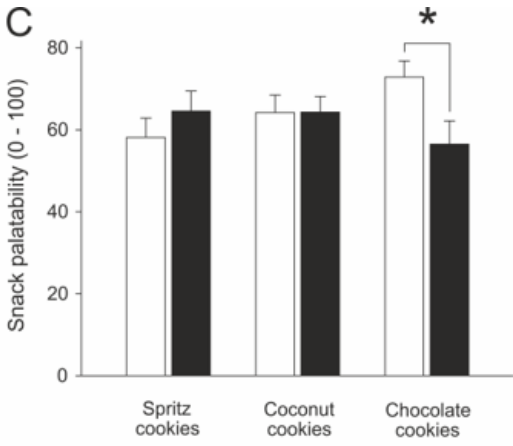
Motivation
Orbitofrontal cortex
Anterior cingulate cortex

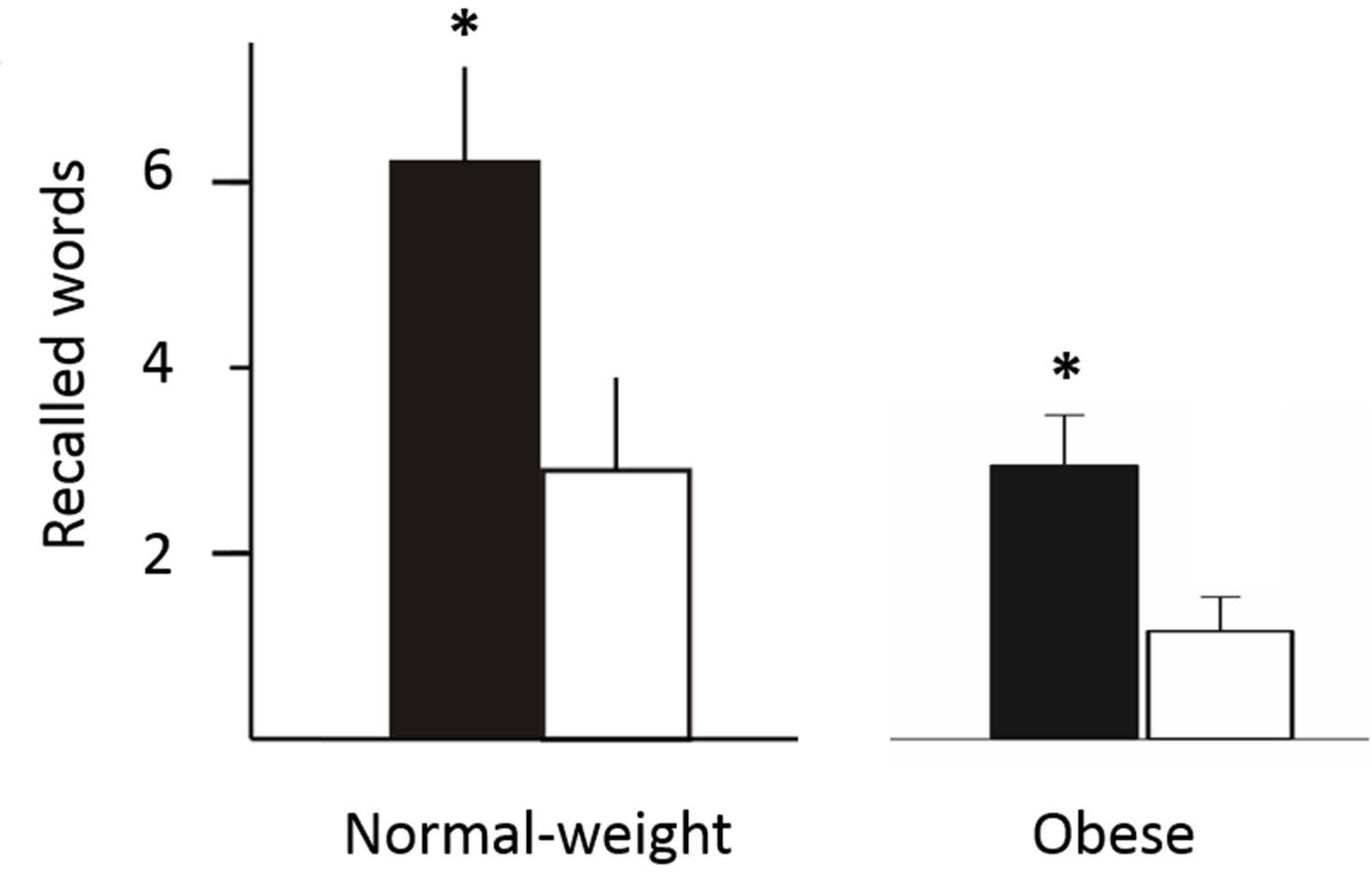

x
20
10
0
-10
-20
y
-26
-16
-6
10
14
24
34
z
-12
-7
-2
8
23
LH
MH
LH& MH

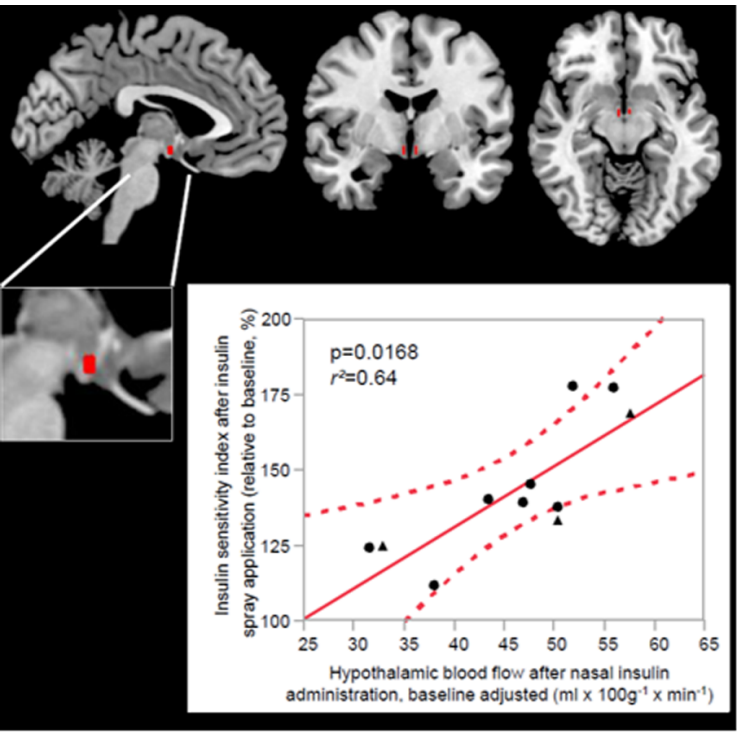

Body weight (kg)
81
80
79
78
*
*
*
0
1
2
3
4
5
6
7
8
follow up
4.5 months
Treatment duration (weeks)
*
15
14
13
12
Body fat (kg)

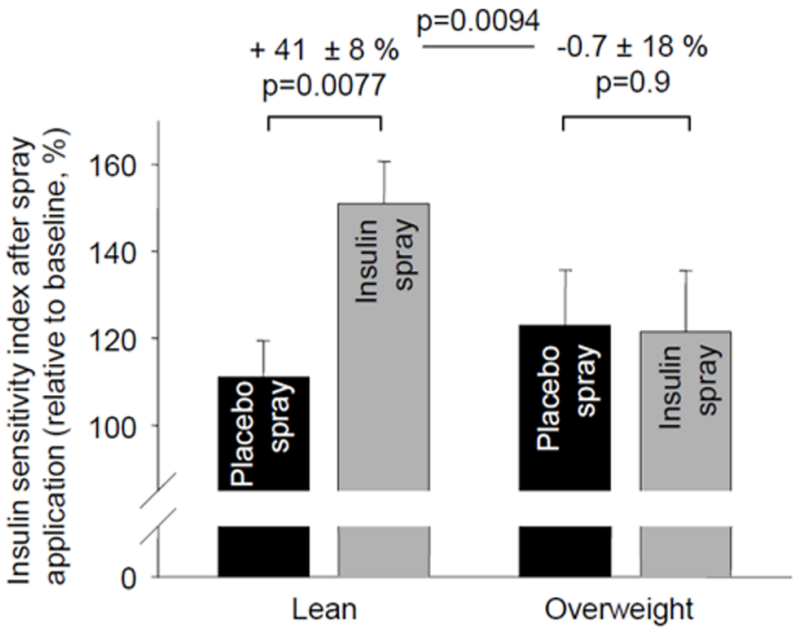

A
Appetite (0 - 100)
90
80
70
60
50
40
30
20
10
0
*
Lunch
Snack
1145
1230
1245
1300
1345
1415
1445
1505
1525
Time

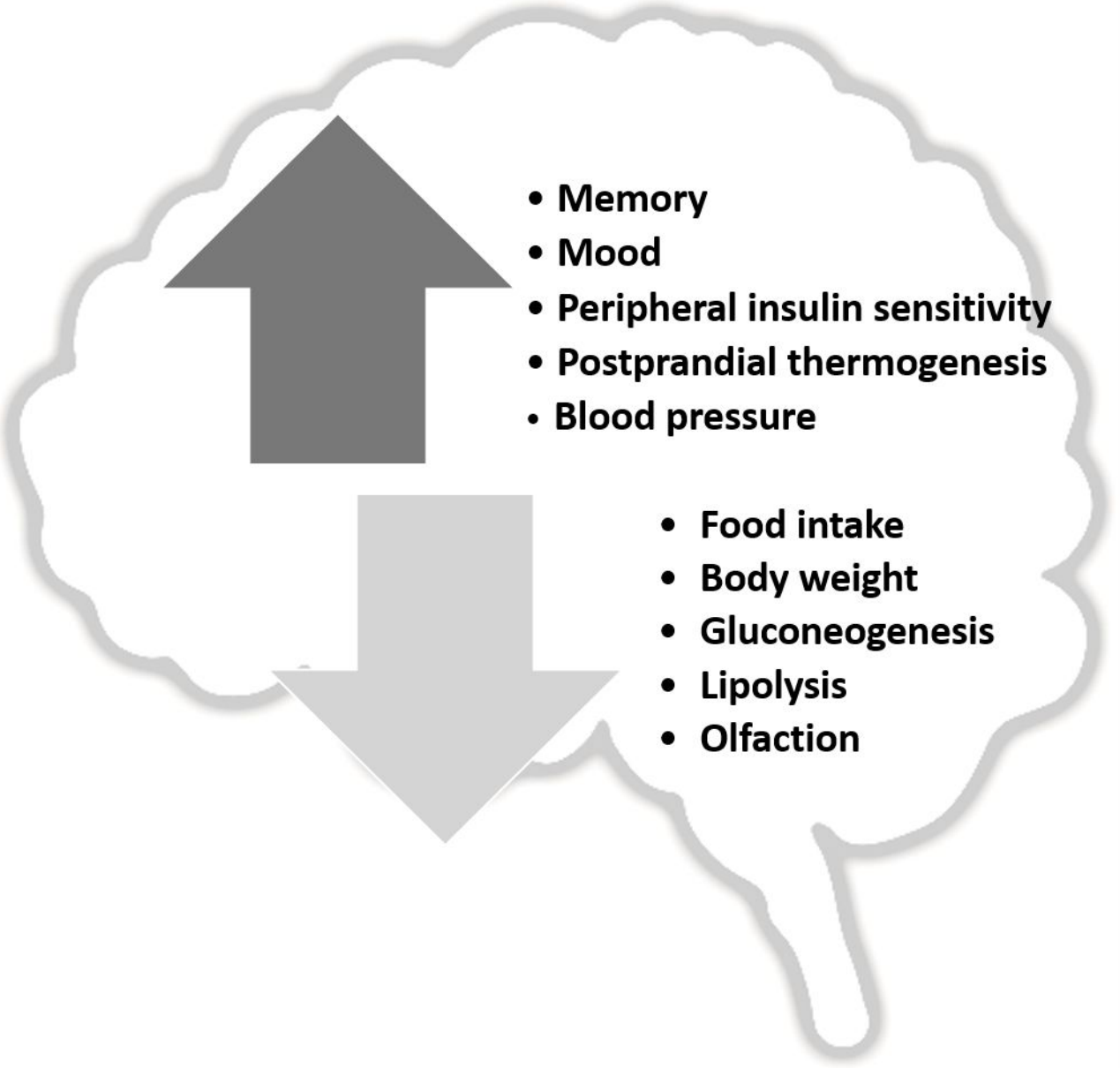

B
Snack intake (kcal)
150
100
50
0
*
Spritz cookies
Coconut cookies
Chocolate cookies

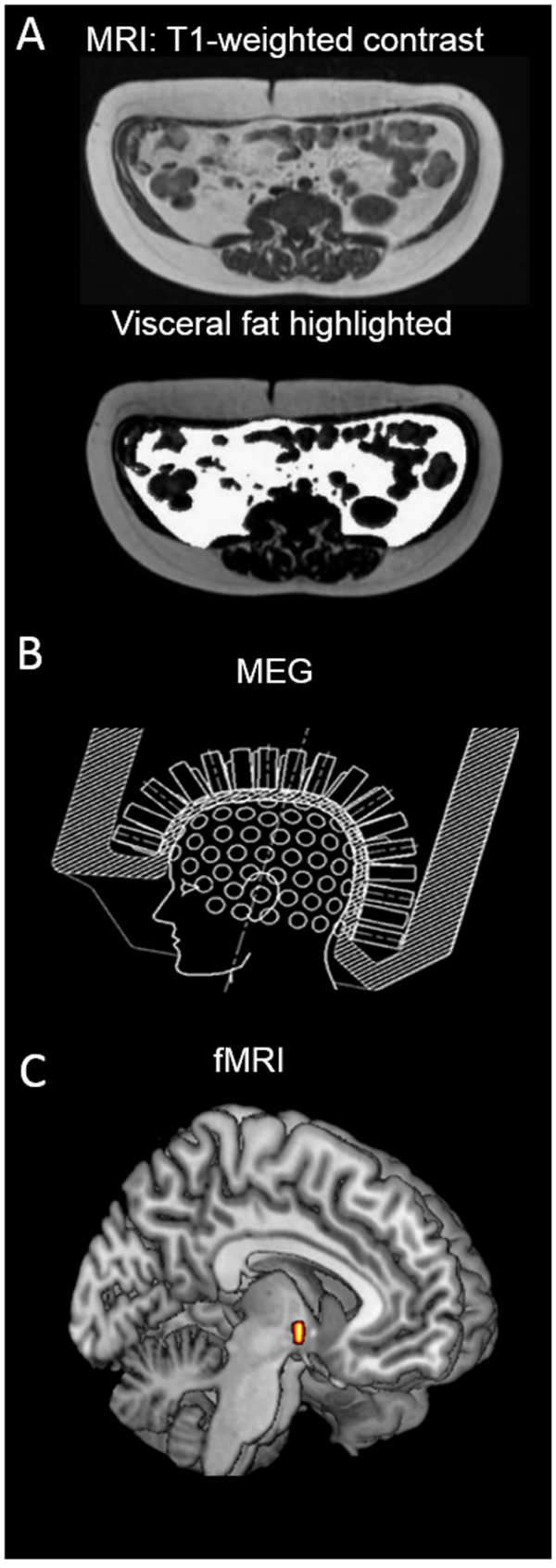

C
Snack palatability (0 - 100)
80
60
40
20
0
*
Spritz cookies
Coconut cookies
Chocolate cookies

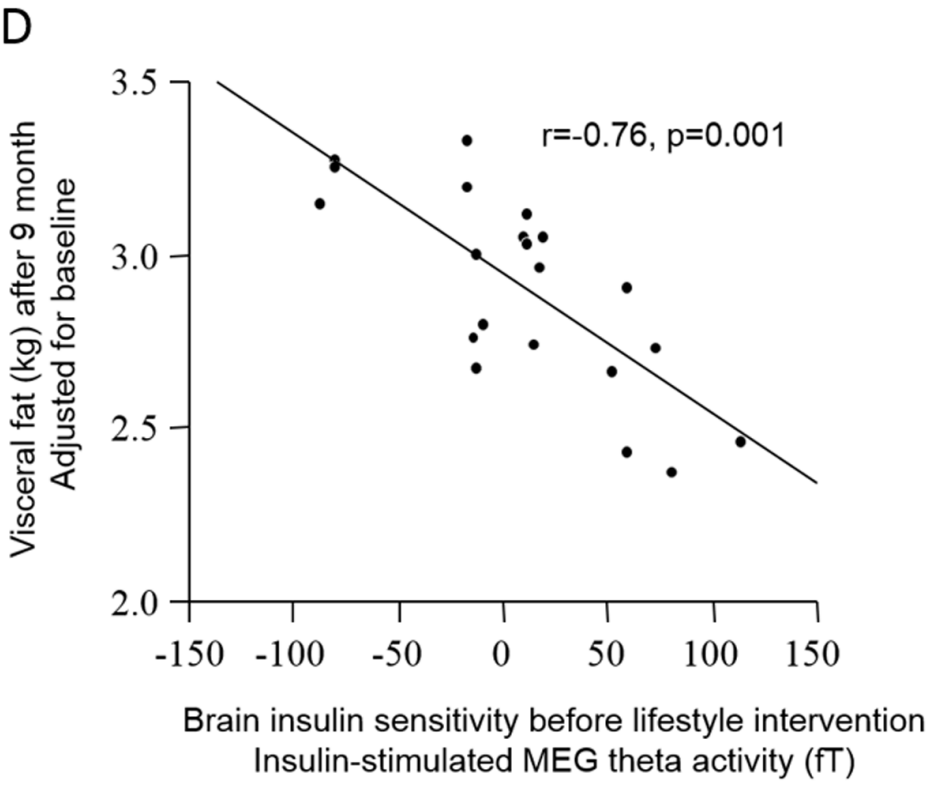

Recalled words
6
4
2
*
*
Normal-weight
Obese

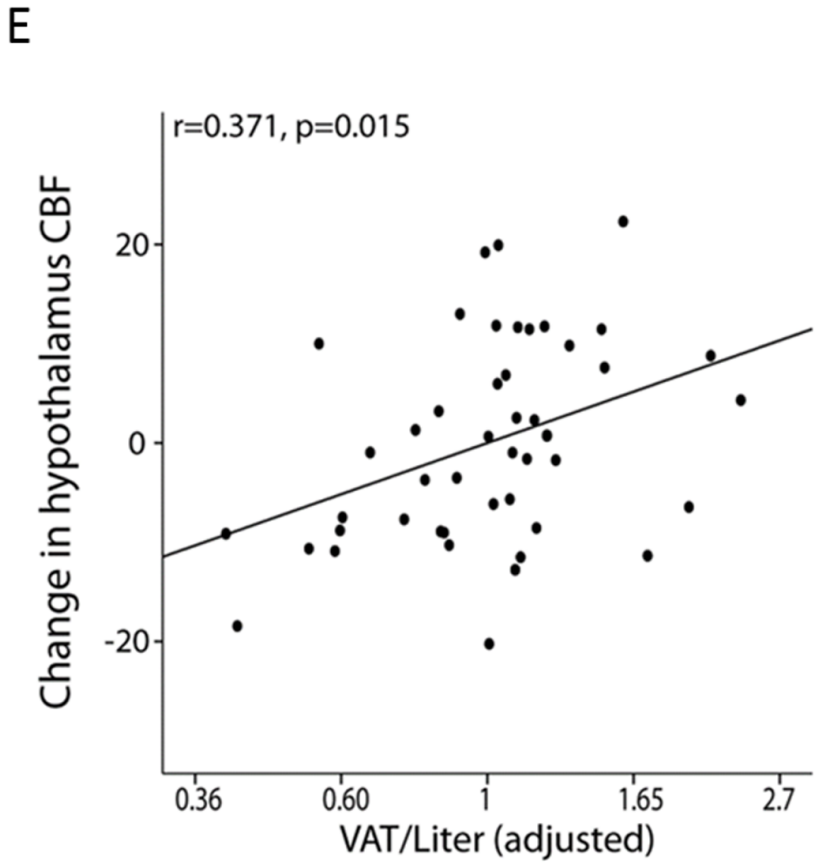

p=0.0168
r^2=0.64
Insulin sensitivity index after insulin spray application (relative to baseline, %)
200
175
150
125
100
25
30
35
40
45
50
55
60
65
Hypothalamic blood flow after nasal insulin administration, baseline adjusted (ml x 100g^{-1} x min^{-1})

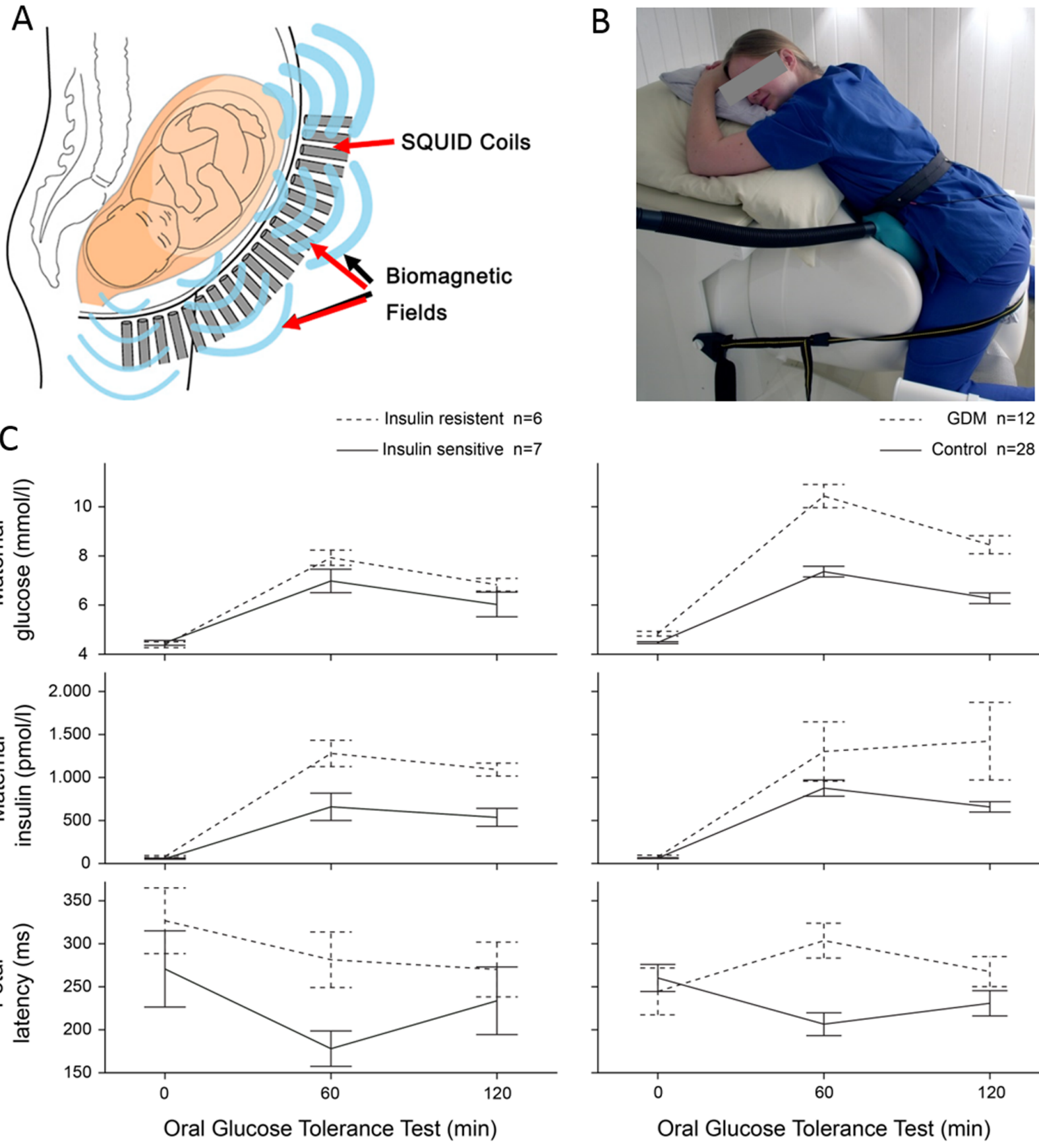

p=0.0094
+ 41 ± 8 %
p=0.0077
-0.7 ± 18 %
p=0.9
Insulin sensitivity index after spray application (relative to baseline, %)
160
140
120
100
0
Placebo spray
Insulin spray
Placebo spray
Insulin spray
Lean
Overweight


• Memory
• Mood
• Peripheral insulin sensitivity
• Postprandial thermogenesis
• Blood pressure
• Food intake
• Body weight
• Gluconeogenesis
• Lipolysis
• Olfaction


A
MRI: T1-weighted contrast
Visceral fat highlighted
B
MEG
C
fMRI


D
r=-0.76, p=0.001
Visceral fat (kg) after 9 month
Adjusted for baseline
3.5
3.0
2.5
2.0
-150
-100
-50
0
50
100
150
Brain insulin sensitivity before lifestyle intervention
Insulin-stimulated MEG theta activity (fT)


E
r=0.371, p=0.015
Change in hypothalamus CBF
20
0
-20
0.36
0.60
1
1.65
2.7
VAT/Liter (adjusted)


A
SQUID Coils
Biomagnetic
Fields
B
C
Insulin resistent n=6
Insulin sensitive n=7
GDM n=12
Control n=28
Maternal glucose (mmol/l)
10
8
6
4
Maternal insulin (pmol/l)
2.000
1.500
1.000
500
0
Fetal latency (ms)
350
300
250
200
150
0
60
120
Oral Glucose Tolerance Test (min)
Oral Glucose Tolerance Test (min)

Overlap between regions affected by cerebral insulin resistance and AD (red dashed circles)

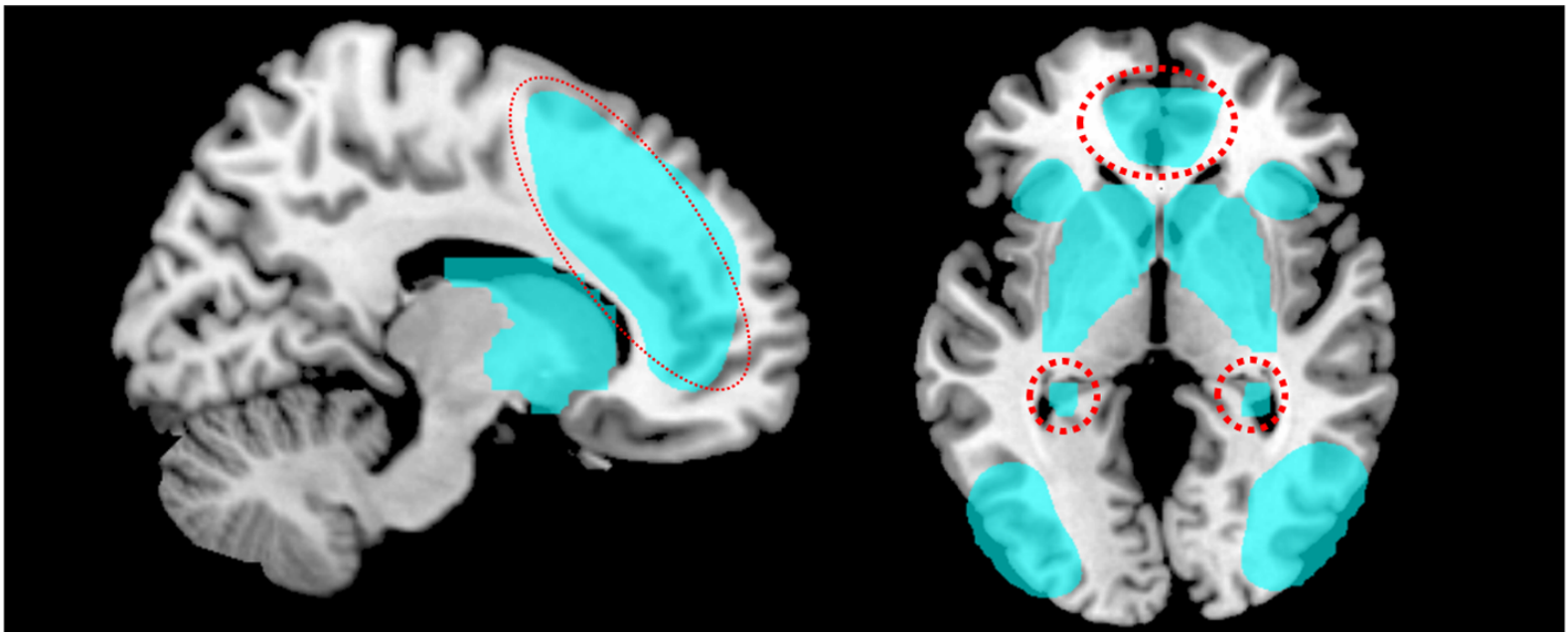


Table 1. Insulin administration techniques for the investigation of brain insulin action		
Method	**Advantage(s)**	**Limitation(s)**
Endogenous stimulation of insulin release		
Mixed-meal tolerance test	The intake of a defined meal stimulates endogenous insulin secretion. The mixture of different nutrients in one meal resembles real-life situations.	A large number of physiological reactions are triggered that might modify insulin secretion or act directly on the brain due to the rewarding properties of food.
Oral glucose tolerance test (oGTT)	The oGTT is a more standardized way of energy intake. After oral ingestion of a 75g glucose solution, blood glucose rises and a number of endocrine factors are released into the circulation, including insulin.	Glucose, insulin and other circulating factors act directly on peripheral tissues, making it difficult to differentiate these peripheral effects from central actions.
Intravenous glucose tolerance test	Insulin secretion is stimulated by an intravenous glucose bolus. This has the advantage of stimulating insulin release without major effects on a number of other endocrine systems.	The route of administration does not reflect the physiological situation. This technique introduces a sharp non-physiological rise in circulating insulin levels.
Exogenous insulin administration		
Hyperinsulinemic euglycemic clamp	Iv. infused insulin continuously reaches the brain, while glucose is kept constant at e.g. normal fasting levels.	Insulin effects are not limited to the brain but occur in most tissues throughout the body, rendering the dissection of peripheral and central effects difficult.
Intranasal Insulin	Insulin enters the nasal cavity and is transported to the CNS, bypassing the blood brain barrier. Hence, systemic exposure is minimized compared to other administration paradigms, disentangling peripheral from central insulin effects.	Very small amounts of the intranasally administered insulin are absorbed into circulation. The route of administration does not reflect the physiological situation.

Table 2. Neuroimaging methods for the investigation of brain insulin action in vivo in humans

Technique	Measured signal/resolution	Methodological details
Functional MRI	Indirect measures of neuronal activity: Cerebral blood flow (CBF) Blood oxygen level dependent signal (BOLD) *Time resolution: seconds* *Spatial resolution: at 3 Tesla approx. 2-3 mm*	BOLD relies on the different magnetic properties of oxygenated and deoxygenated hemoglobin. Due to increases in brain activity, the ratio between oxy- to deoxyhemoglobin changes after an enhanced release of oxygen and increased local CBF. The subsequent decrease in the concentration of deoxygenated hemoglobin, which is paramagnetic, attenuates the local distortion of the magnetic field. Using arterial spin labelling, the direct change in CBF can be measured, providing an absolute quantification of the neural signal (ml/100g brain tissue/min). Arterial blood water flowing into the brain is marked (magnetically 'labeled') by a radiofrequency pulse. The decay of that signal is then measured as a proxy for neural activity.
PET	Probe tissue concentrations of particular molecules of interest using radioactive tracers *Time resolution: seconds* *Spatial resolution: approx. 0.5-1 cm*	Uses the application of radioactive tracers and measures signals by detection of gamma rays. PET typically uses isotopes with a short half-live, which are incorporated either into compounds normally used by the body as glucose or water or into molecules that bind to receptors. Examples: cerebral blood flow by H2 ^{15}O; glucose metabolism by ^{18}F fluorodeoxyglucose (FDG)
EEG	Neuronal activity *Time resolution: <1ms* *Spatial resolution: >1mm*	Measures electric activity of neurons, mostly originating within the cortex, using electrodes attached to the head.
MEG	Neuronal activity *Time resolution: <1ms* *Spatial resolution: >1mm*	Measures the magnetic field generated by electric activity, mostly originating within the cortex, by superconducting sensors distributed in a helmet covering the whole head.

Abbreviations: EEG, electroencephalography; MEG, magnetoencephalography; MRI, magnetic resonance imaging; PET, position emission tomography