OBESITY AND DIABETES: IMPLICATIONS FOR BRAIN-IMMUNOMETABOLISM

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OBESITY AND DIABETES: IMPLICATIONS FOR BRAIN-IMMUNOMETABOLISM

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EDITORIAL
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Editorial: Obesity and Diabetes: Implications for Brain-Immunometabolism

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Keywords: obesity, energy homeostasis, diabetes, neurodegeneration, inflammation

Editorial on the Research Topic

Obesity and Diabetes: Implications for Brain-Immunometabolism

Obesity affects a quarter of the worldwide population and is a risk factor for numerous pathologies (Gregg and Shaw, 2017). It leads to chronic and systemic low-grade inflammation, which impairs the neuronal circuitries dedicated to controlling the hosts' energy homeostasis. While some of the molecular mechanisms underlying obesity started to unfold, the pathology remains intractable (Valdearcos et al., 2015). The 15 articles composing this collection investigate and discuss crucial mechanistic aspects underlying the neurometabolic changes observed in the central nervous system of obese or diabetic individuals.

Hypothalamic microglia undergo various morphological and functional changes in response to high-fat diet. Initially, the resident microglia are activated and, as diet-induced obesity persists, bone marrow-derived myeloid cells gradually replace the brain resident microglia. Mendes et al. and Macedo et al. reviewed the mechanisms underlying such changes to hypothalamic microglial cells. Also, they assess the various roles played by microglia in this context and how one could target aspects of this cascade to develop therapeutic strategies. Next, Rahman et al. reviewed the mechanisms by which glia–glia crosstalk drive overnutrition-induced hypothalamic inflammation.

Rajchgot and colleagues examined the molecular mechanisms by which chronic hyperglycemia leads to overactive spinal microglia. They also analyzed how such changes sensitized nociceptor neurons and lead to diabetic pain neuropathy (Rajchgot et al.).

Gaspar and Velloso review the major findings that support the involvement of hypoxia-inducible factor in the metabolic and energy regulation during the progression of obesity. Metabolic diseases constitute risk factors for the development of neurodegenerative diseases, although the underlying mechanisms behind such effects are not fully clarified.

de Mello and collaborators reviewed recent evidence linking defects in insulin signaling and autophagy to neurodegeneration and discuss potential interventions targeting these pathways (de Mello et al.). Adipokines mediates the communication between the peripheral and central nervous systems. Forny-Germano et al. reviewed the actions of leptin and adiponectin, with emphasis on how altered signaling of these adipokines lead to cognitive dysfunction and augmented the risk of developing Alzhimer's disease in obese individuals.

Using functional magnetic resonance imaging, Rucker and Ikuta revealed a negative association between body mass index (BMI) and pituitary gland functional connectivity. Their data

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highlight the importance of dopamine-rich brain regions and the pituitary gland in feeding behavior and body mass gain. Next, Lizarbe et al. analyzed the effect of a high-fat diet on the cortex, the hippocampus, and the hypothalamus metabolic profiles. 1H-magnetic resonance spectroscopy showed that high-fat diet exposed mice have reduced levels of synaptic and glial proteins compromising hippocampal-dependent spatial memory (Lizarbe et al.). Gomes et al. used a rodent model of Parkinson's disease induced by the administration of 6-hydroxydopamine. In addition to the expected motor impairments, they found no impact on the animals' body mass, food intake, and glucose homeostasis.

Inflammation associated with metabolic disorders breakdown the blood–brain-barrier (BBB), increasing its permeability to peripheral immune cells. Van Dyken and Lacoste reviewed the effects of obesity and diabetes-induced inflammation on BBB permeability as well as roles played by leptin and insulin resistance in this process. The authors also discuss the broader implications of neural inflammation, including its connection to Alzheimer's disease, multiple sclerosis, and the gut microbiome. The role of the crosstalk between intestinal immune cells and the enteric nervous system in the control of blood glucose was addressed by Bessac et al. The authors focused on the gut-brain axis as a major pathway for the nutritional stated of the body.

Vagus nerve and brain cholinergic signaling are important in the regulation of metabolic homeostasis and immune function; relevant aspects of this regulation were reviewed with a specific focus on obesity-associated conditions (Chang et al.). In this review, the authors outlined accumulating preclinical evidence for the therapeutic efficacy of cholinergic stimulation in alleviating obesity-associated inflammation, neuroinflammation, and metabolic derangements. Thus, a rationale for further therapeutic developments using pharmacological and bioelectronics cholinergic modulation for clinical benefit was proposed.

Albeit being not long-lasting, pharmacotherapy and bariatric surgeries were demonstrated to be useful tools in the management of morbid obesity. It is well-accepted that lifestyle changes, such as physical exercise, have beneficial effects controlling the BMI, brain energy metabolism, and have neuro-protective effects. The benefits of physical exercise were reviewed by de Oliveira Bristot et al., giving a particular attention on the muscle-neuron crosstalk, mediated by irisin, peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator 1-alpha (PGC-1α) and mitochondrial uncoupling protein (UCP). Deep brain stimulation (DBS) consists of delivering electrical impulses to specific brain regions modulating neuronal circuit is an approved non-pharmacological therapy for movement disorders. Formolo et al. reviewed the potential effectiveness of DBS for for the treatment of obesity.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the writing and editing of the editorial manuscript.

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MINI REVIEW
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Inflammation and Gut-Brain Axis During Type 2 Diabetes: Focus on the Crosstalk Between Intestinal Immune Cells and Enteric Nervous System

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The gut-brain axis is now considered as a major actor in the control of glycemia. Recent discoveries show that the enteric nervous system (ENS) informs the hypothalamus of the nutritional state in order to control glucose entry in tissues. During type 2 diabetes (T2D), this way of communication is completely disturbed leading to the establishment of hyperglycemia and insulin-resistance. Indeed, the ENS neurons are largely targeted by nutrients (e.g., lipids, peptides) but also by inflammatory factors from different origin (i.e., host cells and gut microbiota). Inflammation, and more particularly in the intestine, contributes to the development of numerous pathologies such as intestinal bowel diseases, Parkinson diseases and T2D. Therefore, targeting the couple ENS/inflammation could represent an attractive therapeutic solution to treat metabolic diseases. In this review, we focus on the role of the crosstalk between intestinal immune cells and ENS neurons in the control of glycemia. In addition, given the growing evidence showing the key role of the gut microbiota in physiology, we will also briefly discuss its potential contribution and role on the immune and neuronal systems.

Keywords: enteric nervous system, immune cells, microbiota, gut-brain axis, diabetes

OVERVIEW OF THE DIFFERENT TYPES OF IMMUNE CELLS IN THE INTESTINE: THE EXAMPLE OF ITS RELATION WITH THE GUT MICROBIOTA

The population of gut microbiota is estimated to 100 trillion microorganisms. Thus, our body is composed of 50% of bacteria and 50% of human cells (Cani, 2016). Precisely, the human body encloses approximatively 4.10^{13} bacteria cells with the largest amount inside the large intestine (10^{11} bacteria cells/g of wet stools) (Sender et al., 2016a). In the last estimation, almost 10 million non-redundant microbial genes have been identified in the human gut (Li et al., 2014). The intestinal tract is subjected to an environmental pressure due to the high microbial load of the luminal content (Sender et al., 2016b). Regarding microbial load, one recent article shows that it is the absolute quantity of microbes and not the proportions of microbes that really matters

(Vandeputte et al., 2017). Depending of the mode of analysis of gut microbiota population (classical relative abundance-based profiling vs. quantitative microbiome profiling), the interpretation of data could be completely different, and maybe falsely interpreted. The study of gut microbiota impacts in physiology is also complicated due to the controversial data present in the literature. In fact, results could differ depending of numerous factors that we have to take into account such as for example dietary environments, but also the composition and the activity of the gut microbes (Cani, 2018a). To illustrate this controversy, *Prevotella copri* and its metabolites could be considered as pro- or anti-diabetic actions depending on the diet or the model used (Cani, 2018a). Therefore, these few examples clearly highlight the fact that we are still at the beginning of the story, and we will need more time to better understand the gut microbiota and its importance in human health.

Nowadays, the impact of gut microbiota in the control of various physiological functions is proposed (Cani, 2018a). Abnormal composition and/or activity of the gut microbiota are associated with the development of numerous pathologies such as cancer, obesity and type 2 diabetes (T2D) (Cani, 2018a; Cani and Jordan, 2018b; Rastelli et al., 2018). Despite the complexity of the crosstalk, a clear link is established between inflammation and modification of the gut microbiota (Stecher, 2015; Cani, 2018a). Here, we will mainly introduce how gut bacteria could modulate the function of intestinal immune cells, and describe the molecular actors involved.

Intestinal bacteria are physically separated from mucosal immune system by a single epithelial cell layer, which the primary function is to absorb nutrients (small intestine) and water (colon). Mucosal immune system prevent microbial invasion and is tightly regulated. One of its major role is to avoid the development of chronic inflammation and the subsequent loss of the intestinal epithelium integrity. Microfold cells or M cells, in the specialized follicle-associated epithelium overlying Peyer Patches (PP), and isolated lymphoid follicles (ILF) are the major cell types that sample bacteria and associated antigens. Processed bacterial-derived antigens are presented locally (i.e., into the PP or ILF) or within the mesenteric lymph nodes that drain dendritic cells to initiate an adaptive immune response (Wells et al., 2017). Both effector- and regulatory-T lymphocytes scattered within the intestinal mucosa are generated in response to commensal bacterial antigens. At steady state, the number and the spectrum of effector-T lymphocytes subsets that are present within the intestinal mucosa are dependent on the host's microbiota. Any pathogen invasion, disruptions of the mucus barrier or of the intestinal epithelium integrity, and/or failure in the regulatory mechanisms of the immune response may result in mucosal inflammation (Barreau and Hugot, 2014; Al Nabhani et al., 2017). Immune mediators released upon inflammation are largely dependent on the nature of the microbes triggering the immune system (Maloy and Powrie, 2011). Intestinal epithelial cells and resident innate immune cells sense pathogens locally. The interactions between the pathogens and the pattern-recognition receptors (PRRs) expressed both by stromal and immune cells trigger rapid production of immune and microbicide mediators (e.g., cytokines, chemokines, bioactive lipids, and cell-autonomous immune effectors), which restrict pathogen growth. In parallel, dendritic cells will mature upon contact with microbe-associated molecular patterns (MAMPs) or when some factors are released by injured tissues, namely the damage associated molecular patterns (DAMPs) (i.e., a process allowing antigen presentation to T cells) (Maloy and Powrie, 2011; Geginat et al., 2015). Regarding the intrinsic properties of mature dendritic cells and their soluble (e.g., cytokines, chemokines) and cellular (stromal, myeloid and lymphoid cells) immune environment, antigen-primed $CD4^+$ T lymphocytes may acquire different effector functions. Indeed, viral or intracellular bacterial infections drive T lymphocyte commitment toward the Th1 phenotype, a process that relies both on the production of IL-12 and IL-18 by myeloid cells and the subsequent IFNγ released by innate lymphoid cells (ILC)1 (Trinchieri, 2003; Bernink et al., 2013). Th1 $CD4^+$ lymphocytes produce high levels of IFNγ but also TNF-α. The clearance of extracellular bacteria and fungi mainly depends on Th17-polarized lymphocytes that produce IL-17, IL-22, IL-21, TNF-α and GMCSF. The differentiation of naïve $CD4^+$ T lymphocytes into Th17 involves an environment enriched in TGFβ, IL-1β and STAT3-inducing cytokines including IL-6 and IL-23 produced by myeloid cells (e.g., dendritic cells and macrophages). IL-23 plays a key role by reinforcing Th17 functional activity and inducing production of GMCSF and TNF-α. The immune polarization of ILCs from group 2 by IL-25, IL-33 and thymic stromal lymphopoietin is a process specific to helminth parasites. This leads to a potent IL-13 release, crucial immune mediator of the anti-parasitic response (Neill et al., 2010). This frontline type 2 cytokine signaling drives the differentiation of T lymphocytes toward the Th2 phenotype, which is characterized by the synthesis of IL4, IL-5 and IL-13 cytokines, that are key to restrict helminth infections. In addition to cytokines, $CD4^+$ T lymphocytes may also produce neuropeptides including opioids, serotonin and vasoactive intestinal peptide (O'Connell et al., 2006; Boue et al., 2011; Souza-Moreira et al., 2011). Within the site of inflammation, most of these neuropeptides as well as chemokines, proteases and bioactive lipids are also produced by endothelial and epithelial cells, migrant and resident innate immune cells.

How make the link between gut microbiota, immunity and metabolic diseases? The development of metabolic disorders is clearly linked with modification of the gut microbiota population, but there are many other factors in the intestine (e.g., bioactive molecules, metabolites, endocrine cells) that are disturbed in pathological state. The diversity of the gut microbiota may not fully explain the pathologic phenotype. In fact, recent data show the importance of specific alterations that may arise at the level of the crosstalk occurring between gut microbes but also luminal and intestinal factors during metabolic disorders (Aron-Wisnewsky et al., 2018; Cani, 2018). One may also mention the controversy regarding microbial diversity and its potential link with the onset of inflammation. This important topic has been recently reviewed and clearly described the potential interaction between microbes and host cells *via* immune factors (Cani, 2018a). Modifications of the balance (i.e., gut

microbiota and immunity) between healthy and pathological situations participate to the establishment of a type 2 diabetic state. For example in this review (Cani, 2018a), it is discussed that the production of the short-chain fatty acids propionate is altered during diabetes. In physiological condition, propionate can bind to GPR-43 located on intestinal lymphocytes. In pathological situations, the decrease of propionate may lead to the lower abundance of specific T cells (mucosal-associated invariant T cells and Treg) in the lamina propria of the gut. Consequently, the gut barrier function could be altered, and some pathogen-associated molecular patterns (PAMPs) can reach the circulation. This is for instance the case for the levels of lipopolysaccharides (LPS) that are significantly increased in the blood, and trigger systemic low-grade inflammation (Cani et al., 2007; Chu et al., 2018) observed in the whole body and can generate the insulin resistance state observed during diabetes (Cani et al., 2012).

INTESTINAL INFLAMMATION AND TYPE 2 DIABETES

Obesity and associated metabolic disorders such as T2D are closely related to a « low-grade » inflammation state as described above (Wellen and Hotamisligil, 2005). In addition to well-described tissues (liver, adipose tissue,. . .) (Hotamisligil, 2006), the intestine may presents an alteration of the gut barrier function thereby leading hyper-permeability, which is associated with a dysbiosis and in some cases with a certain degree of inflammation at the level of the gut (for review see Slyepchenko et al., 2016). Indeed, in response to a high-fat diet (HFD) that induces insulin resistance, it has been proposed that the intestinal tract may develop an inflammatory response characterized by an increase in pro-inflammatory cytokines expression such as TNF-α, IL-1β, IL-6 (Ding et al., 2010; Kim et al., 2012). Some studies have also reported an increase in myeloperoxidase activity (de La Serre et al., 2010) and/or a decrease in IL-18 and IL-22 expression (Everard et al., 2014a; Wang et al., 2014), two cytokines involved in the intestinal barrier homeostasis and the defense against pathogens. However, it is important to note that the degree of inflammation observed during obesity and diabetes does not reach the same magnitude as the one observed during intestinal inflammatory bowel diseases or severe infection. Nevertheless, besides HFD, data also support that the genetic deletion of leptin (ob/ob) or leptin receptors (db/db) in mice leads to the development of obesity and T2D with a significant increase in pro-inflammatory cytokines (TNF-α, IL-1β, IFNγ, IL-6) in the portal vein, as well as an altered gut barrier (Brun et al., 2007). In addition to metabolic consequences, deletion of leptin receptors also alters immunological response against *Entamoeba histolytica* in mice (Guo et al., 2011) as well as in humans (Duggal et al., 2011). The direct link between intestinal inflammation and metabolic disorders is observed in mice knocked-out for TLR5, a component of the innate immune system. TLR5-deficient mice exhibit a diabetic phenotype associated with intestinal inflammation including increase in pro-inflammatory cytokines (IL-1β, TNF-α) and reduction of the length of the colon, one hallmark of intestinal inflammation (Vijay-Kumar et al., 2010; Chassaing et al., 2014).

What are the "local" consequences of this gut inflammation? All mice models above display alterations of the intestinal homeostasis suggesting as close interrelation between "inflammation, microbiota and metabolism" (Geurts et al., 2014). Because of this inflammatory environment, diabetic mice exhibit modifications of intestinal absorptive capacity characterized by an increase of glucose and/or lipids absorption (Ferraris and Vinnakota, 1995; Petit et al., 2007; Mao et al., 2013) that alters the energetic metabolism. Indeed, HFD diabetic mice develop hyperglycemia and hyperinsulinemia (Ding et al., 2010), glucose intolerance and insulin resistance correlated with a decrease in mucus thickness and increased intestinal permeability (Cani et al., 2008; Everard et al., 2013). This is associated with alteration of the gut microbiota composition and activity (Everard et al., 2011, 2014b; Kim et al., 2012; Molinaro et al., 2017) have shown that gut colonization of germ-free mice induces a bi-phasic inflammation that participates to a bi-phasic impairment of glucose metabolism. Interestingly, recent data have also shown in humans a link between gut microbiota and the accumulation of T cells in the gut of obese people following the ingestion of a high-fat diet (Monteiro-Sepulveda et al., 2015). Both the blunted insulin signaling in enterocytes and the altered localization of the glucose transporter GLUT2 in the enterocytes of obese subjects were linked to the nature of cytokines produced by mucosal T cells (Monteiro-Sepulveda et al., 2015). Conversely, in obese and type 2 diabetic patients another study has shown a decreased number of mucosal-associated invariant T cells (MAIT) producing high levels of Th1 and Th17 cytokines (Magalhaes et al., 2015).

Therefore, the molecular partners linking inflammation, gut microbiota and metabolic disorders could be multiple, but the incretin hormones may represent a real potential of interest. The intestinal tract has also the capacity to produce incretins such as Glucagon-Like Peptide-1 (GLP-1) or Gastric Inhibitory Polypeptide (GIP) in response to nutrients and/or bacterial factors (Cani et al., 2013; Rastelli et al., 2018). HFD and ob/ob mice display an alteration of gut endocrine system including the reduction of GIP- and GLP-1-producing cells and a decrease in proglucagon and GIP expression associated with the alteration of incretins secretion in response to an oral glucose load (Morgan et al., 1988; Anini and Brubaker, 2003; Richards et al., 2016; Shimazu-Kuwahara et al., 2017).

In line with these observations, we have demonstrated that prebiotic treatment in diabetic mice restores the level of GLP-1 to improve glucose metabolism (Cani et al., 2006). Moreover, a chronic exposure to TNF-α alters the release of GLP-1 in HFD mice by acting directly on GLP-1-producing cells (Gagnon et al., 2015). Conversely, a 2 weeks treatment with etanercept, an anti-TNF-α drug, reduces hyperglycemia and hyperinsulinemia of HFD mice. Nowadays, this tripartite concept developed in mice

is considered as a potential target to treat metabolic disorders (Geurts et al., 2014).

GUT-BRAIN AXIS AND TYPE 2 DIABETES

The gut-brain axis is shown as a major signaling pathway controlling glucose homeostasis, and its disturbance is associated with a T2D phenotype (Abot et al., 2018a,b). In normal conditions, glucose is detected by intestinal glucose sensors (such as SGLT1, GLUT2, TASR1/2) present on intestinal cells (enteroendocrine and brush cells, enteric glial cells and neurons) which inform the whole body from the presence of glucose *via* the release of various factors (i.e., gut hormones, neurotransmitters, metabolites) (Fournel et al., 2016). Then, intestinal glucose detection generates an afferent nervous message that provokes an increase of hypothalamic nitric oxide (NO) release (Fournel et al., 2017). In turn, this increase of hypothalamic NO release favors the entry of glucose in tissues *via* an activation of the autonomic nervous system (Fournel et al., 2017). In db/db mice, intestinal inflammation perturbs the detection of glucose by enteric glucose sensors (Duparc et al., 2011). In this case, the intestine sends an aberrant nervous message that fails to increase hypothalamic NO release (Duparc et al., 2011). Accordingly, the insulin resistance state observed during T2D is linked to alteration of gut-brain axis. Recently, we have discovered that the enteric nervous system (ENS) is implicated in the maintenance of glycemia in the whole body *via* the brain. One of the main functions of ENS is to regulate contractions of intestinal smooth muscle cells. In physiological conditions, the reduction of intestinal contractions provokes a decreased fed hyperglycemia (Fournel et al., 2017; Abot et al., 2018b). The modifications of mechanical contractions are detected by the hypothalamus, which controls glucose utilization (Fournel et al., 2017). During T2D, intestinal inflammation is clearly associated with a dysfunction of ENS neurons (Gonzalez-Correa et al., 2017), which could favor duodenal hyper-contractility (Abot et al., 2018b). Our group has demonstrated that duodenal hyper-contractility participates to the establishment of hyperglycemia of HFD mice by modulating the hypothalamic NO release (Fournel et al., 2017).

Numerous pathologies that link inflammation, gut microbiota and ENS have been described (Greenwood-Van Meerveld et al., 2017). Along those lines, recent data have shown that specific neurodegenerative diseases (i.e., multiple sclerosis, Parkinson disease) were also characterized by gastrointestinal comorbidities. In these patients, the specific alterations in gut motility and other pre-motor symptoms are linked with changes in the gut microbiota composition and activity (Endres and Schafer, 2018). Whether the couple microbiota and metabolites influencing the intestinal neuronal system may explain the onset of the disease remains to be proven, but it is becoming clear that the ENS can be considered as a cellular target to treat inflammatory diseases (T2D, inflammatory bowel diseases and ulcers).

Therefore, targeting the intestinal inflammation and/or the gut microbiota to restore the glucose and mechano-sensors in type 2 diabetic patients presents a real potential of scientific interest, but remains to be determined.

HOW THE INFLAMMATION MODULATES THE ENTERIC NERVOUS SYSTEM?

Environmental changes related to the inflammatory processes that include a large spectrum of innate and adaptive immune responses, may affect ENS including myenteric plexus controlling gut motility (Cani et al., 2006).

Intestinal motility is regulated by ENS that includes intrinsic primary afferent neurons (IPAN), interneurons and choline acetyltransferase (ChAT)-producing excitatory and NO synthase (NOS)-producing inhibitory motor neurons (Abot et al., 2018a). In the context of immune-mediated regulation of intestinal motility, *muscularis* macrophages residing in the myenteric plexus have been largely studied (Mikkelsen, 2010; Muller et al., 2014; De Schepper et al., 2017). However, cytokines, proteases or neuropeptides produced by mucosal immune cells may also modulate the activity of sensory neurons.

IPANs with their axons extending throughout intestinal mucosa and their cell bodies connected with interneurons which synapse with motor neurons may thus constitute central players in the immune-modulation of the intestinal motility. Accordingly, inflammatory cytokines including TNF-α, IL-1β or IL-6 as well as neuromediators released by innate and adaptive immune cells upon intestinal inflammation alter intrinsic and extrinsic enteric sensory neuron activities (Kindt et al., 2010; Basso et al., 2014; Boue et al., 2014; Brierley and Linden, 2014; Buckley et al., 2014; Hughes Moretta et al., 2014; Mawe, 2015). A slower small-bowel transit and reduced smooth muscle contractility have been reported in patients with inflammatory bowel diseases (Rao et al., 1987; Mawe, 2015). In experimental colitis, intestinal motility is either enhanced or reduced depending on the Th1 or Th2 types of inflammation. Th2-driven colitis induces smooth muscle hyper-contractility and augments intestinal transit while Th1-driven colitis is rather associated with hypo-contractility and reduced intestinal transit (Zhao et al., 2003; Shea-Donohue et al., 2012). Moreover, chronic intestinal inflammation often results in increased innervation together with a reduction of the neuronal activation threshold (Villanacci et al., 2008; Brierley and Linden, 2014). The expression of TNF-α receptors (TNFR1, TNFR2) on enteric neurons also suggest that the neuronal activity maybe modulated directly by the inflammatory environment (Chandrasekharan et al., 2013; Gougeon et al., 2013). Actually, TNF-α has the capacity to modulate electrophysiologic properties of neurons *via* a potentiating effect by increasing the action of cholinergic agonists (carbachol) on gut motility (Rehn et al., 2004). This property of TNF-α could explain the negative impact of cytokines on gut motility. In a molecular point of view, the effect of TNF-α is mediated by an upregulation of the Cyclo-Oxygenase

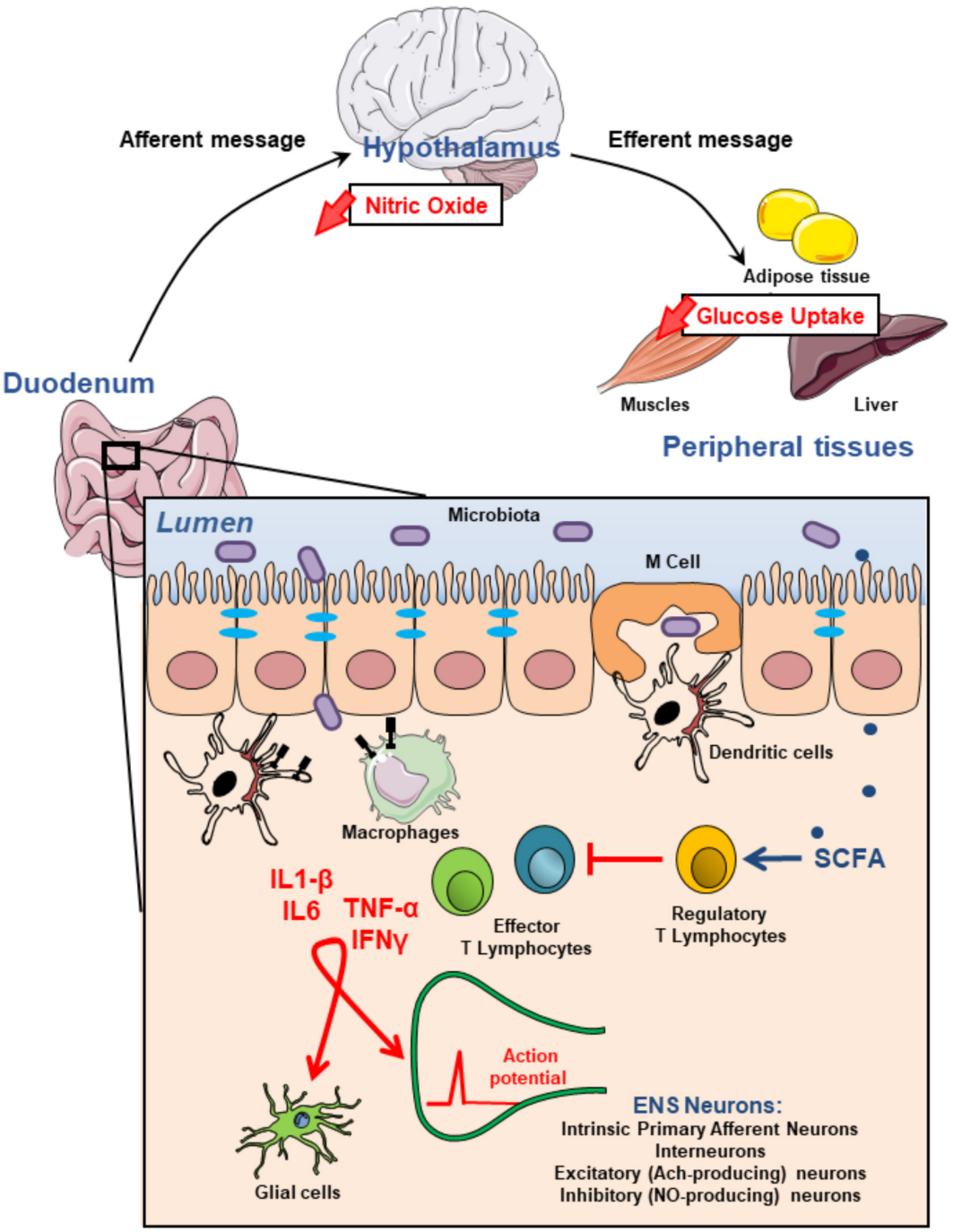

FIGURE 1 | Modulation of the enteric nervous system activity by intestinal immune system may impact on glycemia regulation. Most microbiota-derived products activate both epithelial cells and innate immune cells through pathogen recognition receptors such as toll-like receptors. They also initiate, mainly *via* microbe sampling through M(icrofold) cells, an adaptive immune response. This physiological immune response against intestinal flora generating pro-inflammatory effector T lymhocytes is tighly regulated by regulatory T lymphocytes which induction is associated with the production of short chain fatty acids (SCFAs) by commensal bacteria. Failure of the immune homeostasis may result in the release of higher amounts of proinflammatory cytokines including TNFα, IL1-β, IL6, IFNγ which, in turn, modulate either directly or through glial cells, enteric nervous system (ENS) activity. These alterations of the ENS contribute to impair gut-brain-peripheral axis leading to hyperglycemia and insulin-resistance.

(COX-2), which in turn increases the production of an arachidonic acid derived-molecule, Thromboxane A2 (TXA_2) (Rehn et al., 2005). In experimental colitis, Chandrasekharan et al. (2013) have also demonstrated that TNF-α has an effect on the apoptosis rate of enteric neurons. Treatment with TNF inhibitors provokes a significant decrease of inflammatory markers expression such as inducible NOS (iNOS). IL-1β is another cytokine that has its receptors on ENS neurons (Gougeon et al., 2013). Similarly to TNF-α, IL-1β may increase iNOS mRNA expression in myenteric neurons (Valentine et al., 1996). IL-1β could increase excitability of neurons by directly acting through its receptors expressed on cell surface. Mechanisms of IL-1β including depolarization of the membrane potential, decreased membrane conductance, and increased discharge of action potentials could explain the diarrhea phase, which appears in obese/diabetic patient (Acosta and Camilleri, 2014) or during intestinal inflammation (Xia et al., 1999).

In addition to neurons, the ENS is composed of glial cells that exert a crucial role in intestinal physiology. Suppression of glial cells alters the function of ENS neurons with an increase in excitatory ChAT neurons and a decrease in inhibitory NOS neurons (Aube et al., 2006). Glial cells are exposed to various molecules depending on the environment including immune factors. Proinflammatory cytokines such as IL-1β and/or low IL-10 concentrations have the capacity to block glial cells proliferation (Ruhl et al., 2001a), highlighting links between immune system and enteric nervous system. Moreover, IL-1β has also the capacity to regulate secretory functions of glial cells by increasing IL-6 synthesis and secretion (Ruhl et al., 2001b). In turn, IL-6 may modulate T cell apoptosis or control gut motility to delay gastric emptying (Atreya et al., 2000; Lang Lehrskov et al., 2018).

SUMMARY/CONCLUSION

T2D is associated with modification of the gut physiology including alterations of the function of immune cells and ENS. The crosstalk between the two partners is under the influence of the gut bacteria, which release bioactive factors that could participate as intermediate signaling molecules. Some evidence suggests that the establishment of T2D phenotypes (i.e., hyperglycemia, insulin resistance) may have a potential enteric origin; this is also the case of some neurological disorders (e.g., multiple sclerosis, Parkinson disease, autism). Deciphering the impact of "immune cells-ENS" communication and its impact on "gut-brain" axis could represent a new therapeutic perspective for diabetic patients (**Figure 1**). Therapeutic strategies have to take into account the importance of intestinal inflammation and its consequence on integrity of ENS. The gut microbiota and its bioactive releasing factors could represent one potential solution.

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REVIEW
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Hypoxia Inducible Factor as a Central Regulator of Metabolism – Implications for the Development of Obesity

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The hypothalamus plays a major role in the regulation of food intake and energy expenditure. In the last decade, it was demonstrated that consumption of high-fat diets triggers the activation of an inflammatory process in the hypothalamus, inducing neurofunctional alterations and contributing to the development of obesity. Hypoxia-inducible factors (HIFs) are key molecules that regulate cellular responses to inflammation and hypoxia, being essential for the normal cell function and survival. Currently, evidence points to a role of HIF pathway in metabolic regulation that could also be involved in the progression of obesity and metabolic diseases. The challenge is to understand how HIF modulation impacts body mass gain and metabolic disorders such as insulin resistance. Distinct animal models with tissue-specific knocking-out or overexpression of hypoxia signaling pathway genes revealed a cell-specificity in the activation of HIF pathways, and some of them have opposite phenotypes among the various HIFs gain- and loss-of-function mouse models. In this review, we discuss the major findings that provide support for a role of HIF pathway involvement in the regulation of metabolism, especially in glucose and energy homeostasis.

Keywords: HIF complex, obesity, metabolic disorders, inflammation, hypothalamus, energy homeostasis

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INTRODUCTION

Obesity has reached epidemic proportions around the world. It is characterized by excessive and abnormal fat mass accumulation, associated with chronic low-grade systemic inflammation, which predispose to metabolic diseases. In 2015, in the adult population, a total of 603.7 million were obese and approximately 1.9 billion were overweight. In addition, childhood obesity has increased considerably raising concern issues on the future of public health in the world (Collaborators et al., 2017; Gregg and Shaw, 2017).

Obesity has a multifactorial and complex etiology, involving the combination of genetic and epigenetic factors, life style, and socioeconomic status, which result in increased food intake, decreased energy expenditure, and also changes in nutrient utilization and metabolism, ultimately leading to a positive energy balance. The aberrant expansion of white adipose tissue, through hyperplasia as well as hypertrophy, promotes insufficient intra-adipose blood perfusion resulting

in hypoxia. These niches of hypoxia are partially responsible for white adipose tissue inflammation and insulin resistance (Jiang et al., 2011; Lee et al., 2011, 2014; Krishnan et al., 2012; Michailidou et al., 2015). Hypoxia-inducible factor-α (HIF-α) is a fundamental transcription factor involved in the oxygen homeostasis that induces inflammation and insulin resistance in obesity. In addition, the abnormal regulation of HIF complex is involved in the development of obesity, fatty liver disease, and type 2 diabetes (Virtue and Vidal-Puig, 2011; Girgis et al., 2012). Moreover, recent data show that HIF has important functions in the hypothalamus, the region of the brain that controls energy balance and metabolism (Lando et al., 2002; Bento and Pereira, 2011; Zhang et al., 2011; Ban et al., 2014).

Currently, the approaches used to treat obesity include behavioral counseling for reducing caloric intake and increasing physical activity, the use of some pharmacological compounds that reduce hunger and caloric harvesting from the gut and bariatric surgery. However, these interventions have not proven effective in providing long term weight loss, highlighting the importance of development of new strategies to tackle obesity (Gonzalez-Muniesa et al., 2017; Narayanaswami and Dwoskin, 2017; Srivastava and Apovian, 2018). Several animal studies manipulating HIF pathways highlight the involvement of this transcriptional factor in the pathogenesis of obesity placing it as a potential target for the treatment of obesity and diabetes. The current challenge is to understand how HIF modulation might impact body weight gain and metabolic disorders such as insulin resistance. Here, we review data that have contributed to expand the current understanding of how HIF regulates whole body metabolism and how it is affected by nutritional and immune signals that are involved in the pathophysiology of obesity and metabolic disorders.

HYPOTHALAMIC REGULATION OF ENERGY EXPENDITURE

The regulation of energy homeostasis is a highly integrated and regulated process aimed at maintaining the stability of body energy stores over time. Neurons located in the hypothalamus are the most important components of the complex system that regulates energy flux in the body. Hypothalamic neurons respond to peripheral signals controlling food intake and energy expenditure on a homeostatic way, and also integrate with limbic and cortical areas to coordinate the hedonic responses to food (Timper and Bruning, 2017; Rossi and Stuber, 2018).

The hypothalamus, located at the base of the forebrain and around the walls of third ventricle, is at a privileged location to receive afferent signals from the periphery through the bloodstream, and also from the brainstem. These signals inform hypothalamic neurons about the magnitude of the energy stores in body. In turn, the neurons respond providing signals that regulate hunger and energy expenditure, as well as systemic glucose and lipid metabolism (Williams and Schwartz, 2011; Kim et al., 2018). The hypothalamus is composed by several nuclei arrayed in a three-dimensional organization, each with distinct neuronal connections and functions, including the arcuate nucleus (ARC), paraventricular nucleus (PVN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN), and lateral hypothalamic area (LHA; Baroncini et al., 2012; Kim et al., 2018).

The role of the hypothalamus in feeding and metabolism was first demonstrated in the 1940s, when severe hypophagia was observed after large lesions centered on the lateral hypothalamus (Hetherington and Ranson, 1940). Conversely, lesions on the VMH and ARC induced hyperphagia and obesity (Marshall and Mayer, 1956; Olney, 1969). Such studies led to the definition of the central role played by the hypothalamic nuclei controlling satiety and hunger (Timper and Bruning, 2017; Kim et al., 2018).

The strategic location of ARC in proximity to the median eminence places its neurons in close contact with a special type of blood/spinal fluid interface that is less restrictive than the classical blood/brain barrier (Haddad-Tovolli et al., 2017). Due to this anatomical particularity, ARC neurons are exposed to systemic oscillations of hormone and nutrient levels providing rapid and dynamic information required for optimal regulation of whole body energy homeostasis. Within the ARC, there are three main neuronal subpopulations: a subpopulation of anorexigenic neurons that co-express cocaine and amphetamine-related transcript (CART) and proopiomelanocortin (POMC; Vrang et al., 1999; Yaswen et al., 1999); and two subpopulations of orexigenic neurons, one co-expressing neuropeptide Y (NPY) and agouti-related protein (AgRP; Hahn et al., 1998; Luquet et al., 2005), and another expressing tyrosine hydroxylase (TH; Zhang and van den Pol, 2015, 2016). ARC neurons communicate with other hypothalamic areas (PVN, DMN, and LHA) involved in appetite regulation.

Hormonal Regulation

The identification of leptin in 1994 (Zhang et al., 1994) is a hallmark that provided advance in the characterization of hypothalamic pathways/mechanisms involved in the regulation of energy homeostasis. Leptin is secreted by adipose tissue and circulates at concentrations proportional to fat mass providing key signals for the regulation of food intake (**Figure 1**). Leptin binds to the long form of the leptin receptor, Ob-Rb, both in POMC and AgRP neurons in the hypothalamus. POMC neurons are activated by leptin, while AgRP neurons are inhibited (Cheung et al., 1997; Williams and Schwartz, 2011; Lee et al., 2018). Insulin is also an important regulator of food intake and energy expenditure. Insulin receptors are distributed all over the brain, particularly in hypothalamic neurons and upon ligand-induced stimulation it activates POMC neurons and inhibit food intake (Woods et al., 1979; Belgardt et al., 2008; **Figure 1**).

Activation of POMC neurons induces the release of α-melanocyte-stimulating hormone (α-MSH) that is an endogenous agonist of the melanocortin 3 and 4 receptors (MC3-R and MC4-R), mostly localized in the PVN (Cowley et al., 2001; Mountjoy, 2015). Conversely, AgRP is an endogenous antagonist of MC4-R in the PVN. The inhibition of AgRP neurons by leptin blocks the activation of PVN neurons, which results in the inhibition of synaptic activation, increasing food intake and consequently decreasing energy expenditure (Ollmann et al., 1997; Mizuno and Mobbs, 1999; Lee et al., 2018).

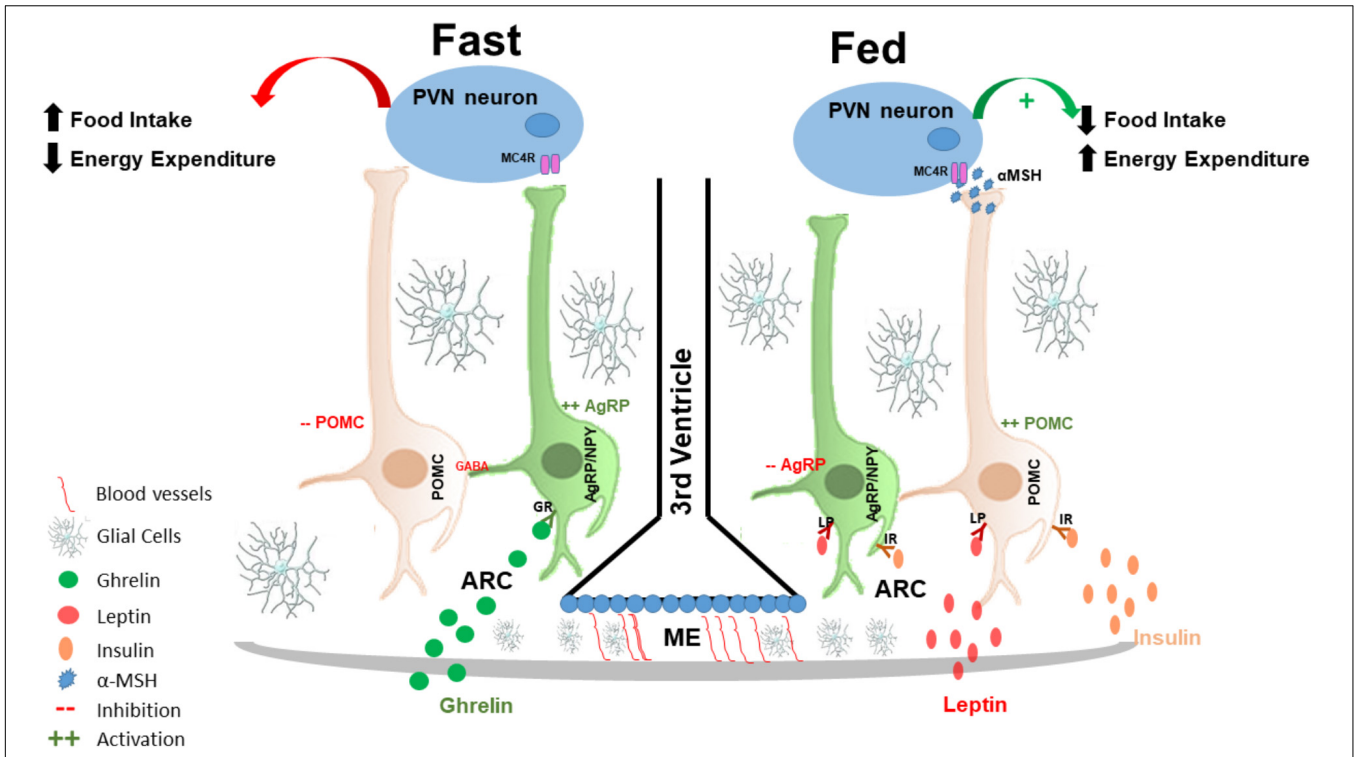

FIGURE 1 | Hypothalamic regulation of energy homeostasis: the hypothalamus senses and integrates peripheral adipostatic hormones that circulate in levels proportionate to nutritional status and adipose tissue stores. Under fasting conditions ghrelin, secreted by the stomach, binds to its receptor in AgRP neurons in the ARC, leading to its activation. AgRP neuronal activation increased the release of AgRP that is an antagonist of MC4R in the PVN neurons; at the same time, AgRP neurons also release of GABA neurotransmitter that inhibited POMC neurons, and consequently increasing appetite and decreased energy expenditures. In the fed state, insulin and leptin act directly on their receptors, both in POMC and AgRP neurons in the ARC of the hypothalamus to control energy homeostasis; insulin and leptin induce inhibition of AgRP and activation of POMC neurons. POMC neuronal activation involves the processing of POMC with formation of α-MSH that is an agonist of MC4R and consequently activating PVN neurons. PVN activation culminates in satiety (decreased food intake) and stimulation of energy expenditure. AgRP, agouti related protein; MC4R, melanocortin 4 receptor; GABA, gamma-aminobutyric acid; POMC, proopiomelanocortin; α-MSH, alpha-melanocyte-stimulating hormone; PVN, paraventricular nucleus.

Ghrelin is yet another hormone that is involved in hypothalamic neuronal regulation. It is primarily produced by the stomach in fasting condition and has the property of inducing food intake, through the activation of ghrelin receptors expressed predominantly in AgRP neurons (Al Massadi et al., 2017). Thus, AgRP/NPY neurons are activated by fasting and ghrelin (Kamegai et al., 2000; Nakazato et al., 2001; Tang-Christensen et al., 2004; Betley et al., 2015), leading to a greater release of AgRP; therefore, stimulating appetite. Furthermore, AgRP neuronal activation also leads to the release of gamma-aminobutyric acid (GABA) and NPY that inhibit POMC neurons (Cowley et al., 2001; Atasoy et al., 2012) providing further increase in food intake.

Nutrient Sensing

There are extensive data indicating that in addition to hormonal factors, hypothalamic neurons can also sense nutrients (glucose, lipids, and some amino acids, such as leucine) to modulate food intake and energy expenditure (Loftus et al., 2000) and also to regulate systemic glucose and lipid levels (Obici et al., 2002b; Chari et al., 2010; Thorens, 2012).

The first demonstration that hypothalamus can sense nutritional status came from studies of Miselis and Epstein (1975), showing that the induction of glucoprivation in hypothalamus increases feeding behavior. Thereafter, two hypothalamic neuronal subpopulations were defined as glucose-excited (GE), found mostly in the lateral ARC, and glucose-inhibited (GI) neurons, found in the medial ARC (Wang et al., 2004; Thorens, 2012). Later on, studies demonstrated that GE are predominantly POMC neurons (Parton et al., 2007), whereas GI are predominantly AgRP (Chalmers et al., 2014). Hypothalamic increase of glucose concentration results in a decrease in AgRP mRNA levels and to an activation of POMC neurons, which leads to decreased food intake, decreased hepatic glucose production and increased insulin secretion (Thorens, 2012).

Energy depletion is sensed by mechanisms that activate catabolic pathways to mobilize cellular components for the provision of energy, such as autophagy and 5′ AMP-activated protein kinase (AMPK). AMPK is a central enzyme for the regulation of whole body metabolism, its activation increases fatty acid oxidation, glucose uptake, and stimulates glycolysis and mitochondrial biogenesis (Ha et al., 2015). In POMC and AgRP neuronal populations, the activation of AMPK acts as

a nutrient sensor and as a regulator of satiety (Claret et al., 2007; Kola, 2008), by altering the expression of orexigenic or anorexigenic neuropeptides. Alterations in hypothalamic glucose concentration act in part by modulating the activity of AMPK which is an important nutrient sensor in several tissues in the body, including the hypothalamus (Minokoshi et al., 2004; Claret et al., 2007; Lockie et al., 2018). Intracerebroventricular (icv) glucose infusion decreases AMPK activity in POMC neurons and consequently reduces food intake (Zhang et al., 2011). However, low glucose concentration induces activation of AMPK (in AgRP neurons) leading to increased food intake and body mass gain, this effect is inhibited by insulin and leptin. Briefly, AMPK is inhibited by high glucose concentrations or by refeeding that decrease the expression of AgRP and NPY (Claret et al., 2007; Chalmers et al., 2014). High glucose concentration can induce depolarization in POMC neurons leading to its activation (Parton et al., 2007). Thus, the combined actions of glucose in AgRP and POMC neurons provide anorexigenic signals through a pathway distinct but yet integrated with the pathway controlled by hormones.

Autophagy is an evolutionary conserved mechanism that provides an alternative form of energy to the starved cell. Some evidence suggests that autophagy is closely involved in the hypothalamic regulation of food intake (Kaushik et al., 2011, 2012; Singh and Cuervo, 2011; Portovedo et al., 2015; Oh et al., 2016). Long-term fasting was shown increase autophagy in AgRP neurons resulting in increased expression of the neuropeptide AgRP and thus, increasing food intake (Kaushik et al., 2011). In POMC neurons, the impairment of autophagy induces an increase in food consumption and body weight gain (Malhotra et al., 2015). Recently, it was demonstrated that autophagy that occurs in hypothalamic neuronal populations is induced/regulated by the activation of AMPK. Under low-glucose availability AMPK-induced autophagy regulates appetite by regulating neuropeptide expression NPY and POMC (Oh et al., 2016).

Fatty acids and the amino acid leucine also regulate food intake via hypothalamus. Enzymes and intermediates of fatty acid metabolism function as energy sensors for hypothalamic response to fats. The icv administration of long-chain fatty acids can trigger a hypothalamic response to inhibit food intake and regulate glucose homeostasis (decrease in plasma insulin and glucose levels and also reduction of hepatic glucose production; Obici et al., 2002a). The mechanism behind regulation of food intake is likely mediated by the decreased expression of orexigenic hypothalamic NPY and AgRP levels (Obici et al., 2002a; Morgan et al., 2004; Chari et al., 2010). A summarized view of the physiological hypothalamic control of food intake and energy expenditure is shown in **Figure 1**.

OBESITY-ASSOCIATED HYPOTHALAMIC DYSFUNCTION

In non-pathological conditions, hypothalamic neurons are programed to respond to hormones, nutrients, and neural signals maintaining whole body energy homeostasis. However, several studies have shown that during the development of obesity, a defective regulation of this system plays a major role in the emergence of a positive energy balance. The main mechanism behind abnormal hypothalamic function in obesity is a low-grade hypothalamic inflammation that is triggered by the increased consumption of dietary fats (De Souza et al., 2005; Cavadas et al., 2016; Guillemot-Legris and Muccioli, 2017; Jais and Bruning, 2017). Activation of Toll-like receptor-4 (TLR4), induction of endoplasmic reticulum stress, and activation of isozyme protein kinase C-theta (PKC-theta) have all been identified as important mediators of the dietary fats induced inflammation (Benoit et al., 2009; Milanski et al., 2009). The consumption of large portions of predominantly saturated dietary fats promotes a rapid (as early as one day) increase in the expression of proinflammatory cytokines in the hypothalamus. Most of this response is dependent on the activation of resident microglia (Thaler et al., 2012; Morari et al., 2014; Valdearcos et al., 2014, 2017). If the consumption of dietary fats is interrupted after short time, the inflammation will disappear without major damage. However, if the consumption of dietary fats persists, peripheral myeloid cells will be recruited to the hypothalamus and the chronicity of inflammation will provoke damage to neurons (Moraes et al., 2009; Souza et al., 2016; Valdearcos et al., 2017). Studies have shown that in diet-induced obesity, the hypothalamic neurons are afflicted by abnormal mitochondrial turnover, abnormal function of the ubiquitin/proteasome system, and abnormal regulation of autophagy (Horvath et al., 2010; Ignacio-Souza et al., 2014; Portovedo et al., 2015; Carraro et al., 2017). Initially, neurons become resistant to the actions of leptin and insulin; however, as inflammation persists, neuronal apoptosis will appear. POMC neurons are more sensitive to the damage inflicted by inflammation and after some time, an imbalance in the orexigenic and anorexigenic hypothalamic neuronal subpopulations can act to boost body mass gain (Souza et al., 2016). Taken together all of these studies suggest that hypothalamic neuroinflammation could be a cause rather than a consequence of diet-induced obesity and metabolic dysfunction. Defining the details involved in the regulation and response to hypothalamic inflammation may help identifying potential targets to prevent and treat obesity.

HIF CANONICAL PATHWAY AND CELLULAR FUNCTIONS

Hypoxia-inducible factors (HIFs) are basic helix-loop-helix transcription factors that act as master regulators of hypoxia activated gene expression, allowing the adaptation to hypoxia (Semenza et al., 1991; Wang et al., 1995). HIF is a heterodimer complex composed by two subunits, α-subunit (oxygen sensitive) and a β-subunit [constitutively expressed, and also called aryl hydrocarbon receptor nuclear translocator (ARNT)]. In mammalian cells, there are three major α subunits isoforms, 1α, 2α (also known as endothelial PAS domain-containing protein 1, EPAS1), and 3α. HIF-1α subunit is ubiquitously expressed and 2α is selectively expressed in tissue-restricted manner (e.g., in vascular endothelial cells and myeloid-derived cells). Both

HIF1α and HIF2α function mainly as transcriptional activators, regulating several biological processes, such as angiogenesis, glucose, fatty acid, cholesterol, and mitochondrial metabolism and inflammation (Greer et al., 2012; Semenza, 2014). HIF3α has very weak transcriptional capacity, compared to other HIFs isoforms. A splicing isoform generated from HIF3α, inhibitory PAS (Per/Arnt/Sim) domain protein (IPAS), can function as a dominant negative regulator of HIF1α. This splicing isoform of HIF3 α can function as a negative feedback loop regulation of adaptive responses to hypoxia/ischemia (Makino et al., 2002).

Under normoxia, the hydroxylation of a proline and asparagine residues suppresses HIF transcriptional activity. Specifically, under normal oxygen conditions, α-subunits are hydroxylated in two conserved proline residues (405 and 531) within the O_2-dependent degradation domain, by prolyl-hydroxylases domain proteins (PHDs; Epstein et al., 2001). The hydroxylation of α-subunit function as a target for von Hippel-Lindau tumor suppressor protein (pVHL), and is subsequently ubiquitylated by the Elongin BC/Cul2/pVHL ubiquitin–ligase complex, a marker for 26S proteasome degradation (Maxwell et al., 1999; Ivan et al., 2001; Jaakkola et al., 2001; Greer et al., 2012). HIF-α subunit is also hydroxylated in an asparagine residue (803) that blocks the association with coactivators. This hydroxylation is mediated by an asparaginyl hydroxylase factor inhibiting HIF-1 (FIH-1; Lando et al., 2002). Together, the oxygen-dependent enzymes, prolyl and asparaginyl hydroxylases, are critical regulatory components of the hypoxic response pathway, regulating both stability and transcriptional activity of HIF complex (Greer et al., 2012; **Figure 2**).

The canonical pathway of HIF activation defines that under low O_2 concentration, α-subunits are no longer hydroxylated by PHDs and neither degraded by the proteasome. Instead, this promotes stabilization of α-subunit that translocates to the nucleus and dimerizes with ARNT/HIF-1β subunit recruiting coactivators such as Creb Binding Protein (CBP)/p300. This transcriptional complex binds to hypoxia-response elements (HREs), within the promoter of target genes, and transactivates gene expression that regulates the adaptive response to hypoxia (Semenza et al., 1991; Greer et al., 2012; Semenza, 2014; **Figure 2**).

In addition to hypoxia, HIF stabilization and transcription activity can be regulated by several other factors, such as reactive oxygen species (ROS), nitric oxide (NO), metabolic intermediates of the tricarboxylic acid (TCA) cycle (such as succinate and fumarate), proinflammatory mediators [tumor necrosis factor-α (TNF-α), interleukine-1β (IL-1β)] and also hormonal factors. The regulation of HIF can occur by increasing its stabilization, by inhibiting both enzymes PHD and FIH, and also by the recruitment and posttranslational modification of cofactors that potentiate the transcriptional complex (Lando et al., 2002).

Hypoxia-inducible factor regulates several genes that promote the adaptation to hypoxia [for example, vascular endothelial growth factor (VEGF), erythropoietin, and glucose transporter-1 (GLUT1)] and also genes that promote a metabolic switch to glycolysis through the transcriptional regulation of key metabolic enzymes [for example, pyruvate dehydrogenase kinase 1 (PDK1), lactate dehydrogenase A (LDHA), and glycogen phosphorylase L (PYGL)]. The transcriptional regulation of these metabolic enzymes by HIF-1 activation under oxygen deprivation stimulates glucose intake, glycolysis, and lactate production to provide ATP. In liver, hypoxia-activated HIF-1 leads to an increase hepatic glucose production by transactivation of phosphoenolpyruvate carboxykinase (PEPCK), a rate-limiting enzyme in gluconeogenesis, gene in liver (Choi et al., 2005).

In obesity, the expansion of adipose tissue (hypertrophy and hyperplasia) creates hypoxia niches that are involved in the inflammatory process and insulin resistance that culminates in hyperglycemia (Halberg et al., 2009; Fujisaka et al., 2013). HIF-1α mRNA and protein are enhanced in adipose tissue in a model of diet-induced obesity, by a mechanism that involves adipogenesis, insulin, and hypoxia (He et al., 2011). Both in obese patients and in animal models of obesity, there is an association between reduced oxygen pressure in adipose depots and macrophage and T cell infiltration (Ye et al., 2007; Pasarica et al., 2009; Fujisaka et al., 2013), highlighting the role of HIF pathway in the development of inflammation.

In the last decade, it has been suggested a broad range of functions including embryonic development, immunity and metabolism (Frede et al., 2006; Semenza, 2014; Corcoran and O'Neill, 2016). When changes in metabolism occur, multiple signals (O_2, CO_2, ROS, immunometabolism, and ATP) emanate from the mitochondria. These signals regulate HIF response, which in turn promote major changes in immune cell function and subsequently affects distinct cellular and organic functions (Peyssonnaux et al., 2007; Jantsch et al., 2008; Corcoran and O'Neill, 2016). Therefore, HIF is a key cellular metabolic sensor all over the body.

Immune Regulation of HIF

Despite the fact that HIF regulation has primarily been thought oxygen concentration, several studies have showed that HIF-1α can have an oxygen-independent regulation, such as in response to hyperglycemia, oxidative stress, and inflammatory proteins (Cramer et al., 2003; Catrina, 2014; Corcoran and O'Neill, 2016). Hypoxia is an important factor in the pathophysiology of a number of human diseases (e.g., obesity, insulin resistance, diabetes, Alzheimer disease, and aging) that are characterized by the presence of inflammatory processes (Eltzschig and Carmeliet, 2011; Iyalomhe et al., 2017). Hypoxia can also lead to the production of oxygen radicals, which, as well, have important implications in these diseases. Nevertheless, HIF-1α can also act as a neuroprotective factor following some neuronal insults, activating survival genes, and promoting the adaptation to oxidative stress and inflammation.

Proinflammatory cytokines (TNF-α and IL-1β) can upregulate HIF-1α, by a mechanism that inhibits PHD enzymes with stabilization of HIF activity; conversely, HIF-1 transcriptional activity can downregulate receptors for inflammatory cytokines, attenuating the neuroinflammation (Dehne and Brune, 2009; Xing and Lu, 2016; Iyalomhe et al., 2017).

The molecular mechanism of HIF activation induced by neuroinflammation involves the activation of TLRs and subsequent activation of the mitogen-activated protein kinase (MAPK) pathway and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kb), which transactivate HIF-1α under

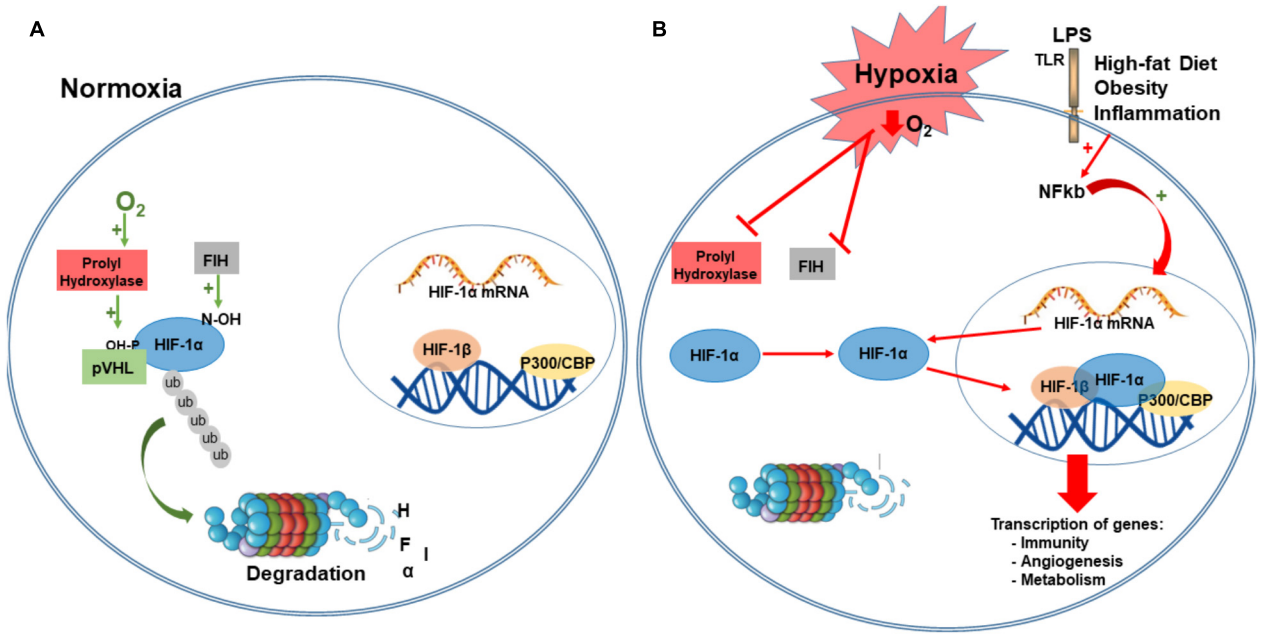

FIGURE 2 | Hypoxia-inducible factor-1 regulation. Under normoxia, HIF-1α is hydroxylated on proline residues 402 and 564 by prolyl hydroxylases (PHD). This hydroxylation is a target for von Hippel-Lindau protein (VHL) binding, a E3 ligase that targets HIF-1α for ubiquitination and proteasomal degradation. Factor inhibiting HIF-1 (FIH-1) hydroxylated an asparagine residue (803) that blocks the binding of the coactivator p300/CBP **(A)**. Under hypoxia, PDH and FIH are inhibited and HIF-1α is stabilized to dimerize with HIF-1β, and binds to target genes at the DNA, recruits p300, and activates transcription of several genes involved in immunity, angiogenesis, metabolism, survival, and proliferation. Immune and inflammatory stimuli can also regulate the HIF pathway, primarily via activation of Toll-like receptors (TRL) and NF-kB activation. NF-Kb is translocated to the nucleus where it regulates the expression of HIF- α mRNA **(B)**. CBP/p300, Creb Binding Protein/p300; LPS, lipopolysaccharide; PHD, prolyl hydroxylases; VHL, von Hippel-Lindau protein; FIH-1, factor inhibiting HIF-1; P-OH, hydroxylation in proline residues; N-OH, hydroxylation in asparaginyl residues; TLR-4, Toll-like receptors; ub, ubiquitin; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells.

normal oxygen tension (Frede et al., 2006; Spirig et al., 2010). Neuroinflammation induced upregulation of HIF-1α suggests the involvement of neuronal survival pathway during inflammatory neurological insults, particularly in the early stages of the process.

In hypothalamus, HIF-1α is increased after short-term high-fat diet consumption, before the onset of body weight gain (Gaspar et al., 2018). The inhibition of HIF signaling in ARC of mice fed a HFD induces hypothalamic inflammation, with increased expression of TLR4, TNFα, and IL-1β, and is associated with an increase in body mass gain and glucose intolerance (Gaspar et al., 2018). Taken together, these results and the fact that consumption of saturated fats is involved in the biogenesis of obesity (Thaler et al., 2012; Valdearcos et al., 2014), it is possible that HIF-1α stabilization is a regulator and a protective factor against saturated fatty acids induced-neuroinflammation, regulating energy expenditure and metabolism.

CENTRAL REGULATION OF METABOLISM BY HIF

The hypothalamus has a critical role in the regulation of energy homeostasis and whole-body metabolism. Changes in hypothalamic nutrient and hormonal sensors play important roles in the genesis of obesity and metabolic disorders. Studies performed at high altitude, in conditions of hypobaric hypoxia, have demonstrated a progressive loss of body mass, caused by the decrease in food intake and thus inducing an energy deficit possibly due to hypobaric hypoxia (Westerterp-Plantenga et al., 1999; Westerterp and Kayser, 2006). In these studies, Wasterterp and colleagues exposed eight men for 31 days to simulated stay in high altitudes with palatable food provided *ad libitum*. They observed a decrease in body mass in 5 ± 2 kg with a 40% reduction in energy intake as a result of a decrease in appetite. Changes in appetite can occur by central mechanisms of energy homeostasis regulation, but also by an increase in leptin secretion (Tschop et al., 1998). Weight loss due to hypobaric hypoxia was also observed in obese patients; in high altitude, obese subjects presented increased leptin levels and higher metabolic rate (Lippl et al., 2010). Populations that live at high altitude had a lower prevalence of impaired fasting glucose and type 2 diabetes compared with low altitude living populations (Santos et al., 2001).

The combination of these studies and the fact that hypothalamus, mainly the ARC, express high levels of both HIF-1α and HIF-2α (Zhang et al., 2011; Gaspar et al., 2018), raised the question of how central HIF activity regulates food intake and whole-body metabolism. HIF-1α is expressed in POMC neurons, in astrocytes and microglial cells within the ARC (Gaspar et al., 2018).

Mice with a neuronal-specific loss for the FIH-1α presented reduced body weight, decreased fat accumulation, elevated

metabolic rate, hyperventilation, enhanced insulin sensitivity, and improved glucose and lipid metabolism; in addition, they are resistant to body weight gain and hepatic steatosis induced by high-fat feeding (Zhang et al., 2010). However, this study does not demonstrate which brain region was responsible for this metabolic regulation, since FIH was deleted in whole-body neuronal cells. FIH neuronal deletion, and consequently increased activity of HIF-1a induces an increased in food intake besides the beneficial effects of whole body weight. FIH is an enzyme that is negative regulator of other proteins such SOCS (Ferguson et al., 2007), that suppress leptin signaling. FIH can also regulate several other proteins such as Notch receptors, p105 and IkBa (Cockman et al., 2006; Coleman et al., 2007), which make a difficult task to for the determination of direct FIH action as a regulator of food intake trough HIF signaling. Since many of these metabolic processes are controlled by neuronal regulatory circuits located in the hypothalamus, at least in part the neuronal HIF-1α activity regulates systemic metabolism through the modulation of afferent neural connections.

Another study showed that HIF is present in the hypothalamus and is upregulated by the local availability of glucose and its metabolites (Zhang et al., 2011). In this study, the authors demonstrate that under glucose availability in HIF can function as a glucose sensor that specifically binds to the promoter of pomc gene, upregulation the POMC expression. HIF loss-of-function specifically in POMC neurons caused increase in food intake, decrease in basal metabolism, and consequently increasing fat mass and weight gain to promote obesity development (Zhang et al., 2011).

Furthermore, endothelial HIF in hypothalamus also has a main role for central regulation of metabolism. The knockdown of endothelium HIF-1α, resulted in impairment of glucose uptake in the ARC and decreased POMC neuronal activity; following caloric deprivation, mice presented increased caloric intake as compared to control (Varela et al., 2017). Taken together, both studies suggested that hypothalamic HIF regulates glucose uptake in the ARC and consequently controls neuronal POMC adaptation to the changing metabolic environment, important for regulation of energy homeostasis mechanisms (**Figure 3A**).

Recently, we demonstrated using bioinformatics analysis, that hypothalamic HIF-1 transcripts are directly correlated with hypothalamic transcripts for proteins involved in inflammation, apoptosis and autophagy; in addition, hypothalamic HIF-1 expression is directly correlated with the phenotype of increased energy expenditure (Gaspar et al., 2018). High-fat diet induced increases in protein expression of hypothalamic HIF-1α; ARC

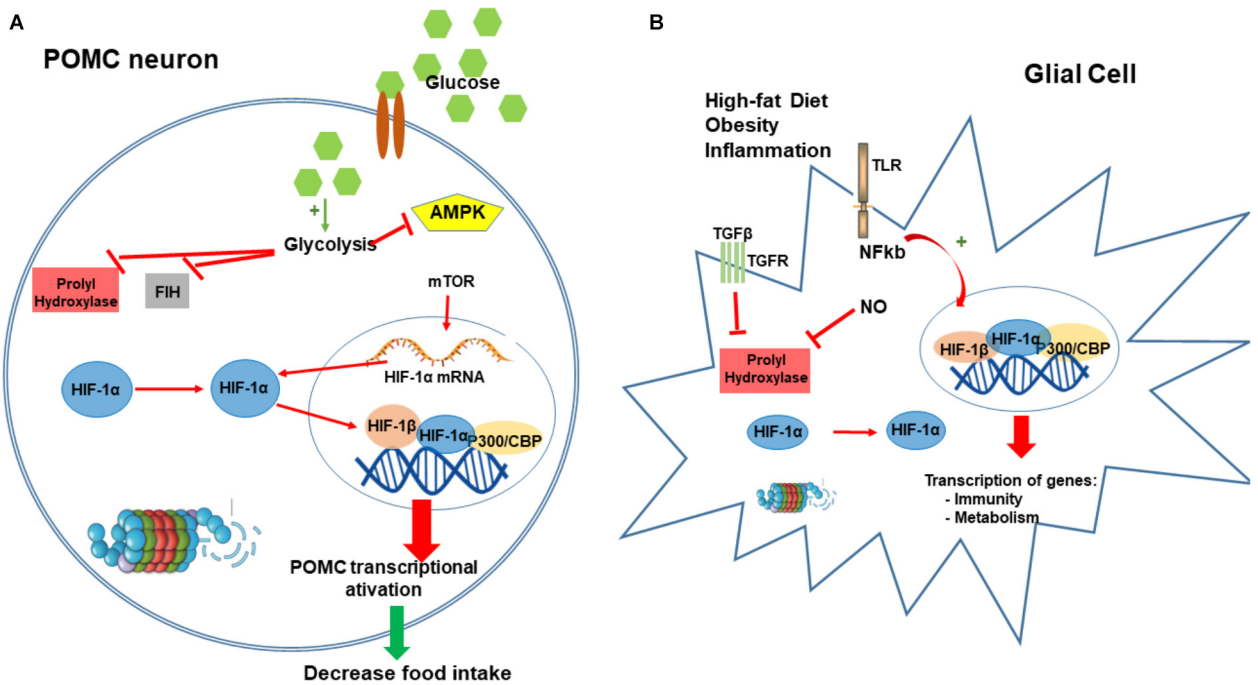

FIGURE 3 | Hypoxia-inducible factor-1 regulation of food intake. HIF in POMC neurons can function as a glucose sensor. After a meal, the increase in neuronal concentration activates neuronal glycolysis producing pyruvate, lactate, and intermediaries from the tricarboxylic acid (TCA) cycle. These metabolites inhibit PHD and FIH enzymes leading to the HIF stabilization. Conversely, these metabolites also block AMPK activation that regulates and increase the expression of HIF-α mRNA. Increase in HIF induces its translocation to the nucleus to bind POMC promoter and consequently stimulating the transactivation of POMC **(A)**. Obesity and consumption of high-fat diet induces an inflammatory condition in the hypothalamus, mostly driven by microglia and glial cells. Our main hypothesis is that inflammation in hypothalamic glial cells (increased TGF-β, TLR4 signaling, and increased NO production) leads to a stabilization of HIF that consequently transactivates several genes involved in the regulation of metabolism and inflammatory process as a compensatory mechanism for the nutritional excess **(B)**. CBP/p300, Creb Binding Protein/p300; PHD, prolyl hydroxylases; FIH-1, factor inhibiting HIF-1; AMPK, 5' AMP-activated protein kinase; POMC, proopiomelanocortin.

inhibition of HIF-1 resulted in further increase in body mass, decreased basal metabolic rate, increased hypothalamic inflammation, and glucose intolerance (Gaspar et al., 2018; **Figure 3B**).

Orexins are hypothalamic neuropeptides involved in the regulatory network of food intake. One of the orexin-responsive genes is HIF-1α; upon orexin stimulus, HIF-1α gene expression is increased, resulting in increased glucose uptake and glycolytic activity. This provides evidence to support the important role of HIF-1 as a transcription factor in the hormone-mediated regulation of hunger (Sikder and Kodadek, 2007).

Also in the liver HIF proteins have important roles in the regulation of glucose and lipid metabolism. Diet-induced obesity induces an upregulation of hepatic HIF-1α (Ochiai et al., 2011), as a compensatory mechanism for metabolic regulation. Mice with liver-specific deletion of HIF-1α exhibited more severe impairment of glucose tolerance and peripheral insulin-resistance than control littermates; these changes were accompanied by a significant reduction of hepatic glucose uptake (Ochiai et al., 2011). Mice with liver specific deletion of HIF-1β, had increased hepatic gluconeogenesis, increased lipogenic gene expression, and impaired glucose tolerance, similar to what happened in type 2 diabetes (Wang et al., 2009).

Despite the importance hypothalamic and hepatic HIF-1α as regulator of energy, glucose, and lipid homeostasis, the increased expression/stability of HIF-1α during the expansion of white adipose tissue is implicated in the inflammatory process and insulin resistance (Ye et al., 2007; Pasarica et al., 2009; Fujisaka et al., 2013; Sun et al., 2013). Both in obese patients and in animal models of obesity, there is an association between reduced oxygen pressure in adipose depots and macrophage and T cell infiltration (Ye et al., 2007; Pasarica et al., 2009; Fujisaka et al., 2013; Lee et al., 2014; Taylor et al., 2014), highlighting the role of HIF pathway in the development of inflammation. However, obese mice deficient in adipocyte-specific HIF2 displayed dysregulation in thermogenesis under cold exposure, as a result of reduced levels of UCP1 and brown adipose tissue impairment (Garcia-Martin et al., 2015). In fact, in brown adipose tissue, HIF complex (isoform 2) is one of the factors that contribute to the upregulation of thermogenesis as an adaptation to obesity (protective effect) through UCP-1 expression (Vucetic et al., 2011; Garcia-Martin et al., 2015).

Thus, HIF-1 can control BAT activity through both direct and indirect mechanisms. In addition, the induction of HIF activity in neuronal cells (neuron-specific FIH null mice) is involved in the regulation of respiration, energy balance, and lipid metabolism (Zhang et al., 2010). Thus, HIF has emerged as an important component of the hypothalamic neuronal machinery involved in the control of body mass and metabolism (Zhang et al., 2011).

In conclusion, HIF has important functions in the regulation of whole body energy homeostasis (both in regulation of food intake, but also in thermogenesis) and also in the hepatic glucose and lipid metabolism. These functions are both directly in the peripheral organs, but also through central nervous system regulation (Zhang et al., 2010, 2011; Gaspar et al., 2016). Considering the positive metabolic results observed in patients under high altitude (hypobaric hypoxia), it is suggested that whole body activation of HIF signaling can have more beneficial than pathological effects against metabolic complications. However, caution should be taken since chronic systemic HIF1α inactivation (genetic or pharmacological manipulation) attenuates WAT expansion and obesity (Sun et al., 2013; Soro-Arnaiz et al., 2016) despite of the HIF1a-dependent beneficial metabolic effects in the hypothalamus. Specifically, in the hypothalamic, HIF-1α is an important nutrient sensor and regulator of energy metabolism; it is also implicated in the regulation of hypothalamic neuroinflammation during the development of obesity. Hypothalamic inhibition of HIF-1α aggravates obesity-associated metabolic phenotype; this highlights the hypothalamic HIF-1α as a potential target for therapeutic intervention against obesity.

CONCLUDING REMARKS

Both human and experimental data support an important role for hypothalamic HIF-1 in the regulation body weight, glucose homeostasis, and liver metabolism. Modulation of specific and selective central/ hypothalamic HIF in obesity can function as a protective mechanism to reduce the negative impact of hypothalamic dysfunction thus, placing tissue specific HIF-1 activation as a potential therapeutic target for the pharmacological management of obesity and type 2 diabetes. Along the role of HIF-1α in the white adipose tissue development, it might be possible that HIF1α actions favoring obesity in WAT overrides that HIF1α-dependent repression of food intake. Nevertheless, further biological, pharmacological, and clinical studies are needed to provide an extended understanding of the mechanisms and molecular players involved in the different cellular responses of HIF in the central mechanisms of energy homeostasis and also during the course of development of obesity and diabetes.

AUTHOR CONTRIBUTIONS

Both authors contributed to the writing, editing, and discussion of the manuscript.

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MINI REVIEW
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Hypothalamic Microglial Activation in Obesity: A Mini-Review

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Emerging data demonstrate that microglia activation plays a pivotal role in the development of hypothalamic inflammation in obesity. Early after the introduction of a high-fat diet, hypothalamic microglia undergo morphological, and functional changes in response to excessive dietary saturated fats. Initially the resident microglia are affected; however, as diet-induced obesity persists, bone marrow-derived myeloid cells gradually replace resident microglia. Genetic and pharmacological approaches aimed at dampening the inflammatory activity in the hypothalamus of experimental models of obesity have proven beneficial to correct the obese phenotype and improve metabolic abnormalities commonly associated with obesity. These approaches provide an experimental proof-of-concept that hypothalamic inflammation is central to the pathophysiology of obesity; understanding the details of the roles played by microglia in this process may help the development of preventive and therapeutic advances in the field. In this review, we discuss the potential mechanisms underlying hypothalamic microglial activation in high-fat induced obesity.

Keywords: inflammation, chemokine, cytokine, brain, metabolism

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INTRODUCTION

The mediobasal hypothalamus (MBH) is a key brain region involved in the central control of caloric intake and energy expenditure. Its function relies on a complex neuronal network that senses the energy status of the body and coordinates responses aimed at maintaining energetic homeostasis. First-order neurons in this network are located in the arcuate nucleus (ARC), sitting close to the median eminence (ME), which is richly irrigated by fenestrated capillaries that secure special permeability to the blood/spinal fluid interface (BSFI) (Rodríguez et al., 2010). The partially leaky ME-BSFI facilitates ARC neuronal and glial cells to rapidly and directly sense minimal changes in peripheral signals, such as hormones (e.g., leptin and insulin) and nutrients, essential to the regulation of whole-body energy homeostasis (Lam et al., 2005; Schaeffer et al., 2013; Timper and Brüning, 2017). Under physiological conditions, feeding leads to the activation of ARC proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) neurons resulting in suppressed food intake and increased energy expenditure. Conversely, fasting leads to the activation of neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons, which antagonize the function of POMC neurons (Schwartz et al., 2000). Over time, the correct function of these neuronal subpopulations leads to body-mass stability. However, the excessive consumption of saturated fatty acids (SFAs) can disrupt the physiological pattern of food intake and energy

expenditure control by triggering an inflammatory response in the hypothalamus, which affects the MBH at the functional and structural levels (De Souza et al., 2005; Velloso and Schwartz, 2011). Leptin resistance emerges as an early functional outcome of hypothalamic inflammation (Dragano et al., 2017; Pan and Myers, 2018), which can be overcome by reducing local inflammatory activity with genetic or pharmacological approaches, or simply by reducing the amount of SFAs in the diet. With time, chronic structural abnormalities can develop, such as changes in neuronal projections, gliosis, and eventually, neuronal death (Moraes et al., 2009; Dorfman and Thaler, 2015). The extension and chronicity of the structural and functional losses of the hypothalamus may explain the relapsing nature of obesity that can resist behavioral, pharmacological, and even surgical therapeutic measures. Thus, understanding the mechanisms involved in the evolution and persistence of hypothalamic inflammation may offer new insights into the development of strategies for the prevention and treatment of obesity.

Studies have shown that diet-induced inflammation initially and preferentially affects the MBH (Thaler et al., 2012). One of the particularities that may explain the anatomical specificity of this process is the nature of the ME-BSFI and the presence of tanycytes in this region, what makes the MBH more sensitive to dietary fats than other circumventricular organs, undergoing a rapid structural, and functional disorganization in mice fed a high-fat diet (HFD) (Ramalho et al., 2018). About the same time that structural and functional changes are occurring in the ME-BSFI, resident microglia undergo morphological and physiological alterations in the ARC region that neighbors the ME (Valdearcos et al., 2014). However, upon persistence of a HFD, bone marrow-derived myeloid cells (BMDM) migrate into the MBH, which is an important condition to secure chronic inflammation (Valdearcos et al., 2017). Approaches that inhibit the migration of BMDM cells to the MBH can reduce hypothalamic inflammation and attenuate diet-induced obesity (DIO). Despite the fact that most studies in this field were performed employing experimental models, there is evidence supporting the presence of inflammation and gliosis in the hypothalamus of obese humans as well (van de Sande-Lee et al., 2011; Thaler et al., 2012), further supporting hypothalamic inflammation as a potential pharmacological target for the obesity treatment. Here, we review the studies that provided progress in the characterization of abnormalities of MBH microglia in obesity.

DIETARY FATS MODIFY HYPOTHALAMIC MICROGLIAL MORPHOLOGY, DISTRIBUTION, AND FUNCTION

Microglial cells are the most abundant brain innate immune cells and are relatively quiescent under baseline conditions. However, when exposed to injury signals, microglia undergo a rapid reaction characterized by morphological and functional changes aimed at enabling them to mount an inflammatory response against the potentially existing treat (Saijo and Glass, 2011).

Studies with rodents have shown that microglia of the MBH are sensitive to SFAs (lauric, palmitic, and stearic acids) (Valdearcos et al., 2014). In the hypothalamus of rodents, consumption of large portions of SFAs promotes a rapid microglial response characterized by the appearance of a number of prolonged processes emanating from a discreetly enlarged cell body (Baufeld et al., 2016). Initially, these morphological changes are restricted to the ARC region nearby the transition between the MBH and ME and are accompanied by increased expression of inflammatory cytokines and other markers of inflammation. The hypothalamic inflammatory response to SFAs occurs in a biphasic mode. During the first week of a HFD, the mRNA and protein levels of hypothalamic cytokines such as TNF-α, IL1-β, and IL-6 increase, as well as chemokines such as MCP-1 and fractalkine (Morari et al., 2014). Upon the persistence, for 2 weeks and longer, of the exposure to SFAs in diets that are particularly rich in stearate and palmitate, the levels of inflammatory markers decrease, only to return after 4 weeks, and then persist for as long as the content of SFAs in the diet is excessive (Thaler et al., 2012).

The biphasic nature of the inflammatory response in the hypothalamus of experimental models of obesity occurs similarly to the classical biphasic inflammatory response in other tissues. One reason behind the biphasic nature of obesity-associated hypothalamic inflammation is that the initial response is mounted by resident microglia; however, as dietary-fat consumption persists, bone marrow-derived myeloid cells gradually replace the MBH microglia (Valdearcos et al., 2017). Thus, at this point, it is important to understand the mechanisms that govern microglia activation, phenotype definition, and the recruitment of bone marrow-derived myeloid cells to the site of injury.

MECHANISMS OF HYPOTHALAMIC MICROGLIA ACTIVATION IN OBESITY

Similar to the resident macrophages of tissues outside the brain, microglia of rodents are capable of sensing and responding to the presence of pathogen-associated molecular patterns (PAMPs) (Kigerl et al., 2014). The systemic injection of a single high-dose of lipopolysaccharide (LPS) promotes activation-like changes in the morphology of hypothalamic microglia, which is detected as early as 8 h after injection, peaking after 24 h and recovering after 7 days (Buttini et al., 1996). This is accompanied by increased expression of MHC-I and inflammatory cytokines (TNF-α, IL-1, and IL-6), and also by the activation of COX-1 and NF-κB (García-Bueno et al., 2009). Interestingly, the same macrophage receptor that is activated in response to LPS, TLR4, can be activated in response to SFAs (Huang et al., 2012).

Under baseline conditions, TLR4 is expressed at low levels in the brain, including the hypothalamus of rodents; however, its expression increases considerably when rodents are fed a HFD (Milanski et al., 2009). In other brain regions, TLR4 expression is involved in medical conditions such as autoimmunity (Kerfoot et al., 2004), HIV infection (Cheung et al., 2017) and Alzheimer's disease (Ledo et al., 2016), illustrating its pleiotropic roles in brain immunity and defense. A detailed screening of dietary fats

that could potentially promote the activation of hypothalamic TLR4 identified long-chain SFAs as the most potent inducers of its association with MyD88, resulting in the activation of NF-κB and JNK and leading to endoplasmic reticulum stress (Zhang et al., 2008). When dietary SFAs are substituted by similar amounts of unsaturated fatty acids (flax seed oil and olive oil), the hypothalamic inflammation is dampened in parallel to the reduced activity of JNK and NF-κB (Cintra et al., 2012). The pharmacological and the genetic inhibition of TLR4 reduce diet-induced hypothalamic inflammation and protect rodents from DIO and glucose intolerance (Milanski et al., 2012). Importantly, contact with large portions of SFAs, and not the presence of obesity *per se*, is required to induce hypothalamic microglial activation; this is evidenced by the fact that genetically obese mice, such as, ob/ob (leptin-deficient), db/db (leptin receptor-deficient) and MC4R-KO, do not present hypothalamic microglia activation when they are fed on chow, despite the evidence that even on this diet they become obese (Gao et al., 2014).

Cell culture approaches were used to investigate additional details involving the activation of microglia by fatty acids. Palmitate and stearate are each capable of inducing a rapid activation of BV2 microglial cells line through a mechanism dependent on TLR4 (Wang et al., 2012). Reactive morphological changes and increased production of inflammatory cytokines, nitric oxide, and reactive oxygen species (ROS) characterize the activation of microglia (Wang et al., 2012). In addition, upon exposure to palmitate, another microglial cell line, THP-1, is capable of producing inflammatory cytokines and inducing neurotoxicity (Little et al., 2012).

As a proof-of-concept for the central implication of microglia in the development of diet-induced hypothalamic inflammation, Valdearcos et al. (2014) used distinct strategies to modify the number of microglia in the hypothalamus. When microglia is increased, the intensity of hypothalamic inflammation induced by SFAs is boosted; conversely, when MBH microglia is reduced hypothalamic inflammation is abrogated. Importantly, a positive relationship exists between the number of hypothalamic microglia and the obese phenotype (Baufeld et al., 2016).

In addition to the direct effects of dietary fats to induce hypothalamic inflammation and microglial activation, some studies have shown that neural afferents from the gut can also trigger an inflammatory response in the hypothalamus (Mulders et al., 2018). The gut inflammatory signals resulting from the consumption of HFD can act through vagal connections to the nodose ganglion to increase hypothalamic microglial cells and trigger inflammation in a mechanism that can be inhibited by ghrelin (Waise et al., 2015). Furthermore, in at least one study, the gut microbial landscape changes promoted by consumption of a HFD was shown to play a role in the development of diet-induced hypothalamic microgliosis (Zhuang et al., 2017).

It is worthwhile mentioning that brain regions other than hypothalamus can also present microglial activation in response to excessive consumption of dietary fats (Erion et al., 2014; Spencer et al., 2017). However, differently of the hypothalamus, microgliosis in other regions is a late event, reinforcing the current concept that hypothalamus is the first brain region affected in response to the consumption of large amounts of dietary fats (Thaler et al., 2012; Carraro et al., 2018). Particularly, in the hippocampus, diet-induced microgliosis has been identified in both human and experimental models of Alzheimer's disease and provides a potential mechanistic link between obesity/type 2 diabetes and the accelerated cognitive and memory decline that is present in this highly prevalent medical condition (Cope et al., 2018).

INTRACELLULAR SIGNALING TARGETS FOR REDUCING MICROGLIA INFLAMMATORY ACTIVATION

Microglial cells express triggering receptor of myeloid cell-2 (TREM2), which is an important negative regulator of NF-κB. *In vitro* studies showed that TREM2 overexpression inhibits neuroinflammation by down-regulating PI3K/AKT and NF-κB signaling in BV2 microglia (Zhong et al., 2017; Li et al., 2018). Curiously, LPS downregulates the expression of TREM2 through the activation of JNK and NF-κB signaling pathways, resulting in a pro-inflammatory vicious cycle (Zhong et al., 2017).

Quercetin, a polyphenolic flavonoid found in many dietary sources, is a molecule studied due to its anti-inflammatory functional properties and also reduces inflammatory responses through inhibition of NF-κB activity. Quercetin can suppress the lipid content inside the palmitic acid-treated microglia through heme oxygenase (HO-1) induction (Kang et al., 2013; Yang et al., 2017a). HO-1 is an antioxidant enzyme that plays a key role in defense mechanisms against oxidative damage and inflammation. In addition, quercetin suppresses LPS-induced nitric oxide (NO) production and inducible NO synthase (iNOS) expression in BV2 cells (Kang et al., 2013).

Alpha-viniferin belongs to the same polyphenol group as quercetin and has anticancer and anti-inflammatory activity. In LPS-BV2 treated cells, α-viniferin suppresses the expression of the proinflammatory genes iNOS and COX-2 in the early stage of inflammation by inhibiting AKT/PI3K-dependent NF-κB activation and inhibits the production of proinflammatory mediators NO and PGE2 in the late stage by stimulating the Nrf2-mediated HO-1 signaling pathway (Dilshara et al., 2014). It is important to highlight that although many *in vivo* and *in vitro* studies have already shown these anti-inflammatory actions, the bioactive effects and bioavailability of these compounds in humans still need to be further investigated (Shanely et al., 2010; Pfeuffer et al., 2013; Lee et al., 2016).

Researchers have also studied other nutrients with functional properties in the context of HFD-induced microglial activation through modulation of NF-κB activity. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major ω-3 PUFAs of fish oil and have exhibited many anti-inflammatory effects in HFD-induced hypothalamic inflammation (Cintra et al., 2012; Nascimento et al., 2016). In the MG6 and BV2 microglial cell lines, EPA and DHA each suppressed the production of pro-inflammatory cytokines TNF-α and IL-6 throughout the inhibition of NF-κB activity and the activation of SIRT1 signaling (Inoue et al., 2017).

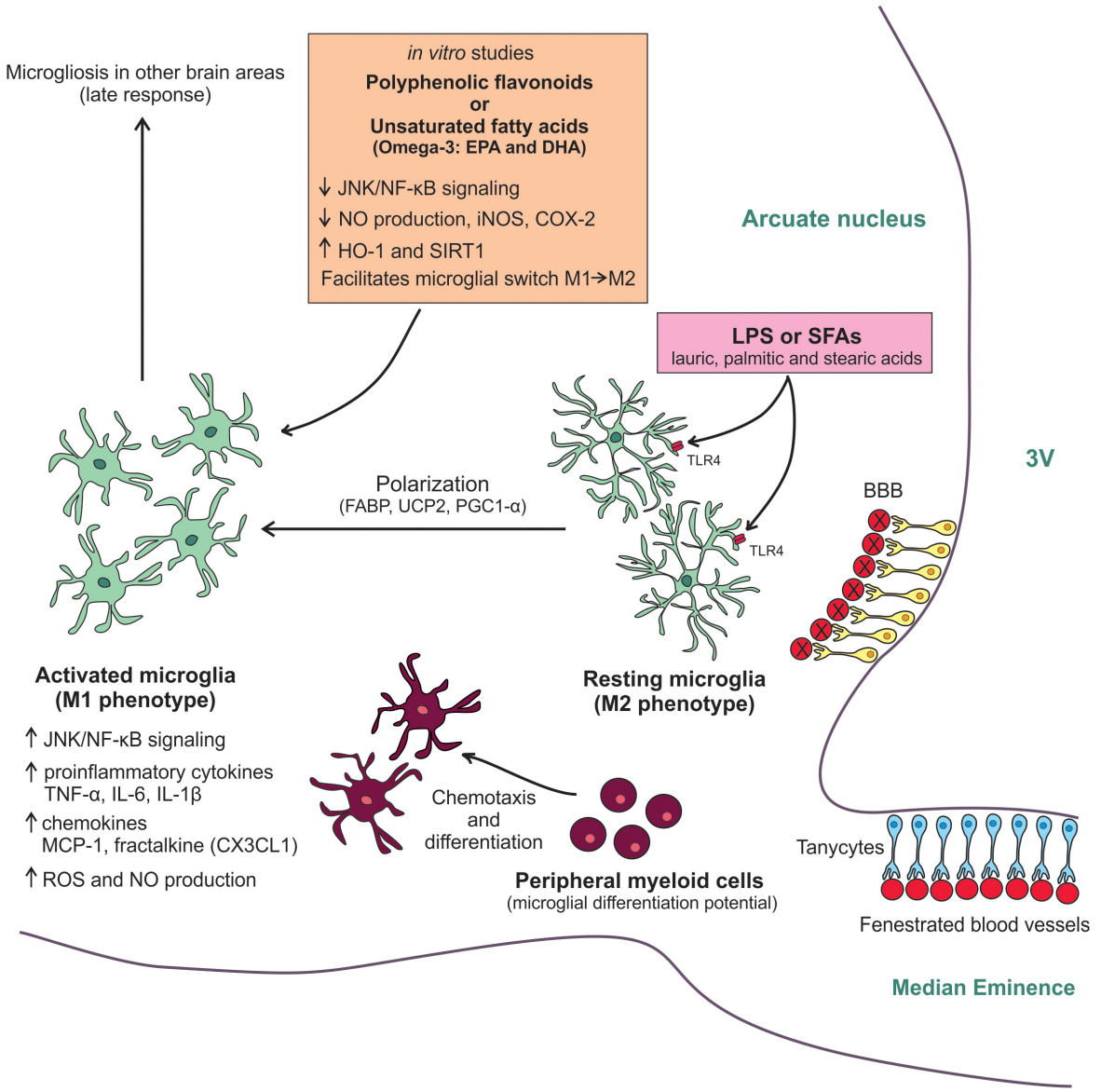

FIGURE 1 | Molecular mechanisms involved in the HFD-induced hypothalamic microgliosis. Diet-induced inflammation initially affects the MBH. Due to its anatomic location, microglial cells of arcuate nucleus are sensitive to LPS and SFAs from the diet. Both molecules activate TLR4 in the microglia membrane resulting in the switching to M1-like pro-inflammatory phenotype. This M2/M1 polarization has an energetic demand and depends on UCP2 activity, which is regulated by FABP, and on PGC-1α activation. The microglia activation results in an increased JNK/NF-kB activity, raised expression of pro-inflammatory cytokines and chemokines, as well as increased ROS and NO production. With the persistence of the high-fat feeding, peripheral myeloid cells are recruited to the neural tissue. These cells have a microglial differentiation potential and can quickly differentiate in activated cells helping resident microglia smother the hypothalamic inflammatory response. The chemotaxis of these peripheral cells is mainly controlled by MCP-1/CCR2 axis and fractalkine. As a late response, the microgliosis can also affect other non-hypothalamic brains areas. Some anti-inflammatory and antioxidants molecules, such as polyphenolic flavonoids and unsaturated fatty acids, have been tested *in vitro* for attenuating the microglial activation. The main benefits found in these studies were the reduction of JNK/NF-kB activity, decreased NO production, as well as iNOS and COX-2 expression, and increased HO-1 and SIRT-1 expression. Besides, these molecules also seem to facilitate the microglial switch M1/M2, reducing the inflammatory response. Further studies are still needed to further test these mechanisms in experimental models.

A few studies also investigated the potential involvement of ROS production as a mechanism of transducing inflammatory signals in microglia. Microglial inflammatory-response activation increases the production of oxidative-stress products. It is known that mitochondria constitute the major source of ROS generation in activated microglia (Park et al., 2015). ROS are capable of modifying microglial pro-inflammatory gene expression by modulating kinase cascades and activating some transcription factors, including MAPK and NF-κB (Pawate et al., 2004). LPS-treated BV2 cells show increased ROS production through NADPH oxidase, which raises the expression of TNF-α, activating NF-κB (Sumi et al., 2010). Otherwise, when these cells

are treated with a NADPH oxidase inhibitor, ROS production decreases (Mander et al., 2006).

Thus, NF-κB and JNK signaling systems play central roles in the transduction of inflammatory signals in microglia. They can be either activated or inhibited by various nutrients, which make them important targets for approaches aimed at dampening HFD-induced hypothalamic inflammation. It is important to consider that due to the complex nature of human diet as compared to experimental diets offered to rodents, further clinical studies will be required in order to confirm the inflammatory nature of the hypothalamic damage in human obesity.

MICROGLIA POLARIZATION

Microglial cells are able to sense changes in the surrounding environment and become activated to either a pro-inflammatory (M1) or an anti-inflammatory (M2) phenotype (Kettenmann et al., 2011; Tang and Le, 2016). Polarized microglia are distinguished from polarized peripheral macrophages by protein expression, phagocytic capacity, and response to injuries (Durafourt et al., 2012; Girard et al., 2013). Considering that HFD leads to the recruitment of peripheral myeloid cells to the MBH, it is important to understand if some differences exist in the molecular mechanisms of polarization between microglial and infiltrated peripheral myeloid cells in the central nervous system (CNS).

Microglial cell polarization has an energetic demand and, thus, depends on uncoupling protein 2 (UCP2) activity, which is regulated by fatty-acid-binding proteins (FABP) (Hotamisligil and Bernlohr, 2015). Mice lacking FABP4 in the hypothalamus have increased UCP2 expression and reduced expression of TNF-α and ionized calcium-binding adapter molecule 1 (Iba1, a marker of microglial activation (Duffy et al., 2017). Pharmacological inhibition of FABP is an interesting therapeutic approach to reducing saturated-fat-induced pro-inflammatory response because it increases UCP2 expression. In addition, the inhibition of hypothalamic UCP2 increases the inflammatory damage inflicted by TNF-α, which places UCP2 as an important component in the polarization machinery of microglia (Degasperi et al., 2008).

Studies have identified a number of compounds that can reduce inflammation in the CNS by suppressing microglia M1 phenotype (Zhou et al., 2014; Orihuela et al., 2016). Conversely, only a few compounds, such as interleukin-4 and interleukin-13, can promote the polarization of microglia to the M2-like anti-inflammatory phenotype (Zhou et al., 2014). In this context, promoting an M1-M2 switch could be an interesting approach to attenuate inflammation of the neural tissue (Hu et al., 2015; Xu et al., 2015). In line, a recent study showed that betulinic acid (BA), a naturally pentacyclic triterpenoid with anti-inflammatory properties, promotes M2 polarization and inhibits M1 polarization in LPS-stimulated BV2 microglial cells and in the cerebral cortex of LPS-injected mice through calmodulin-dependent protein kinase kinase β (CaMKKβ)-dependent AMP-activated protein kinase (AMPK) activation (Li et al., 2018). In another study, Xu et al. (2015) showed that telmisartan, a potent angiotensin II receptor blocker, also prevents LPS-induced microglia activation throughout M2 microglia polarization through CaMKKβ-dependent AMPK activation. Another mechanism that leads to microglia M1/M2 polarization depends on PGC-1α inhibition, which can be induced by LPS. Some compounds, like resveratrol, a natural polyphenol with anti-inflammatory effects, has beneficial effects on microglia activation throughout PGC-1α activation, inhibiting the M1-like phenotype while stimulating the M2-like phenotype (Yang et al., 2017b).

Although a microglial switch offers a promising avenue for reducing HFD-induced microglial inflammation, further characterization of microglial phenotypic behavior, especially its temporal and spatial evolution, is critical in electing potential therapeutic approaches. Furthermore, it is important to consider that microglial polarization is a dynamic and natural reaction to saturated-fat overconsumption and its complete blockage could impair microglial activity in resolving inflammation.

THE RECRUITMENT OF PERIPHERAL MYELOID CELLS TO THE HYPOTHALAMUS IN DIO

Peripheral myeloid cells are recruited to MBH in mice fed a HFD and contribute to the development of obesity-associated hypothalamic microgliosis (Morari et al., 2014; Valdearcos et al., 2017). The fenestrated nature of the BSFI surrounding the MBH is regarded as an important factor involved in this process (Ramalho et al., 2018). Numerous studies showed that bone-marrow-derived microglia (BMDM) can infiltrate the brain parenchyma in distinct types of brain injury (Ajami et al., 2007; Davoust et al., 2008; Prinz and Mildner, 2011). Valdearcos et al. (2017) demonstrated that myeloid cells infiltrating the MBH in mice fed a HFD expressed neither typical microglial markers nor markers indicative of a bloodborne origin. Thus, it was initially suspected that these cells could represent a non-microglial population of brain-resident myeloid cells.

Under normal conditions, BMDM does not renew microglial cells, but under certain circumstances in which cells present in systemic circulation cross the BSFI, they can undergo differentiation into microglia. Thus, the adult bone marrow contains a subpopulation of cells displaying a microglial differentiation potential that can be mobilized promptly at any time (Davoust et al., 2008); however, which factors induce this specific type of monocyte chemotaxis into the MBH in the context of dietary excess is currently under investigation.

Fractalkine (CX3CL1), a chemokine that signals through the CX3CR1 receptor, is involved in this earlier activation of hypothalamic inflammation in HFD-fed mice (Morari et al., 2014). Another chemokine axis, MCP-1/CCR2, has also been described as an important potential mediator of the migration of monocytes into affected areas in brain diseases (Deshmane et al., 2009). Under chronic stress, e.g., a long period of high-fat feeding,

the BMDM are recruited to the paraventricular nucleus of the hypothalamus (PVN) throughout, after the activation of the MCP-1/CCR2 axis (Ataka et al., 2013). In this study, the authors blocked the recruitment by peripheral administration of a CCR2 antagonist, which resulted in a reduction of local inflammation, providing evidence of an important role of the MCP-1/CCR2 axis in diet-induced hypothalamic inflammation.

Although the origin and identity of myeloid cells that migrate to the MBH after HFD consumption are unknown, these cells displayed morphological features of resident microglia (Valdearcos et al., 2017). The BMDM, as resident microglia, can be clearly distinguished from other CNS-infiltrating macrophages, based on its CD45 expression (Priller et al., 2001). In addition, BMDM can proliferate in the neural tissue when inflammation is induced, whereas infiltrating macrophages do not (Wirenfeldt et al., 2007).

Another important question still unanswered refers to the mechanism that induces the recruitment of BMDM into the peripheral circulation because it precedes recruitment to the CNS. SDF-1/CXCR4 interaction is required to recruit BMDM out of the bone marrow toward systemic circulation (Katayama et al., 2006). SDF-1 expression, in turn, is regulated by brain stimuli through sympathetic pathways, throughout β3-adrenergic receptors on bone marrow stromal cells (Lilly et al., 2011). Thus, it is possible that the signal that triggers the whole process is mediated by autonomic connections between the hypothalamus and the bone marrow; however, further studies are needed to clarify this important issue.

In conclusion, the current data are reshaping the concept that resident microglia constitutes a stable brain-cell population that is not affected by the recruitment of BMDM (Matsumoto and Fujiwara, 1987; Lassmann et al., 1993). Certain types of brain injury, including the damage caused in the hypothalamus by the excessive consumption of SFAs, can recruit BMDM, which seem to be an important event to modulate the inflammatory response. Particularly, after consumption of a HFD, it seems the recruitment of peripheral myeloid cells to the hypothalamus occurs in an attempt to help the resident microglia smother the inflammatory response. As this response happens at the beginning of HFD overconsumption, exploring which mechanisms are involved and how it alters hypothalamic neurocircuits involved in metabolic control represents a new frontier for proposing a viable therapeutic approach before the damage is established. **Figure 1** provides a summarized view of the mechanisms involved in the regulation of hypothalamic microglia in obesity.

OUTSTANDING QUESTIONS

(1) How do different subsets of microglia participate in the HFD-induced hypothalamic inflammation?
(2) Could microglia from other brain areas also migrate to the MBH contributing to the local inflammation?
(3) Is the recruitment of BMDM triggered by hypothalamic outputs? If so, which mechanisms are involved?
(4) Is there inflammation and microglial activation in the hypothalamus of obese humans? If so, is there any possibility of pharmacologically targeting this inflammation to treat human obesity?

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All authors wrote the paper and approved the final version of the manuscript.

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REVIEW
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Impact of Metabolic Syndrome on Neuroinflammation and the Blood–Brain Barrier

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Metabolic syndrome, which includes diabetes and obesity, is one of the most widespread medical conditions. It induces systemic inflammation, causing far reaching effects on the body that are still being uncovered. Neuropathologies triggered by metabolic syndrome often result from increased permeability of the blood–brain-barrier (BBB). The BBB, a system designed to restrict entry of toxins, immune cells, and pathogens to the brain, is vital for proper neuronal function. Local and systemic inflammation induced by obesity or type 2 diabetes mellitus can cause BBB breakdown, decreased removal of waste, and increased infiltration of immune cells. This leads to disruption of glial and neuronal cells, causing hormonal dysregulation, increased immune sensitivity, or cognitive impairment depending on the affected brain region. Inflammatory effects of metabolic syndrome have been linked to neurodegenerative diseases. In this review, we discuss the effects of obesity and diabetes-induced inflammation on the BBB, the roles played by leptin and insulin resistance, as well as BBB changes occurring at the molecular level. We explore signaling pathways including VEGF, HIFs, PKC, Rho/ROCK, eNOS, and miRNAs. Finally, we discuss the broader implications of neural inflammation, including its connection to Alzheimer's disease, multiple sclerosis, and the gut microbiome.

Keywords: blood–brain barrier, neuroinflammation, obesity, diabetes, brain homeostasis

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INTRODUCTION

Metabolic syndrome is one of the most wide-spread diseases in North America, with a prevalence of 34% amongst adults in the United States (Aguilar et al., 2015). It is perhaps the leading avoidable cause of premature death (Hennekens and Andreotti, 2013). Metabolic syndrome is an umbrella term encompassing conditions such as obesity, dyslipidemia, hyperglycemia, and hypertension. These often occur together and result in insensitivity to hormones such as leptin, adiponectin, and insulin (Kaur, 2014). Insulin resistance underlies type 2 diabetes mellitus (T2DM). While the causes of metabolic syndrome are complex, high fat diet (HFD), inactive life styles and genetic predispositions are important risk factors. The components of metabolic syndrome result in wide-ranging effects, many of which impact the central nervous system (CNS) and result in neurodegenerative diseases linked to dysfunction of the BBB. Metabolic syndrome also alters blood

pressure and arterial stiffness which, in turn, can damage the BBB. However, given that this review focuses on the effects of inflammation on the BBB, this point will not be discussed.

The BBB is a conserved structure preventing and controlling the passage of most blood components into the central nervous system (CNS) (Andreone et al., 2015; Chow and Gu, 2015; O'Brown et al., 2018). Endothelial cells (ECs) joined together by tight junctions (TJs) form its basic structure (Reese and Karnovsky, 1967), while the basement membrane (BM), pericytes, and astrocytes perform supporting and regulatory functions (Andreone et al., 2015; Daneman and Prat, 2015). The integrity of the BBB is central to neural health, and many autoimmune neurological disorders are associated with its breakdown (Garg and Smith, 2015; Zhao et al., 2015; Chakraborty et al., 2017). BBB opening has been largely linked to inflammation. The maturation and invasion of leukocytes, the release of cytokines, and destruction of targeted cells are effects associated with CNS inflammation that can quickly lead to neuronal damage (Varatharaj and Galea, 2017). Although a sealed BBB normally prevents the passage of immune cells into the CNS, inflammation induces BBB opening by altering its various components (Ransohoff et al., 2015), as we will discuss in this review.

Components of the BBB

In peripheral organs like the liver or kidneys, capillaries contain fenestrae, little gaps in the vessel wall that allow passage of nutrients, proteins, and even cells into surrounding tissues (Claesson-Welsh, 2015; Augustin and Koh, 2017). In most brain areas, however, ECs are connected by TJs that prevent proteins and cells from crossing the vessel wall. Endothelial TJs are formed by the transcellular proteins claudin, occludin, and junctional adhesion molecules (JAMs) (Daneman and Prat, 2015). Claudins are particularly important for barrier function, as loss of claudins greatly increases barrier permeability (Hartsock and Nelson, 2008). Occludins are highly enriched at the BBB, but they do not appear to be essential to barrier function, as occludin-deficient mice retain normal BBB functioning. Nevertheless, occludins are implicated in calcium flux across the BBB (Hartsock and Nelson, 2008). JAMs, particularly JAM4, regulate passage of leukocytes across the BBB and paracellular permeability. In addition to transcellular proteins, zona occludens-1 (ZO-1), ZO-2, and ZO-3 are important for localizing claudins to the TJs and for connecting them to the cytoskeleton (Hartsock and Nelson, 2008; Daneman and Prat, 2015).

Due to a lack of plasmalemma vesicles, brain ECs have very low rates of transcytosis (Ben-Zvi et al., 2014; Andreone et al., 2017; Chow and Gu, 2017). They display low expression of leukocyte adhesion molecules (LAMs) and of ligand-specific transporters. Passage across the BBB is mostly controlled by these transporter proteins. The luminal and abluminal sides possess different proteomic profiles, allowing the BBB to isolate the vascular and perivascular environments (Daneman and Prat, 2015). Many of these transporters, such as the glucose transporter-1 (GLUT1) are passive, allowing their substrates to flow down their concentration gradients (Zhao et al., 2015). Other transporters consume energy, using ATP hydrolysis to fuel the transport of substrates against their concentration gradient. *P*-glycoprotein, for instance, removes toxins from the brain, including β-amyloid (Aβ), a major player in AD (Deo et al., 2014). This tight control on transport across ECs creates strict regulation and polarization of the vascular and perivascular environments (Chow and Gu, 2015; Daneman and Prat, 2015).

Other structures involved in the BBB include astrocytes, pericytes, and the BM. The latter is made of collagens, laminin, nidogen, heparin, and other glycoproteins. It forms a further barrier in the BBB, but can be disrupted by matrix metalloproteinases (Thomsen et al., 2017). Pericytes are embedded within the endothelial BM and form direct connections with ECs. They are involved in the regulation of angiogenesis and vascular stability, and in controlling the BBB (Armulik et al., 2010; ElAli et al., 2014). Astrocytes are the most abundant glial cell type and play important roles in neurovascular regulation. Their processes ensheathe parenchymal blood vessels with their endfeet. They regulate vasomotor responses and cerebral blood flow in response to changes in neural activity in a given region (Attwell et al., 2010; Petzold and Murthy, 2011; Lacoste and Gu, 2015). They also release factors regulating the maturation and maintenance of the BBB (MacVicar and Newman, 2015; Zhao et al., 2015).

Inflammation and BBB Disruption

CNS Immune Privilege and Leukocyte Invasion

Unlike most organs, the healthy CNS contains very few immune cells, parenchymal microglial cells being the sole population. In the healthy CNS, microglia are maintained without reconstitution from the bone marrow and have no contact with plasma proteins, allowing the CNS to maintain an immunosuppressed environment (Ransohoff and Engelhardt, 2012). The CNS tightly controls, and generally prevents, the passage of immune cells into the perivascular space. This 'CNS immune privilege' is largely accomplished by the BBB, which limits leukocyte extravasation (i.e., diapedesis) across the endothelium (Zhao et al., 2015). Undue leukocyte extravasation into neural spaces can result in autoimmune disorders such as multiple sclerosis (MS) (Ransohoff and Engelhardt, 2012).

Leukocyte extravasation requires interactions between adhesion molecules on ECs and leukocytes. LAMs expressed by ECs include P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Su et al., 2012). The selectins bind to P-selectin glycoprotein ligand (PSGL-1), while ICAM-1 and VCAM-1 bind to α4-integrins on leukocytes. After the initial binding event, immune cells roll along the vessel wall releasing chemokines that strengthen their binding interactions. This dramatically reduces cell motility, allowing immune cells to crawl along the vessel in search of a point for extravasation (Engelhardt, 2008; Engelhardt and Ransohoff, 2012). Most transmigration occurs via a paracellular route, which depends on interactions with platelet endothelial cell adhesion molecule (PECAM) and JAM-A (Muller, 2011; Engelhardt and Ransohoff, 2012; Tietz et al., 2018). It can also happen transcellularly, generally when the leukocyte is unable to find an endothelial junction and is strongly

activated. The immune cell extends pseudopods and passes right through the endothelium. This phenomenon involves clustering of ICAM-1 and can be inhibited by blocking PECAM. Calveolin-1 plays an important role, especially in the migration of Th1 cells (Lutz et al., 2017). Microtubules are also critical in both types of movement, transporting molecules involved in extravasation to required sites (Muller, 2011).

Leukocyte adhesion molecules are suppressed in the neurovasculature, limiting the opportunity for leukocyte invasion (Chow and Gu, 2015; Daneman and Prat, 2015). The BM forms a second barrier to immune entry. To pass through, leukocytes must be helped by matrix metalloproteinases, such as MMP-9, which clear away membrane filaments. Inflammatory factors open the BBB by upregulating these various factors (Engelhardt and Ransohoff, 2012).

A process called immunosurveillance monitors antigens in the brain and mounts an immune response if foreign bodies are detected. This largely takes place in the cerebrospinal fluid (CSF). Immune cells enter the ventricles through the choroid plexus (CP), a structure in the cerebral ventricles that produces CSF (Ghersi-Egea et al., 2018). There, they are exposed to antigen presenting cells (APC) and drain through meningeal lymphatic vessels into the to the deep cervical lymph node (Louveau et al., 2018). Cells activated in the CSF activate a pro-inflammatory signaling pathway that triggers opening of the BBB and allows leukocyte infiltration, as reviewed elsewhere (Ransohoff and Engelhardt, 2012). Proper functioning of the CP is highly important for neuronal health (Gelb et al., 2018). More recently, a direct route of immune cell infiltration was identified between the bone of the skull and brain meninges by means of microscopic vascular channels (Herisson et al., 2018).

Inflammation and the BBB

Inflammation acts through various pathways to affect gene expression. NF-κB, one of the most important factors, is activated by pro-inflammatory cytokines such as TNF-α and IL-1β. Toll-like receptor-4 (TLR4) binds to microbial molecular patterns and activates myeloid differentiation primary response gene 88 (My88), which also leads to NF-κB activation (Lawrence, 2009). The JAK\STAT pathway is activated by a wide variety of signaling molecules, including interferons (IFNα/β/γ), interleukins (IL-2/3/4/5/6 etc.), and growth factors. These ligands bind to their receptors, leading to activation of STATs which affect gene regulation (O'Shea et al., 2015). Mitogen-activated protein kinase (MAPK) has a role in three separate pathways, including extracellular-signal-regulated kinases (ERKs), Jun amino-terminal kinases (JNK), and p38/stress-activated protein kinases (SAPKs). JNK leads to the activation of c-Jun and c-Fos, which dimerize and form activator protein 1 (AP-1) (Morrison, 2012). Receptors involved in inflammatory responses include cytokine and growth factor receptors, receptor tyrosine kinases, G-coupled protein receptors, and integrins (Morrison, 2012).

Reactive oxygen species are important mediators of inflammation. They are produced as a natural biproduct of aerobic respiration in the mitochondria, through NADPH oxidase activity in phagocytes, and through uncoupled nitric oxide (NO) production (Mittal et al., 2014). Through their oxidative activity, they promote the formation of disulfide bonds between cysteine residues on proteins, affecting protein function (Mittal et al., 2014). In particular, signaling pathways including NF-κB, JNK, and JAK/STAT can be altered by ROS (Nakano et al., 2006; Ray et al., 2012; Hoesel and Schmid, 2013), leading to upregulation of inflammatory cytokines such as transforming growth factor beta (TGF-β), IL-1, IL-6, IL-18, and TNF-α (Elmarakby and Sullivan, 2012). This leads to further inflammation and leukocyte infiltration.

Increased inflammation leads to disruption of the BBB (**Figure 1** and **Table 1**). TJ proteins, including claudin-5, ZO-1, occludins, and caveolin are downregulated (Chehade et al., 2002; Argaw et al., 2009; Pfeiffer et al., 2011; Sajja et al., 2014; Yoo et al., 2016; Varatharaj and Galea, 2017; Xu et al., 2017). Transcytosis is also altered, together with downregulation of *P*-glycoprotein, leptin, and amino acid transporters (Ransohoff et al., 2015), but upregulation of influx transporters for TNF-α, lysosomal enzymes, Aβ, and monoamines (Varatharaj and Galea, 2017). Fibrin is transported across the disrupted BBB and deposits as insoluble fibrin, a key activator of immune responses (Davalos and Akassoglou, 2012). Interestingly, a recent study successfully targeted fibrin with 5B8 antibody, which inhibited fibrin-induced inflammation without affecting clotting (Ryu et al., 2018). In response to inflammation, leukocyte extravasation increases, with upregulation of VCAM-1 and ICAM-1 (Chai and Song, 2014), as well as P and E-selectin (Carvalho-Tavares et al., 2000). Upregulated MMPs degrade the BM, allowing leukocyte passage (Thomsen et al., 2017). Astrocyte endfeet are disrupted, impairing their ability to maintain BBB integrity (Fan et al., 2014). Astrocyte gene expression shifts toward a pro-inflammatory and cytotoxic state, including production of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and prostaglandins (Zamanian et al., 2012). TNF-α further promotes inflammatory responses, inducing extravasation of macrophages by acting through NF-κB (Skelly et al., 2013). TNF-α and IL-1β both induce expression of chemokines CXCL1 and CCL2, which are involved in immune cell recruitment to brain ECs. Other pathways become affected by pro-inflammatory states. For example, Wnt/β-catenin is regulated by NF-κB activation, as reviewed elsewhere (Ma and Hottiger, 2016). Inhibition of Wnt/β-catenin leads to increased expression of VCAM and Caveolin-1, proteins critical for transmembrane trafficking (Lengfeld et al., 2017). Overall, inflammation increases BBB permeability, promoting leukocyte extravasation, increasing diffusion of solutes across the BBB, and allowing entry of pathogens and toxins into the CNS. This further stimulates inflammatory responses in a vicious cycle.

Conditions that increase systemic inflammation impacts many of the systems in the body, including the brain. As such, inflammation is increasingly being implicated in neuropathology, as we will explain below.

OBESITY AND THE BBB

Obesity is the excess accumulation of body fat caused by an imbalance between energy intake and consumption. It results

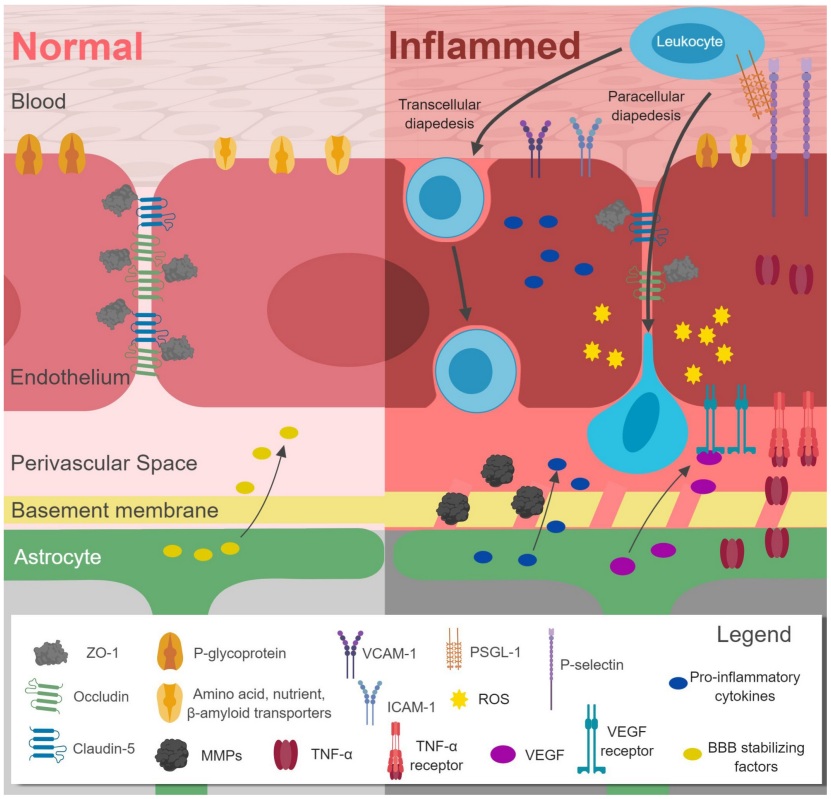

FIGURE 1 | Effects of inflammation on the blood–brain-barrier (BBB). Downregulation of claudin-5, occludin, ZO-1, leptin and amino acid transporters, and P-glycoprotein occurs in conjunction with upregulation of VCAM-1, ICAM-1, P-selectin, MMPs, and pro-inflammatory cytokines. Leukocyte extravasation (diapedesis) increases. Astrocytic gene expression shifts away from BBB stabilizing factors toward VEGF and pro-inflammatory cytokines. The basement membrane is also disrupted. *Figure made with BioRender*.

in high levels of adipose tissue, a hormonally active tissue that secretes adipokines and cytokines. As the number of adipose cells increase, more of these regulating proteins are secreted, causing tissues to develop resistance to their effects. For example, leptin resistance prevents proper satiety so that hunger still occurs despite excess fat. Insulin resistance prevents proper intake and use of blood glucose, causing hyperglycemia. These hormones, and their disruption, affect nearly every organ in the body, making obesity a contributing factor in many diseases, including cardiovascular disease, fatty lipid disease, and neurological disorders like AD (Blüher, 2013).

Obesity and Cerebral Inflammation

The effects of obesity are largely mediated through inflammation (**Figure 2**). High fat diet (HFD) is commonly used to study obesity in mice, and neural inflammation can be seen even before significant weight gain in these murine models. Saturated fatty acids (SFAs), like palmitate, play an important role in activating these early inflammatory pathways. SFAs interact with toll-like receptor 4 (TLR4) and activate myeloid differentiation primary response gene 88 (MyD88) (Milanski et al., 2009), which leads to activation of NF-κB. This pathway is linked to inhibition of anorexic hormones insulin and leptin, partially through increased expression of NF-κB-induced expression of suppressor of cytokine signaling 3 (Kievit et al., 2006; Zhang et al., 2008). NF-κB also upregulates pro-inflammatory cytokines including Il-1β, TNF-α, and IL-6 (Yudkin et al., 1999; De Souza et al., 2005; Zhang et al., 2005; Hommelberg et al., 2009; Lawrence, 2009; Jais and Brüning, 2017), causing a decrease in TJ protein expression and an decrease in BBB integrity (Gustafson et al., 2007). Metabolic, housekeeping, and structural genes are downregulated in mice with diet-induced obesity (Ouyang et al., 2014b). Amplified ROS production results from increased mitochondrial respiration, upregulated expression of NADPH oxidase, and the action of inflammatory cytokines (Zhang et al., 2005; Tucsek et al., 2014; Jais and Brüning, 2017). They further promote cytokine expression and oxidative stress (De Souza et al., 2005; Dorfman and Thaler, 2015). Microglia are activated and upregulate their Fcγ receptors, priming them for response to IgGs (Tucsek et al., 2014). Immune cells are also activated, and macrophages infiltrate the brain parenchyma (Stranahan et al., 2016).

One of the earliest regions to experience HFD-induced inflammation is the hypothalamus (Prada et al., 2005). As a major control center for energy and weight balance, its impairment contributes to the development of obesity (Jais and Brüning, 2017). Hypothalamic inflammation precedes weight gain by a few days in mice on HFD (Thaler et al., 2012). As described above, SFAs promotes leptin and insulin resistance through the

TABLE 1 | The effects caused by inflammation, obesity, and T2DM on the BBB.

Effects of inflammation on BBB	
Downregulation of TJ proteins claudin-5, ZO-1, and occludin	Chehade et al., 2002 ; Argaw et al., 2009; Pfeiffer et al., 2011; Sajja et al., 2014; Yoo et al., 2016; Varatharaj and Galea, 2017; Xu et al., 2017
Downregulation of *P*-glycoprotein and of leptin and amino acid transporters	Ransohoff et al., 2015
Upregulation of influx transporters for TNF-α, lysosomal enzymes, Aβ, monoamines	Varatharaj and Galea, 2017
Transport of fibrin and deposition as insoluble fibers	Davalos and Akassoglou, 2012; Ryu et al., 2018
Upregulation of VCAM-1 and ICAM-1	Chai and Song, 2014
Upregulation of P and E-selectins	Carvalho-Tavares et al., 2000
Upregulation of MMPs	Thomsen et al., 2017
Disruption of astrocytic endfeet	Fan et al., 2014
Shift in astrocytic gene expression toward pro-inflammatory cytokines	Zamanian et al., 2012
Increased extravasation of macrophages	Skelly et al., 2013
Dysregulation of Wnt/β-catenin pathway	Ma and Hottiger, 2016; Lengfeld et al., 2017
Effects of obesity on BBB	
Increased activation of NF-κB through TLR4 mediated SFA signaling	Milanski et al., 2009
Upregulation of pro-inflammatory cytokines	Yudkin et al., 1999; De Souza et al., 2005; Zhang et al., 2005; Hommelberg et al., 2009; Lawrence, 2009; Jais and Brüning, 2017
Downregulation of metabolic and housekeeping genes	Ouyang et al., 2014b
Amplified ROS production	Zhang et al., 2005; Tucsek et al., 2014; Jais and Brüning, 2017
Activation of microglia	Tucsek et al., 2014
Infiltration of macrophages into the parenchyma	Stranahan et al., 2016
Insensitivity to anorexic hormones leptin and insulin	Kievit et al., 2006; Zhang et al., 2008
Disruption of the hypothalamus	Prada et al., 2005; Horvath et al., 2010; Jais and Brüning, 2017
Disruption of the hippocampus and cognitive impairment	Kanoski et al., 2010; Davidson et al., 2012, 2013; Hargrave et al., 2016
Effects of T2DM on BBB	
Increased ROS production	Wang et al., 2012
Upregulation of pro-inflammatory cytokines	Elmarakby and Sullivan, 2012
Downregulation of TJ proteins	Chehade et al., 2002; Argaw et al., 2009; Sajja et al., 2014; Yoo et al., 2016; Xu et al., 2017
Increased permeability of BBB	Hawkins et al., 2007; Fujihara et al., 2016
Upregulation of VCAM-1 and ICAM-1	Janelidze et al., 2017
Thickening of the BM	Junker et al., 1985
Increased MMP activity	Hawkins et al., 2007; Thomsen et al., 2017
Increased levels of AGEs	Kowluru et al., 2004

NF-κB pathway, impairing the hypothalamus' ability to lower hunger and regulate blood sugar (Boden and Shulman, 2002; Jais and Brüning, 2017). Long term HFD reduces the number of synapses on hypothalamic neurons and increases neural apoptosis (Horvath et al., 2010).

High fat diet also leads to cognitive deficits (Pistell et al., 2010). Several studies by Davidson et al. (2012, 2013) have looked at the impact of HFD on the hippocampus, a major center for learning and memory (Kanoski et al., 2010; Hargrave et al., 2016). In one, mice were challenged to determine if they could associate external landmarks with a food reward, an operation dependent on the hippocampus. Mice on HFD were less able to make the association compared with controls fed on a regular diet (Hargrave et al., 2016). Further experiments tested their ability to discriminate between similar stimuli. While mice with diet-induced obesity performed as well as the control in the hippocampus-independent test, they performed poorly on the hippocampus-dependent discrimination test (Davidson et al., 2012, 2013). Interestingly, cognitive impairment occurred before obesity developed, and while it was a good predictor of obesity, the latter was a poor predictor of impairment. Even further, cognitive deficits could be identified before significant BBB leakiness occurred (Davidson et al., 2013). This raises questions about the development and time course of BBB pathology. Cognitive deficits and corresponding BBB disruption can be detected as early as 24 days after HFD (Davidson

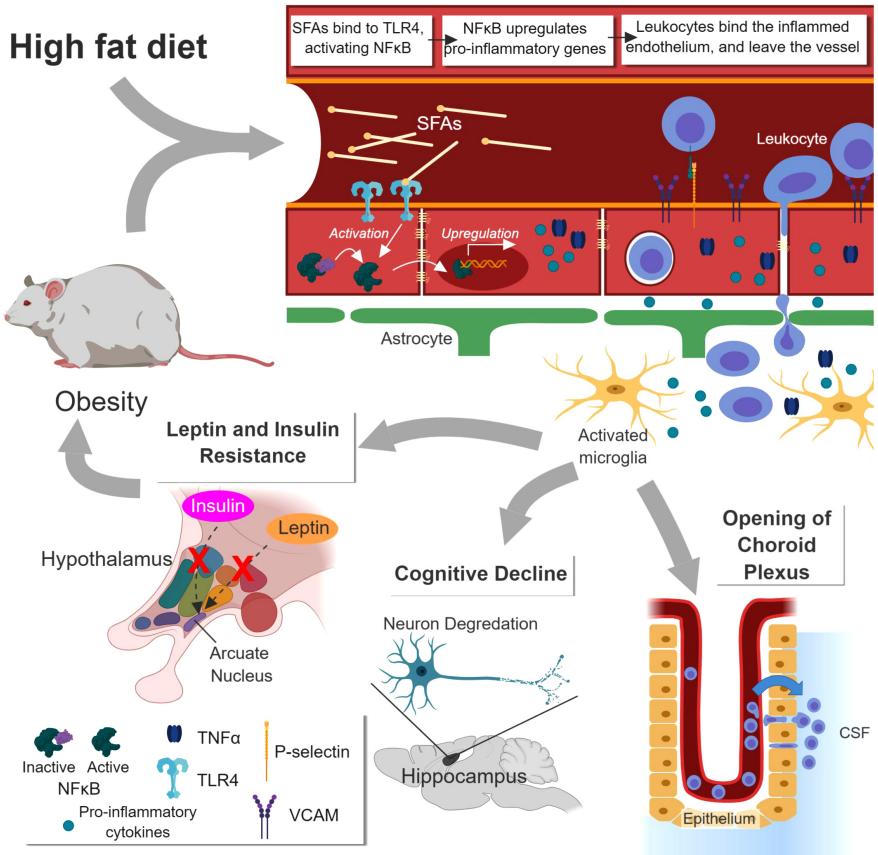

FIGURE 2 | Effects of obesity on the BBB and the brain. Increased saturated fatty acids (SFA) concentration caused by high fat diet (HFD) or obesity enhance NF-κB-mediated inflammation at the BBB via TLR4 receptor. This causes increased leukocyte extravasation, release of pro-inflammatory cytokines, and activation of microglia. The hypothalamus is disrupted, leading to leptin and insulin insensitivity and greater obesity. Disruption of the hippocampus leads to cognitive impairment. Opening of the choroid plexus leads to enhanced leukocyte influx into the cerebrospinal fluid (CSF), more antigen sampling, and greater risk of immune response. *Figure made with BioRender*.

et al., 2013). However, plasma triglyceride and free fatty acid levels do not appear significantly altered that early. There are a number of possible contributing factors. Cognitive deficits were associated with elevated blood glucose levels, and obese rats show decreased GLUT1, a deficiency correlated to impaired cognitive performance (De Vivo et al., 1991). Insulin resistance may also play a role (Hargrave et al., 2016). Indeed, insulin resistance of brain ECs is known as type III diabetes, and it is strongly associated with AD. It is possible that increased Aβ levels may play a role, as this is caused by increased levels of circulating fats and is associated with BBB disruption (Hargrave et al., 2016). None of these factors, however, have been definitively linked with early BBB decline in response to HFD.

Many studies on BBB integrity have looked at regulatory regions like the hypothalamus or memory and learning regions like the hippocampus. A few, however, have looked at the CP, a structure found in the ventricles of the brain that produces the CSF. While it doesn't possess a tight barrier, the CP largely controls the flow of immune cells into the CSF, hence playing a major role in brain immunosurveillance (Schulz and Engelhardt, 2005; Engelhardt, 2006; Ransohoff and Engelhardt, 2012; Ghersi-Egea et al., 2018). In one study, rats exposed to HFD showed a decrease in TJ integrity and corresponding increase in CP leakiness (Kanoski et al., 2010). Another study showed decreased passage of insulin-like growth factor-1 (IGF-1), a neuroprotective factor related to neuropathologies, across the CP barrier (Dietrich et al., 2007). These point to a direct link between neuroimmune disorders and HFDs.

Obesity, Leptin, and Cerebral Inflammation

Many obesity-associated pathologies result from increased production of adipocytes. These, in addition to being the body's predominant fat storage cell, secrete a number of signaling molecules called adipokines. One of these, leptin, is produced by adipose tissue when the body's energy needs are met, acting on the hypothalamus to increase satiety. An increase in adipocyte number leads to higher secretion of leptin, however, despite the high plasma concentration of leptin in obese individuals, they continue to feel hunger. Indeed, the brain develops a leptin

resistance, preventing the hormone from exerting its usual effect. Leptin resistance, and factors causing it, has been reviewed elsewhere (Sáinz et al., 2015).

One of the primary possible causes for leptin resistance is ineffective transport across the BBB. Leptin receptors at the BBB are responsible for modulating leptin transport into the parenchyma [although they do not necessarily transport it (Banks and Lebel, 2002)], and they seem to be easily saturated. Obese mice have higher plasma levels of leptin than WT counterparts, but similar levels of leptin in the CSF, suggesting that leptin transport, while not diminished by obesity, does not increase with higher plasma concentrations of leptin (Burguera et al., 2000; Nave et al., 2003). Administering leptin into the plasma of an obese mouse has no effect, but delivering it directly to the brain produces a robust leptin response (Van Heek et al., 1997). Further research, however, has shown that leptin transport can be reduced in obesity. Mice treated with triglycerides in milk displayed a 44% decrease in leptin transport across the BBB compared to mice given fat-free milk (Banks et al., 2004). Increased plasma triglyceride levels are a key feature of obesity (Musunuru, 2010) and would play an important role in leptin resistance.

Leptin has been implicated in many roles beyond signaling satiety, including modulation of immunity. For example, endothelial leptin signaling plays a role in leukocyte extravasation in the spinal cord and other CNS areas. Knocking out leptin receptors prevents this migration, preserves TJ integrity, and attenuates the progression of experimental autoimmune encephalomyelitis (EAE), a mouse model of MS (Lang and Ratke, 2009). Leptin also acts directly on immune cell populations. It promotes the development and activation of Th1 leukocytes, cells associated with the development of EAE (Lord et al., 1998; Matarese et al., 2001b). Conversely, the Th2 immune response, which plays a protective role in EAE, is diminished by leptin (Zamvil and Steinman, 1990; Matarese et al., 2001b). Increased leptin also correlates with a decreased number of Tregs, a regulatory T cell that inhibits inflammation and immune response (Matarese et al., 2005; De Rosa et al., 2007).

These effects of leptin on immune cells are at least partially mediated by the mTOR signaling pathway (Galgani et al., 2010; Procaccini et al., 2012). mTOR-mediated leptin signaling leads to a reduction in Treg production, and Tregs will themselves release leptin to control their own responsiveness (De Rosa et al., 2007; Procaccini et al., 2010). mTOR activation also enhances the development and survival of CD4+CD25- (non-regulatory, helper) T cells by upregulating Bcl-5, a factor involved in T cell differentiation (Galgani et al., 2010). This induced shift toward a pro-inflammatory environment has implicated leptin as an essential player in the development of EAE. Indeed, EAE will not develop in leptin deficient mice (Matarese et al., 2001a; Ouyang et al., 2014a). This also accounts for evidence suggesting increased risk of contracting MS amongst obese adolescents (Hedström et al., 2016). On the other hand, leptin acts on astrocyte leptin receptors to enhance beneficial EAE immune response and decrease disease severity (Mishra et al., 2013). Thus, leptin has a multifaceted role in neuroinflammation, the details of which remain to clarified.

TYPE 2 DIABETES

An important consequence of obesity is type 2 diabetes mellitus (T2DM). Caused by loss of insulin sensitivity in adipocytes, muscles, and other insulin-dependent cells, T2DM results in a loss of effective glucose control (Pandey et al., 2015). If patients do not regulate their sugar levels through diet and/or exercise, they easily become hyperglycemic. This results in increased inflammation, perturbed metabolic pathways, and a number of complications including retinopathy, nephropathy, neuropathy, and degradation of the BBB (Ennis and Keep, 2007; Campos, 2012; Blake and Trounce, 2014; Mapanga and Essop, 2016).

T2DM and Inflammation

As in HFD models of obesity, T2DM mediates many of its effects through inflammation (Brownlee, 2005) (**Figure 3**). The hyperglycemia caused by T2DM leads to increased mitochondrial respiration in ECs, pericytes, and astrocytes. This promotes ROS production and oxidative stress. Enhanced ROS activate NF-κB, activator protein-1 (AP-1), and STAT pathways (Wang et al., 2012). This, in turn, leads to upregulation of inflammatory cytokines (Elmarakby and Sullivan, 2012). ROS also interfere with astrocyte communication by inhibiting the folding of gap junction proteins. Damage to astrocytes can be rescued using antioxidants and chaperone proteins (Gandhi et al., 2010). The damage caused by ROS also eventually leads to pericytes loss, BBB breakdown, and astrocytic endfeet degeneration (Brownlee, 2005). Left untreated, this inflammation can lead to cognitive impairment, just as observed in obesity models (Luchsinger et al., 2007).

The impact of T2DM-induced inflammation in the BBB is readily measurable at the molecular level. T2DM downregulates TJ proteins including claudin-5, ZO-1, occludin, and caveolin (Chehade et al., 2002; Argaw et al., 2009; Sajja et al., 2014; Yoo et al., 2016; Xu et al., 2017). This allows greater influx of blood components into the perivascular space, as shown by studies on albumin and (14)C sucrose penetration (Hawkins et al., 2007; Fujihara et al., 2016). CAMs, including ICAM-1 and VCAM-1, are upregulated (Janelidze et al., 2017). The BM also becomes thicker (Junker et al., 1985), an effect associated with angiopathy leading to increased vascular permeability (Roy et al., 2015). BM thickening is promoted by activation of PKC, AGEs, and growth factors such as TGF-β and connective tissue growth factor. It leads to a compositional change in the extracellular matrix. The upregulation of fibronectin, collagen IV, and laminin compromises cell attachment to the BM and BBB permeability, while downregulation of heparin sulfate proteoglycans removes anionic protein binding sites, destabilizing the BM (Roy et al., 2010; Chronopoulos et al., 2011). MMP activity also increases, and while its protease activity does not offset the increased BM synthesis, it is important for leukocyte extravasation (Hawkins et al., 2007; Thomsen et al., 2017).

The rate at which inflammation causes damage is brain region-dependent. A longitudinal study by Huber et al. (2006) found that increased BBB permeability first occurred in the midbrain, 28 days after T2DM was induced. This was followed by the hippocampus, the cerebral cortex, and the basal ganglia

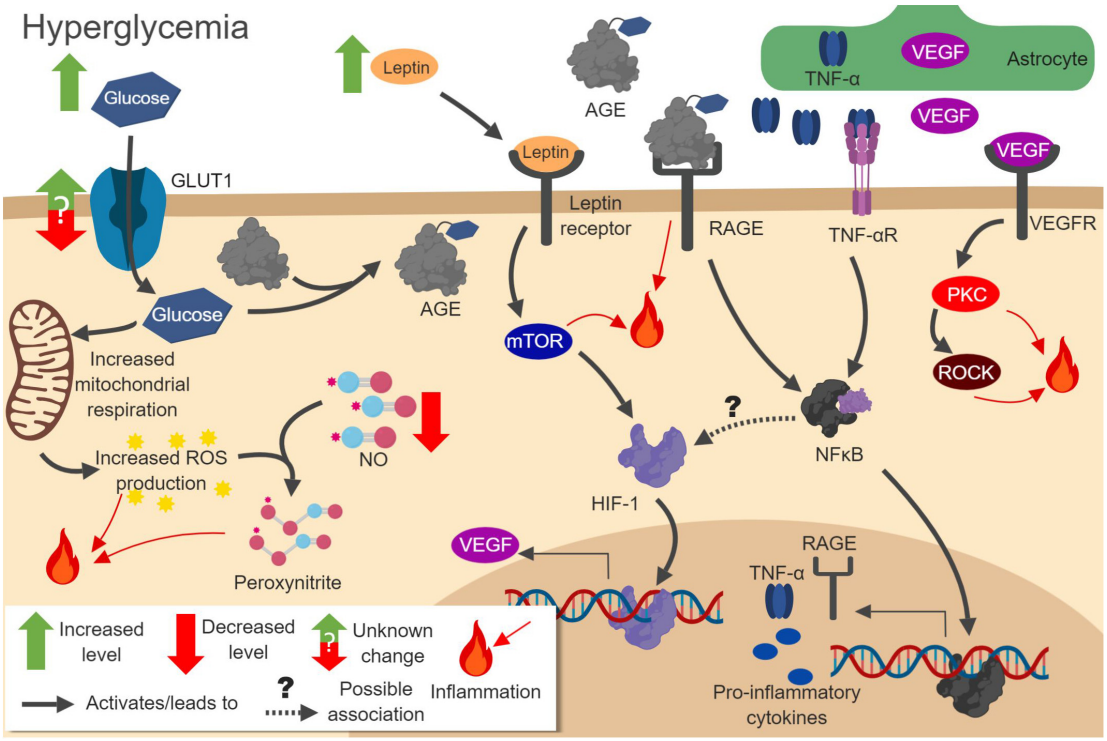

FIGURE 3 | Pathways involved in hyperglycemia-induced neuroinflammation. Increased glucose concentration in cells leads to amplified oxidative respiration and reactive oxygen species (ROS) production. ROS react with NO to produce peroxynitrite. Increased glucose also leads to formation of advanced glycation end products (AGEs), which act on RAGE to increase NF-κB activation. Activated NF-κB increases pro-inflammatory gene expression, including RAGE itself and cytokines. Increased leptin leads to mTOR and HIF-1 pathway activation, increasing vascular endothelial growth factor (VEGF) production. VEGF released from astrocytes activates protein kinase C (PKC) and Rho-associated kinase (ROCK), which further promotes inflammation. *Figure made with BioRender*.

(Huber et al., 2006). Whole brain MRI scans found most brain atrophy in the hippocampus and the temporal, frontal, and limbic gray matter. Damage was also seen in frontal and temporal white matter, but to a lesser extent (Moran et al., 2013). Interestingly, damage to the microvasculature continues even after the hyperglycemia is controlled (Huber et al., 2006). This is partially due to the accumulation of advanced glycation end products (AGEs), which have detrimental effects on the brain (Ceriello, 2012).

Inflammatory Mediators in T2DM

Hyperglycemia and Insulin

Pathways mediating the effects of hyperglycemia are complex and not fully understood. Most seem to be an immediate result of increased glucose levels. Unlike many organs, the brain endothelium uses GLUT1 as glucose transporter, which is not directly regulated by insulin (Shah et al., 2012). In theory, hyperglycemia would lead to increased glucose absorption by the BBB. Whether this actually happens is a matter of debate. Much of the early evidence suggested that hyperglycemia lowered GLUT1 (*Slc2a1*) expression, normalizing brain glucose transport (Pardridge et al., 1990; Cornford et al., 1995). However, other research suggested that there was no difference in glucose transport between normoglycemic and hyperglycemic rats (Pelligrino et al., 1992; Jacob et al., 2002). The answer seems to depend largely on the conditions, models, and brain region. For example, one study found downregulated GLUT1 in the retina, but not in the cerebral cortex (Badr et al., 2000). Another found decreased GLUT1 expression in chronic, but not acute, hyperglycemia (Duelli et al., 2000). This controversy has been reviewed in depth (Shah et al., 2012; Prasad et al., 2014). A study by Jais et al. (2016) found that GLUT1 production and glucose uptake decreased in mice on a HFD. However, the resulting inflammation caused upregulation of VEGF, which led to recovery of GLUT1 levels (Jais et al., 2016). This opens new doors for the study of signaling processes involved in BBB response to hyperglycemia.

Perhaps more important than glucose transport is brain glucose concentration. Here, the preponderance of evidence shows increased glucose uptake through the BBB (Shah et al., 2012). This higher glucose concentration and metabolic rate explains the inflammatory phenotype described above. Note that this has no bearing on the GLUT1 debate. While GLUT1 is the most important glucose transporter, there are many others (GLUT3, GLUT4, GLUT8, and SGLT1) that can modulate neural glucose levels. Few studies have been done on human glucose levels, and their results are mixed, suggesting either no change or an increase in glucose concentration (Kreis and Ross, 1992; Seaquist et al., 2005). Some of these have been done on T1DM

patients, but not T2DM (Shah et al., 2012). More work is needed to address this gap.

Surprisingly, studies suggest that altered insulin signaling does not play a critical role in type 2 diabetic pathology. For example, in T1DM, where pancreatic insulin production has been cut off and insulin levels are very low, hyperglycemic patients display the same oxidative stress as their type 2 counterparts (Garg et al., 2014; Yorulmaz et al., 2015). Cell transport and permeability are similarly disrupted in each type. Mice lacking brain endothelial insulin receptors maintain normal BBB function and permeability (Kondo et al., 2004). T2DM patients, on the other hand, display insulin resistance. Thus, despite higher insulin levels, patients with type 2 diabetes show little reactivity to insulin, making them functionally equivalent to patients with type 1 diabetes.

Role of VEGF

The effects of hyperglycemia involve stimulation of *vascular endothelial growth factor* (VEGF), a factor classically associated with both angiogenesis and vascular permeability.

Vascular endothelial growth factor stimulates vasculogenesis, resulting in immature, unstable vessels. Signaling molecules such as Angiopoietin-1 promote vessel maturation and stabilization (Yancopoulos et al., 2000). VEGF also regulates angiogenesis in response to hypoxia via hypoxia-induced factors (Masoud and Li, 2015).

Because VEGF increases BBB permeability, it is associated with inflammation. For example, chronic intracerebral infusion of VEGF in mice was found to upregulate ICAM-1 and major histocompatibility complex I and II, increasing the opportunity for immune response (Proescholdt et al., 1999). Treatment with VEGF also disrupts claudin-5, occludin, and ZO-1, important TJ proteins (Fischer et al., 2002; Argaw et al., 2009). When given after stroke, VEGF triggers rapid (within minutes) stimulation of caveolae-mediated transcytosis, increasing transcellular transport and permeability (Feng et al., 1999; Chen et al., 2002).

Studies of VEGF expression in T2DM have focused on diabetic retinopathy. Increased VEGF production was found in response to hyperglycemia, an effect linked to the increased retinal vascularization (Cai et al., 2014; Capitão and Soares, 2016; Caprnda et al., 2017). The effects of hyperglycemia on VEGF expression in the rest of the brain are less known. One study found that cultured astrocytes exposed to higher glucose had increased VEGF expression (Wang et al., 2012). Astrocytic VEGF is released directly into the parenchyma, allowing it to act on the abluminal VEGF receptors of the endothelium.

The effects of plasma VEGF depend to a certain extent on the state of the BBB. VEGF receptors are located on the abluminal side of blood vessels, so VEGF must be able to cross the barrier in order to exert its effects (Qu-Hong et al., 1995). Since the BBB is more permeable in diabetic conditions, the pro-angiogenic and vascular permeability effects of VEGF are most clearly observed in those conditions. A study found that inhibiting early VEGF release from ECs could promote stroke recovery in diabetic mice (Reeson et al., 2015). Interestingly, the most commonly prescribed drug for acute management of stroke, rtPA, generates adverse side effects including increased BBB permeability (Suzuki et al., 2016), in great part via activation of VEGF signaling and increased transcytosis (Benchenane et al., 2005; Turner and Vink, 2012).

One of the upstream players regulating VEGF is HIF-1, a transcriptional factor normally upregulated in hypoxic conditions, which increases oxygen supply, glucose transport, and blood vessel formation (Fischer et al., 2002; Argaw et al., 2009; Balamurugan, 2016). It is also upregulated in response to hyperglycemia and to the resulting advanced glycation endproducts (AGEs) and ROS (Treins et al., 2001). Inhibiting HIF-1 activity reduced the hyperglycemic increase in BBB permeability and decreased expression of VEGF (Yan et al., 2012). Mechanisms by which hyperglycemia can dysregulate HIF-1 are reviewed elsewhere (Catrina, 2014). Further upstream, mTOR regulates HIF-1 activity. Inhibiting mTOR lessens the severity of BBB disruption and decreases the upregulation of both HIF-1 and VEGF (Land and Tee, 2007; Wei et al., 2016). As discussed previously, mTOR shifts the immune system away from Tregs and stabilizes Th1 and Th2 cell, priming the immune system for reaction (Matarese et al., 2001b). mTOR is responsive to nutritional status and blood sugar levels. HFD is linked to higher mTOR activity, which is associated with a more active immune system and higher rates of autoimmunity. mTOR is regulated by leptin which, like in obesity, is upregulated in diabetes due to higher levels of fat and blood glucose (Galgani et al., 2010; Bandaru and Shankar, 2011). NF-κB has also been suggested to mediate TNF-α stimulated upregulation of HIF-1 in muscle cells (Remels et al., 2015). This connection remains to be elucidated in the brain context.

Downstream, VEGF activates PKC, enzyme involved in endothelial permeability (Fleegal et al., 2005; Titchenell et al., 2012; Shao and Bayraktutan, 2013). PKC-β plays an especially important role, and hyperglycemia promotes dysfunction in its pathway, causing an increase in NADPH oxidase and MMP-2, and a decrease in occludin (Shao and Bayraktutan, 2013). PKC-β regulates the Rho-kinase (ROCK)/myosin light chain kinase (MLCK) pathway, which plays an important role in vascular smooth muscle tone. ROCK signaling inhibits expression of endothelial nitric oxide synthase (eNOS), which reduces the availability of NO, an effect associated with endothelial dysfunction. ROCK also inhibits eNOS phosphorylation and its resulting activation by inhibiting Akt activity (protein kinase B), as reviewed elsewhere (Yao et al., 2010). Pathological RhoA/ROCK activation in ECs also promotes an association between caveolin-1 and eNOS and their translocation to membrane caveolae compartments (Zhu et al., 2003, 2013). There, eNOS is inhibited (Ju et al., 1997; Drab et al., 2001; Ming et al., 2002), impairing BBB permeability (Siddiqui et al., 2011). Pathological activation of ROCK also promotes oxidative stress (Rivera et al., 2007; Soliman et al., 2012). Pharmacological blockade of ROCK reduces hyperpermeability via inhibition of oxidative stress in ECs (Allen et al., 2010; Gibson et al., 2014). Interestingly, it has been recently demonstrated that genetic or pharmacological blockage of ROCK prevents BBB breakdown following brain injury, opening new possibilities for treatment (Sadeghian et al., 2018).

Role of eNOS and NO

Nitric oxide (NO) plays autocrine and paracrine roles in the blood stream. It is synthesized by nitric oxide synthases (NOS), enzymes including neuronal NOS (nNOS), inducible NOS (iNOS), and eNOS. The latter constitutively releases a small amount of NO from ECs, which maintains smooth muscle tone (Kumar et al., 2017). It is also implicated in BBB permeability modulation and angiogenesis (Murohara et al., 1998). eNOS is activated by phosphorylation of serine 1177 in humans. This is modulated by VEGF signaling through a number of pathways. VEGF activates phospholipase C, which induces IP_3 synthesis and release of Ca^{2+} into the cytoplasm, followed by activation of calmodulin. This pathway leads to activation of PKC, AMP-dependent kinase (AMPK), and Akt (protein kinase B). VEGF can also activate Akt through PI3K. PKC, Akt, and AMPK can all phosphorylate eNOS.

Depending on the model of inflammation used, eNOS has been found to be up or down-regulated. Inflammatory and stroke models show overexpression of NO leading to detrimental effects (Murohara et al., 1998; Mohammadi et al., 2011). Diabetic models, however, show decreased eNOS expression (Safiah Mokhtar et al., 2013). This partly involves ROCK signaling, but control of eNOS is very complex. For example, insulin activates eNOS through the PI3K/Akt pathway. In hyperglycemia, however, the high amount of glucose increases the activity of the hexosamine pathway, which increases the concentration of UDP-β-*N*-acetylglucosamine (UDP-GlcNAc). This factor glycosylates members of the Pl3K/Akt pathway, limiting eNOS activation and subsequent NO activity (Patti et al., 1999; Federici et al., 2002). AGEs can also limit the production of NO by modifying gene expression, glycosylating eNOS, or quenching NO (Xu et al., 2003; Rojas et al., 2010). This creates a strong case for lowered eNOS activity in diabetes.

Nitric oxide has a half-life of a only few seconds but can diffuse freely through the cytoplasm and cell membranes, making it an effective signaling molecule for the local environment (Pacher et al., 2007). NO directly activates cGMP production, which decreases both P-selectin in endothelium and its ligand, β-2 adhesion molecule, in neutrophils. eNOS null mice show persistent upregulation in inflammatory pathways, with 9-10 times more leukocytes binding to the endothelium following an inflammatory stimulus (Cirino et al., 2003). eNOS is also associated with downregulation of MMPs (Tronc et al., 2000).

Nitric oxide plays an inflammatory role in an oxidative environment. It reacts with superoxide to form peroxynitrite, a highly reactive oxidant responsible for many detrimental effects, including disruption of DNA and protein structure (Pacher et al., 2007). Many studies have implicated peroxynitrite as a key player in BBB degradation (Mayhan, 2000; Tan et al., 2004; Parathath et al., 2006; Phares et al., 2007). The peroxynitrite-dependent IFN-γ pathway is sufficient to increase BBB permeability (Phares et al., 2007), and blocking peroxynitrite or superoxide ameliorates inflammation-induced BBB disruption (Mayhan, 2000; Tan et al., 2004). Peroxynitrite also activates latent MMPs (Rajagopalan et al., 1996) and inhibits the MMP inhibitor, TIMP (Frears et al., 1996), causing degradation of the BM.

The role of NO is still somewhat unclear. One study found inactivation of eNOS attenuated the downregulation of claudin-5 and occludin and decreased the BBB permeability caused by VEGF signaling from reactive astrocytes (Argaw et al., 2012). However, this report did not specify whether peroxynitrite was involved. The same can be said for studies that connected eNOS activation with increased BBB permeability in connection with VEGF activation or induced stroke (Fukumura et al., 2001; Mohammadi et al., 2011). Clarification of the precise mechanism responsible for these effects is necessary to evaluate their significance. In addition, peroxynitrite has a multifaceted role, with one study suggesting it protected neurons against nitric-oxide mediated apoptosis (García-Nogales et al., 2003). Thus, more work is needed to elucidate the role of NO in inflammation-induced BBB breakdown.

Role of AGEs

AGEs, primary mediators of diabetes-associated pathologies, result from hyperglycemia. Glucose combines with a protein to form a Schiff base that undergoes further modification to become an AGE. AGEs alter protein structure and function, affecting enzyme activity, reducing protein half-life and weakening ligand binding (Vlassara and Palace, 2002). Certain AGEs can cross-link proteins, creating matrixes that inhibit normal protein function. Their production leads to ROS formation that cause damage to the cell by oxidative stress. DNA and lipids are vulnerable to AGE formation. For review see (Ahmed, 2005; Ceriello, 2012).

AGEs act on a variety of receptors, the most common of which is the receptor for AGE (RAGE). RAGE activates an immune response by modulating a number of cytokines, including pro-inflammatory cytokines IL-8, TGF-β1, TNF-α, interferon γ (IFN-γ), IL-2, and IL4, and basement membrane modifying proteins COL-1, COL-III, and MMP-2 (Shimizu et al., 2013; Serban et al., 2016). Many of RAGE's effects are mediated through NF-κB (Ahmed, 2005). NF-κB activation leads to increased expression of IL-6, IL-1α, and TNF-α. It promotes coagulation and vasoconstriction through the upregulation of tissue factor, thrombomodulin, and endothelin-1. It also increases VCAM-1 and ICAM-1 expression, allowing for enhanced penetration of leukocytes (Lawrence, 2009; Hoesel and Schmid, 2013). Perhaps most importantly, NF-κB promotes further expression of RAGE, creating a positive feedback loop. This has implicated RAGE in metabolic memory, a phenomenon in which oxidative damage and inflammatory gene expression continue even after hyperglycemia has been normalized (Ceriello, 2012). In one study, as little as 2 months of hyperglycemia before glucose control was re-established was enough to cause permanent changes to NF-κB activity (Kowluru et al., 2004). Hyperglycemia greatly alters gene expression, and many oxidation controlling genes are downregulated. These modifications may persist after glucose levels are normalized, further contributing to metabolic memory (Ceriello, 2012). As a result, the inflammatory state induced by hyperglycemia can persist long after blood glucose levels are controlled, increasing the severity of hyperglycemia.

Role of miRNAs

Micro RNAs (miRNAs) have also been shown to play a role in BBB disruption. miRNAs are short, 15–20 nucleotide-long RNAs that bind to messenger RNAs, preventing their translation or marking them for degradation (Hammond, 2015). They are increasingly recognized to play an important role in many pathologies, including inflammatory diseases. Pro-inflammatory cytokines TNFα and IFNγ have been shown to downregulate several miRNAs. One of them, miR-125a-5p, regulates barrier tightness and prevents leukocyte extravasation (Reijerkerk et al., 2013). Another, miR-143, is associated with T2DB. It downregulates oxysterol-binding protein-related protein 8, impairing the ability of insulin to induce Akt activation. This creates a miRNA-linked mechanism for insulin insensitivity (Li et al., 2018). miR-143 also downregulates PUMA, a pro-apoptotic molecule. PUMA is associated with BBB damage and permeability, and it decreases TJ protein expression (Bai et al., 2016). These studies open a new mechanistic approach to inflammation, necessitating further investigation.

IMPACT OF METABOLIC SYNDROME ON NEURAL HEALTH

Connections to Aging

The inflammatory effects of metabolic syndrome described above are, in many respects, comparable to effects of aging. While aging is a very complex process not well understood to date, its features appear to be the products of inflammation caused by cell senescence and deterioration, with the accumulation of AGEs and other harmful waste products (Lucke-Wold et al., 2014). Metabolic syndrome often accompanies the aging process, with 46.7% prevalence amongst those over 60 compared to 18.3% amongst those aged 20–39 (Aguilar et al., 2015). It leads to less healthy aging and poorer quality of life. Hypertension and cardiovascular disease have long been known to increase risk of ischemic stroke, and obesity and diabetes are also known risk factors (Lucke-Wold et al., 2014). Metabolic syndrome is also associated with reduced cognitive performance and exacerbates the risk and outcomes of neurodegenerative disease, such as AD and MS (Hassing et al., 2009).

Both obesity and diabetes, through the mechanisms described above, lead to increased neuroinflammation, essentially accelerating the aging process. For example, AGEs produced because of diabetes contributes to production of ROS which causes protein and DNA damage, cell death, and the activation of immune pathways (Ahmed, 2005). High concentrations of leptin induced by obesity lead to immune cell activation (Lord et al., 1998; Matarese et al., 2001b). The damage caused to endothelial cells by increased inflammation and ROS are more susceptible to binding platelets and forming clots (McEwen, 2014).

Astrocytes succumb to the pressures of oxidative stress and telomeric replication exhaustion, becoming unable to meet their role of maintaining ion and neurotransmitter homeostasis, and the BBB (Bhat et al., 2012). The BBB begins to decay, with occludin and ZO-1 significantly reduced (Mooradian et al., 2003), and endothelial cell structure becoming less robust (Lee et al., 2012). Meanwhile, inflammatory pathways are increasingly active. In their review on aging and metabolic syndrome, Lucke-Wold et al. (2014) described a positive feedback loop in which activated microglia produce ROS, leading to cell death and increased local glutamate levels. This, in turn, leads to increased secretion of pro-inflammatory cytokines and further microglial activation. This process would be interrupted in a younger brain by astrocytes buffering excess glutamate (Lucke-Wold et al., 2014).

Connections to Alzheimer's Disease

Neuroinflammation is implicated in many neurological disorders, and inflammation caused by metabolic syndrome increases the likelihood and severity of these pathologies. The link between obesity, diabetes and AD is now well established and has been studied extensively (Nguyen et al., 2014; Pugazhenthi et al., 2017). Both obesity and diabetes can lead to insulin resistance in the CNS, a disorder known as type 3 diabetes (de la Monte and Wands, 2008). The resulting lack of insulin and insulin-like growth factors signaling is identical to that seen in AD. Both lead to neuronal death and glial activation. They also result in mitochondrial dysfunction, oxidative stress, and increased ROS levels. The inflammasome, a protein complex leading to pro-inflammatory cytokine secretion, caspase-1, and proteolytic cleavage, gets upregulated and increases inflammation (Pugazhenthi et al., 2017). As described above, this leads to degradation of the BBB and the influx of leukocytes. Aβ formation is upregulated in AD, and the damaged BBB is less able to remove it, causing accumulation in the parenchyma (Bell and Zlokovic, 2009; Zlokovic, 2011; Sagare et al., 2012). In addition, leptin has been implicated in AD pathogenesis. It decreases Aβ production by blocking β-secretase and increasing Aβ uptake, and it deactivates glycogen synthase kinase beta, the protein primarily responsible for tau hyperphosphorylation (Folch et al., 2015). Although leptin levels increase in obesity, insensitivity prevents it from fulfilling its function. Lastly, adiponectin, an anti-inflammatory molecule with protective effects against AD, is decreased in obesity (Letra et al., 2014).

Connections to Multiple Sclerosis

An association exists between MS and T1DM, however, this is more related to their common origin as autoimmune diseases, and less to an inflammatory environment created by T1DM (Tettey et al., 2015). Links between MS and T2DM are more tenuous. In their review, Tettey et al. (2014) reported evidence for vascular comorbidities in MS, including T2DM. An epidemiological study was unable to find any difference in MS prevalence between diabetic, hypertensive, or hyperlipidemic populations and the general population from 1984 to 2006 (Marrie et al., 2012). More recently, Hou et al. (2017) showed a small, albeit significant, association between T2DM and MS incidence. Another study focusing on MS patients between found that diabetes, obstructive lung disease and hypertension affected clinical outcomes such as walking speed, self-reported disability, and depression (Conway et al., 2017). However, none of these studies provide strong evidence that T2DM increases risk of contracting MS.

As with T2DM, investigations on links between obesity and MS have produced mixed results. The prevalence of obesity amongst MS patients has been found either increased (Slawta et al., 2003), decreased (Markianos et al., 2013; Pinhas-Hamiel et al., 2015), or unchanged (Khurana et al., 2009). A systemic review by Wens et al. (2013) found evidence for increased risk of cardiovascular disease in MS, but the precise connection remains unclear. However, several studies have associated obesity during adolescence with a higher risk of contracting MS (Hedström et al., 2012, 2016). A mendelian randomization study by Mokry et al. (2016) found significant association between genetically induced obesity and MS, with low risk of pleiotropic effects (i.e., the genes in question influence the two conditions through separate pathways).

Possible mechanistic links between obesity and MS has been reviewed elsewhere (Guerrero-García et al., 2016; Palavra et al., 2016). These focus on a few factors, including a possible confounding role played by serum levels of vitamin D, dyslipidemia, and adipokines such as leptin. An increased inflammatory state may be a very important link between obesity, T2DM, and MS. HFDs have been shown to open the CP, which would allow for greater immune sampling and, presumably, greater immune sensitivity (Kanoski et al., 2010). In addition, systemic inflammation would open the BBB, allowing for easier immune penetration (Varatharaj and Galea, 2017). These effects would decrease the threshold required for autoimmune activation, allowing the stimulation of myelin reactive cells.

METABOLIC SYNDROME AND THE GUT MICROBIOME-BBB AXIS

The gut microbiome has been implicated as an important player in neural health, as well as with obesity and T2DM. Imbalance of the microbiome, known as dysbiosis, has long been known to play a role in the development of metabolic syndrome and obesity (Barlow et al., 2015). For example, germ free (GF) mice gain significantly less weight than specific-pathogen-free (SPF) counterparts, despite higher food intake. Reintroducing bacteria to the GF mice results in further weight gain (Bäckhed et al., 2004). Substantial work has explored the specific contributions of various bacterial phyla to obesity and diabetes, as reviewed elsewhere (Barlow et al., 2015).

More recently, dysbiosis has been shown to be comorbid with neuropathologies including autism spectrum disorder, Parkinson's disease, MS, and chronic pain (Martin et al., 2018). The microbiota communicate with the brain by interacting with endocrine and enterochromaffin cells, which secrete hormones and neuromodulatory molecules. They also directly signal the brain through peptides, inflammatory molecules, and bacterial metabolites (Martin et al., 2018). Aberration of these signaling pathways leads to neural dysfunction, and gut microbiota profiles have been linked to specific brain profiles, including the size of structures like the hypothalamus, caudate nucleus, and hippocampus (Fernandez-Real et al., 2015; Labus et al., 2017). GF mice showed cognitive deficits, as did *Citrobacter rodentium* infected mice exposed to acute stress (Gareau et al., 2011; Fernandez-Real et al., 2015). In humans, female subjects put on a probiotic milk product for 4 weeks had altered neural activity in affective, viscerosensory, and somatosensory cortices (Tillisch et al., 2013). The microbiota also influences the BBB. GF mice display increased BBB permeability compared to SPF mice, with downregulation of claudin-5 and occludin. Exposure of GF mice to the SPF microbiota decreased BBB permeability and upregulated TJ proteins (Braniste et al., 2014). These findings highlight the importance of the gut microbiota for neural health, but many questions remain unresolved. The microbiota-to-BBB connection has been discovered very recently, and more work is required to elucidate the pathways involved. In addition, most of the work in this area has been done on rodents, and translation to humans remain unclear (Martin et al., 2018).

THE POSITIVE EFFECTS OF INFLAMMATION

Despite all the harmful consequences of inflammation, it still serves vitally important functions in the brain that are worth mentioning. Interestingly, many of these functions center around MS, a disease notorious for autoimmune attack driven by inflammation. For example, myelin reactive T cells are normally implicated in neural decline, but when they were injected into rats following partial crush injury of the optic nerve, the rats retained 300% more retinal ganglion cells (Moalem et al., 1999). This beneficial response is specific to anti-myelin T cells. Immune deficient mice given CNS trauma showed improved recovery following treatment with myelin reactive cells, whereas treatment with ovalbumin reactive cells did not have any effect (Yoles et al., 2001). These effects seem to come largely from Th1 and Th2 cells. One study immunizing mice with Th1 and Th2 myelin reactive cells opened the BBB using an antiseptic approach, instead of pertussis toxin, to induce EAE. Instead of a pathological response, Th1 and Th2 both accelerated re-vascularization and healing (Hofstetter et al., 2003), suggesting Th1 and Th2 in a non-inflammatory environment are beneficial for EAE recovery. Another study observed the progression of MS in the mouse brain. Areas with initial oligodendrocyte and myelin loss had high phagocyte count but low B or T cell count. Areas with complete demyelination had high T cell count, an observation apparently connected to oligodendrocyte regeneration (Henderson et al., 2009). Several mechanisms underlying this effect have been explored, and a likely candidate is the release of neurotrophins from activated immune cells that beneficially affect brain recovery. These include compounds like neural growth factor, which modulates B cell proliferation, immunoglobulin production, and cell survival, and brain derived neurotrophic factor, involved in neuronal survival (Hohlfeld et al., 2006; Schwartz and Raposo, 2014).

Such studies contradict the idea that autoreactive T cells are accidental biproducts of failed T cell sorting in the thymus. Indeed, Schwartz and Raposo (2014) defined "protective autoimmunity" as an essential component of CNS health. In their model, an active population of

CNS-reactive T cells are essential for proper neuronal development and protection against neurodegenerative diseases such as AD. These cells must be carefully controlled by Tregs, which limit the immune response. While low Treg activity will result in chronic inflammation, high activity of Tregs would lead to neurodegenerative diseases. Such model implies that effective treatment for diseases like MS cannot unselectively suppress the immune system. Instead, it must suppress aspects of the immune system that are destructive, while promoting those that are regenerative.

CONCLUSION

Metabolic syndrome and the resulting insulin and leptin resistance and hyperglycemia have pro-inflammatory effects with profound consequences on the BBB. Breakdown of the BBB leads to immune infiltration into the parenchyma and neuronal death. This carries many implications, depending on the brain region affected. Impact on the hypothalamus leads to hormonal disbalance (Boden and Shulman, 2002; Horvath et al., 2010; Jais and Brüning, 2017), damage to the hippocampus leads to cognitive decline (Davidson et al., 2012), and injury to the CP leads to increased immune sensitivity (Kanoski et al., 2010). Many signaling pathways have been implicated in these processes, including VEGF, PKC, RhoA/ROCK, HIF, mTOR, eNOS, AGEs, and miRNA, all of which intersect each other (Fischer et al., 2002; Cirino et al., 2003; Land and Tee, 2007; Yao et al., 2010; Reijerkerk et al., 2013; Shao and Bayraktutan, 2013; Balamurugan, 2016; Serban et al., 2016). Anti-inflammatory drugs target these pathways, but because of the complexity of the picture, it is difficult to predict what side effects they may produce. Given that a certain amount of inflammation is necessary (Moalem et al., 1999), it is vital to carefully select drug targets that will modulate the patient's immune response in a protective way. Resolving metabolic memory may be one of the more relevant therapeutic approaches, as it causes continued inflammation even after blood glucose levels are resolved (Ceriello, 2012). Furthermore, the effects of glucose on GLUT1 expression levels in the human brain have not been fully resolved. Since hyperglycemia is one of the most important factors causing the conditions described above, understanding why its flux across the barrier increases is essential to understanding and preventing its effects. miRNAs remain a largely unexplored factor in systemic inflammation. Given the increasingly important role miRNAs play in cellular physiology, further study is warranted. Finally, the microbiota has been implicated as a key player in BBB integrity, but remains poorly understood. More work is needed to elucidate this connection.

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PVD prepared the draft following BL's instructions. BL chose the theme, edited and corrected the manuscript.

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REVIEW
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Interglial Crosstalk in Obesity-Induced Hypothalamic Inflammation

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Glial cells have recently gained particular attention for their close involvement in neuroinflammation and metabolic disorders including obesity and diabetes. In the central nervous system (CNS), different types of resident glial cells have been documented to express several signaling molecules and related receptors, and their crosstalks have been implicated in physiology and pathology of the CNS. Emerging evidence illustrates that malfunctioning glia and their products are an important component of hypothalamic inflammation. Recent studies have suggested that glia–glia crosstalk is a pivotal mechanism of overnutrition-induced chronic hypothalamic inflammation, which might be intrinsically associated with obesity/diabetes and their pathological consequences. This review covers the recent advances in the molecular aspects of interglial crosstalk in hypothalamic inflammation, proposing a central role of such a crosstalk in the development of obesity, diabetes, and related complications. Finally, we discuss the possibilities and challenges of targeting glial cells and their crosstalk for a better understanding of hypothalamic inflammation and related metabolic dysfunctions.

Keywords: obesity, diabetes, hypothalamus, inflammation, glia, chemokine, metabolism

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INTRODUCTION

The hypothalamus is a critical brain structure and consists of three major areas such as the periventricular, the medial, and the lateral hypothalamic area. The median eminence (ME) is located in the middle–basal hypothalamus and the dorsal side of the third ventricle, and has blood vessels without barrier, which act as a window to release hypothalamic metabolic signals (Peruzzo et al., 2000; Elizondo-Vega et al., 2015). Nuclei of each area are metabolically important and play crucial roles in diverse functions including nutrient sensing, appetite control, and energy metabolism (Coll and Yeo, 2013; Elizondo-Vega et al., 2015). The hypothalamus receives metabolic signals from periphery through hormonal and cellular communication, letting the hypothalamus to control whole-body metabolism (Alquier and Kahn, 2004). However, disturbance of this mechanism is a major concern in a broad range of metabolic diseases including obesity and diabetes. Rising evidence suggests that

hypothalamic inflammation particularly in the arcuate nucleus (ARC), a part of periventricular area, is a common pathological feature related with obesity and diabetes (Valdearcos et al., 2015). It affects the endocrine system and disturbs nutritional homeostasis, which causes metabolic alterations and subsequent pathologies. In animal experiments, high-fat diet (HFD)-induced chronic inflammation in the hypothalamus has been documented to modulate feeding behavior, energy expenditure, insulin secretion, and hepatic glucose production, all of which have been associated with dysregulation of glucose and fatty acid (FA) metabolism in obesity and diabetes (Cai and Liu, 2011). It has been reported that hypothalamic inflammation is observed within first 3 days of HFD onset with increased level of inflammatory markers (Thaler et al., 2012; Baufeld et al., 2016). An animal study of neonatal overnutrition revealed that hypothalamic inflammation is age- and sex-dependent, which is observed only in male animals with significant weight gain at postnatal day 150 (Argente-Arizon et al., 2018). The possible reason of such differences is the elevated level of circulating estrogens in female animals, probably inhibiting the effects of FAs on inflammation. As in other brain regions, the hypothalamus also contains various types of non-neuronal cells such as microglia, astrocytes, tanycytes, and NG2-positive glial cells (oligodendrocyte progenitor cells), which all play important physiological roles as metabolic sensors in the hypothalamus (Garcia-Caceres et al., 2012; Elizondo-Vega et al., 2015; Djogo et al., 2016; Freire-Regatillo et al., 2017). Emerging evidence suggests that glial cells may be a key component of pathological hypothalamic inflammation as well. Notably, wide structural changes have been observed in hypothalamic astrocytes in animal models of obesity and type 2 diabetes (Horvath et al., 2010). This study suggests that astrocytes contribute to synaptic rewiring by altering the glial ensheathment of neurons, which may disturb the ability of neurons to receive direct nutritional signals from the periphery. In line with this thought, it has been proposed that hypothalamic inflammation is a potential cause of leptin and insulin resistance in the hypothalamus of rodents (Thaler et al., 2012; Andre et al., 2017) and patients with obesity and diabetes (Schur et al., 2015; Kreutzer et al., 2017).

In recent years, glial cells have become a focus of modern neuroscience research, for instance, with respect to neurodevelopment and brain health/disease (Barres, 2008). They are homeostatic regulators in the central nervous system (CNS) and play a critical role by providing metabolic support for neurons. In addition, they maintain extracellular pH, protect neurons from any insults, as well as participate in neurovascular coupling, neurotransmitter uptake, synaptic formation and pruning, and phagocytosis (Jha and Suk, 2013; Jha et al., 2014). Moreover, glial cells participate in these functions by communicating with and influencing other glial cell types in a mutual manner, ultimately playing a crucial role in brain development, function, and diseases (Jha et al., 2018). This interglial communication occurs through various cell surface molecules and the release of signaling molecules such as cytokines, chemokines, adenosine triphosphate, and growth factors (Tanuma et al., 2006; Pascual et al., 2012; Rocha et al., 2012; Liddelow et al., 2017). It has been reported that glial crosstalk plays a crucial role in the development and progression of neuropathologies depending on the level of their secreted molecules following various CNS insults (Tian et al., 2012). Among different glial cell types, microglia and astrocytes are the primary innate immune cells and key responders to a variety of CNS insults such as pathogens, ischemia, and chemogenic factors, which further lead to the activation of these glial cells and ensue the production of increased levels of proinflammatory mediators, for example, cytokines, chemokines, reactive oxygen species, and nitric oxide (NO) (Tanuma et al., 2006; Iizumi et al., 2016; Hou et al., 2017; Liddelow et al., 2017). These mediators are responsible for chronic inflammation and resultant pathologies in the CNS. However, several molecular targets such as anti-inflammatory cytokines and neurotrophic factors have been identified in other brain areas where the inflammatory response due to microglia and astrocytic activation has been reduced in Alzheimer's disease (Mandrekar-Colucci et al., 2012), Parkinson disease (PD) (Subramaniam and Federoff, 2017), and epilepsy (Benson et al., 2015). It has been reported that activated microglia-derived IL-10 reduces inflammatory responses in the stratum, which has been proposed as a crucial target to enhance the neuroprotection in an experimental model of PD (Schwenkgrub et al., 2013; Joniec-Maciejak et al., 2014).

Emerging evidence highlights that microglia, astrocytes, and their interaction play an important role in the regulation of immune and inflammatory responses in the CNS (Farina et al., 2007; Ransohoff and Brown, 2012; Kirkley et al., 2017). In the context of metabolic inflammation in the hypothalamus, the interaction between these glial cell types has been reported to be a decisive contributor to progression and severity of neurometabolic diseases (Kim et al., 2018). In addition, glial interaction modulates their activation states, particularly through the expression of cell surface receptors including cytokine and chemokine receptors, and activation of intracellular signaling pathways (Zhang et al., 2017; Kim et al., 2018; Valdearcos et al., 2018), potentially amplifying the inflammatory activation in the hypothalamus under diabetes and obesity conditions (Rahman et al., 2018). A recent study has revealed that inflammatory activation of astrocytes by lipid droplets and the potential crosstalk of these astrocytes with microglia in free FA (FFA)-rich environments might be implicated in the obesity-induced pathological hypothalamic inflammation and subsequent metabolic complications (Kwon et al., 2017).

This review discusses the recent advances in the molecular aspects of interglial crosstalk in hypothalamic inflammation, particularly astrocyte–microglia crosstalk, with extended discussion on the potential crosstalks between microglia–astrocyte and tanycyte–astrocyte, suggesting a central role of the interglial crosstalk in the development of obesity, diabetes, and their complications, based on the data currently available in the literatures (**Figure 1**). Finally, we also discuss the possibilities and challenges of targeting glial cells and their crosstalk for a better understanding

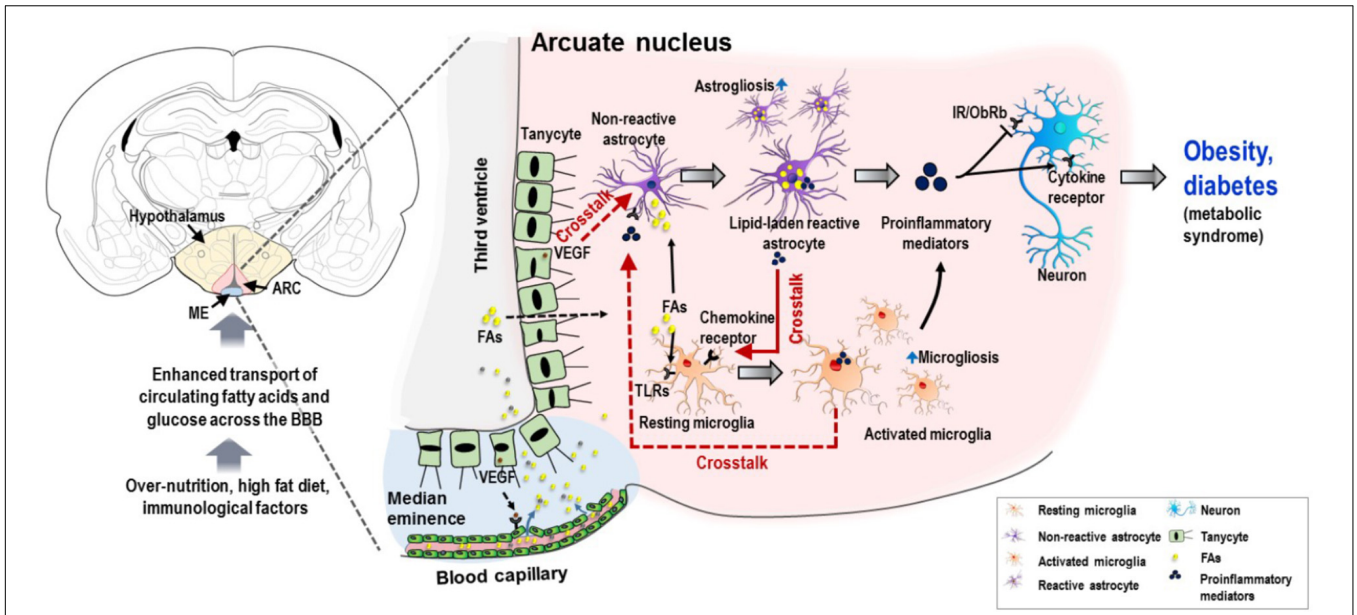

FIGURE 1 | Schematic representation of potential interglial crosstalk in hypothalamic inflammation and the ontology of obesity and diabetes. Overnutrition, HFD, immunological factors, and increased circulating nutrients (FAs and glucose), arising in a temporal sequence, appear to induce glial malfunctions and pathological hypothalamic inflammation. The excessive circulating nutrients are transported into the hypothalamus through the permeable blood capillaries. Tanycytes, a specialized ependymal cell type located in the lateral walls and surface of the third ventricle, allow the passage of these excessive nutrients particularly FAs from the circulation to the hypothalamic ARC. This may be due to the expression of VEGF in tanycytes, which targets microvessel loops and promotes microvessel permeability. FAs can activate both microglia and astrocytes, primarily by binding to TLRs expressed in microglia. Similarly, FAs cause accumulation of lipid droplets in astrocytes, leading to inflammatory activation of astrocytes and release of proinflammatory mediators, which may further activate microglia by molecular interaction enhancing the inflammatory processes. In addition, activated microglia and VEGF-rich tanycytes may also contribute to chronic hypothalamic inflammation by their secreted molecules and subsequent crosstalk with astrocytes. This proinflammatory mechanism impairs insulin and leptin signaling in neurons, leading to cellular stress responses. This condition negatively affects the proper functioning of hypothalamic neuronal circuits, which leads to metabolic dysregulation associated with obesity and diabetes. Solid red arrows, reported glial crosstalk; dotted red arrows, possible crosstalk. BBB, blood–brain barrier; ARC, arcuate nucleus; FA, fatty acid; TLR, toll-like receptor; VEGF, vascular endothelial growth factor; IR, insulin receptor; ObRb, leptin receptor b.

of hypothalamic inflammation and its related metabolic dysfunctions.

CHEMOKINE-MEDIATED ASTROCYTE–MICROGLIA CROSSTALK

Chemokines are a superfamily of structurally related small (most of them between 8 and 14 kDa) chemotactic cytokines, i.e., they promote leukocyte migration, and are implicated in various inflammatory pathological processes and infectious diseases. In the healthy brain, different cell types including glial cells or neurons constitutively express many chemokines and their receptors, and their expression levels are found to be increased in activated astrocytes and microglia (Le Thuc et al., 2017). Among those, some chemokines such as monocyte chemoattractant protein-1 (MCP-1/CCL2), stromal cell-derived factor (SDF-1/CXCL12), and fractalkine/CX3CL1 and their receptors have been shown to be expressed in the hypothalamus (Banisadr et al., 2005), and their association with obesity-induced inflammatory responses and metabolic complications have been reported (Morari et al., 2014; Poon et al., 2016).

The chemokine MCP-1/CCL2 is expressed in glial cells such as astrocytes and microglia, and central delivery of MCP-1 has been shown to promote hypothalamic inflammation and metabolic dysregulation as well (Le Thuc et al., 2016). Intriguingly, a recent study has demonstrated that astrocyte-derived MCP-1 is crucial for the onset and/or progress of the obesity-induced glial cell-mediated hypothalamic inflammation (Kwon et al., 2017). *In vitro* experiment using primary hypothalamic astrocytes has revealed that the cells accumulate lipid droplets under FFA-rich conditions with their increased inflammatory reactivity, mimicking obese conditions. The lipid-laden astrocytes produce large amounts of proinflammatory mediators such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and MCP-1, suggesting that under obese conditions these lipid-laden astrocytes may trigger the onset of hypothalamic inflammation. Of note, treatment of microglia with fatty astrocytes-conditioned medium markedly increased the chemotactic activity of microglia with upregulation of CCR2 (receptor for MCP-1/CCL2), and treatment of anti-MCP-1 antibody diminished the chemotactic activity. These results indicate that the fatty astrocytes-derived inflammatory chemokine MCP-1 enhances microglial chemotactic activity by binding to its receptor, and the recruited microglia may interact with the neighboring astrocytes, amplifying the hypothalamic inflammatory responses (**Figure 2**). It has been reported that mice fed with HFD show the increased number of microglia in ARC region of the hypothalamus.

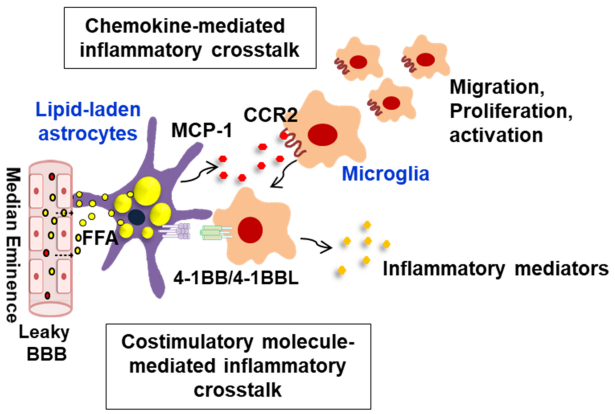

FIGURE 2 | Schematic representation of interglial crosstalk via chemokines and costimulatory molecules in hypothalamic inflammation under obese conditions. In the hypothalamus, glial cells such as astrocytes and microglia can directly respond to peripheral excess nutrient signals, such as FAs, through a unique vascular system (fenestrated capillaries) and damaged BBB and these glial cells become reactive to release inflammatory mediators. In obese environments rich in FAs, astrocytes accumulate lipid droplets, and they release the proinflammatory chemokine MCP-1, which promotes microglia migration, proliferation, and activation. The lipid-laden astrocytes can also directly interact with the migrated microglia through membrane-bound costimulatory receptors/ligands such as 4-1BB/4-1BBL, thus reinforcing the inflammation. This glial cell-mediated inflammatory crosstalk may be crucial for obesity-induced hypothalamic inflammation. BBB, blood–brain barrier; FFA, free fatty acid; MCP-1, monocyte chemoattractant protein-1; CCR2, C–C chemokine receptor type 2.

The study suggested that the increased number of microglia in the ARC might be due to increased infiltration of bone marrow-derived monocytes/macrophages into the CNS (Thaler et al., 2012; Buckman et al., 2014; Valdearcos et al., 2014). Given that the chemokine MCP-1 disrupts the integrity of the blood–brain barrier (BBB) (Yao and Tsirka, 2014), the chemokine MCP-1 may also act in obesity as a signal to recruit bone marrow-derived monocytes/macrophages into the hypothalamus.

In recent years, chemokines such as SDF-1/CXCL12 and fractalkine/CX3CL1 have been shown to be associated with obesity-induced hypothalamic inflammation. SDF-1 is constitutively expressed in hypothalamic astrocytes, microglia, as well as in neurons (Banisadr et al., 2003). SDF-1 and its receptors CXCR4 and CXCR7 have been shown to be upregulated in the hypothalamic nuclei by HFD intake, which is accompanied by an increase of orexigenic neuropeptides (Poon et al., 2016). Moreover, central administration of CXCL12 increases caloric intake, stimulates the expression of orexigenic neuropeptides, and reduces novelty-induced locomotor activity, suggesting that SDF-1 might have an important role in mediating the HFD-induced neuronal and behavioral effects. The crosstalk between astrocytic SDF-1 and microglial CXCR4 has been shown to participate in the development of neuroinflammation and the pathogenesis of neuropathic pain hypersensitivity (Luo et al., 2016). Fractalkine/CX3CL1 is predominantly expressed and released by neurons, and its receptor CX3CR1 is expressed in microglia (Tian et al., 2012). Fractalkine and its receptor play a pivotal role in the recruitment, infiltration, and proinflammatory polarization of leukocytes and microglial cells that are involved in the regulation of neuroinflammatory conditions (Cardona et al., 2006; Cardona et al., 2008), indicating that the chemokine acts as a mediator in the crosstalk between leukocytes and microglia. Fractalkine has been shown to exert both anti-inflammatory (Zujovic et al., 2000) and proinflammatory properties (Huang et al., 2006; Denes et al., 2008), however, recent studies support the proinflammatory role of fractalkine in obesity-induced hypothalamic inflammation. In obesity-prone mice, the inhibition of hypothalamic fractalkine reduces obesity-related inflammatory activity as demonstrated by the reduction of TNF-α and IL-1β levels and the decreased recruitment of bone marrow-derived cells to the hypothalamus. This model also prevents the development of obesity and glucose intolerance (Morari et al., 2014), indicating that fractalkine is a key player in diet-induced early hypothalamic inflammation. Chemokines such as SDF-1 and fractalkine are considered interesting therapeutic targets to fight against obesity-induced inflammatory complications; however, detailed molecular mechanisms underlying the chemokine-mediated interglial crosstalk in obesity-induced hypothalamic inflammation remain to be further investigated.

INTERGLIAL CROSSTALK MEDIATED BY COSTIMULATORY MOLECULES

The contribution of glia–glia interaction to hypothalamic inflammation is strongly influenced by their potential for mutual interaction with the inflammatory microenvironment. Substantial evidence points to reactive glia as a pivotal factor during the obesity-induced hypothalamic inflammation process. As mentioned earlier, astrocytes and microglia in the hypothalamus are often found in close proximity to each other, indicating a potential reciprocal contact-dependent communication between them, facilitating their inflammatory crosstalk.

Several immune signaling costimulatory molecules (ligands/their receptors, membrane-bound proteins), which are mostly expressed in immune cells, glial cells, and neurons, and their crosstalk has been implicated in CNS inflammatory pathologies. For instance, different TNF receptor superfamily (TNFRSF) members and their ligands such as TWEAK/FN14 and CD40/CD40L contribute to the pathogenesis of Alzheimer's disease and multiple sclerosis, which have a strong inflammatory component and glia involvement (Aarts et al., 2017). CD40/CD40L interaction has been reported to enhance the inflammatory response in multiple sclerosis, and genetic ablation or antibody-mediated inhibition of the CD40/CD40L interaction reduces the severity of experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis (Gerritse et al., 1996; Grewal et al., 1996; Aarts et al., 2017). The 4-1BB (also known as TNFRSF9 or

CD137) receptor/ligand system may be another potent mediator in controlling neuroinflammatory pathologies. It has been reported that mice with 4-1BB ligand (4-1BBL) deficiency display profoundly less microglial activation following EAE induction compared with wild-type animals, and, thus, the gene deficiency reduces the severity of this neuroinflammatory pathology (Yeo et al., 2012). These costimulatory receptors/ligands are, therefore, considered to be attractive therapeutic candidates for diverse neuroinflammatory diseases. However, studies on the association of these receptors/ligands system with obesity-induced hypothalamic inflammation are currently limited.

Nonetheless, there is recent evidence supporting the occurrence of interglial crosstalk through 4-1BB/4-1BBL interaction in the hypothalamus under obese conditions (Kim et al., 2018). 4-1BB is a member of the TNFRSF and a membrane-bound costimulatory protein, which is highly expressed in activated T cells as well as NK cells (Vinay and Kwon, 2011). It has also been reported to be expressed by some nonimmune cells such as adipocytes and endothelial cells (Drenkard et al., 2007; Kim et al., 2011) and delivers potent costimulatory or inflammatory signals upon activation. 4-1BB binds to its ligand 4-1BBL, which is highly expressed in antigen-presenting cells such as macrophages. In addition, the 4-1BB receptor/ligand system can transmit bidirectional signals between two interacting cells, a process referred to as forward and reverse signaling (Shao and Schwarz, 2011). Although most studies are focused on 4-1BB/4-1BBL-mediated immune regulations, a few recent studies have shown that the 4-1BB/4-1BBL-mediated bidirectional signal can promote obesity-induced adipose tissue inflammation by the recruitment of adipocytes/macrophages and inflammation in skeletal muscle by myotubes/macrophages recruitment (Tu et al., 2012; Le et al., 2013). Intriguingly, astrocytes express 4-1BB, whereas microglia express 4-1BBL (Reali et al., 2003; Yeo et al., 2012), and these molecules are likely to have the potential to mediate the crosstalk between astrocytes and microglia. Indeed, it has been shown that mice fed with HFD display an increased expression of 4-1BB and 4-1BBL transcripts in the hypothalamus when compared with lean controls (Kim et al., 2018), supporting the idea that these molecules may be responsible for the crosstalk between astrocytes and microglia in obese conditions. Using mouse primary astrocytes and BV-2 mouse microglial cells *in vitro*, the involvement of these molecules in glial cells-mediated hypothalamic inflammatory responses was assessed in a recent study (Kim et al., 2018). First, obesity-related factors such as lipopolysaccharide, FFA, glucose, and adipose tissue-conditioned medium treatment increased the transcript levels of 4-1BB in astrocytes and 4-1BBL in microglia, mimicking the conditions of obesity. Second, stimulation of 4-1BB on astrocytes delivered an inflammatory signal to increase astrocyte reactivity, leading to an increased production of proinflammatory mediators such as TNF-α, IL-6, and MCP-1 at the levels of mRNA and protein. Third, 4-BBL stimulation of microglial cells increased microglial activation, enhanced the release of proinflammatory mediators such as MCP-1 and ICL-6, and decreased the level of an anti-inflammatory cytokine IL-10. This was mediated through activation of the mitogen-activated protein kinase pathway. Fourth, an increased production of inflammatory cytokines was observed in the co-culture of glial cells with augmented glial activation markers. More importantly, inhibition of 4-1BB/4-1BBL crosstalk with a neutralizing antibody decreased the inflammatory responses in the co-cultured glial cells. Moreover, 4-1BB-deficient astrocytes revealed a reduction in the inflammatory responses when co-cultured with microglia, compared with those astrocytes/microglia isolated from wild-type animals. Finally, consistent with these findings, an *in vivo* study revealed that 4-1BB deficiency reduces HFD-induced hypothalamic inflammation, which is accompanied by the reduction of glial activation markers and the level of inflammatory cytokines. Taken all together, these findings suggest that 4-1BB and/or 4-1BBL signaling plays a crucial role in the interglial crosstalk in hypothalamic inflammatory responses under obese conditions (**Figure 2**) and might be a potential therapeutic target for the inhibition of obesity-induced hypothalamic inflammation and associated metabolic complications.

TANYCYTES AND THEIR POTENTIAL CROSSTALK

Tanycytes are specialized ependymal cells. Anatomically, this cell type is located in the lateral walls and surface of the third ventricle. Tanycytes maintain the nutrient transport from the circulation to the energy-sensing ARC of the hypothalamus (Mullier et al., 2010; Rahman et al., 2018). According to their location and morphological distribution, tanycytes are classified as two major subtypes (α- and β-tanycytes), which are further subdivided into α1-, α2-, β1-, and β2-tanycytes (Rizzoti and Lovell-Badge, 2017). Specifically, α-tanycytes reside dorsally, while β-tanycytes occupy the ventral side wall and surface of the third ventricle in the ME. Previous studies revealed that HFD increases the proliferation of β2-tanycytes with postnatal neurogenesis in the ME. It is also found that the new born neurons are derived from the β2-tanycytes, and inhibition of tanycyte division and differentiation decreases the HFD-induced body weight gain (Lee and Blackshaw, 2012; Lee et al., 2012). These findings suggest that β2-tanycytes-derived neurogenesis might contribute to energy homeostasis in response to HFD. In the hypothalamus, tanycytes are a source of vascular endothelial growth factor (VEGF) (Langlet, 2014). It has been reported that in mice under food deprivation condition, tanycytes express VEGF-A, which targets microvessel loops to promote microvessel permeability and to reorganize tight junction complexes in the ME and ARC (Langlet et al., 2013). This, in turn, may facilitate the communication between circulating metabolites and brain parenchyma. In a previous study, microglia-secreted VEGF has been shown to regulate the pathogenic activities of astrocytes in an EAE mouse model of multiple sclerosis (Rothhammer et al., 2018). In line with this thought, tanycytes-derived VEGF can be a potential contributor to overnutrition-induced hypothalamic inflammation by interacting with astrocytes. It has been reported that mice fed a HFD show an early loss

of the structural organization of β1-tanycytes (Ramalho et al., 2018) in the ME. In this study, immunofluorescence analyses revealed that consumption of an HFD alters the expression and spatial distribution of different proteins including insulin-like growth factor-binding protein 2, the most specific marker of β1-tanycytes, involved in the organization of the BBB. This may facilitate the transport of excess lipid-rich nutrients into the hypothalamus. A previous animal study revealed that hypothalamic tanycytes also accumulate lipid droplets in normal condition (Brion et al., 1982). A recent study using a microscopy analysis of the lipid droplet distribution in the hypothalamus has demonstrated numerous lipid droplets in tanycytes in response to HFD or obese condition (Hofmann et al., 2017). Further research is required to better understand the acute and chronic effects of HFD on tanycyte characteristics in energy metabolism and the role of tanycyte interaction with other glial cells in hypothalamic inflammation and subsequent pathologies.

CONCLUSION AND FUTURE PERSPECTIVES

Accumulating evidence suggests that hypothalamic glial cells respond to diet rich in FAs. In particular, long-chain FAs, such as palmitic acid, have been implicated in microglial activation and subsequent initiation of intracellular inflammatory pathways through toll-like receptors (Milanski et al., 2009; Valdearcos et al., 2014). It has been reported that mice fed with HFD show an activation and proliferation of both microglia and astrocytes in the hypothalamus, and activation of these glial cells play a crucial role in the alteration of local neuronal circuits, which has been suggested to promote obesity (Horvath et al., 2010; Andre et al., 2017). An *in vitro* study using primary microglia and astrocytes isolated from mice brains have revealed that only microglia, but not astrocytes, respond to FAs (Valdearcos et al., 2014). Indeed, FA-mediated inflammatory activation of primary microglia cultures isolated from whole mouse brains or of BV-2 mouse microglial cells is associated with an increased release of proinflammatory mediators, such as TNF-α, IL-1β, and IL-6 (Wang et al., 2012). These findings suggest that HFD-induced inflammatory activation of astrocytes in the hypothalamus might be due to microglial crosstalk with astrocytes through interaction between microglia-secreted proinflammatory mediators and related receptors expressed in astrocytes. In contrast, a previous study reported that FAs, such as palmitic acid, stearic acid, and lauric acid, can activate inflammatory signaling leading to the release of cytokines in cultured astrocytes isolated from rat brain (Gupta et al., 2012). These previous findings suggest that HFD-induced hypothalamic inflammation might be mediated by both glial cell types through their direct or indirect molecular interaction. The contrast in the data in the literature might be due to differences in experimental conditions or species. Further studies are required to explain these discrepancies.

Several *in vivo* and *in vitro* studies provided in the last few years a new view on the roles of microglia–astrocyte interaction involved in the pathogenesis of neuroinflammatory and degenerative diseases (Jha et al., 2018). In addition, bidirectional communication between microglia and astrocytes has been reported to mediate prolonged inflammation via their released molecules such as TNF-α, NO, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived hydrogen peroxide (H_2O_2), and transforming growth factors following CNS insults (Jha et al., 2018). Similarly, in the context of metabolic inflammation in the hypothalamus, several animal studies have revealed an increased level of such molecules in the hypothalamus and their role in pathological hypothalamic inflammation associated with obesity and diabetes (Duparc et al., 2011; Dalvi et al., 2017; Katashima et al., 2017; Mendes et al., 2018), but the molecular mechanisms underlying the interglial crosstalk in relation to chronic inflammation in the hypothalamus need to be further highlighted in future research. Glial cells and their inflammatory activation in response to fat-rich diet are closely involved in chronic hypothalamic inflammation and leptin/insulin resistance, which have drawn growing attention to the role of these glial cells in metabolic dysregulation associated with obesity and diabetes. Various secreted molecules from malfunctioning glia and signaling from cell surface receptors have been proposed as a potential mechanism of hypothalamic inflammation. Recent studies have provided a significant insight into our understanding of the complex roles of glial cells and their crosstalk in overnutrition-induced chronic hypothalamic inflammation and its pathological consequences. The complex heterogeneous nature of each glial cell type has made a challenging task to gain a clear insight into the interglial crosstalk in CNS physiology as well as pathologies. Further investigations will be needed for a better understanding regarding the functional significance of the interglial crosstalk in such pathological hypothalamic inflammation. Recently established innovative tools, such as isolation technique of high-purity glial cell populations from rodents and humans, optogenetics, chemogenetics, nanotechnology, single-cell multi-omics, induced pluripotent stem cells, and organoids may accelerate preclinical research.

Diverse glia-secreted molecules including cytokines, chemokines, and gliotransmitters, as well as related receptors expressed in specific glial cell types, can be possible targets for a further clarification of the interglial crosstalk in chronic hypothalamic inflammation. A recent study has demonstrated that restoring the feeding pattern by blocking gap-junction hemichannels in the CNS prevents HFD-induced obesity in mice (Sasaki et al., 2018). It has been suggested that blockade of hemichannels inhibits microglial release of small molecules, without attenuating acute inflammatory cytokine induction. In line with these findings, the authors speculated that HFD-induced obesity can be mediated via the gap junction hemichannel pathway, which affects the timing of meal and the inflammatory cytokine signaling pathway, thereby revealing a new therapeutic target in the CNS for the treatment of obesity. As gap-junction hemichannels control the release of diverse microglial molecules, it is important to understand their association with interglial crosstalk in both acute and chronic phase of obesity. Current and future research may boost the feasibility of glia-based therapeutic strategies targeting the interglial crosstalk and related molecules (either secreted or

cell surface molecules) to prevent and alleviate hypothalamic inflammation and metabolic diseases.

AUTHOR CONTRIBUTIONS

All authors have made a substantial intellectual contribution to this work, and approved submission of the manuscript. RY and KS formulated the focus of this review. MR, M-SK, and I-KL conducted the literature review and participated in the discussion. MR, RY, and KS wrote the manuscript.

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Neurochemical Modifications in the Hippocampus, Cortex and Hypothalamus of Mice Exposed to Long-Term High-Fat Diet

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Metabolic syndrome and diabetes impact brain function and metabolism. While it is well established that rodents exposed to diets rich in saturated fat develop brain dysfunction, contrasting results abound in the literature, likely as result of exposure to different high-fat diet (HFD) compositions and for varied periods of time. In the present study, we investigated alterations of hippocampal-dependent spatial memory by measuring Y-maze spontaneous alternation, metabolic profiles of the hippocampus, cortex and hypothalamus by ^{1}H magnetic resonance spectroscopy (MRS), and levels of proteins specific to synaptic and glial compartments in mice exposed for 6 months to different amounts of fat (10, 45, or 60% of total energy intake). Increasing the dietary amount of fat from 10 to 45% or 60% resulted in obesity accompanied by increased leptin, fasting blood glucose and insulin, and reduced glucose tolerance. In comparison to controls (10%-fat), only mice fed the 60%-fat diet showed increased fed glycemia, as well as plasma corticosterone that has a major impact on brain function. HFD-induced metabolic profile modifications measured by ^{1}H MRS were observed across the three brain areas in mice exposed to 60%- but not 45%-fat diet, while both HFD groups displayed impaired hippocampal-dependent memory. HFD also affected systems involved in neuro- or gliotransmission in the hippocampus. Namely, relative to controls, 60%-fat-fed mice showed reduced SNAP-25, PSD-95 and syntaxin-4 immunoreactivity, while 45%-fat-fed mice showed reduced gephyrin and syntaxin-4 immunoreactivity. For both HFD levels, reductions of the vesicular glutamate transporter vGlut1 and levels of the vesicular GABA transporter were observed in the hippocampus and hypothalamus, relative to controls. Immunoreactivity against GFAP and/or Iba-1 in the hypothalamus was higher in mice exposed to HFD than controls, suggesting occurrence of gliosis. We conclude that different levels of dietary fat result in distinct neurochemical alterations in the brain.

Keywords: glucose, insulin, diabetes, brain metabolism, synaptic dysfunction, gliosis

INTRODUCTION

The ever-increasing life expectancy has led to a dramatic increase in the prevalence of age-associated disorders namely metabolic syndrome and type 2 diabetes (T2D), and neurodegenerative disorders, such as Alzheimer's disease (AD). Notably, there is a growing body of epidemiological evidence suggesting that both metabolic syndrome and T2D increase the risk of developing age-related cognitive decline, mild cognitive impairment, vascular dementia, and AD (Frisardi et al., 2010; Duarte, 2015).

In addition to the many components of metabolic syndrome that may affect brain function, brain insulin resistance is a proposed mechanistic link between T2D and AD. Indeed, post-mortem analyses of brains from dementia patients revealed that insulin receptors are downregulated, and pointed toward a major role of neuronal insulin resistance in the etiology of AD (Steculorum et al., 2014; Duarte, 2015). Insulin receptors are widely distributed in the brain (Havrankova et al., 1978; Hill et al., 1986). Insulin and insulin-like growth factor 1 (IGF-1), which have converging signaling pathways in the brain, regulate the neuronal control of energy and glucose homeostasis by acting on hypothalamic nuclei (Steculorum et al., 2014), and also regulate cognitive processes through its actions on neurotransmission, synaptic plasticity, and neurogenesis within cortical and hippocampal circuits (Fernandez and Torres-Alemán, 2012).

A number of components of the metabolic syndrome such as hyperglycemia, microvascular complications, insulin resistance, dyslipidemia and hypertension occur in diabetes, and are risk factors for cognitive dysfunction (Duarte, 2015; Moheet et al., 2015). Although this combination of factors with potential impact on the central nervous system makes the mechanisms of diabetes-induced brain dysfunction difficult to study, research on different rodent models of diabetes have found that diabetic conditions lead to synaptic deterioration that results in defective neurotransmission and synaptic plasticity (Duarte et al., 2009, 2012a; Calvo-Ochoa et al., 2014), as well as neuroinflammation and astrogliosis (Saravia et al., 2002; Baydas et al., 2003; Duarte et al., 2009, 2012a; Calvo-Ochoa et al., 2014).

Changes in the cellular integrity of neurons and astrocytes are likely reflected on brain metabolic regulation, as well as metabolic profiles. Indeed, studies employing MRS, which allow to quantify brain metabolite profiles in a non-invasive manner, have generally identified reduced levels of the putative neuronal marker *N*-acetylaspartate, as well as increased in *myo*-inositol content (a proposed marker of astrocyte density) in brains of subjects with both AD and diabetes, relative to healthy individuals (reviewed in Duarte, 2016). Accordingly, altered metabolite profiles have been also reported in the brain of diabetes and HFD-induced obesity models (van der Graaf et al., 2004; Duarte et al., 2009; Wang et al., 2012; Lizarbe et al., 2018; Ribeiro et al., 2018).

In the present study, we tested the hypothesis that the severity of the metabolic syndrome is related to the degree of neurochemical alterations, as well as brain function deficits. For that, mice were fed diets containing different amounts of fat during 6 months, which lead to different degrees of glucose intolerance and insulin resistance (Soares et al., 2018). We then evaluated spatial memory performance, metabolic profiles of the hippocampus, cortex and hypothalamus, and levels of proteins specific to synaptic and glial compartments.

Abbreviations: Aβ, amyloid β; AD, Alzheimer's disease; ANOVA, analysis of variance; CRLB, Cramér-Rao lower bound; GABA, γ-aminobutyrate; HFD, high-fat diet; IGF-1, insulin-like growth factor 1; LSD, least significant difference; MOPS, 3-(*N*-morpholino)propanesulfonic acid; MRS, magnetic resonance spectroscopy; NAAG, *N*-acetylaspartylglutamate; TBS, Tris-buffered saline; T2D, type 2 diabetes; VOI, volume of interest.

MATERIALS AND METHODS

Animals

Animal experiments were performed with the approval by the local ethics committee (Service de la Consommation et des Affaires Vétérinaires, Epalinges, Switzerland). Sample size estimation was based on previous MRS experiments (Gapp et al., 2017; Lizarbe et al., 2018). Eleven-week-old male C57BL/6J mice were purchased from Charles River (L'Arbresle, France), and housed in groups of 3–4 on a 12-h light-dark cycle with lights on at 07:00, room temperature at 21–23°C, and humidity at 55–60%. Mice were randomly split in three groups (n = 11/group), and dietary interventions started after 1 week of acclimatization to the facility, i.e., at 3 months of age. During 6 months, mice were fed standardized diets containing 10%, 45%, or 60% of kilocalories from lard-based fat (D12450B, D12451, D12492, Research Diets, New Brunswick, NJ, United States), as previously detailed (Soares et al., 2018). Food and tap water were provided *ad libitum*. After this 6-month housing period, the following procedures were carried out sequentially with 2 days of interval: behavior experiments, one MRS scan session, glucose tolerance tests, tissue collection.

Behavioral Tasks

Exploratory behavior and locomotor activity of mice was evaluated over a 5-min period in the dark, using a squared open field arena measuring 30 cm × 30 cm and 25 cm high, divided in four squares of 15 cm × 15 cm. The number of crossings in these squares and the number of rearing movements with forepaws were quantified as horizontal and vertical activity, respectively. Rearing with the forepaws pressed against the walls was not considered.

Spontaneous alternation was observed in a Y-maze (Duarte et al., 2012a), with each arm of 30 cm long, 5 cm wide, and 25 cm height, converging to an equal angle. Mice were placed at the bottom of one arm in the Y-maze and were allowed to explore freely all three arms for a single 8-min session in the dark. The recorded spontaneous alternation behavior was used to access hippocampal-dependent spatial memory. If the mouse remembers the arm it has just explored, it will then enter the other arm of the maze instead. Complete spontaneous alternation was defined as successive entries into the three arms and expressed as fraction of the possible alternations in the

respective test. In additionally to the open field test, the number of entries in the arms of the maze also allowed to access locomotor activity.

Magnetic Resonance Spectroscopy (MRS)

All experiments were carried out in a 14.1 T magnet with a horizontal bore of 26 cm (Magnex Scientific, Abingdon, United Kingdom), equipped with a 12-cm internal diameter gradient coil insert (400mT/m, 200μs), and interfaced to a DirectDrive console (Agilent Technologies, Palo Alto, CA, United States). Radio frequency transmission and reception were achieved with a home-built quadrature surface coil composed of two geometrically decoupled single-turn loops of 12 mm inner diameter resonating at 600 MHz. Spontaneously breathing mice were anesthetized with 1–1.5% isoflurane (Animalcare Ltd., York, United Kingdom) in a 1:1 O_2:air mixture, and fixed in a home-built mouse holder with a bite bar and two ear inserts. Body temperature was maintained at 37°C by warm water circulation. Respiration and temperature were continuously monitored using a MR-compatible system (Small Animal Instruments, Inc., Stony Brook, NY, United States). Volumes of interest (VOI) were placed in the dorsal hippocampus (1.2 mm × 2.0 mm × 2.1 mm), cortex (0.8 mm × 4.0 mm × 1.6 mm) or hypothalamus (1.8 mm × 2.7 mm × 1.8 mm) according to anatomical landmarks in T_2-weighted fast-spin-echo images. Field homogeneity in the VOI was achieved with FAST(EST)MAP (Gruetter and Tkác, 2000). Spectra were acquired using SPECIAL with echo time of 2.8 ms and repetition time of 4 s (Mlynárik et al., 2006). We accumulated 210, 320, and 400 scans for the cortex, hippocampus, and hypothalamus, respectively. The order of scanning the regions was random.

The concentrations of brain metabolites were determined with LCModel (Stephen Provencher Inc., Oakville, ON, Canada), including a Mac spectrum in the database and using the unsuppressed water signal measured from the same VOI as internal reference (Gapp et al., 2017). The following metabolites were included in the analysis: alanine, ascorbate, aspartate, creatine, γ-aminobutyrate (GABA), glutamine, glutamate, glutathione, glycine, glycerophosphorylcholine, glucose, lactate, *myo*-inositol, *N*-acetylaspartate, *N*-acetylaspartylglutamate (NAAG), phosphorylethanolamine, phosphorylcholine, phosphocreatine, *scyllo*-inositol, taurine. The Cramér-Rao lower bound (CRLB) was provided by LCModel as a measure of the reliability of the quantification for each metabolite. In most measured spectra, *scyllo*-inositol was below the detection limit, and was excluded from subsequent data analyses. Alanine in the hypothalamus was also below the detection limit. Remaining metabolites had CRLB below 30%. Given the substantial overlap between phosphorylcholine and glycerophosphorylcholine signals, total choline levels are reported.

Three mice (one on 45% fat diet, two on 60% fat diet) were excluded from the study because of abnormally high glutamine concentration in all brain regions investigated, which suggests the occurrence of congenital portosystemic shunting (Cudalbu et al., 2013).

Glucose Tolerance Test

After a 6-h fast starting at 07:00, a blood sample was collected to determine glucose and insulin levels. Then mice were given an oral load of glucose (1.5 g/kg), and blood glucose was measured after 15, 30, 60, 90, and 120 min from the tail tip with the Breeze glucometer (Bayer, Zürich, Switzerland).

Tissue Collection

Mice were briefly anesthetized with isoflurane (2% in air). Glycemia under isoflurane anesthesia was measured using the Ascensia Contour glucometer (Bayer). A blood sample was collected from the descending aorta, and then mice were decapitated. The brain was rapidly removed, and brain regions were dissected in ice-cold phosphate-buffered saline (PBS; in mmol/L: 137 NaCl, 2.7 KCl, 1.5 KH_2PO_4, 8.1 Na_2HPO_4, pH 7.4). Brain and plasma samples were stored at −80°C until further processing.

Western Blot

Tissue samples were homogenized with a needle sonicator in lysis buffer [in mmol/L: 150 NaCl, 1 ethylenediaminetetraacetic acid (EDTA), 50 tris(hydroxymethyl)aminomethane (Tris)-HCl, 1% (v/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, 0.5% (w/v) sodium dodecylsulfate (SDS), pH 8.0] containing protease inhibitors (Roche, Switzerland). The homogenate was then maintained in constant agitation for 2 h at 4°C. After centrifugation at 3,000 × *g* for 10 min at 4°C to remove major debris, the supernatant was saved. Total protein content was determined with the bicinchoninic acid assay (kit from Pierce, Thermo Fisher Scientific, Göteborg, Sweden).

Samples were then diluted in NuPAGE-LDS sample buffer [in mmol/L: 141 Tris-base, 106 Tris-HCl, 50 dithiothreitol, 0.51 EDTA, 0.22 Coomassie Brilliant Blue G-250, 0.175 phenol red; 2% (w/v) lithium dodecylsulfate, 10% glycerol, pH 8.5] and heated for 2 min at 95°C, 2–20 μg of protein were loaded in precast NuPAGE Novex 4–12% polyacrylamide gradient gels (Invitrogen, Thermo Fisher Scientific), along with molecular weight standards (Precision Plus Protein standards from Bio-Rad, Sundbyberg, Sweden), and electrophoresis was carried out at 125 V in a 3-(*N*-morpholino)propanesulfonic acid (MOPS) running buffer (in mmol/L: 50 MOPS, 50 Tris Base, 1 EDTA, 0.1% SDS, pH 7.7).

Proteins were transferred onto 0.45 μm polyvinylidene difluoride membranes (GE Life Sciences, Sweden) in a buffer composed of 192 mmol/L glycine, 25 mmol/L Tris and 20% (v/v) methanol (pH 9.2). The membranes were subsequently blocked for 90 min in 5% (w/v) low-fat milk (VWR, Stockholm, Sweden) solution prepared in Tris-buffered saline (TBS) (in mmol/L: 20 Tris, 137 NaCl, pH 7.46) with 0.1% (v/v) tween-20 (TBS-T), and were then incubated with primary antibodies (**Table 1**) over night at 4°C. After three 15-min washes in TBS-T containing 1% (w/v) milk, membranes were incubated with the respective secondary antibodies conjugated with horseradish peroxidase (**Table 1**)

TABLE 1 | Antibodies employed in the present study.

Antigen	Host	Dilution	Supplier	Molecular weight (kDa)*
Primary antibodies:				
Gephyrin	R	1:500	Abcam (#ab181382)	90–95
GFAP	R	1:2,000	Abcam (#ab68428)	49–51
Iba-1	G	1:500	Sigma-Aldrich (#SAB2500041)	15–20
PSD-95	R	1:2,000	Abcam (#ab76115)	90–95
SNAP-25	R	1:5,000	Abcam (#ab109105)	25–26
Synaptophysin	R	1:10,000	Abcam (#ab32127)	34–36
Syntaxin-1	R	1:1,000	Sigma-Aldrich (#S1172)	34–39
Syntaxin-4	R	1:1,000	Abcam (#ab184545)	31–39
vGAT	R	1:1,000	Abcam (#ab42939)	53–55
vGluT1	R	1:2,000	Abcam (#ab180188)	61–62
vGluT2	R	1:1,000	Abcam (#ab216463)	63–65
HRP-conjugated secondary antibodies:				
Rabbit IgG	D	1 : 10 000	Abcam (#ab6802)	
Goat IgG	D	1 : 10 000	GeneTex (#GTX232040-01)	

*HRP, horseradish peroxidase; D, donkey; G, goat; R, rabbit. *Molecular weight range observed in the present study.*

for 1 h at room temperature. After the second antibody, the membranes were washed three more times in TBS-T and developed in the ChemiDoc (Bio-Rad) using the SuperSignal West Pico PLUS Chemiluminescent substrate (Thermo Scientific).

Biochemical Analyses

Plasma corticosterone and leptin were assayed with ELISA kits from Abcam (Cambridge, United Kingdom). Plasma insulin concentration was determined using the mouse insulin ELISA kit from Mercodia (Uppsala, Sweden).

Statistical Analysis

All results were analyzed using analysis of variance (ANOVA), followed by independent comparisons with the Fisher's least significant difference (LSD) test. For the analyses of metabolite concentrations, the three brain regions were analyzed together as repeated measures, and *post hoc* analyses were only performed in case of significant effects of fat or fat-region interaction at $P < 0.05$. Results are presented as mean ± SEM unless otherwise stated.

RESULTS

Chronic exposure to HFD resulted in development of obesity, mild hyperglycemia, glucose intolerance, and hyperinsulinemia (**Figure 1**). Weight gain was proportional to the dietary fat content ($F = 64.2$, $P < 0.001$, **Figure 1A**). Fed glycemia was modified by HFD ($F = 9.1$, $P = 0.001$, **Figure 1B**), being significantly increased in 60%-fat fed mice compared to controls (+56 ± 14%, $P < 0.001$) and to 45%-fat fed mice (+45 ± 13%, $P = 0.002$). Mice under 10%- and 45%-fat diets showed similar fed glycemia. After fasting for 6 h, mice showed a glycemia that was proportional to the amount of fat in the diet ($F = 23.1$; $P < 0.001$, **Figure 1C**), while insulinemia was similarly increased in both HFD groups compared to controls ($F = 9.2$, $P < 0.001$, **Figure 1D**). The diet also had an effect on the fasting glycemia to plasma insulin ratio ($F = 3.6$, $P = 0.041$) that is commonly taken as an indicator of insulin sensitivity. In particular, compared to controls (10%-fat fed mice), the ratio of glucose to insulin was reduced by 94 ± 42% ($P = 0.034$) and 95 ± 42% ($P = 0.033$) in mice from the 45%-fat or 60%-fat groups, respectively. An oral glucose tolerance test after this fasting period revealed an impairment of blood glucose clearance that was also proportional to the amount of fat present in the diet ($F = 14.6$, $P < 0.001$, **Figure 1E**). This is especially clear when looking at the glycemia at 2 h post the glucose administration ($F = 13.5$, $P < 0.001$, **Figure 1F**), with 60%-fat fed mice displaying increased glycemia compared to both 45%-fat fed mice (+32 ± 14%, $P = 0.033$) and controls (+109 ± 21%, $P < 0.001$). HFD also affected the circulating concentrations of corticosterone ($F = 4.2$, $P = 0.025$, **Figure 1G**) and leptin ($F = 19.0$, $P < 0.001$, **Figure 1H**). In *post hoc* tests, corticosterone was only significantly different between mice under diets with 10% and 60% fat (+32 ± 11%, $P = 0.007$), while leptin was similarly increased in both HFD groups compared to controls.

Spatial Memory

Exposure to HFD resulted in impaired spatial memory performance, measured as spontaneous alternation in the Y-maze ($F = 3.8$, $P = 0.030$, **Figure 2A**). In particular, there was a reduction of the Y-maze spontaneous alternation in mice consuming both the 45%-fat diet (−11 ± 5%, $P = 0.028$) and 60%-fat diet (−10 ± 4%, $P = 0.025$), relative to controls. Diet also had an effect on the number of entries in the maze arms ($F = 8.2$, $P = 0.001$), but not resulting in major impairment of exploratory behavior, which was also assessed in the open-field test (**Figure 2B**). Nevertheless, mice exposed to both 45%-fat and 60%-fat diets showed a reduction in locomotor activity, that is reduced number of crossing events between quadrants of the arena ($F = 5.5$, $P = 0.008$), and reduced vertical explorations of the arena's space, that is rearing events ($F = 3.8$, $P = 0.030$). Nevertheless, the observed reduction activity did not impair the analysis of spontaneous alteration behavior as it is normalized for the number of possible complete alternations, and there were sufficient arm entries in the Y-maze (**Figure 2A**).

Metabolic Profiles

Proton spectra were acquired with an average SNR of 18 ± 3 (standard deviation across all brain areas). Typical spectra are shown in **Figure 3**. Linewidths at half-height were 11.2 ± 3.0 Hz, 16.2 ± 4.0 Hz and 13.6 ± 2.2 Hz (mean with standard deviation) in the hippocampus, cortex and hypothalamus, respectively.

The amount of fat in the diet significantly impacted the levels of creatine ($F = 13.7$, $P < 0.001$, **Figure 4A**), which also varied across analyzed brain areas (region effect $F = 55.1$, $P < 0.001$; interaction $F = 0.9$, $P = 0.506$). Although phosphocreatine levels were not impacted by HFD exposure (**Figure 4B**), the content

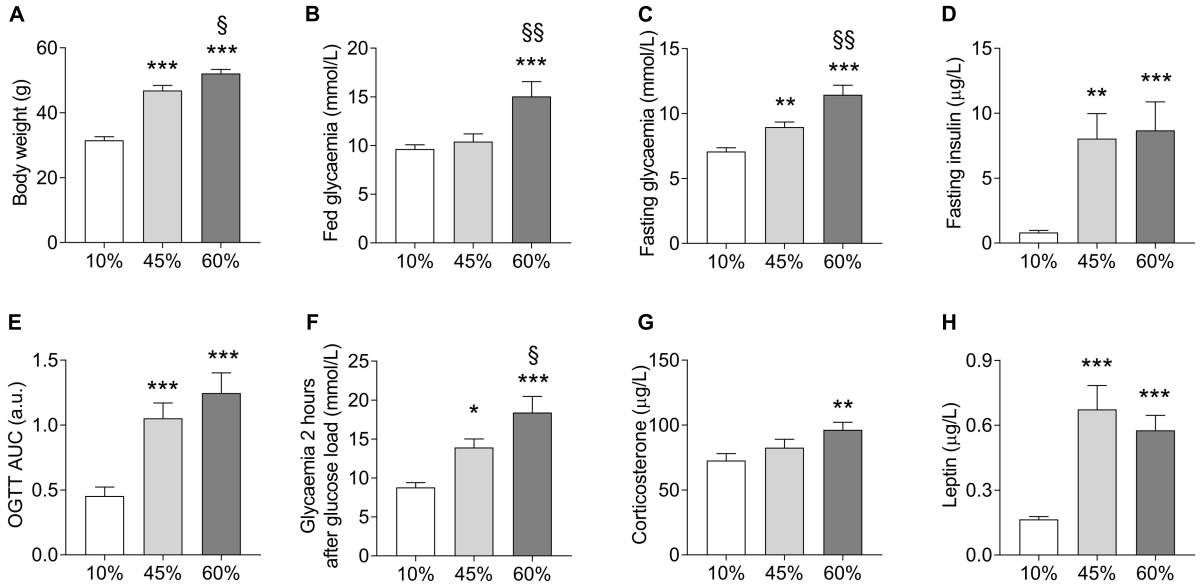

FIGURE 1 | HFD-induced changes on body weight **(A)**, fed glycemia **(B)**, glycemia **(C)**, and plasma insulin **(D)** after a 6-h fasting, area under the curve (AUC) of an oral glucose tolerance test (OGTT) with 1.5 g/kg of glucose **(E)** and glycemia measured 2 h after the glucose load **(F)**, and plasma corticosterone **(G)** and leptin **(H)** concentrations. Data are mean ± SEM. Symbols indicate significant differences in LSD tests after significant ANOVA, comparing HFD fed mice (45% fat or 60%-fat) and controls (10%-fat; $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$), or the diets containing 45% and 60% fat ($^{§}P < 0.05$, $^{§§}P < 0.01$).

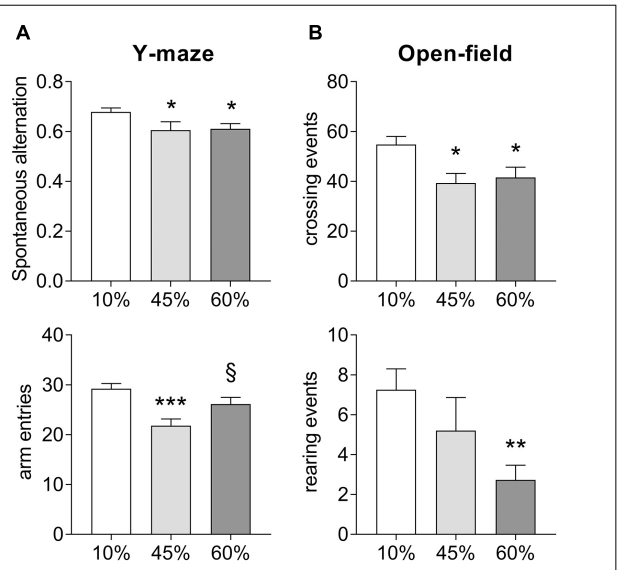

FIGURE 2 | Impact of HFD exposure on the performance in the Y-maze **(A)** and the open-field arena **(B)**. Data are mean ± SEM. Symbols indicate significant differences in LSD tests after significant ANOVA, comparing HDF fed mice (45% fat or 60%-fat) and controls (10%-fat; $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$), or the diets containing 45% and 60% fat ($^{§}P < 0.05$).

of fat was associated to reductions of the phosphocreatine-to-creatine ratio ($F = 13.1$, $P = 0.009$, **Figure 4C**), which suggests impaired energy metabolism. This effect was particularly prominent in the cortex (−31%, $P = 0.003$) and hypothalamus (−20%, $P = 0.050$), and not significant in the hippocampus (−13%, $P = 0.303$) of the 60%-fat group compared to controls.

High-fat diet caused a general increase of glutamine levels ($F = 7.9$, $P = 0.050$, **Figure 4D**), and there was also an effect of brain region on glutamine levels (region $F = 33.3$, $P < 0.0001$; interaction $F = 0.9$, $P = 0.717$), and *post hoc* analyses revealed a significant increase the hippocampus of mice fed 60%-fat diet relative to controls (+21 ± 10%, $P = 0.036$), as well as relative to mice fed 45%-fat diet (+27 ± 10%, $P = 0.011$). Interestingly glutamate levels were not significantly affected by the diet (**Figure 4E**). Brain concentrations the neuro-transmitting amino acids GABA (**Figure 4F**), glycine (**Figure 4G**), and aspartate (**Figure 4H**) also remained unaltered by HFD exposure.

The levels of the neuronal marker *N*-acetylaspartate showed a significant diet-region interaction (interaction $F = 6.7$, $P = 0.006$; region $F = 44.2$, $P < 0.001$; fat $F = 4.5$, $P = 0.066$, **Figure 4I**). *Post hoc* analyses identified an increase of *N*-acetylaspartate concentration in the hippocampus of mice exposed to 60%-fat diet compared to controls (+11 ± 4%, $P = 0.007$), and compared to 45%-fat fed mice (+18 ± 4%, $P < 0.001$).

N-Acetylaspartylglutamate levels were modified by fat content in the diet ($F = 12.8$, $P = 0.015$, **Figure 4J**) and varied across brain areas ($F = 20.4$, $P < 0.001$), without interaction ($F = 0.5$, $P = 0.918$). However, no significant changes were detected in *post hoc* analyses between controls and mice in either HFD group. However, significant differences were observed between 45%-fat and 60%-fat groups in the cortex (+36 ± 22%, $P = 0.005$) and hippocampus (+46 ± 20%, $P = 0.046$).

We found a diet-region interaction effect on the concentration of glutathione (interaction $F = 9.8$, $P = 0.047$; region $F = 10.9$, $P = 0.006$; diet $F = 2.7$, $P = 0.247$, **Figure 4K**). *Post hoc* analyses showed a significant increase of hippocampal glutathione levels

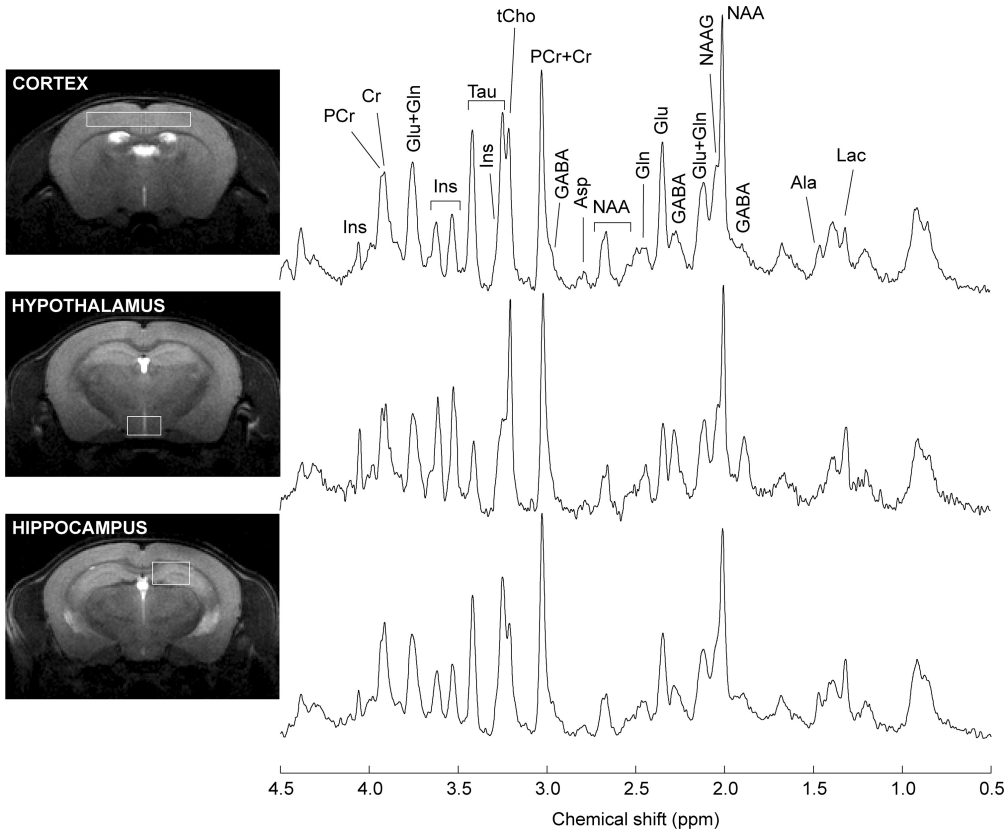

FIGURE 3 | Typical spectra recorded from the mouse hippocampus, cortex and hypothalamus. Spectra were acquired with SPECIAL at 14.1 T. For signal enhancement of the displayed spectra, a Gaussian apodization (gf = 0.08, gfs = 0.02) was applied prior Fourier transformation. Lac, lactate; Ala, alanine; GABA, γ-aminobutyrate; NAA, *N*-acetylaspartate; NAAG, *N*-acetylaspartylglutamate; Glu, glutamate; Gln, glutamine; Asp, aspartate, Cr, creatine; PCr, phosphocreatine; tCho, total choline-containing compounds; Tau, taurine; Ins, *myo*-inositol.

in the 60%-fat group compared to controls (+35 ± 13%, $P = 0.011$). The concentrations of the antioxidant Asc were not significantly modified by HFD exposure (**Figure 4L**).

There was a significant diet-region interaction effect on the concentration of *myo*-inositol (interaction $F = 4.9$, $P = 0.029$; region $F = 45.4$, $P < 0.001$; diet $F = 5.2$, $P = 0.071$, **Figure 4M**). *Post hoc* analyses indicated that the increase of *myo*-inositol was exclusive to the hypothalamus (+18 ± 5%, $P < 0.001$ for mice exposed to 60%-fat diet vs. controls; and +18 ± 5%, $P < 0.001$ vs. 45%-fat).

High-fat diet caused an increase of taurine levels ($F = 1.3$, $P = 0.047$, **Figure 4N**), which also varied across the three brain regions ($F = 85.9$, $P < 0.001$) without diet-region interaction ($F = 0.2$, $P = 0.788$). *Post hoc* analyses showed a significant difference of hippocampal taurine content between the mice exposed 60%-fat diet and controls (+12 ± 0.5%, $P = 0.022$).

The concentration of total choline-containing compounds was affected by HFD exposure ($F = 6.8$, $P = 0.001$, **Figure 4O**) and varied across brain regions ($F = 68.1$, $P < 0.001$) without interaction between the two factors ($F = 1.8$, $P = 0.149$). The content of total choline was significantly higher in mice exposed to 60%-fat diet in the hippocampus (+17 ± 6%, $P = 0.007$ vs. controls; +14 ± 6%, $P = 0.018$ vs. 45%-fat) and hypothalamus (+16 ± 4%, $P < 0.001$ vs. controls; +15 ± 4%, $P < 0.001$ vs. 45%-fat), but not in the cortex.

A significant diet-region interaction effect was observed on phosphorylethanolamine levels (interaction $F = 8.7$, $P = 0.022$; region $F = 19.8$, $P < 0.0001$; fat $F = 3.2$, $P = 0.252$, **Figure 4P**). *Post hoc* analyses identified a 40 ± 16% reduction of phosphorylethanolamine concentration in the cortex of mice exposed to 45%-fat diet compared to controls ($P = 0.017$). Such reduction in mice exposed to the 60%-fat diet did not reach statistical significance (−24 ± 17%, $P = 0.148$). The concentration of phosphorylethanolamine in the hypothalamus was different between the two HFD groups ($P = 0.009$).

Brain glucose levels were increased with the amount of fat in the diet ($F = 12.6$, $P = 0.037$, **Figure 4Q**) with no region ($F = 0.8$, $P = 0.513$) or interaction ($F = 4.4$, $P = 0.146$) effects. Compared to control mice, mice exposed to 60%-fat diet displayed a glucose concentration increase of 84 ± 25% in the hippocampus ($P < 0.001$) and of 41 ± 20% in the cortex ($P = 0.049$), but negligible in the hypothalamus (+16 ± 21%, $P = 0.442$).

We observed a significant diet-region interaction effect on the levels of lactate (interaction $F = 11.9$, $P = 0.005$, region $F = 10.1$, $P = 0.002$, fat $F = 6.9$, $P = 0.080$,

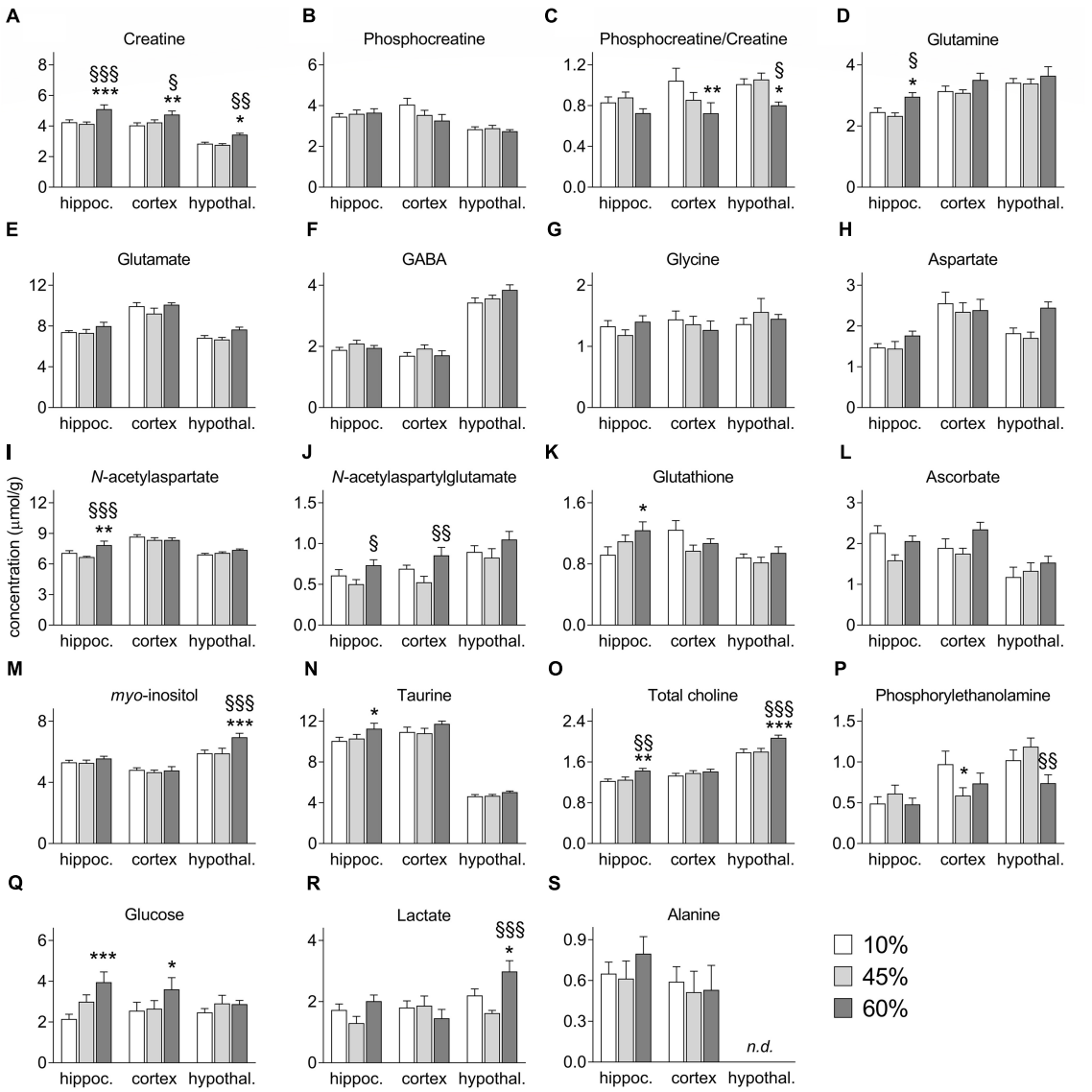

FIGURE 4 | Concentrations of metabolites in the hippocampus (hippoc.), cortex and hypothalamus (hypothal.) of mice fed diets containing 10%, 45%, and 60% fat. Panels **(A–S)** show alterations of each metabolite as presented in the text. Data are mean ± SEM (µmol/g). Symbols indicate significant differences in LSD tests after significant ANOVA, comparing HFD fed mice (45% fat or 60%-fat) and controls (10%-fat; $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$), or the diets containing 45% and 60% fat ($^{\S}P < 0.05$, $^{\S\S}P < 0.01$, $^{\S\S\S}P < 0.001$).

Figure 4R). *Post hoc* analyses identified an increase of lactate concentration in the hypothalamus of mice exposed to 60%-fat diet compared to controls (+36 ± 16%, $P = 0.025$), and compared to 45%-fat fed mice (+84 ± 21%, $P < 0.001$). Levels of alanine were not significantly modified by HFD exposure (**Figure 4S**).

Synaptic Degeneration

In order to infer on synaptic degeneration, we measured the density of proteins located in synapses (**Figure 5**). Levels of synaptophysin were not significantly impacted by HFD exposure in any of the brain regions investigated (hippocampus $F = 2.4$, $P = 0.112$; cortex $F = 1.5$, $P = 0.250$; hypothalamus $F = 0.4$, $P = 0.661$), although one may notice a tendency for reduced synaptophysin content in the hippocampus of HFD mice relative to controls ($P = 0.054$ and $P = 0.090$ for 45% and 60%-fat versus controls, respectively). Also levels of syntaxin-1 were similar across experimental groups in the hippocampus ($F = 0.9$, $P = 0.414$), cortex ($F = 1.8$, $P = 0.240$) and hypothalamus ($F = 0.3$, $P = 0.764$). The synaptosomal-associated protein SNAP-25 was modified in the hippocampus of HFD-exposed mice ($F = 6.8$, $P = 0.029$), but not in the cortex or hypothalamus. In particular, SNAP-25 in the hippocampus on mice the fed 60%-fat diet was reduced by 25 ± 8% ($P = 0.021$) when compared to controls and by 26 ± 8% ($P = 0.017$) when compared to 45%-fat fed mice. Levels of syntaxin-4, which is another protein

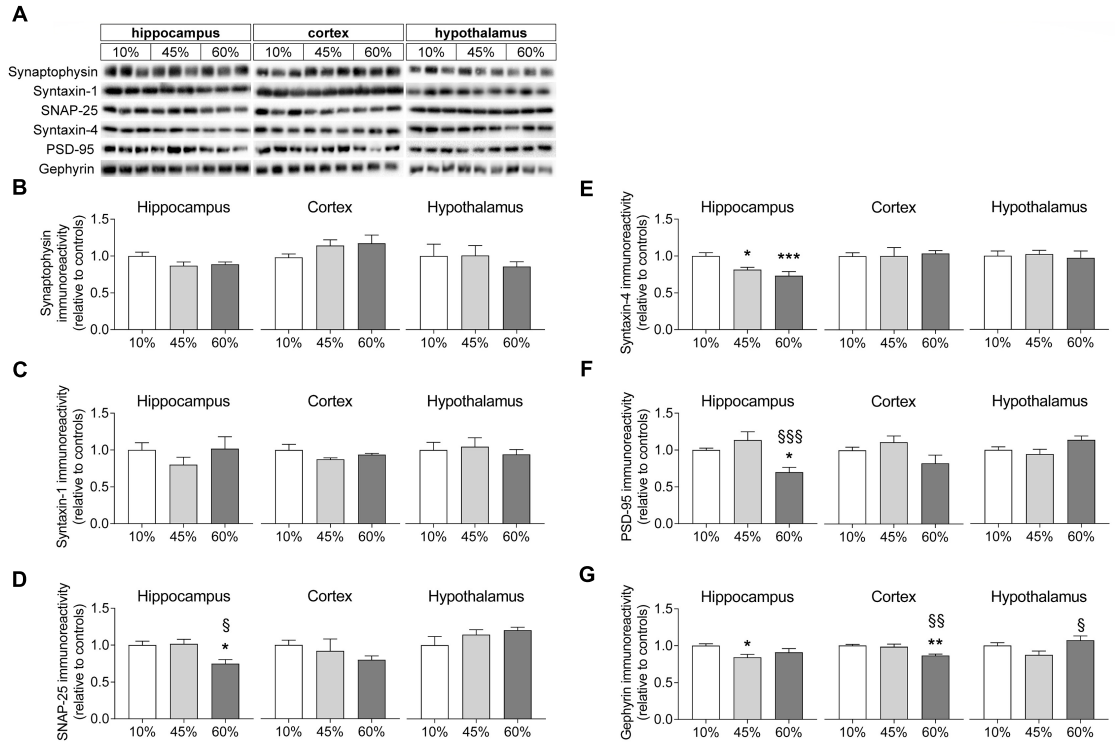

FIGURE 5 | HFD-induced changes on levels of synaptic proteins. **(A)** Shows typical Western blot of synaptophysin **(B)**, syntaxin-1 **(C)**, SNAP-25 **(D)**, syntaxin-4 **(E)**, PSD-95 **(F)**, and gephyrin **(G)**. Samples from three mice of each group were loaded in SDS-PAGE gels (2 μg of protein of syntaxin-1 and synaptophysin, 5 μg of protein for the remaining proteins). Measured immunoreactivity was normalized to the average of the three controls (10%-fat group, white bar) to allow pooling data from different gels. Data are mean ± SEM of $n = 6$. Symbols indicate significant differences in LSD tests after significant ANOVA, comparing HFD fed mice (45% fat or 60%-fat) and controls (10%-fat; $^{*}P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.001$), or the diets containing 45% and 60% fat ($^{§}P < 0.05$, $^{§§}P < 0.01$).

of the exocytosis machinery of nerve terminals but mainly occurring in processes of astrocytes (Tao-Cheng et al., 2015), were reduced in the hippocampus ($F = 9.2$, $P = 0.002$) but not cortex ($F = 0.1$, $P = 0.932$) or hypothalamus ($F = 0.1$, $P = 0.887$). In particular, when compared to controls, syntaxin-4 in the hippocampus was reduced by 19 ± 5% ($P = 0.011$) and 27 ± 7% ($P < 0.001$) in mice fed 45%-fat and 60%-fat diets, respectively.

Proteins from the post-synaptic region of synapses were also modified by HFD feeding. Diet impacted the postsynaptic density protein PSD-95 levels in the hippocampus ($F = 8.1$, $P = 0.002$) but not cortex ($F = 2.9$, $P = 0.076$) or hypothalamus ($F = 3.3$, $P = 0.067$). PSD-95 levels were reduced in the hippocampus of mice fed the 60%-fat diet by 30 ± 7% ($P = 0.012$) when compared to controls, and by 38 ± 13% ($P < 0.001$) when compared to mice fed the 45%-fat diet. Levels of gephyrin, another protein from the post-synaptic zone, varied with the amount of fat in the diet in the hippocampus ($F = 3.8$, $P = 0.044$), cortex ($F = 8.8$, $P = 0.003$) and hypothalamus ($F = 3.9$, $F = 0.035$). Compared to controls, the 45%-fat diet caused a significant reduction of gephyrin levels in the hippocampus (−16 ± 5%, $P = 0.014$). In the cortex, 60%-fat diet mice showed reduced gephyrin relative to the other groups (−13 ± 3%, $P = 0.002$ vs. controls; −12 ± 4%, $P = 0.004$ vs. 45%-fat diet). In the hypothalamus there was a reduction of −18 ± 8% in gephyrin levels in mice fed 45%-fat diet versus 60%-fat diet ($P = 0.011$).

We further measured the relative levels of vesicular glutamate and GABA transporters (vGluT1/2 and vGAT; **Figure 6**). The amount of fat in the diet was associated with a reduction of vGluT1 levels in the mouse hippocampus ($F = 7.9$, $P = 0.005$) and hypothalamus ($F = 4.3$, $P = 0.033$) but not in the cortex ($F = 1.5$, $P = 0.297$). In the hippocampus, both 45%-fat and 60%-fat fed mice showed significantly reduced vGluT1 levels when compared to controls (−38 ± 9%, $P = 0.001$ for 45%-fat diet; −22 ± 7%, $P = 0.032$ for 60%-fat diet). The same was observed in the hypothalamus (−39 ± 14%, $P = 0.015$ for 45%-fat diet; −32 ± 16%, $P = 0.039$ for 60%-fat diet). HFD exposure impacted levels of vGluT2 in the mouse cortex ($F = 10.3$, $P = 0.001$) and hypothalamus ($F = 3.9$, $P = 0.043$) but not in the hippocampus ($F = 0.3$, $P = 0.748$). In the cortex, mice fed 60%-fat diet showed increased cortical vGluT2 relative to the other groups (+25 ± 6%, $P < 0.001$ vs. controls; +24 ± 7%, $P = 0.001$ vs. 45%-fat diet). In the hypothalamus, we observed a significant reduction of vGluT2 levels in mice fed 45%-fat diet (−28 ± 8%, $P = 0.014$ vs. controls). Levels of vGAT were affected by HFD exposure in the hippocampus ($F = 3.6$, $P = 0.044$) and hypothalamus ($F = 3.6$, $P = 0.049$). The hippocampus of mice exposed to both 45%-fat and 60%-fat diet showed less vGAT levels than controls (−25 ± 9%, $P = 0.024$

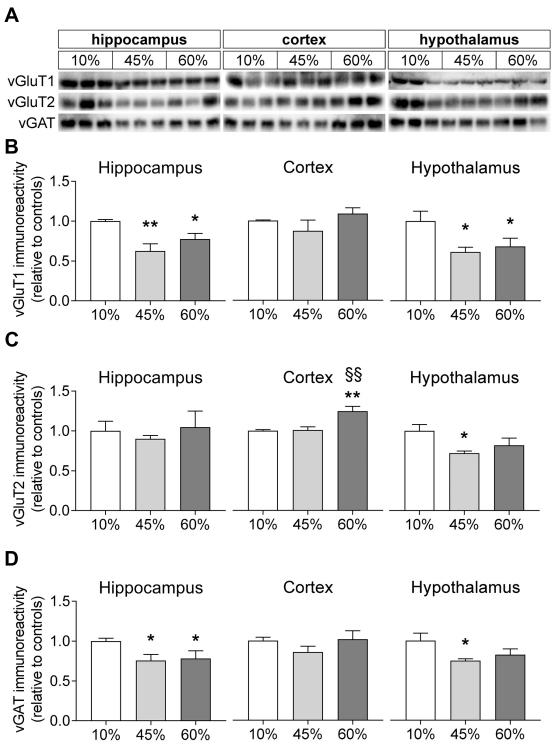

FIGURE 6 | HFD-induced changes on levels of vesicular glutamate and GABA transporters. **(A)** Shows representative Western blots of vGluT1 **(B)**, vGluT2 **(C)**, and vGAT **(D)**. Samples from three mice of each group were loaded in SDS-PAGE gels (20 μg of protein). Measured immunoreactivity was normalized to the average of the three controls (10%-fat group, white bar) to allow pooling data from different gels. Data are mean ± SEM of $n = 6$. Symbols indicate significant differences in LSD tests after significant ANOVA, comparing HFD fed mice (45% fat or 60%-fat) and controls (10%-fat; $^{*}P < 0.05$, $^{**}P < 0.01$), or the diets containing 45% and 60% fat ($^{§§}P < 0.01$).

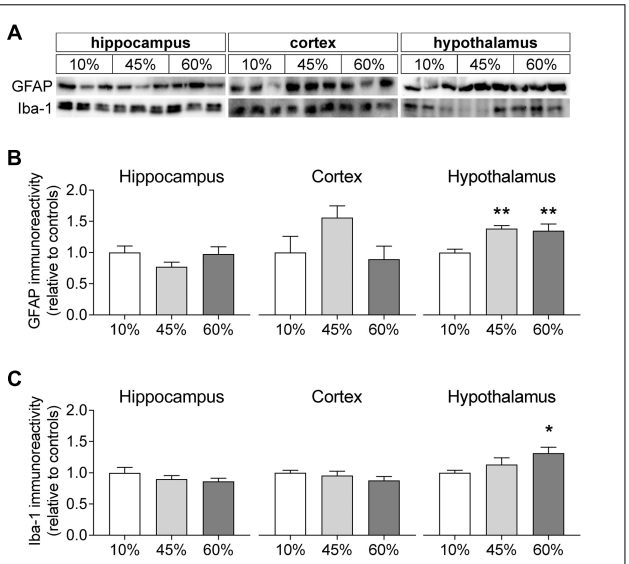

FIGURE 7 | HFD-induced changes on levels of glia-specific proteins. **(A)** Shows typical Western blot of GFAP **(B)** and Iba-1 **(C)**. Samples from three mice of each group were loaded in SDS-PAGE gels (20 μg of protein). Measured immunoreactivity was normalized to the average of the three controls (10%-fat group, white bar) to allow pooling data from different gels. Data are mean ± SEM of $n = 6$. Symbols indicate significant differences in LSD tests after significant ANOVA, comparing HFD fed mice (45% fat or 60%-fat) and controls (10%-fat; $^{*}P < 0.05$, $^{**}P < 0.01$). No significant differences were observed between the two HFD groups.

for 45%-fat diet; −22 ± 10%, $P = 0.042$ for 60%-fat diet). In the hypothalamus, the reduction of vGAT was significant only in mice exposed to 45%-fat diet (−25 ± 10%, $P = 0.018$ vs. controls).

Gliosis Markers

To infer on the modification of glial compartments, we measured the levels of glia-specific proteins (**Figure 7**). The glial fibrillary acidic protein (GFAP) that is specific to astrocytes was modified by fat levels in the diet in the hypothalamus ($F = 7.8$, $P = 0.005$), but not in the hippocampus ($F = 1.6$, $P = 0.235$) and cortex ($F = 2.6$, $P = 0.155$). Relative to controls, the hypothalamus of mice exposed to 45%-fat diet displayed a 38 ± 7% increase of GFAP immunoreactivity ($P = 0.003$), and a similar increase was observed for mice on the 60%-fat diet (+35 ± 12%, $P = 0.005$). Also the ionized calcium-binding adapter molecule 1 (iba-1), a protein present in microglial cells, showed increased levels in the hypothalamus ($F = 3.5$, $P = 0.045$), but not in the hippocampus ($F = 1.1$, $P = 0.352$) and cortex ($F = 1.1$, $P = 0.360$). In particular, there was a 31 ± 10% increase of iba-1 immunoreactivity in the hypothalamus of mice exposed to 60%-fat diet compared to controls ($P = 0.014$).

DISCUSSION

The present results show that mice chronically exposed to a lard-based HFD develop glucose intolerance associated to reduced insulin sensitivity, impaired hippocampal-dependent spatial memory, and neurochemical alterations in the hippocampus, cortex and hypothalamus. While metabolic profiles of the hippocampus, cortex and hypothalamus were modified in mice fed 60%-fat but not 45%-fat diet, when compared to controls, memory dysfunction was observed in both HFD groups. Accordingly, levels of proteins required for adequate synaptic function were found modified in mice fed diets containing fat at either 45% or 60%, relative to controls. This indicates that synaptic dysfunction may occur in the brain upon diet-induced obesity in the absence of major changes of metabolite concentrations. Nevertheless, this does not exclude the presence of alterations in the rate of certain metabolic pathways.

Modifications of Energy Metabolism

A general reduction of the phosphocreatine-to-creatine ratio was prominent in 60%-fat fed mice, when compared to the other groups, which suggests a disruption of mechanisms regulating metabolism and maintaining the cellular energy

status in the brain. HFD exposure resulted in changes of glucose concentration in the hippocampus and cortex that were parallel to the changes in fed glycemia. However, this was not observed in the hypothalamus, where glucose levels were similar in all diets tested. This suggests that glucose transport and metabolism is differently adapted to the mild hyperglycemia state of 60%-fat fed mice, likely related to the glucose-sensing ability of the hypothalamus. In line with this, the hypothalamus of mice exposed to 60%-fat, but not the hippocampus and cortex, displayed higher levels of lactate than the other experimental groups. The measurement of basal metabolite concentrations, however, does not provide insight into metabolic pathways, namely glycolysis and tricarboxylic acid cycle. By employing ^{13}C MRS with ^{13}C-labeled glucose we have previously demonstrated that energy metabolism is altered in the hypothalamus upon high-fat feeding (Lizarbe et al., 2018). Modifications of brain energy metabolism were reported in rats fed a fat-rich diet for 3 weeks, namely reduced glucose utilization and increased astrocytic metabolism (Melø et al., 2006). Recently, we also reported that a rat model of lean T2D, the Goto-Kakizaki rat, displays impaired neuronal oxidative metabolism and reduced glutamate-glutamine cycle rate, as well as exacerbated oxidative metabolism in astrocytes (Girault et al., 2018). The relative contributions of fatty acids and glucose as available brain energy substrates, and of neurodegeneration-associated metabolic changes to brain energy dysfunction deserve further investigation. Previous experiments suggest that HFD exposure increases the utilization of fat in detriment of glucose oxidation, at least in the hypothalamus (Lizarbe et al., 2018). This observation, together with the present findings of higher lactate and lower glucose concentrations, leads us to speculate that long-term HFD exposure results in increased glycolysis in for lactate production while other available substrates, such as fats, are metabolized in the tricarboxylic acid cycle.

Osmolarity Regulation

Changes of the phosphocreatine-to-creatine ratio were due to increases in the creatine concentration rather than decreases of phosphocreatine (see **Figures 4A–C**), which is in line with the relevance of creatine levels for osmolarity regulation. Osmolarity control in the brain is considered to occur via the concentrations of not only creatine but also taurine and *myo*-inositol, since hyper-osmolarity causes their accumulation (Gullans and Verbalis, 1993). Also *N*-acetylaspartate, which is present in large concentrations in neurons, has been proposed to act as a brain osmolyte (Taylor et al., 1994; Gotoh et al., 1997), and is effectively released to the interstitial space upon osmotic challenges (Sager et al., 1997; Bothwell et al., 2001). Accordingly, taurine and *N*-acetylaspartate in the hippocampus, and *myo*-inositol in the hypothalamus were also increased upon exposure to 60% but not 45% fat in the diet. It should be noted that these modifications in brain osmolytes follow the plasma glucose concentration in the fed state, as well as corticosterone levels, which were significantly higher in 60%-fat fed mice than the other groups. Indeed, in addition to chronic hyperglycemia, it cannot be excluded that glucocorticoid-mediated signaling plays a role in diabetes-induced impaired brain metabolism and function (discussed in Girault et al., 2018).

Metabolic Indications of Neurodegeneration

N-Acetylaspartate reductions are often associated to impaired mitochondrial integrity in neurons, as well as lipid and myelin biosynthesis (Duarte et al., 2012b). Metabolism of *N*-acetylaspartate is compartmentalized between neurons and oligodendrocytes and serves for myelination. *N*-acetylaspartate is mainly stored in neurons, being synthesized from mitochondrial acetyl-CoA and Asp (Baslow, 2003), and its de-acetylation occurs in oligodendrocytes (Kirmani et al., 2002), where it can replenish both acetyl-CoA and oxaloacetate (through aspartate) pools providing carbon skeletons for both energy production and local lipid synthesis, required in the myelination process. In line with this role of *N*-acetylaspartate, Canavan disease is characterized by accumulation of *N*-acetylaspartate due to impaired de-acetylation in oligodendrocytes, which is associated to defects in myelin synthesis and deposition (Chakraborty et al., 2001). Indeed, it was recently suggested that high-fat feeding has an impact on myelination, namely by promoting the loss of oligodendrocyte progenitor cells and mature oligodendrocytes (Yoon et al., 2016).

A significant increase of hippocampal glutathione levels was observed in the 60%-fat group relative to controls, while there was a tendency for reduced glutathione levels in the cortex. It should be noted that the levels of glutathione measured by MRS include both reduced and oxidized forms, which are not distinguishable. Although lower ratio of reduced-to-oxidized glutathione has been reported in short-term HFD feeding (e.g., Alzoubi et al., 2018), no change of brain levels of oxidized and reduced glutathione, or their ratio upon long-term HFD exposure (Li et al., 2013). It should be noted however that Li et al. (2013) reported a non-significant increase of 15–20% of hippocampal reduced glutathione in high- versus low-fat-fed mice, in line with the present observation. It is likely that a total glutathione increase in the hippocampus might reflect a compensatory mechanism to oxidative stress upon chronic HFD exposure. In fact, the brain of HFD-fed mice displays impaired redox homeostasis, including: (1) enhanced activity of enzymes that generate free radicals, such as xanthine oxidase or NADPH oxidase; (2) reduced activity of enzymes with antioxidant action, namely glutathione peroxidase and catalase; (3) increased concentration of free radicals; (4) and oxidative damage to lipids and proteins (e.g., Morrison et al., 2010; Li et al., 2013; Charradi et al., 2017; Liu et al., 2017; Alzoubi et al., 2018; Maciejczyk et al., 2018). Accordingly, the hippocampus of mice fed a 60%-fat diet for 4 months also shows impaired signaling mediated by the transcription factor NRF2 which protects against brain oxidative damage (Morrison et al., 2010). Namely, Morrison et al. (2010) reported HFD-induced reductions of NRF2 protein levels, NRF2-DNA binding activity, and levels of the NRF2 responsive proteins in the hippocampus.

Interestingly, impaired cellular redox regulation in the brain has been proposed to impact oligodendrocyte proliferation (consistent with increased levels of *N*-acetylaspartate, discussed above), which has implications on the maintenance of myelin sheets around axons, and thus brain connectivity (see Corcoba et al., 2016 and references there in).

The content of total choline was significantly higher in hippocampus and hypothalamus, but not cortex. The main contributors to the choline peaks in MRS are the water-soluble glycerophosphorylcholine and phosphorylcholine, which are involved in membrane lipid metabolism (Duarte et al., 2012b). Namely, it is precursor of phosphatidylcholine and, in turn, of sphingomyelin, which is necessary for adequate myelination of axons (Oshida et al., 2003), and is implicated in immune responses (Li et al., 2015). In contrast to phosphorylcholine, a HDF-induced reduction of phosphorylethanolamine concentration was observed in the cortex. It is interesting to note that besides these changes in the concentration of phosphorylethanolamine in the cortex, no other significant metabolic alterations were observed between mice fed the diet containing 45% of fat and those in the control group. Altogether, these results point to alterations on phospholipid metabolism with possible implications on plasma membrane permeability, maintenance of action potentials and conduction of electric impulses, and on events that involve membrane deformation, fusion and fission, such as transcellular signaling, vesicular release of neurotransmitters, gliotransmitters or neuromodulators, mitochondrial dynamics, and autophagy (Aureli et al., 2015; Krols et al., 2016; Lauwers et al., 2016; van Echten-Deckert and Alam, 2018). In addition, lipid metabolism is important for membrane reorganization of astrocytes and microglia in neuroinflammation, which have been proposed to be reflected on MRS-detected changes of choline-containing compounds (discussed in Duarte et al., 2012b). In our study, total choline increased with HFD in the hippocampus and hypothalamus, while increased levels of GFAP and Iba-1, which are signs of inflammation, were exclusively observed in the hypothalamus. These results support the notion that MRS markers do not distinguish neuroinflammation from neurodegeneration and other processes (Zahr et al., 2014; Pardon et al., 2016).

Taurine is an amino acid that, although it is present at 1 μmol/g in the human brain, it reaches relatively large concentrations in the rodent brain (above 5 μmol/g in rats and above 8 μmol/g in mice; Duarte, 2016), thus playing a major role as osmolyte (Duarte et al., 2009 and references therein). Taurine is released from both neurons and glial cells (Hada et al., 1998), and acts as an agonist at receptors of the GABAergic and glycinergic neurotransmission systems (Albrecht and Schousboe, 2005). In the present study, HFD exposure caused a reduction of proteins associated to inhibitory neurotransmission (gephyrin and vGAT), suggesting that increased taurine might be a compensatory mechanism for the loss of inhibitory tone. Moreover, taurine is transported into the mitochondrial matrix where it buffers pH to the optimal value for isocitrate dehydrogenase, which is one of the key enzymes of the tricarboxylic acid cycle regulating energy metabolism and oxidative phosphorylation, contributes to stabilize the pH gradient across the inner-membrane, and thus helps preserving mitochondrial function and preventing oxidative damage (Hansen et al., 2010). Therefore, this HFD-induced increase of taurine levels in the is likely a beneficial adaptation of the brain.

Microgliosis and Astrogliosis

Increased levels of *myo*-inositol have been suggested to represent astrogliosis in the neurodegeneration-associated inflammatory process (discussed in Duarte et al., 2012b). The HFD-associated increased *myo*-inositol of the present study was exclusive to the hypothalamus. Interestingly, the hypothalamus was also the sole brain region to show significantly HFD-associated increased levels of glial specific proteins, namely GFAP and Iba-1 that are present in astrocytes and microglia, respectively. This overexpression of GFAP and Iba-1 is thus indicative of HFD-associated hyper-reactivity of astrocytes (astrogliosis) and microglia (microgliosis), as we reported recently (Lizarbe et al., 2018). Brain disorders are accompanied by neuroinflammation with astrogliosis and microgliosis generally in response to damage of nearby neuronal processes, but gliosis might become deleterious for the neurons upon chronicity (López-Valdés and Martínez-Coria, 2016). A transient increase in levels of pro-inflammatory cytokines was observed in the hypothalamus within 1–3 days of HFD exposure (e.g., Thaler et al., 2012; Waise et al., 2015), which might constitute a protective or reparative inflammatory process. In contrast, the hypothalamic gliosis in chronic HFD exposure (present study) is likely neurotoxic and has been suggested to participate in the genesis of obesity-associated diabetes since it may cause alterations of food intake, energy expenditure, insulin secretion, hepatic glucose production and metabolism of glucose and fats (Rahman et al., 2018).

The hippocampus and cortex of HFD-exposed mice did not show signs of gliosis, when compared to controls (no significant increase in levels of *myo*-inositol, GFAP or Iba-1). Despite the general consensus that neuroinflammation and astrogliosis occur in insulin resistance and T2D (Saravia et al., 2002; Baydas et al., 2003; Duarte et al., 2009, 2012a; Calvo-Ochoa et al., 2014), conflicting results have been reported regarding astrogliosis in memory-related brain areas upon HFD exposure. This is likely due to the distinct study designs, diet compositions, fat origin, age and/or species. Recent studies do not find HFD-induced increased levels of GFAP in the rat hippocampus (Raider et al., 2016; Ribeiro et al., 2018). In contrast, Tarantini et al. found that mice exposed to a 60%-fat diet from 3 to 8 months of age show mild behavior deficits and neuroinflammation, which are exacerbated by exposure to a cellular stress induced by deletion of the transcription factor *nrf2* that regulates the expression of antioxidant proteins, thus protecting against oxidative damage (Tarantini et al., 2018). A particularly interesting study by Tsai et al. (2018) found that GFAP levels increase in the hippocampus of mice exposed to 60%-fat diet for 3 months, relative to controls, and that HFD consumption leads to a reduction of the length and ramification of astrocytic processes in the CA1 and CA3 regions but not the dentate gyrus of the hippocampus. This retraction of astrocytic processes is of importance for the

metabolic support that astrocytes should provide to neurons and their synapses (Sonnay et al., 2017), and in fact Tsai et al. (2018) also reported that HFD reduced levels of the astrocytic glutamate carriers GLT-1 and GLAST in the hippocampus, without changes of glutamine synthase levels. Soontornniyomkij et al. (2016) also found that 5 months of HFD exposure does not change levels of glutamine synthetase in the hippocampus of 5-month-old mice, although HFD increases the density of this protein in aged mice. Altogether, these observations suggest impaired glutamate-glutamine cycle rate that, in turn, may result in glutamatergic dysfunction and consequent accumulation of glutamine in astrocytes. Consistent with impaired glutamate release, we observed reduced levels of vesicular glutamate transporters, mainly vGluT1. However, care should be taken when interpreting changes on levels of carriers and enzymes because they may not reflect changes of their activity in the brain *in vivo*.

Moreover, as mentioned above, observations from different diet-induced obesity studies should be compared with caution. For example, in contrast to the reduced levels of glutamate carriers caused by a 60% HFD (Tsai et al., 2018), hippocampal slices prepared from mice fed a 45%-fat diet for only 2 months displayed enhanced glutamate uptake, increased density of glutamate carriers and reduced levels of the glutamate degrading enzymes glutamine synthetase and GABA-decarboxylase, compared to slices from controls (Valladolid-Acebes et al., 2012). Based on our results, we believe that the exposure to diets containing fat at 45% or 60% elicits different degenerative processes rather than resulting from a simple dose-response phenotype.

Glutamine synthesis takes place in astrocytes (Sonnay et al., 2017). In our study, HFD caused indeed an increase of glutamine levels in the 60%-fat group (but not the 45%-fat) relative to controls, which was particularly significant in the hippocampus. In line with our observations, increased hippocampal glutamine-to-creatine ratio was recently reported in Wistar rats exposed to a 60%-diet from 8 to 12 months of age (Ribeiro et al., 2018). In young adult Fisher 344 rats under a 60%-fat diet for 5 months, Raider et al. (2016) also observed a tendency for higher hippocampal glutamine concentration, and a significantly increased glutamine-to-glutamate ratio, relative to controls. This study, however, reported lower levels of *myo*-inositol and creatine in high versus low fat-fed rats (Raider et al., 2016), contrasting to the findings of the present study (discussed above).

Dysfunction in Excitatory and Inhibitory Synapses

N-Acetylaspartylglutamate (NAAG) levels increased in the cortex and hippocampus with HFD exposure. Similar NAAG changes were observed in HFD-exposed rats (Raider et al., 2016). NAAG is synthetized from ATP-dependent condensation of *N*-acetylaspartate and glutamate in neurons, packed into synaptic vesicles of presynaptic terminals, including those of pyramidal neurons in the cortex and limbic system, is co-released with glutamate, and acts as an antagonist of the glycine site of the NMDA receptor (Duarte and Xin, 2018 and referenced therein). Importantly, NAAG also acts on the metabotropic type II glutamate receptor mGluR3 inhibiting the release of neurotransmitters, such as glutamate, GABA and glycine (Neale, 2011). In HFD-exposed mice, increased NAAG levels would thus reduce glutamatergic neurotransmission by both inhibiting the activity of NMDA receptors and augmenting the negative feedback of glutamate release through its agonist action on presynaptic mGluR3 receptors. High NAAG concentration might prevent glutamate excitotoxicity, which is important when astrocytes have impaired glutamate clearance (see above), but on the other hand sufficient glutamatergic activity is necessary for proper function, including memory performance.

Consistent with reduced rate of the glutamate-glutamine cycle and with synaptic dysfunction in general, we observed a reduction in the levels of some pre- and post-synaptic proteins and of vesicular glutamate and GABA transporters in mice exposed to diets containing either 45% or 60%, relative to controls. We measured the density of two presynaptic proteins that are target SNARE (Soluble *N*-ethylmaleimide-sensitive factor Attachment protein REceptor) proteins required for vesicle fusion, namely syntaxin-1 and SNAP-25 (Söllner et al., 1993), and the density of synaptophysin that is a major component of presynaptic vesicles (Valtorta et al., 2004). SNAP-25 was reduced in the hippocampus of mice fed 60%-fat diet, when compared to controls, and there was also a tendency for hippocampal synaptophysin levels to be reduced with increasing fat content in the diet. Similar results were found for the density of vGluT1 and vGAT in the hippocampus, which suggest a reduction of the number of excitatory and inhibitory synaptic vesicles that are available for neurotransmission. Deficits in gliotransmission cannot be excluded since vesicular transporters are present in both neurons and astrocytes (see Schubert et al., 2011 and references therein). Therefore, we further measured the levels of syntaxin-4. While syntaxin-1 is mostly located in neurons, namely in the plasma membrane of the presynaptic terminal, syntaxin-4 is particularly concentrated in glial cells, namely in peri-synaptic astrocytic processes (Schubert et al., 2011; Tao-Cheng et al., 2015). In fact, the level of syntaxin-4 was also found reduced in the hippocampus of mice from either HFD group, confirming also a dysfunction at the level of gliotransmission. We further measured the density of PSD-95 that is associated to excitatory postsynaptic densities (Hunt et al., 1996), and gephyrin that is present at the inhibitory postsynaptic density, where it interacts with glycine and γ-aminobutyric acid type A ($GABA_A$) receptors (Maric et al., 2011). In the hippocampus, HFD also led to a reduction of PSD-95 (60%-fat group only) and of gephyrin (only significant for 45%-fat), compared to controls. Reduced level PSD-95 in the hippocampus of 45%-fat fed mice (for 2 weeks) relative to controls was also reported previously (Arnold et al., 2014). This synaptic dysfunction might be an important contributor for the hippocampal-dependent spatial memory impairment that was measured through the reduced spontaneous alternation in the Y-maze. In the cortex, only the levels of gephyrin were reduced in 60% fat fed mice versus controls, suggesting reduced inhibitory tone by the GABAergic system. In the hypothalamus, only vesicular

neurotransmitter transporters and not the remaining measured synaptic proteins were reduced by HFD exposure, suggesting reduced neurotransmission capacity in both orexigenic and anorexigenic neurons (Meister, 2007; Moraes et al., 2009).

CONCLUSION

In sum, increasing the dietary amount of lard-based fat from 10 to 45% or 60% of the total energy intake leads to obesity accompanied by increased fasting blood glucose and insulin (pointing toward reduced insulin sensitivity), reduced glucose tolerance and increased plasma leptin. However, compared to controls (10%-fat) increased fed glycemia and plasma corticosterone were only observed in the 60%-fat fed mice. Exposure to both 45%-fat and 60%-fat diets resulted in deterioration of systems involved in neuro- and gliotransmission in the hippocampus, and in impaired spatial memory performance. Metabolic alterations measured by ^{1}H MRS were generally observed in mice exposed to 60%-fat but not 45%-fat diet. Therefore, we conclude that different features of the metabolic syndrome result in distinct neurochemical alterations in the brain, all likely contributing for memory impairment. Moreover, chronically increased blood glucose and/or corticosterone appear to be main drivers for changes in brain metabolite levels.

AUTHOR CONTRIBUTIONS

JD designed the study and wrote the manuscript. All authors performed the experiments, analyzed the data, and revised the manuscript.

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Glucose Homeostasis Is Not Affected in a Murine Model of Parkinson's Disease Induced by 6-OHDA

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There is a mutual relationship between metabolic and neurodegenerative diseases. However, the causal relationship in this crosstalk is unclear and whether Parkinson's disease (PD) causes a posterior impact on metabolism remains unknown. Considering that, this study aimed to evaluate the appearance of possible changes in metabolic homeostasis due to 6-hydroxydopamine (6-OHDA) administration, a neurotoxin that damage dopaminergic neurons leading to motor impairments that resemble the ones observed in PD. For this, male Wistar rats received bilateral 6-OHDA administration in the dorsolateral striatum, and the motor and metabolic outcomes were assessed at 7, 21, or 35 days post-surgical procedure. Dexamethasone, a diabetogenic glucocorticoid (GC), was intraperitoneally administered in the last 6 days to challenge the metabolism and reveal possible metabolic vulnerabilities caused by 6-OHDA. Controls received only vehicles. The 6-OHDA-treated rats displayed a significant decrease in locomotor activity, exploratory behavior, and motor coordination 7 and 35 days after neurotoxin administration. These motor impairments paralleled with no significant alteration in body mass, food intake, glucose tolerance, insulin sensitivity, and biochemical parameters (plasma insulin, triacylglycerol, and total cholesterol levels) until the end of the experimental protocol on days 35–38 post-6-OHDA administration. Moreover, hepatic glycogen and fat content, as well as the endocrine pancreas mass, were not altered in rats treated with 6-OHDA at the day of euthanasia (38th day after neurotoxin administration). None of the diabetogenic effects caused by dexamethasone were exacerbated in rats previously treated with 6-OHDA. Thus, we conclude that bilateral 6-OHDA administration in the striatum causes motor deficits in rats with no impact on glucose and lipid homeostasis and does not exacerbate the adverse effects caused by excess GC. These observations indicate that neurodegeneration of dopaminergic circuits in the 6-OHDA rats does not affect the metabolic outcomes.

Keywords: glucocorticoid, glycemia, lipids, liver, pancreatic islets, Parkinson's disease

INTRODUCTION

The first description of Parkinson's disease (PD) was made by James Parkinson in the early nineteenth century (Pearce, 1989). It is considered the second most prevalent neurodegenerative disease in the world, affecting approximately 1% of the world population over 60 years (Schapira, 2009). The disease is characterized by a slow and progressive loss of dopaminergic neurons from the substantia nigra resulting in a gradual loss of motor functions (Stern et al., 2012). At the diagnosis period, approximately 40% of the dopaminergic neurons are intact and the presence of dopamine in the striatum is restricted to approximately 20–30% of the normal condition (Riederer and Wuketich, 1976). The primary etiology of the disease is not yet well known. However, epidemiological studies associate the etiology with several risk factors such as neuronal aging, genetic and environmental factors, viruses and inadequate eating habits (Marttila et al., 1977; Gorell et al., 1997; Mattson, 2003; Steenland et al., 2006; Goldman, 2010).

A classic animal model of PD is obtained through the neurotoxin 6-hydroxydopamine (6-OHDA), a substance that does not cross the blood-brain barrier and therefore requires its administration to be performed directly on the substantia nigra *pars compacta* (SNpc) or on the striatum (Jackson-Lewis et al., 2012). As demonstrated for the first time by Ungerstedt (1968), an intranigral injection of 6-OHDA in rats rapidly eliminates about 60% of tyrosine hydroxylase (TH)-positive neurons in this brain area, with the subsequent loss of TH-positive terminals in the striatum (Blandini et al., 2008). However, when administered at the striatum, the neurotoxin promotes a slower and progressive degeneration and subsequent loss of neurons in the SNpc, which also occurs in PD context (Sauer and Oertel, 1994; Przedborski et al., 1995; Lee et al., 1996).

Several studies have suggested an association between metabolic-related diseases (i.e., diabetes *mellitus*) and neurodegenerative diseases. Some authors have suggested that diabetes *mellitus* type 2 (T2DM) is one of the risk factors for the development of neurodegenerative diseases such as PD (Hu et al., 2007; Klimek et al., 2015; Pagano et al., 2018). In addition, a higher prevalence of T2DM was reported in patients with PD (Pressley et al., 2003). Although there are some associations between metabolic outcomes and PD, or vice versa, an observational study reports that the prevalence of T2DM in patients with PD is similar to the prevalence of individuals without PD (Becker et al., 2008). Scigliano et al. (2006) reported a lower prevalence of DM2 in patients diagnosed with PD; whereas no differences were found in the risk of developing PD between eutrophic patients and diabetic patients (Simon et al., 2007). However, it is known that PD can damage several brain areas and may affect regions involved in metabolic control such as the hypothalamus (Dayan et al., 2018).

The pathophysiological process of PD is complex since it may involve environmental, genetic and inflammatory risk factors that are not yet well understood. In addition, remains unknown whether the brain damage observed in PD can negatively affect peripheral metabolic aspects such as glycemia, lipidemia, glucose tolerance, and peripheral insulin sensitivity. Thus, there is no definition of which the causal factor is; whether the metabolic dysfunctions favor the development of PD or, if the contrary is true, or if both conditions can occur. Thus, due to the high prevalence of metabolic diseases and PD in elderly populations and the lack of knowledge about the relationship between PD and metabolic outcomes, we aimed to investigate the possible impact of murine model of motor dysfunctions caused by 6-OHDA on glycemic and lipid homeostasis. We also challenged these rats with dexamethasone, a diabetogenic drug, to verify how much these 6-OHDA-treated rats are vulnerable or not to glucocorticoid (GC) adverse effects. Our hypothesis is that rats with motor deficits caused by 6-OHDA administration will exhibit deterioration of glucose tolerance and insulin sensitivity and that these changes will be exacerbated when GCs were administered. Overall, we demonstrated that motor impairments caused by 6-OHDA treatment did not parallel with peripheral metabolic parameters either predispose rats to the diabetogenic GC effects.

MATERIALS AND METHODS

Ethics Statement

The experimental protocol was approved by the Federal University of Santa Catarina Committee for Ethics in Animal Experimentation (Approval ID: 4468101116) in accordance with the Brazilian National Council for Animal Experimentation Control (CONCEA).

Animals

Thirty-six adults male Wistar rats (starting weight 280–350 g) were housed in a temperature and humidity-controlled environment and kept on a 12-h light–dark cycle (lights on 06:00 – 18:00 h). All rats had *ad libitum* access to food (commercial standard chow for rats, Nuvilab CR-1; Nuvital, Brazil) and filtered tap water. The animals were supplied by the Federal University of Santa Catarina's Animal Breeding Center.

Experimental Design and Groups

Animals were acclimatized for a period of 8 weeks before being randomly assigned in two groups, followed by a new distribution in four experimental groups, as will be described in detail. Animals were submitted to a baseline rotarod assessment protocol and those who were successful in this test were included in the study and underwent stereotaxic surgery. On the first day, only 12% responded adequately to the rotarod test (**Figure 1A**). The rate of successful increased in the second test day and, on day 3, approximately 91% successfully achieve the inclusion criteria on the rotarod test. Approximately 8% did not adapt to the test and therefore did not follow to the surgical procedure. As shown in **Figure 1B**, rats were submitted to the open field test on the 7th and 35th days after the surgery and to a second test, rotarod test, on the 7th, 21st, and 35th postoperative days. The intraperitoneal (i.p.) glucose tolerance test (ipGTT) was performed on the 8th, 22nd, and 36th days of the experimental protocol, while the i.p. insulin tolerance test (ipITT) was applied on the

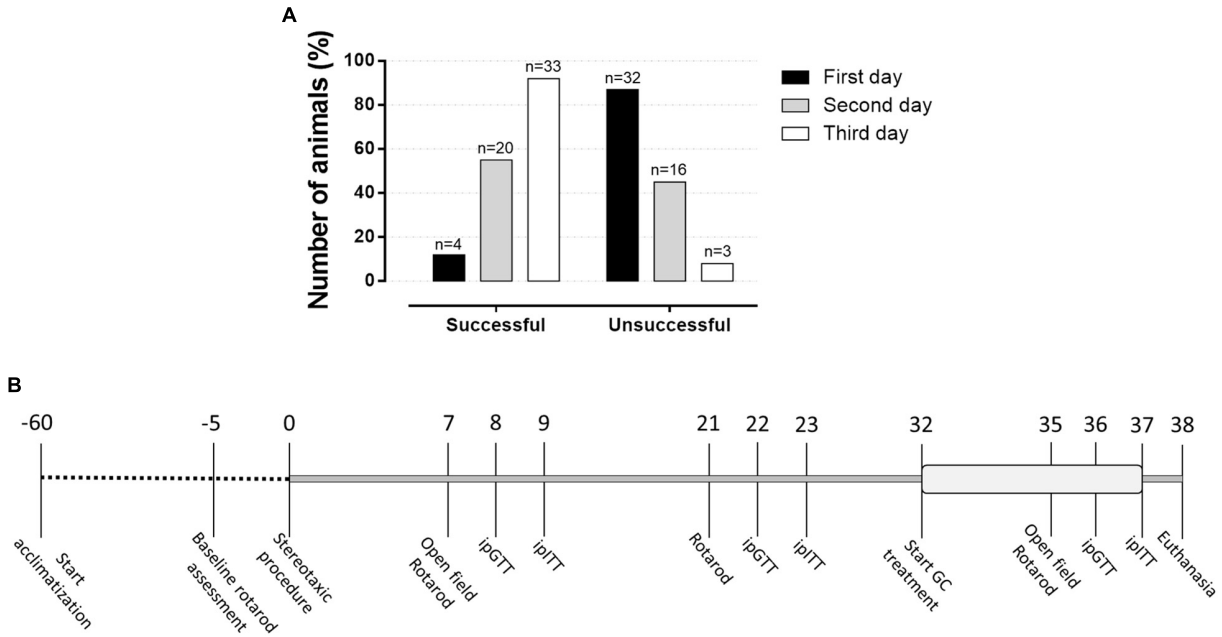

FIGURE 1 | Percentage of animals responsive to the rotarod basal motor protocol and experimental design. **(A)** This test was applied before to initiate the surgeries to identify those rats able to follow in the study. Around 12% of the animals responded to the rotarod test in the first attempt, which achieves approximately 91% of success on the third attempt day. **(B)** The number above line means days along experimental protocol with the respective experimental approaches applied in the study.

9th, 23rd, and 37th postoperative days. The treatment with dexamethasone started on the 32nd day, which lasted until the 37th day of the experimental protocol (6 days). On day 38, the animals were euthanized, and plasma and organs were collected for further biochemical analysis as described in the next sections.

Groups were defined as follow: initially, rats were randomly allocated in two experimental groups: (1) Control group (Control) (n = 17): rats treated only with vehicle via the intra-striatal administration (described in the next section), and (2) 6-hydroxydopamine group (6-OHDA) (n = 16): rats treated with 6-hydroxydopamine via the intra-striatal administration (described in the next section). Then, half of the animal from the Control and 6-OHDA groups were treated with dexamethasone composing the following groups, respectively: (3) Dexamethasone group (Dexa) (n = 9): received i.p. injection of dexamethasone (as described in other section), and (4) 6-hydroxydopamine+dexamethasone group (6-OHDA + Dexa) (n = 8): treated as 6-OHDA and Dexa groups. This experimental protocol was performed in two different sets of animals with half of the number of animals on each occasion.

Surgical Procedure

Before any surgical procedure, rats were anesthetized with ketamine/xylazine [75/10 mg/kg body mass (b.m.), i.p.]. 6-OHDA (20 μg in 3 μL and an infusion rate of 1 μL/min) (Sigma-Aldrich, St. Louis, MO, United States) was injected bilaterally into the dorsolateral striatum (DLS) at the coordinates: AP: +0.2 mm, ML: ±3.5 mm and DV: −4.8 mm to bregma (Paxinos and Watson, 1997), using a Hamilton 10 μL syringe with a 26-gauge needle connected to a 30-gauge cannula, whereas control rats received vehicle (saline solution). Then, the cannula was left untouchable for an additional minute to prevent backflow. This experimental procedure has been used in rats to generate motor impairments that resemble PD motor impairments (Rampersaud et al., 2012; Hegazy et al., 2017).

Dexamethasone Treatment

We used dexamethasone to generate an *in vivo* metabolic challenging considering the diabetogenic properties of this GC as previously demonstrated by several studies (Giozzet et al., 2008; Angelini et al., 2010; Rafacho et al., 2014). Dexamethasone disodium phosphate (Decadron®, Aché, Campinas, SP, Brazil) was daily injected, through i.p. vial, between 07:30 and 08:30 h, at a dose of 1 mg/kg, b.m., for the last 6 consecutive days.

Motor Tests

Open Field

This test was performed under a red-light (12 lux) condition and with controlled sound, temperature, and humidity. Animals were placed in an arena (100 × 100 cm) during 5 min for free exploration. The arena had smooth sidewalls, measuring 40 cm height, formed by odorless white painted metal and the base was made of concrete coated with black contact paper. The square base had 5 × 5 quadrants, each square with 400 cm^2 (20 × 20 cm). All experiments were recorded (Logitech C270, Lausanne, Switzerland), and the following parameters were analyzed: (1) number of squares crossed by the animal,

which represents the locomotor activity; (2) number of rearing behavior, which represents the exploratory activity; (3) number of quadrants crossed peripherally or centrally, which allows identifying anxiety-related behaviors.

Rotarod

This test was applied to evaluate the motor coordination and balance of the animals. The equipment was prepared at three speeds: 12, 20, and 24 revolutions per minute (rpm). The change of speed occurred manually and involved shutdown of the equipment for each speed change. Thus, there was a brief interruption of the test between the rotational changes. All animals were exposed individually to the three different speed levels, consecutively. The baseline assessment protocol consisted of three trials in a constant 12 rpm speed. In the end, rats that remained in the rotarod for 1 min were included in the study and were underwent stereotaxic surgery (**Figure 1A**). This baseline assessment was initiated 5 days before stereotaxic surgery and was extended for three consecutive days (**Figure 1B**). On the 7th, 21st, and 35th postoperative days, animals were positioned in the rotarod apparatus, aiming to remain up to 2 min in each speed. The residence time (latency to fall) of each rat at each stage was measured with a conventional digital chronometer as an indicator of balance and motor coordination.

Measurements of *in vivo* Metabolic Parameters

Verification of body mass was performed every 3 days from the beginning of the experimental protocol to the day of euthanasia in a digital electronic balance (TECNAL; Piracicaba, SP, Brazil). Food intake was monitored as described for body mass. The determination of food intake was performed by weighing the remaining chow discounted from the total of that deposited 24 h before and the mass difference represents the daily amount ingested per cage. The average amount of food ingested per animal was obtained by the following formula [(total chow (g) ingested in the cage/number of rats per cage)/individual rat mass (g)] × 100. The results were expressed as grams (g) of food ingested per 100 g of body mass. During the dexamethasone treatment both body mass and food intake were daily measured, but for aesthetic reasons, only the initial and final measures of food intake were used in the line graph.

I.p. Glucose Tolerance Test (ipGTT)

The i.p. glucose tolerance test (ipGTT) was performed on 8th, 22nd, and 36th days of the experimental protocol in fasted (08 h) rats, with the experiments occurring around 16:00. The rats had the tip of the tail cut (no more than 1 mm) for blood collection. The first drop was discarded, and the second drop was used for the determination of blood glucose (time 0) using a glucometer (Accu-Chek Performa; Roche Diagnostics, GmbH, Mannheim, Germany). Immediately after, a 50% glucose solution pre-warmed at 36°C (2 g/kg, b.m.) was administered by i.p. injection and blood samples were collected from the tail tip at 30, 60, and 120 min for blood glucose measurements as described before (Giozzet et al., 2008; Motta et al., 2015). Area-under-glucose-curve (AUC) was obtained after normalization by the initial value.

I.p. Insulin Tolerance Test (ipITT)

The i.p. insulin tolerance test (ipITT) was performed on 9th, 23rd, and 37th days of the experimental protocol in fed rats, with the experiments occurring around 16:00. Blood collection at min 0 occurred as for ipGTT. Then, the animals received an i.p. injection of recombinant human insulin pre-warmed at 36°C (Biohulin® 1 IU/kg b.m.). Blood samples were posteriorly collected from the tail tip at 10, 20, and 40 min for blood glucose measurements (Giozzet et al., 2008; Protzek et al., 2016). AUC was obtained from normalized blood glucose values (expressed as % from min 0).

Euthanasia and Biochemical Analysis

On the 38th day, 24 h after the last dexamethasone administration, animals received a halothane super dosage (1 mL) (Tanohalo®, Cristália, Curitiba, PR, Brazil). Blood samples were collected through heart puncture with EDTA-NaF containing syringes (Glistab – Labtest; Lagoa Santa, MG, Brazil) and centrifuged at 400 *g* for 10 min at 21°C (Eppendorf 5810R). The plasma was separated and stored at −80°C until the posterior determination of insulin, total cholesterol, and triacylglycerol, according to manufacturer instructions and previously published data (dos Santos et al., 2014; Motta et al., 2015). Animals were then continuously perfused through the left ventricle with 20 mL of 0.9% NaCl containing 0.1% heparin followed by infusion of 200 mL of 4% paraformaldehyde (pH 7.4, Sigma-Aldrich). Organs of interest were carefully removed and weighed.

Liver Glycogen and Hepatic Triacylglycerol Content

Hepatic glycogen content was determined by a phenol-based assay according to a previous publication (Giozzet et al., 2008). Determination of hepatic triacylglycerol content was performed according to a previous publication (Gonçalves-Neto et al., 2014). The same hepatic lobe was used for collection of the fragments for all animals.

Hepatic Morphology

After perfusion, liver fragments (from the same lobe in all animals) were collected and fixed in 10% buffered formalin, pH 7.4, for 24 h, dehydrated and embedded in paraffin. Representative tissue sections (5 μm) were obtained on a rotating microtome (Leica) and placed on glass slides. Afterward, the slides were submitted to the staining procedure of Hematoxylin and Eosin for further morphological evaluation to verify the presence of lipids and glycogen according to Gonçalves-Neto et al. (2014).

The Relative Mass of the Endocrine Pancreas

After perfusion, pancreas fragments (splenic region) were collected and fixed in 4% paraformaldehyde for 24 h, dehydrated

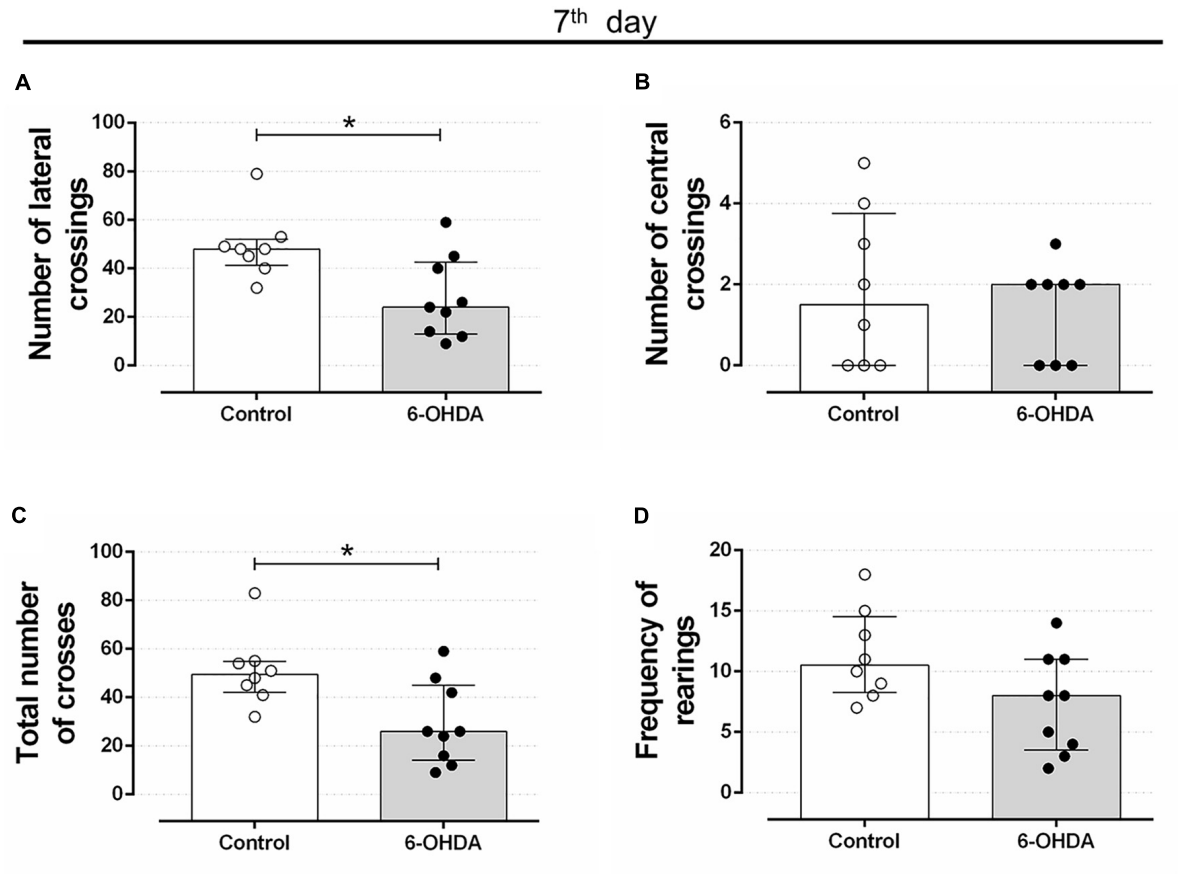

FIGURE 2 | Effect of 6-OHDA on the 7th day of the experimental protocol, in motor parameters evaluated in the open field test. **(A)** number of lateral crossings; **(B)** number of central crossings; **(C)** the total number of crosses (central + lateral); **(D)** average frequency of rearings. Data are median and interquartile range as they are asymmetrically distributed (nonparametric). *Indicates a significant difference compared to the respective control group using unpaired Mann–Whitney. $n = 8–9$, $p < 0.05$.

and embedded in paraffin. Representative tissue sections (5 μm) were obtained on a rotating microtome (Leica) and placed on glass slides. Afterward, the slides were submitted to the staining procedure of hematoxylin and eosin and later scanned by the AxioScan automatic slide scanner (ZEISS, Oberkochen, Germany) for quantification of the relative endocrine pancreatic mass and density of pancreatic islets. To obtain the relative mass data, the total area of the pancreas and pancreatic islets were calculated using the ZEN software (ZEISS). The sum of the areas corresponding to the pancreatic islets was divided by the total pancreas area and multiplied by 100 to obtain the percentage of endocrine pancreas area. To obtain the density of pancreatic islets the following relation was used: islet density = (total number of islets in section × 1000) divided by the total area of the pancreas analyzed. The result was expressed by the number of islets per 1000 μm^2 of pancreas area (Protzek et al., 2014).

Statistical Analysis

All analyses were performed using GraphPad Prism Version 6.01 software (GraphPad Inc., La Jolla, CA, United States). The results were expressed as the mean ± SD or mean ± SEM for parametric data or median and interquartile ranges for non-parametric data. The symmetry of the data was tested by Kolmogorov–Smirnov, Shapiro–Wilk, and D'Agostino and Pearson omnibus normality tests. It was considered symmetric if approved by at least one of three tests. Analysis of variance (two-way ANOVA) followed by Tukey's *post hoc* test was used for multiple comparisons of parametric data or Kruskal–Wallis followed by Dunn's *post hoc* when the variables presented an asymmetric distribution, while the categorical data were evaluated by the Person Chi-square test. Significance was set at $p < 0.05$.

RESULTS

Characterization of the 6-OHDA Model

Central Administration of 6-OHDA Caused Reduction in Locomotor Activity in the Open Field Test

Rats treated with 6-OHDA exhibited motor impairment on the 7th day after stereotaxic surgery. The number of lateral and

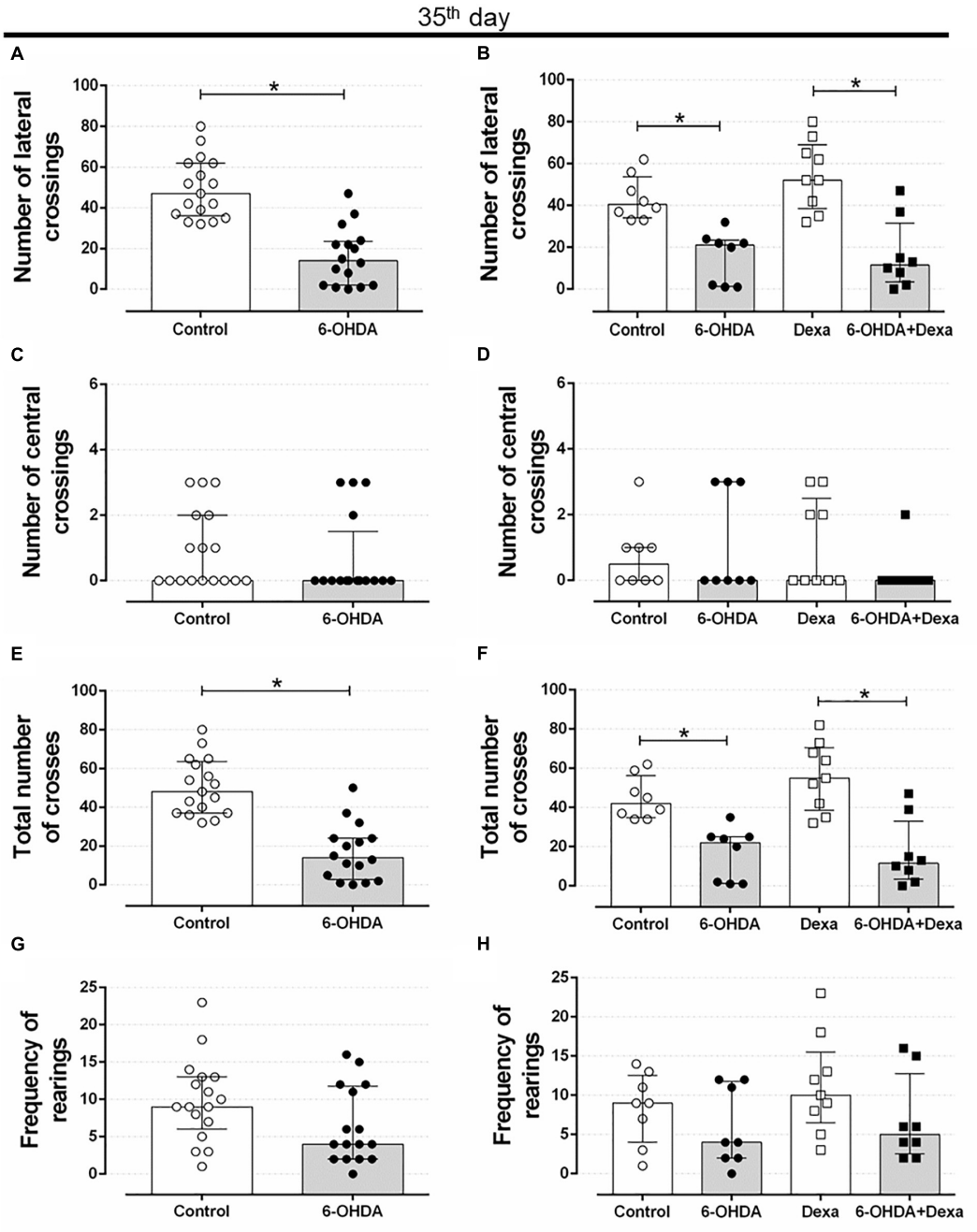

FIGURE 3 | Effect of 6-OHDA on the 35th day of the experimental protocol, in motor parameters evaluated in the open field test. **(A)** number of lateral crosses without distinction of dexamethasone treatment; **(B)** number of lateral crossings; **(C)** number of central crosses without distinction of dexamethasone treatment; **(D)** number of central crossings; **(E)** total number of crosses (central + lateral) without distinction of dexamethasone treatment; **(F)** total number of crosses (central + lateral); **(G)** frequency of rearings without distinction of dexamethasone treatment; **(H)** frequency of rearings. Data are median and interquartile range as they are asymmetrically distributed (nonparametric). *Indicates a significant difference compared to the respective control groups using unpaired Mann–Whitney in **(A,C,E,G)**, and Kruskal–Wallis with Dunn's *post hoc* test in **(B,D,F,H)**. n = 16–17 in **(A,C,E,G)**, and 8–9 in **(B,D,F,H)**, $p < 0.05$.

total crosses significantly reduced around 43% in the 6-OHDA group of rats, compared with the Control group (**Figures 2A,C**, respectively). No significant alterations were observed in the number of central crosses and in the frequency of rearing behavior (**Figures 2B,D**, respectively).

Motor impairment addressed on the open field test persisted until the end of experimental protocol (35 days after stereotaxic surgery and on the 3rd day of GC treatment) in rats treated with 6-OHDA, as observed by the significant reduction in the number of lateral crosses (62%) and in the number of total crosses (67%)

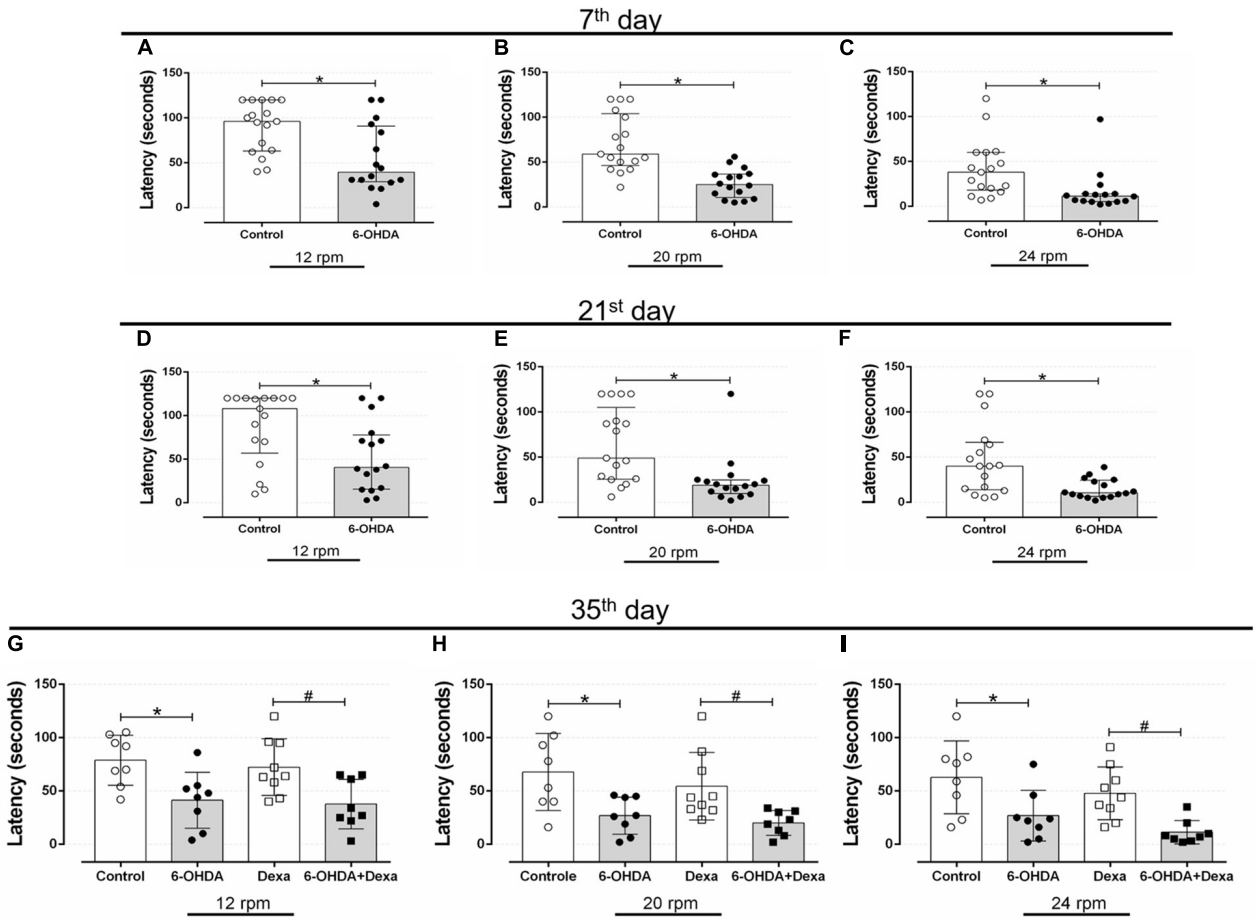

FIGURE 4 | Effect of 6-OHDA injection, on the 7th, 21st, 35th and day of the experimental protocol, in motor parameter evaluated in the rotarod test d. **(A–C)** Latency to fall in seconds at speeds of 12, 20, and 24 revolutions per minute (rpm) at the 7th, **(D–F)** 21st, and **(G–I)** 35th day. Data are median and interquartile range as they are asymmetrically distributed (non-parametric) in **(A–F)**, and mean ± SD in **(G–I)**. * and # Indicates a significant difference compared to the Control and Dexa groups, respectively, using unpaired Mann–Whitney in **(A–F)**, and ordinary two-way ANOVA with Tukey's *post hoc* test in **(G–I)**. n = 16–17 in **(A–F)**, and 8–9 in **(G–I)**, $p < 0.05$.

when compared with the Control group values (**Figures 3A,E**). This motor impairment was not attenuated or exacerbated by the GC treatment reinforcing the 6-OHDA effect (**Figures 3B,F**). There were no significant differences in the number of central crosses as well as in the number of rearing behavior in both 6-OHDA and 6-OHDA + Dexa groups compared to their respective Control groups (**Figures 3C,D,G,H**). Again, treatment with dexamethasone (Dexa and 6-OHDA + Dexa groups) exerted no impact on these parameters.

Central Administration of 6-OHDA Caused Reduction in Motor Coordination and Balance Impairments

Rotarod is considered a gold-standard method to evaluate motor coordination in rodents. Bilateral administration of 6-OHDA into the dorsolateral striatum resulted in impairment of the motor coordination of the animals. At the rate of 12 rpm (**Figure 4A**), the 6-OHDA group had a significant 39% reduction in the latency to rotarod fall on the 7th postoperative day, when compared to the Control group. In addition, the 6-OHDA group also showed a significant reduction of 63 and 60% at the speed of 20 and 24 rpm (**Figures 4B,C**, respectively).

The latency to fall from the rotarod equipment was very similar at day 21. The reduction in the latency to rotarod fall in the 6-OHDA group was of 40, 62, and 68% at the 12, 20, and 24 rpm speeds, respectively, demonstrating the significant persistence of motor impairment in relation to the Control group (**Figures 4D–F**).

The same pattern of latency to fall was observed on the 35th day of the experimental protocol (3rd day of GC treatment). The 6-OHDA group remained with a significant reduction of 46% in the rotarod permanence at 12 rpm (**Figure 4G**), 59% at 20 rpm (**Figure 4H**), and 54% at 24 rpm (**Figure 4I**), compared with the Control group. Treatment with dexamethasone did not influence the latency time to fall from rotarod, as observed in **Figures 4G–I**. Altogether, these findings confirm that bilateral administration of 6-OHDA in male rats is enough to cause motor dysfunctions.

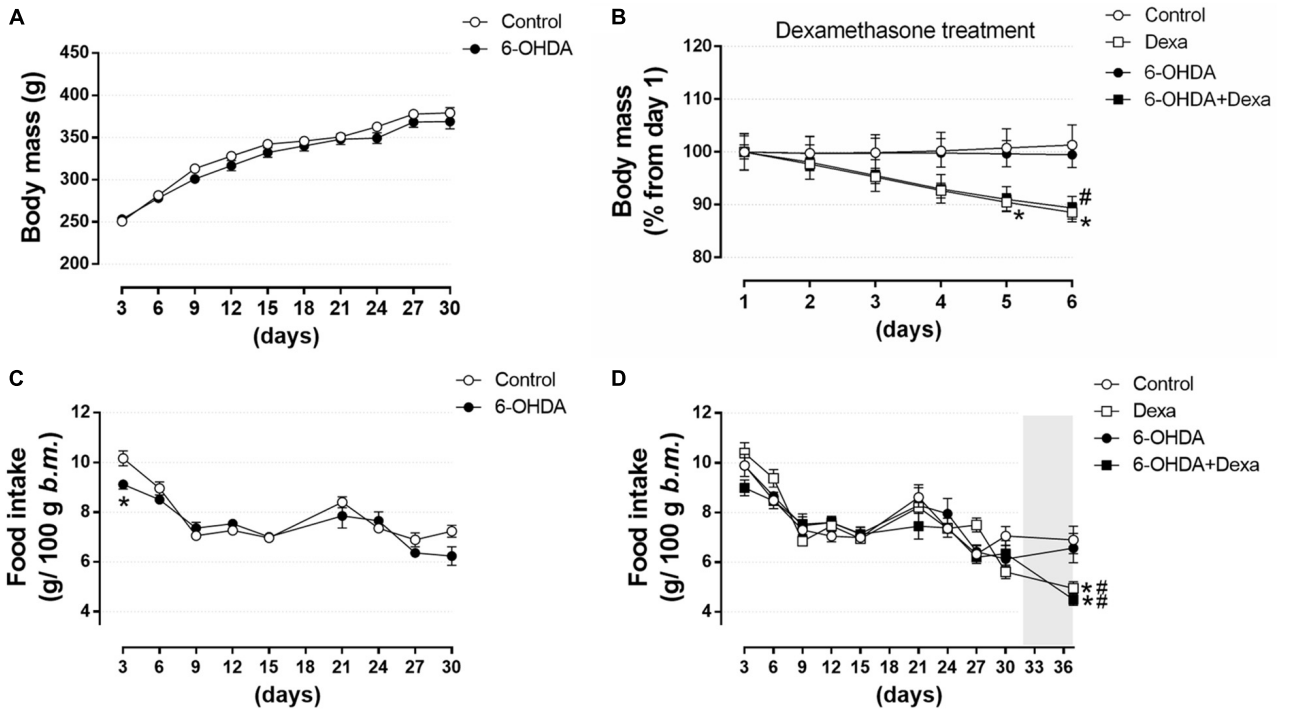

FIGURE 5 | Body mass and food intake over 36 days. **(A)** Body mass of Control and 6-OHDA groups in the first 30 days. **(B)** Normalized body mass (from the 1st day of treatment) of the Control, Dexa, 6-OHDA and 6-OHDA + Dexa groups during the 6 days of dexamethasone treatment. **(C)** Food intake of Control and 6-OHDA groups during the first 30 days. **(D)** Food intake of Control, Dexa, 6-OHDA, and 6-OHDA + Dexa groups during the whole experimental protocol. Data are mean ± SEM (SEM were applied only for aesthetic reasons). * and # Indicates a significant difference compared to the Control and 6-OHDA groups, respectively, using unpaired Student's *t*-test in **(A,C)**, and ordinary two-way ANOVA with repeated measures in **(B,D)**. n = 16–17 in **(A,C)**, and 8–9 in **(B,D)**, $p < 0.05$. The light gray color in **(D)** means the period of dexamethasone treatment (six consecutive days).

Central Administration of 6-OHDA Did Not Impact on Body Mass Gain and Food Intake

To verify whether motor dysfunctions resulted from treatment with 6-OHDA was associated to any alteration on murinometric parameters, and whether the treatment with dexamethasone was able to reveal any metabolic vulnerability in those animals, several parameters typical of metabolic studies were addressed. There were no differences in body mass between the Control and 6-OHDA groups during the first 30 days, before the beginning of dexamethasone treatment (**Figure 5A**). In the 32nd day, the metabolic challenge was initiated by treatment with dexamethasone. From this moment this parameter was analyzed in the four experimental groups. Both the Dexa and 6-OHDA + Dexa groups presented a significant reduction of ∼11% in body mass at the end of the dexamethasone treatment (Δ 44 g and 39 g, respectively) (**Figure 5B**). Food intake was similar between Control and 6-OHDA groups when analyzed during the first 30 days of the experimental protocol, except for a transient reduction in the consumption of the 6-OHDA group in the first measure of the experimental protocol, when compared to the Control group (**Figure 5C**). In the 32nd day the metabolic challenge with dexamethasone started, and from this moment on, the parameter was analyzed in the four experimental groups. Both the Dexa and 6-OHDA + Dexa exhibited a significant reduction in food intake of 25 and 32%, respectively, at the end of dexamethasone treatment when compared to their respective controls (**Figure 5D**).

Central Administration of 6-OHDA Did Not Alter Glucose Tolerance and Insulin Sensitivity

To verify whether motor impairment caused by 6-OHDA may be associated with glucose homeostasis disturbances, we proceeded with analyzes of metabolic parameters to identify possible alterations associated with the neuromotor outcome. Considering glucose intolerance may anticipate the elevation in blood glucose, we assessed the glucose tolerance on the 8th, 22nd, and 36th days, and the insulin sensitivity on the 9th, 23rd, and 37th days of the experimental protocol. Rats treated with 6-OHDA exhibited a mild increase of blood glucose levels 30 min after an i.p. glucose *bolus* than the Control group (**Figure 6A**). The blood glucose values were 332 ± 17 mg/dL for the Control group and 388 ± 20 mg/dL for 6-OHDA group at min 30. However, this isolated alteration did not influence the area-under-curve (AUC) values, which revealed no changes in the overall glucose tolerance between the groups (**Figure 6C**). To verify whether motor impairment might be associated with an early change in insulin sensitivity, we performed ipITT on the 9th day of the experimental protocol. The insulin sensitivity remained unaltered in the 6-OHDA group compared

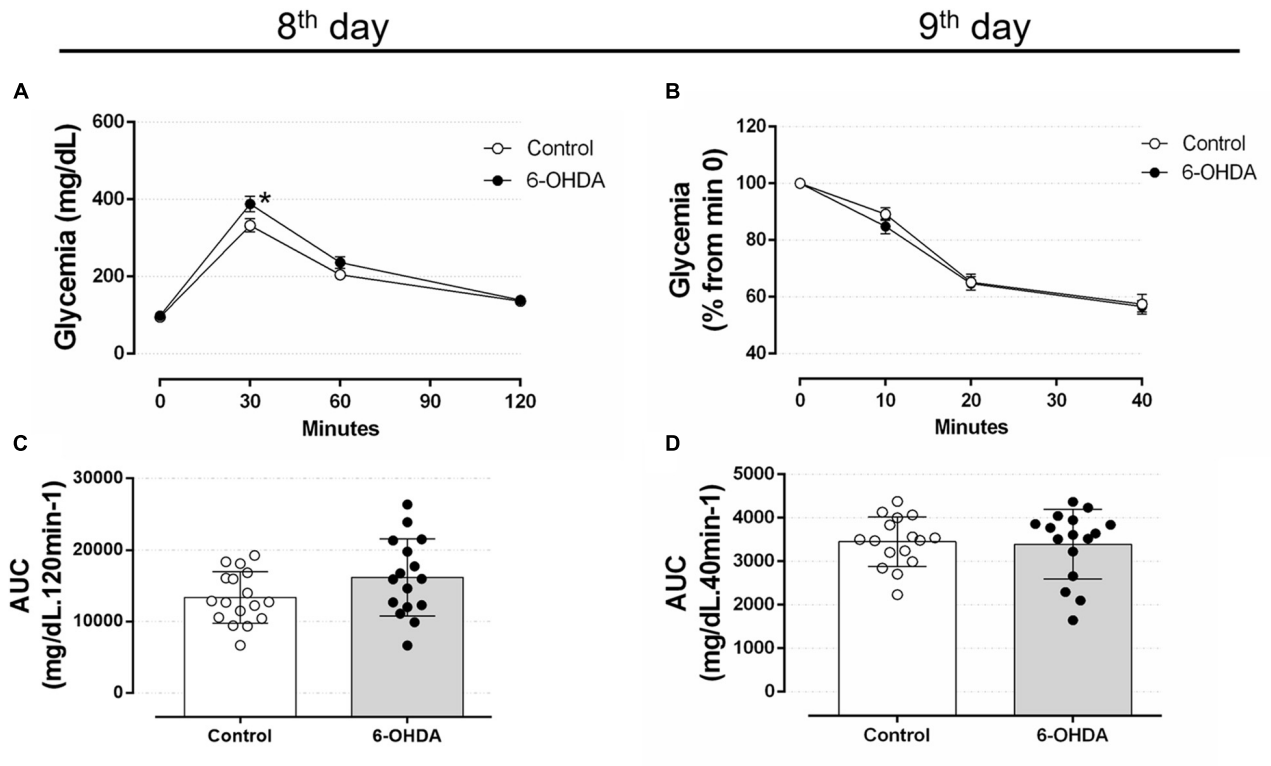

FIGURE 6 | Blood glucose values during the intraperitoneal glucose and insulin tolerance test on the 8th and 9th day of the experimental protocol. **(A)** Blood glucose values during the ipGTT on the 8th day. **(B)** Blood glucose values (normalized from min 0) during the ipITT on the 9th day. **(C)** Area-under-curve during ipGTT and **(D)** during ipITT. Data are mean ± SEM in **(A,B)**, and mean ± SD in **(C,D)** (SEM were applied only for aesthetic reasons). *Indicates a significant difference compared to the Control using ordinary two-way ANOVA with repeated measures. Unpaired Student's *t*-test was applied in **(C,D)**. $n = 16–17$, $p < 0.05$.

with the Control group (**Figures 6B,D**). The absolute fed blood glucose values on baseline of ipITT were 118 ± 7 and 121 ± 8 mg/dL for the Control and 6-OHDA groups, respectively ($n = 16–17$, NS).

The GTT values obtained in the 22nd postoperative day revealed no differences between the 6-OHDA and Control groups at 30, 60, and 120 min after intraperitoneal glucose load (**Figures 7A,C**). In addition, insulin sensitivity remained unaltered in the 23rd postoperative day in the 6-OHDA group when compared with the Control group (**Figures 7B,D**). The absolute fed blood glucose values on baseline of ipITT were 114 ± 5 and 112 ± 7 mg/dL for the Control and 6-OHDA groups, respectively ($n = 16–17$, NS).

On the 36th day of the experimental protocol (5th day of dexamethasone treatment), treatment with GC resulted in glucose intolerance in the Dexa and 6-OHDA + Dexa groups when compared with the respective Control and 6-OHDA groups, as observed at 30, 60, and 120 min (**Figure 8A**) and in the AUC values (**Figure 8C**). Again, motor dysfunction caused by 6-OHDA did parallel with normal glucose tolerance even after 36 days postoperative and was associated with no exacerbation in the glucose intolerance caused by GC treatment. Glucose intolerance caused by dexamethasone treatment occurred parallelly to a significant decrease in the insulin sensitivity as observed by the ipITT data obtained on the 37th day (**Figures 8B,D**). 6-OHDA administration did not affect insulin sensitivity *per se* or exacerbated the action of GC treatment. The absolute fed blood glucose values on baseline of ipITT were 116 ± 5, 119 ± 7, 155 ± 39, and 180 ± 34 mg/dL for the Control, 6-OHDA, Dexa and 6-OHDA + Dexa groups, respectively ($n = 8–9$, $p < 0.05$ for dexamethasone-treated rats when compared with their respective 6-OHDA groups; only dexamethasone effect).

Central Administration of 6-OHDA Did Not Affect Plasma Insulin, Triacylglycerol, and Total Cholesterol Levels

To verify whether motor impairment caused by bilateral 6-OHDA administration could be associated with alteration in peripheral glucose and lipid metabolism, we evaluated the insulinemia and some circulating lipid levels. As observed in **Figures 9A,B**, rats receiving dexamethasone (Dexa and 6-OHDA + Dexa groups) exhibited an increase in plasma insulin levels compared with their respective control groups due to GC effect. Motor dysfunction induced by 6-OHDA was not associated with changes in insulinemia compared to the Control group (**Figure 9A**) and did not exacerbate the elevation in insulinemia caused by GC. In a very similar fashion, groups treated with dexamethasone showed an increase in plasma triacylglycerol levels, compared to

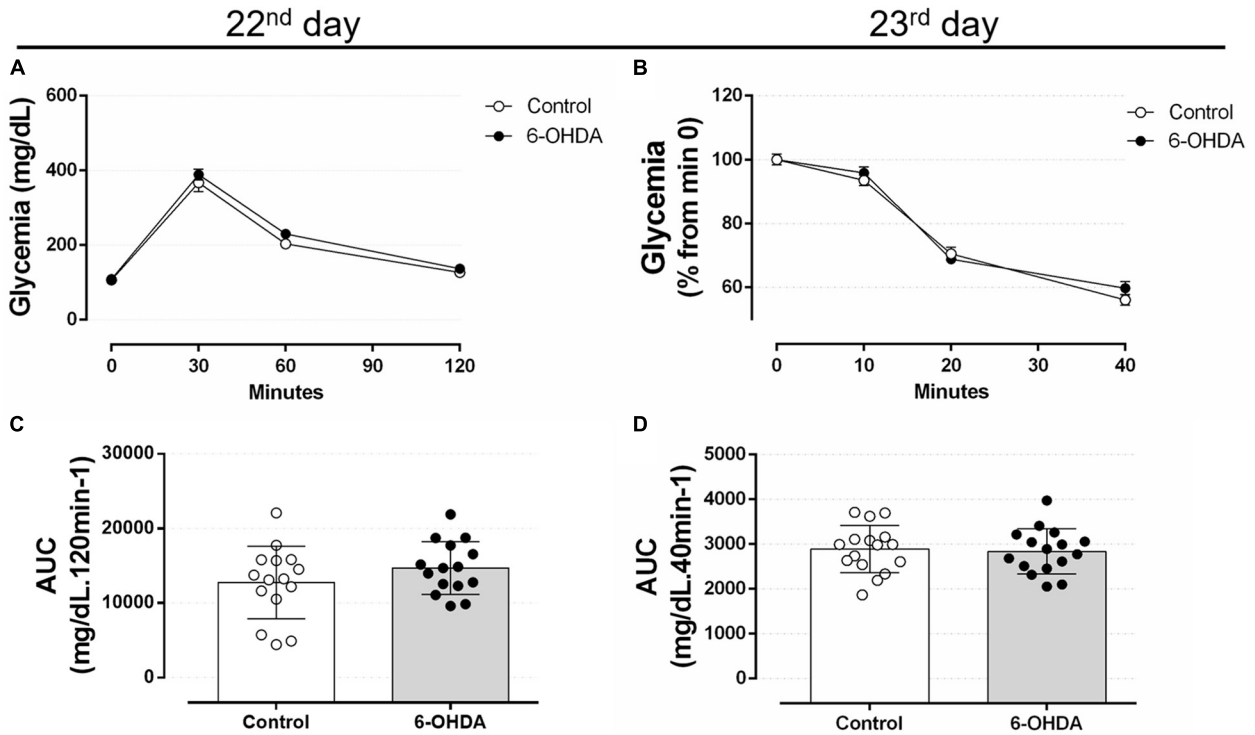

FIGURE 7 | Blood glucose values during the intraperitoneal glucose and insulin tolerance test on the 22nd and 23rd day of the experimental protocol. **(A)** Blood glucose values during the ipGTT on the 22nd day. **(B)** Blood glucose values (normalized from min 0) during the ipITT on the 23rd day. **(C)** Area-under-curve during ipGTT and **(D)** during ipITT. Data are mean ± SEM in **(A,B)**, and mean ± SD in **(C,D)** (SEM were applied only for aesthetic reasons). Ordinary two-way ANOVA with repeated measures was applied in **(A,B)**, whereas unpaired Student's *t*-test was applied in **(C,D)**. $n = 16–17$, $p < 0.05$.

their respective control groups (Control and 6-OHDA groups) (**Figure 9B**). No influence of 6-OHDA treatment *per se* was observed on this parameter. In addition, the four experimental groups evaluated did not show differences in plasma cholesterol levels (118, 108, 110, and 105 mg/dL for Control, Dexa, 6-OHDA and 6-OHDA + Dexa groups, respectively).

Central Administration of 6-OHDA Did Not Alter Hepatic Lipid and Glycogen Contents

The accumulation of ectopic lipids is a common outcome in the contexts of metabolic diseases. Thus, to verify whether treatment with 6-OHDA influenced these parameters, we collected hepatic fragments to perform biochemical and histological analyses. As illustrated in **Figures 10A,B**, central administration of 6-OHDA did not influence the content of triacylglycerol and glycogen in the liver, respectively. GC-treated groups (Dexa and 6-OHDA + Dexa groups) showed higher hepatic triacylglycerol and glycogen contents when compared with the Control and 6-OHDA groups.

The morphological analysis of the liver sections revealed a significant phenotype of glycogen deposition and microvesicle lipids distribution in both the dexamethasone-treated groups (**Table 1** and **Figures 10D,F**). Administration of 6-OHDA did not impact on lipid and glycogen content (**Table 1** and **Figure 10E**). As expected, none of the liver sections from the Control group exhibited signs of glycogen and triacylglycerol contents (**Table 1** and **Figure 10C**).

Central Administration of 6-OHDA Did Not Alter Adiposity and Metabolic Organ Masses

After perfusion and euthanasia of the animals, the relative mass of omental, epididymal, and retroperitoneal fat depots and metabolic organs were quantified. Neither 6-OHDA nor GC administration altered the visceral adiposity as observed in **Table 2**. Liver and spleen masses remained unaltered too. It was only observed a reduction in the adrenal gland mass in the 6-OHDA + Dexa group compared to the 6-OHDA group.

Central Administration of 6-OHDA Did Not Impact on Endocrine Pancreas Mass

Increase in the endocrine pancreas mass is an adaptive compensatory response to a reduction in peripheral insulin sensitivity. Thus, we evaluated whether motor dysfunction could be associated with the impairment of such expected adaptation in GC-treated rats. No differences were observed in the relative mass of the pancreas between the four groups (**Figure 11A**). The increase in the relative pancreatic islet mass caused by GC treatment was not affected by 6-OHDA treatment (**Figures 11B,D–G**). The increase of the relative mass was accompanied by a no significant increase of islet density

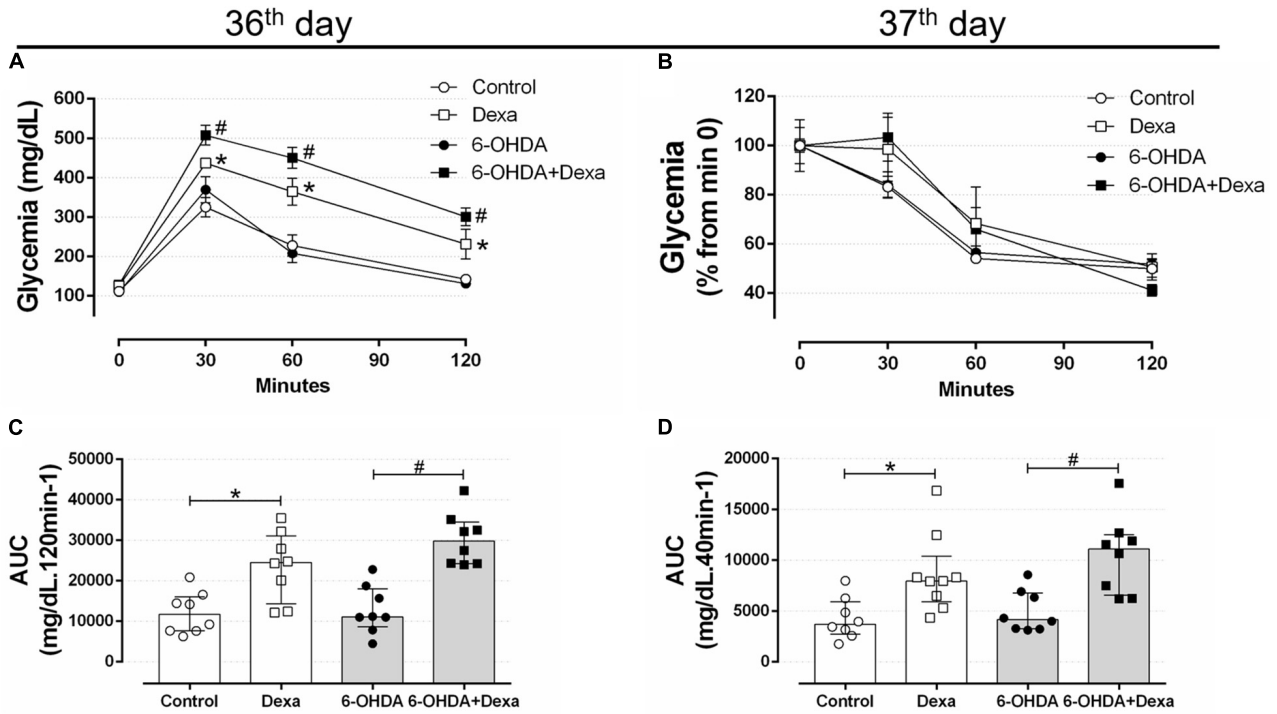

FIGURE 8 | Blood glucose values during the intraperitoneal glucose and insulin tolerance test on the 36th and 37th day of the experimental protocol. **(A)** Blood glucose values during the ipGTT on the 36th day. **(B)** Blood glucose values (normalized from min 0) during the ipITT on the 37th day. **(C)** Area-under-curve during ipGTT and **(D)** during ipITT. Data are mean ± SEM in **(A,B)** (SEM were applied only for aesthetic reasons), and median and interquartile range as they are asymmetrically distributed (nonparametric) in **(C,D)**. * and # Indicates a significant difference compared to the Control and 6-OHDA groups, respectively, using ordinary two-way ANOVA with repeated measures in **(A,B)**, or Kruskal–Wallis with Dunn's *post hoc* test in **(C,D)**. $n = 8–9$, $p < 0.05$.

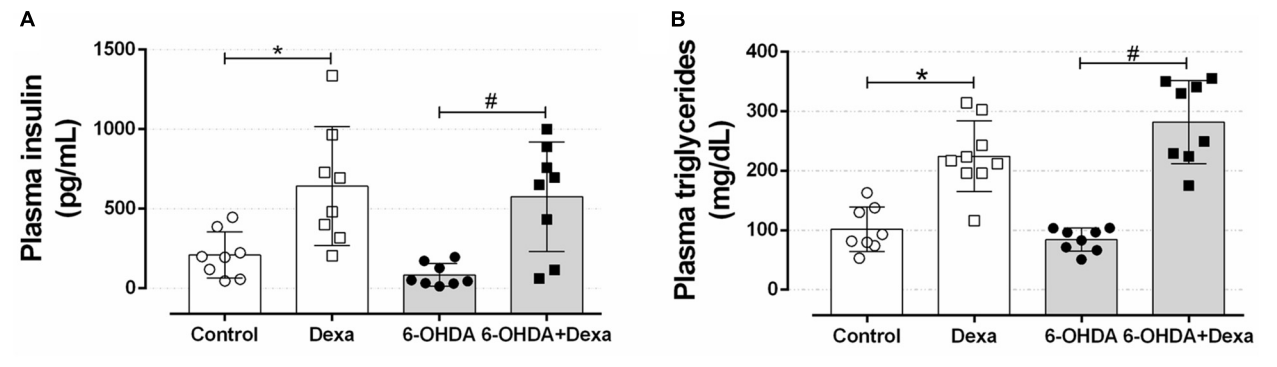

FIGURE 9 | Circulating parameters. **(A)** Plasma insulin and **(B)** triacylglycerol concentration at the day of euthanasia on the 38th day of the experimental protocol. Data are median and interquartile range as they are asymmetrically distributed (nonparametric). * and # Indicates a significant difference compared to the respective Control and 6-OHDA groups using Kruskal–Wallis with Dunn's *post hoc* test. $n = 8–9$, $p < 0.05$.

(number of islets per 1000 μm^2 of pancreas section) in the four experimental groups (**Figure 11C**).

DISCUSSION

In the present study, we investigated the impact of bilateral administration of 6-OHDA on the striatum of rats on metabolic parameters to verify whether a persistent motor dysfunction could affect the glucose and lipid homeostasis in a later period. Overall, we observed that central administration of 6-OHDA reproduced the motor dysfunction (i.e., impairment of locomotion and motor coordination) as evidenced in the open field and rotarod tests, which was persistent for the entire period of the experiment. This motor dysfunction was not associated with alteration of glucose tolerance and insulin sensitivity or circulating lipids levels and did not exacerbate the classical adverse effects of excessive GCs.

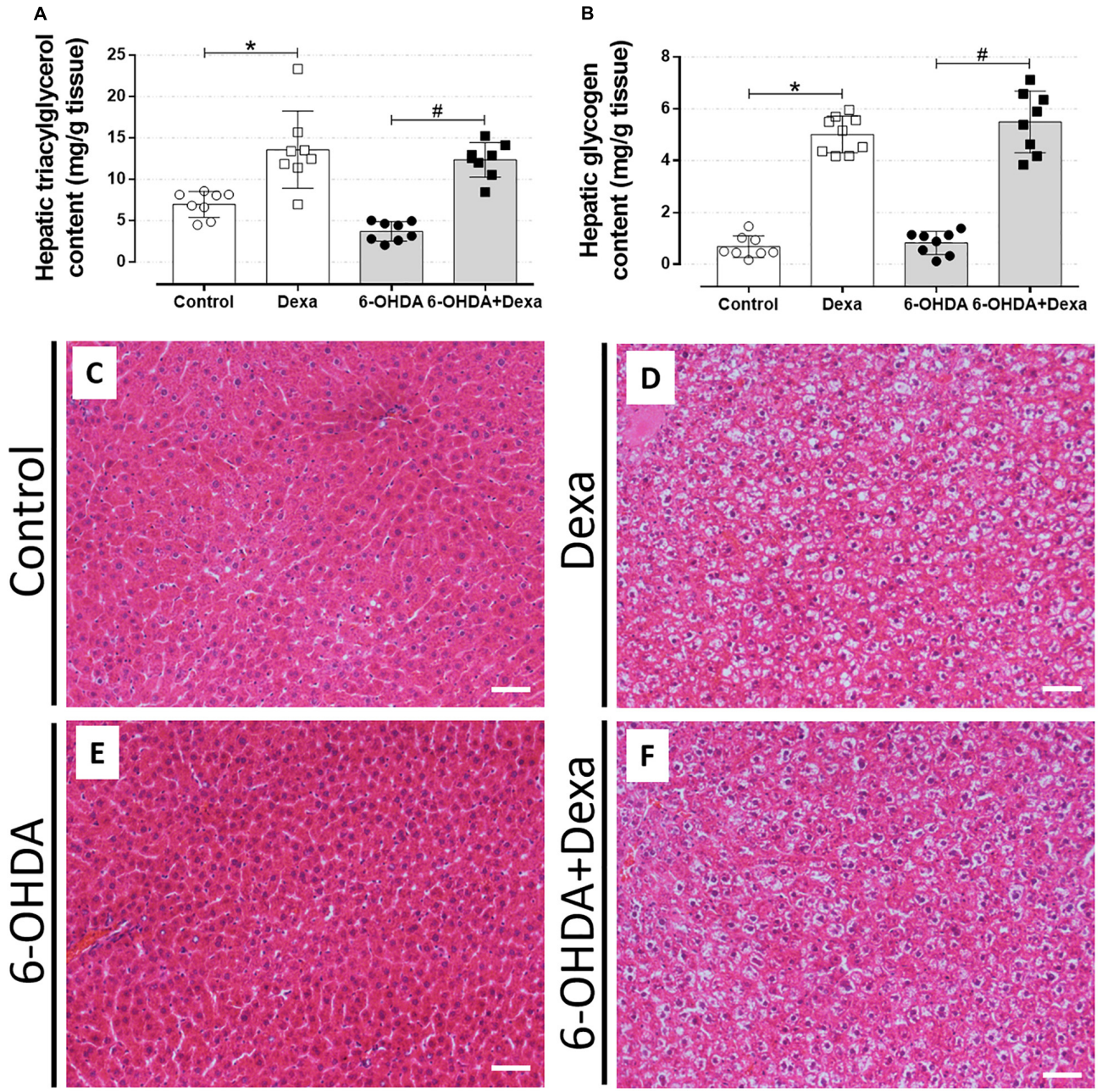

FIGURE 10 | Liver parameters. **(A)** Hepatic triacylglycerol and **(B)** glycogen content at the day of euthanasia on the 38th day of the experimental protocol. Data are mean ± SD. * and # indicate a significant difference compared to the respective Control and 6-OHDA groups using ordinary two-way ANOVA with Tukey's *post hoc* test. $n = 8–9$, $p < 0.05$. **(C–F)** Representative liver sections from all groups in 200x magnitude stained with Hematoxylin and Eosin. $n = 8–9$. Scale bar in **(C–F)** represents 25 μm.

To achieve a persistent motor dysfunction, we selected the PD rat model of bilateral 6-OHDA administration (Sauer and Oertel, 1994; Przedborski et al., 1995; Lee et al., 1996). With this approach occurs a neuronal lesion that gradually evolves from the nigrostriatal pathway, 1 day after the stereotaxic procedure, to the nigral cells, 1 week after injection, reaching the maximum degeneration between 2 and 3 weeks (Sauer and Oertel, 1994; Przedborski et al., 1995; Blandini et al., 2007). In addition, the dose of 20 μg administered in the striatum promotes a loss of dopaminergic cells varying between 20 and 85% in the SNpc region and 60–90% in the striatum region up to 2 weeks after the surgical procedure (Przedborski et al., 1995; Lee et al., 1996; Kirik et al., 1998; Blandini et al., 2007).

In human PD there is an extensive loss of dopaminergic neurons from the SNpc, resulting in a decline in dopamine concentrations of the striatum, which results in several motor

TABLE 1 | An indication of the presence of lipid and/or glycogen deposition.

	Lipids	Glycogen
Control	0/8	0/8
Dexa	8/9*	8/9*
6-OHDA	1/8	1/8
6-OHDA+Dexa	8/8#	8/8#

*Numbers indicate the presence of phenotype per total number of sections analyzed. Each section represents a different rat. * and # indicates a significant difference compared to the respective Control and 6-OHDA groups, respectively using Pearson Chi-square test, $p < 0.05$, $n = 8–9$.*

deficiencies including rigidity, tremor, and loss of postural reflexes (Riederer and Wuketich, 1976; Beal, 2005). In *in vivo* studies with rodents, dopaminergic loss resulting from the action of the 6-OHDA toxin and confirmation of motor impairments can be detected through analyses of motor behaviors. In our study, infusion of 6-OHDA into the dorsolateral striatum of rats resulted in motor impairment such as decreased locomotor activity at 7 and 35 days after 6-OHDA administration (**Figures 2**, **3**). These results are supported by numerous previous studies. Bilateral administration of 20 μg of 6-OHDA in the dorsolateral striatum of rats promotes significant deficits in locomotor activity addressed in the open field test, 21 days after 6-OHDA administration (Matheus et al., 2016). Similarly, motor impairments were observed with unilateral administration of 10 μg of 6-OHDA in the striatum, which included a decrease in locomotion and exploratory behavior of male Wistar rats, observed 3 weeks after treatment with the 6-OHDA neurotoxin (Hegazy et al., 2017). Locomotor and exploratory deficits can also be achieved with bilateral 6-OHDA administration of 15 μg in the striatum of rats (Rampersaud et al., 2012).

In the present study, the 6-OHDA-treated rats also exhibited impaired motor coordination (**Figure 4**). This motor dysfunction is supported by several studies. For instance, the motor coordination was impaired after 1 week of unilateral 6-OHDA administration of 28 μg or bilateral 6-OHDA administration of 20 μg in rats (Monville et al., 2006; Kumar et al., 2012). These findings support our results demonstrating the validity of our experimental animal model. It is important to emphasize that 6-OHDA-lesioned rats displayed motor dysfunctions for the entire experimental protocol duration in the present study, corroborating findings from previous studies (Rampersaud et al., 2012; Matheus et al., 2016).

Although we were not searching for the impact of GC treatment on central outcomes, there is evidence suggesting that 7 days of treatment with dexamethasone (1 mg/kg b.m.) in 6-OHDA-treated mice may partially inhibit microglial activation by attenuating the production of proinflammatory cytokines (Kurkowska-Jastrzebska et al., 2004). This anti-inflammatory action of GCs could attenuate the degeneration of dopaminergic neurons in *in vivo* models of PD improving some motor deficits. In our study, no apparent attenuation that could be attributable to GCs were observed in the behavioral tests performed on the 35th day of the experimental protocol (4th day of exposure to GC). This apparent discrepancy can be explained, at least in part, because in this latter study with mouse the GC was administered previously to 6-OHDA lesion.

Murinometric data revealed no impact of 6-OHDA administration on body mass and on food intake in the present study (**Figure 5**). The nigrostriatal dopaminergic system plays an essential role in the regulation of appetite and body weight (Ungerstedt, 1971; Fibiger et al., 1973; Lénárd, 1977). In addition, there is evidence in mouse showing that hypothalamic arcuate nucleus (ARC) possesses TH-positive neurons and that these neurons play an orexigenic role in energy homeostasis (Zhang and van den Pol, 2016). Evidence obtained in animal models suggests that dopaminergic lesions greater than 95% induce adipsia and hypophagia (Ungerstedt, 1971; Sakai and Gash, 1994). However, in the present study, the animals were submitted to a lesion in the striatum that is expected to produce a slower progression of motor symptoms, which may have prevented any relevant influence on the control of food intake. Studies have shown that patients with PD tend to lose weight with disease progression, presenting a lower body weight when compared to the corresponding age-control population. Explanations for weight loss of PD patients include difficulty in chewing and swallowing, increased time required to eat, decreased olfactory and palatability, depression, and increased energy requirements intake due to muscular rigidity and involuntary movements (Beyer et al., 1995; Lorefält et al., 2006). Whether neurodegeneration in dopaminergic neurons outside striatum (i.e., hypothalamic ARC) in PD are also affected, and how much this can influence the metabolism from PD patients, is a question that merit investigation. Dayan et al. (2018) demonstrated that PD patients with symptoms of autonomic impairments exhibit disrupted thalamic-striatal-hypothalamic functional connectivity, suggesting the possible involvement

TABLE 2 | Organ masses (g/100 g body mass) on the day of euthanasia.

	Control	Dexa	6-OHDA	6-OHDA + Dexa
Omental fat	0.17[0.12;0.23]	0.17[0.16;0.24]	0.15[0.11;0.19]	0.16[0.09;0.24]
Epididymal fat	1.67[1.51;1.76]	1.85[1.48;1.87]	1.42[1.35;1.57]	1.68[1.18;2.25]
Retroperitoneal fat	1.58[1.43;2.12]	1.32[1.16;1.70]	1.38[1.12;1.53]	1.51[1.11;1.88]
Liver	4.15[3.83; 4.49]	4.92[4.24;5.05]	4.19[3.25;4.35]	4.46[4.11;4.93]
Spleen	0.18[0.16;0.19]	0.15[0.13;0.17]	0.18[0.17;0.19]	0.14[0.13;0.17]
Adrenals	0.02[0.01;0.02]	0.01[0.01;0.01]	0.02[0.01;0.02]	0.01[0.01;0.01]#

Results are expressed as a median ± interquartile range. The (#) indicates a significant difference compared to the 6-OHDA group using Kruskal–Wallis with Dunn's post hoc test. ($n = 8$, $p < 0.05$; see main text for a detailed description).

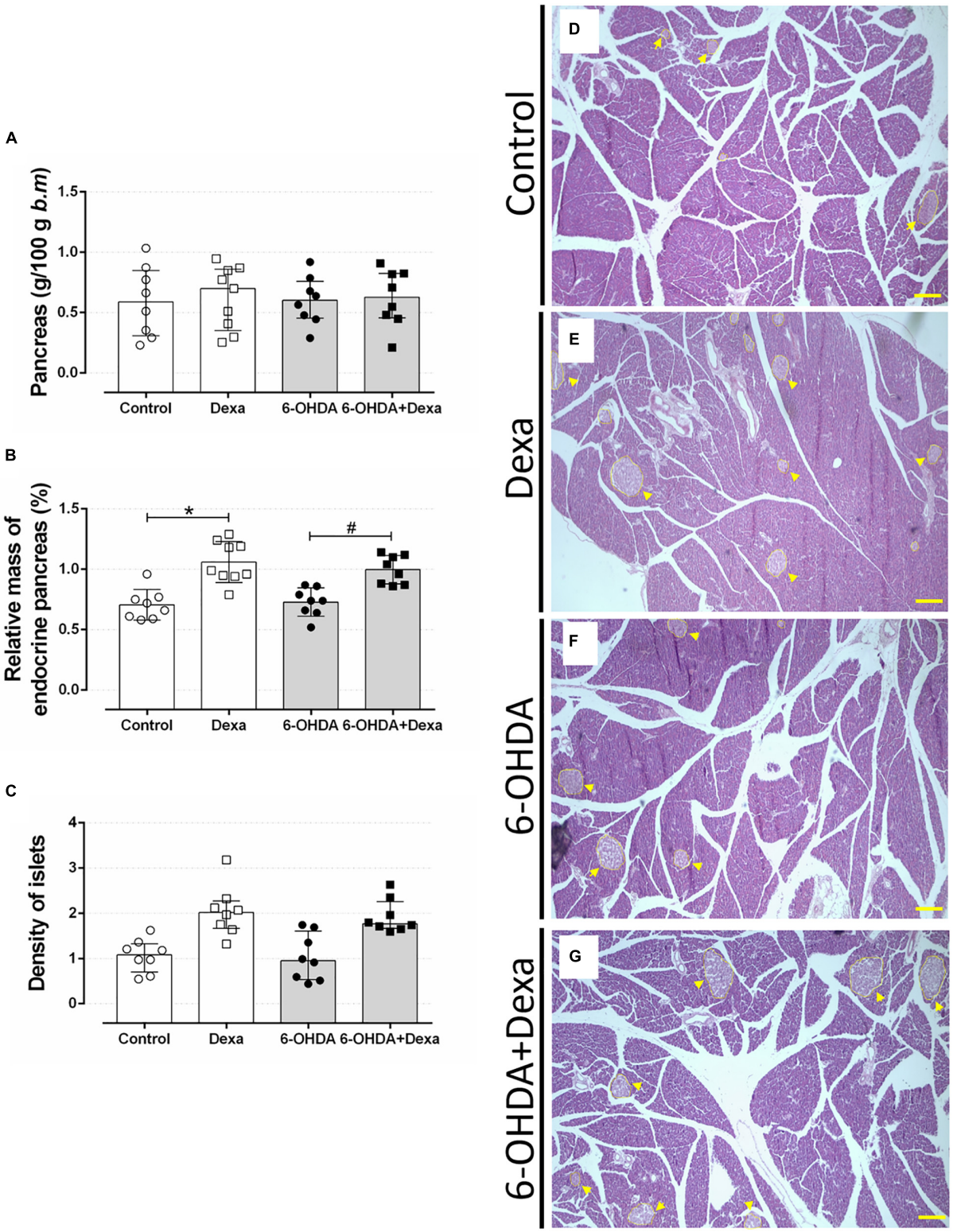

FIGURE 11 | Mass of endocrine pancreas. **(A)** The relative pancreas mass, **(B)** relative pancreatic islet mass, and **(C)** the islet density (islet number per 1000 μm^2 pancreas section) at the day of euthanasia on the 38th day of the experimental protocol. Data are mean ± SD. * and # Indicate a significant difference compared to the respective Control and 6-OHDA groups using ordinary two-way ANOVA with Tukey's *post hoc* test. $n = 8–9$, $p < 0.05$. **(D–G)** representative pancreas sections from all groups in 40x magnitude stained with Hematoxylin and Eosin. $n = 8–9$. Scale bar in **(D–G)** represents 200 μm. Yellow arrowheads are pointing to pancreatic islets.

of neural circuits that regulate autonomic function. Reduction in both body mass and food intake is a well-known adverse effect of high GC doses, as previously demonstrated in rats (Giozzet et al., 2008; dos Santos et al., 2014; Protzek et al., 2014). Numerous mechanisms by which GCs act on body mass and appetite includes: (i) suppression of osteoblastogenesis, as well as apoptosis of osteoblasts, decreasing bone density (Manolagas and Weinstein, 1999); (ii) increase of lipolysis in adipose tissue and catabolic effect on muscle tissue (Franco-Colin et al., 2000); (iii) decrease of expression of the orexigenic peptides NPY and AgRP (Liu et al., 2011); (iv) increase of circulating anorexigenic hormones insulin and leptin (Caldefie-Chézet et al., 2001; Giozzet et al., 2008; dos Santos et al., 2014; Protzek et al., 2014); and (v) by a negative water balance (Thunhorst et al., 2007).

Glucose tolerance and insulin sensitivity remained unaltered during the entire experimental protocol, despite the presence of consistent motor dysfunction caused by 6-OHDA administration (**Figures 6–8**). Some studies suggested the possible association between PD and impairment in insulin signaling. A study with rats submitted to the unilateral 6-OHDA administration of 12 μg in SNpc demonstrated an increase in serine phosphorylation of the insulin receptor substrate 2 that parallels with a depletion of dopamine (White, 2002; Morris et al., 2008). However, functional parameters reflecting the balance of glucose metabolism were not investigated by the authors. The presence of motor dysfunction caused by 6-OHDA did not exacerbate the known adverse effects of GC on glucose tolerance and insulin sensitivity in our study (**Figures 6–8**). The negative action of GC on glucose tolerance and in peripheral insulin sensitivity has been described in several clinical and preclinical studies (for a detailed review, refers to Pasieka and Rafacho, 2016). GCs treatment may alter glucose homeostasis through elevation of hepatic glucose output, reduction of skeletal muscle glucose uptake and an increase of adipose tissue lipolysis (Pasieka and Rafacho, 2016). Decreased insulin sensitivity is commonly accompanied by an increase in plasma insulin concentrations and an increase in the relative mass of pancreatic islets (Kahn et al., 1993), events observed in our GC-treated rats (**Figures 9**, **11**). Although we did not evaluate the *in vivo* insulin secretion under ipGTT experiment, the suggestive insulin hypersecretion observed by our hyperinsulinemic rats was not enough to prevent glucose intolerance and this is supported by previous studies (Giozzet et al., 2008; Angelini et al., 2010; Protzek et al., 2014; dos Santos et al., 2014), an effect that was not exacerbated in 6-OHDA-treated rats.

The impairment in glucose homeostasis may be followed by an imbalance in lipid homeostasis. Consistent with the diabetogenic effects of GCs, dexamethasone-treated rats had increased levels of circulating triacylglycerides, with a parallel increase in hepatic fat content (**Figures 9B**, **10A** respectively). There is evidence demonstrating that GCs may increase circulating and hepatic triacylglycerol levels by inhibiting lipoprotein lipase in adipose tissue (Franco-Colin et al., 2000) and upregulating hepatic lipogenic enzymes (Motta et al., 2018). The presence of motor dysfunctions seemed to be innocuous on these lipidic measures. The relative masses of metabolic organs, except for the hypotrophy of adrenal gland mass in Dexa + 6-OHDA group, were unaltered and the perfusion process may have masked the visualization of these changes since these parenchymas retained fixative solution used for the perfusion procedure.

One of the limitations of our study resides in the fact that we did not appreciate the TH distribution or quantification by immunohistochemistry or western blot experiments, respectively. However, there is evidence for a sustained lesion in the striatum and in the SNpc up to 8 weeks after unilateral (Perlbarg et al., 2018) or bilateral (Oliveira et al., 2017; Fernandes-Junior et al., 2018) administration of 6-OHDA in rats. No motor function parameters were evaluated in these aforementioned studies demonstrating that not all studies include both analyses (motor analysis and TH immunostaining) to confirm the effectiveness of 6-OHDA administration.

The relationship between neurodegeneration and metabolic outcomes are complex and is difficult to predict which comes first. This crosstalk is influenced by numerous factors, which turn difficult to precisely define the causal factor(s). For instance, there is evidence linking the severity of neurodegeneration caused by 6-OHDA with a previous metabolic status (i.e., diet-induced obesity) in rats (Morris et al., 2010). Similarly, there is also evidence linking the inflammation of hypothalamus triggered by Aβ oligomers infusion with posterior disruption of metabolic homeostasis (Clarke et al., 2015), suggesting a relationship between central and peripheral components. With our current experimental design, we defined the independent factor (treatment with 6-OHDA) and then considered all metabolic outcomes as dependent variables, and by doing this we tried to isolate as much as possible the causal factor.

Recent evidence demonstrated the relationship between metabolic disorders and consequent negative prognostic for PD. For instance, coexistence of T2DM with PD lead to a faster motor progression and cognitive decline (Pagano et al., 2018) as well as reduction in the baseline dopamine transport availability in the caudate and ventral striatum, cortical thinning in the inferomedial temporal lobe that is also associated with reduced cognitive performances (Chung et al., 2018) when compared to patient with only PD. In addition, the content of the tau and α-synuclein in the cerebrospinal fluid is higher in patients with both PD and T2DM in relation to patients only with PD, and these markers are already upregulated in T2DM patients compared with healthy controls (Pagano et al., 2018), indicating a relationship between PD-pathology markers and T2DM. This mutual relationship is reinforced when considering the benefits of some antidiabetic drugs (i.e., agonists of glucagon-like peptide-1 receptor and inhibitors of dipeptidyl peptidase-4) exerts on the neurodegenerative process in patients with PD (Ashraghi et al., 2016).

A recent study conducted by Ter Horst et al. (2018) revealed that bilateral deep brain stimulation targeting the border of the nucleus accumbens (NAc) core and ventral anterior limb of the internal capsule ameliorates some metabolic aspects of patients with diabetes (i.e., improves the peripheral insulin sensitivity). This study also demonstrated that pharmacological dopamine deletion decreases insulin sensitivity, whereas optogenetic activation of NAc dopaminergic-receptor-1 neurons improves glucose tolerance in mice, suggesting that striatal dopamine signaling plays a role in glucose metabolism.

CONCLUSION

Our findings let us conclude that bilateral 6-OHDA administration in the dorsolateral striatum causes sustained motor impairments with no impact on glucose and lipid homeostasis and does not exacerbate the adverse effects caused by excess GC in glucose and lipid metabolism. These observations suggest that on this two-way road between PD and peripheral metabolism, it is probable that dopaminergic circuit dysfunction has a minor impact on metabolic outcomes.

DATA AVAILABILITY STATEMENT

The datasets generated to support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

AR, EM, FG, and RP contributed to the experimental design. FG, FdS, MB, and RF conducted the experiments. AR and FG contributed with analytic tools and data analysis, performed the data collection, and wrote up the manuscript. AR, DZ, and RP supplied reagents and materials. AR, EM, and FG contributed to the discussion of the experimental findings. All authors read and approved the manuscript's final format.

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REVIEW
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The Role of Leptin and Adiponectin in Obesity-Associated Cognitive Decline and Alzheimer's Disease

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Cross-talk between adipose tissue and central nervous system (CNS) underlies the increased risk of obese people to develop brain diseases such as cognitive and mood disorders. Detailed mechanisms for how peripheral changes caused by adipose tissue accumulation in obesity impact the CNS to cause brain dysfunction are poorly understood. Adipokines are a large group of substances secreted by the white adipose tissue to regulate a wide range of homeostatic processes including, but not limited to, energy metabolism and immunity. Obesity is characterized by a generalized change in the levels of circulating adipokines due to abnormal accumulation and dysfunction of adipose tissue. Altered adipokine levels underlie complications of obesity as well as the increased risk for the development of obesity-related comorbidities such as type 2 diabetes, cardiovascular and neurodegenerative diseases. Here, we review the literature for the role of adipokines as key mediators of the communication between periphery and CNS in health and disease. We will focus on the actions of leptin and adiponectin, two of the most abundant and well studied adipokines, in the brain, with particular emphasis on how altered signaling of these adipokines in obesity may lead to cognitive dysfunction and augmented risk for Alzheimer's disease. A better understanding of adipokine biology in brain disorders may prove of major relevance to diagnostic, prevention and therapy.

Keywords: adipokine, leptin, adiponectin, obesity, Alzheimer's disease, cognitive decline

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INTRODUCTION

Lifestyle, nutrition and lack or inefficient exercise to control body weight is making the global population more susceptible to obesity. Food with high sugar and carbohydrate contents are in general cheaper and more readily available for the current busy lives. These factors contribute to a widespread epidemic of overweight and obesity, metabolic disorders characterized by the accumulation of peripheral and/or visceral adipose tissue (Hendrickx et al., 2005; Hill et al., 2012). Obesity is a well established risk factor for a number of other chronic disorders, including type 2 diabetes, cardiovascular diseases, arthritis and some types of cancer (Elias et al., 2005; Haslam and James, 2005; Lengyel et al., 2018). More recently, attention has been drawn to the impact of obesity on central nervous system (CNS) functioning, as growing evidence indicate that the obese population are more susceptible to some neurological conditions. Overweight and, in particular, central obesity during midlife have been associated to a higher risk of developing

cognitive disorders, including Alzheimer's disease (AD), later in life (Kivipelto et al., 2005; Rosengren et al., 2005; Whitmer et al., 2005, 2008). In addition, overweight and obese individuals are at higher risk for developing mood disorders, such as major depression disorder (MDD) and bipolar disorder (BD) (Luppino et al., 2010; reviewed in Mather et al., 2009; Vogelzangs et al., 2010; Vannucchi et al., 2014; Mansur et al., 2015). These evidences indicate that changes in the organism that accompany overweight and obesity can ultimately lead to CNS dysfunction. However, the pathophysiological mechanisms and molecular players underlying this connection are poorly known.

The obese body accumulates adipose tissues broadly classified in white (WAT) and brown adipose tissue (BAT) (Caron et al., 2018). Initially considered a fat storage organ, it is now established that the WAT is an endocrine organ secreting a group of substances that act in a pleiotropic manner exerting autocrine, paracrine or endocrine effects on processes in the periphery and CNS (Ahima, 2012). This class of WAT-derived substances are named adipocytokines or adipokines. Adipokines comprehend a wide range of molecules including hormones, cytokines and growth factors (Lehr et al., 2012), and exert a variety of distinct functions in the organism ranging from control of metabolism homeostasis to immune system regulation and behavior (Kiliaan et al., 2014; Fasshauer and Blüher, 2015). Abnormal production and secretion of adipokines resulting from aberrant accumulation of WAT in obesity leads to dysregulation of important homeostatic systems, resulting in the complications of obesity and in an increased risk for comorbidities such as insulin resistance and type-2 diabetes, hypertension, atherosclerosis and other cardiovascular diseases, and neurological disorders such as depression and AD (Kiliaan et al., 2014).

In the following sections, we review the literature for studies on the role of adipokines as the possible mediators of signals from the periphery to the CNS in obesity-associated brain dysfunctions. Specifically, we will focus on two of the most abundant and well studied adipokines – leptin and adiponectin – and their interaction with cognitive processes of the CNS in health and disease.

ADIPOKINES IN OBESITY AND RELATED DISEASES

Disproportional accumulation of white adipose tissue in overweight and obesity is accompanied by a generalized change in the circulating levels of several adipokines. Adipose dysfunction and adipokine dysregulation are thought to be responsible for or, at least, be an important contributor to the increased risk of obese people to develop a number of related diseases. For instance, increased levels of proinflammatory adipokines such as interleukin (IL)-1β, IL-6, TNFα and leptin, and decreased levels of anti-inflammatory adipokines, such as adiponectin, in obesity produce a chronic state of low-grade inflammation which promotes the development of insulin resistance and type-2 diabetes, hypertension, atherosclerosis and other cardiovascular diseases, and some types of cancer (Friedemann et al., 2012; Odegaard and Chawla, 2013; Hotamisligil, 2017). Moreover, since adiponectin also acts as an insulin-sensitizing hormone in muscle and liver, lower levels of adiponectin further contribute to peripheral insulin resistance in obesity (Liu et al., 2016; Saltiel and Olefsky, 2017). Lastly, increased circulating levels of leptin in obesity lead to hypothalamic leptin resistance, turning down anorexigenic and energy expenditure signals and further contributing to aggravate obesity (Waterson and Horvath, 2015).

The CNS is not exempt from negative effects of obesity, as adipose dysfunction associated with obesity have been linked to altered brain metabolism, neuroinflammation, neuronal dysfunction, brain atrophy, impaired mood and cognitive decline (Luppino et al., 2010; Ahima et al., 2017; Arshad et al., 2018). Studies associating obesity to morphometric changes in brain structure are somehow controversial. While the vast majority demonstrates obesity to be associated with lower gray matter and whole brain volumes (Pannacciulli et al., 2006; Gunstad et al., 2007; Raji et al., 2010; Yokum et al., 2011; Marqués-Iturria et al., 2013; Veit et al., 2014), fewer publications showed no association (van Boxtel et al., 2007; Sharkey et al., 2015). This controversy has been recently addressed in a meta-analysis study which found obesity to be consistently associated with lower gray matter volumes in brain areas associated with executive functions, including medial prefrontal cortex, left temporal lobe and bilateral cerebellum. These findings were further validated in the same study in an independent dataset (García-García et al., 2018).

Obesity has also been linked to cognitive disorders. Obese individuals are under greater risk to develop age-related cognitive decline, vascular dementia, mild cognitive impairment (MCI) and AD (Frisardi et al., 2010). Furthermore, animal models of obesity also develop cognitive decline (Winocur et al., 2005; Kleinert et al., 2018; McLean et al., 2018). Mechanisms proposed to underlie obesity-associated risk for cognitive disorders include development of brain inflammation (Nguyen et al., 2014; Heneka et al., 2015) and central insulin resistance (de la Monte et al., 2009; Kim and Feldman, 2015). Peripheral inflammation in obesity results from secretion of proinflammatory cytokines by adipocytes and adipose tissue-resident activated macrophages (Lee and Lee, 2014). Proinflammatory cytokines such as TNFα, interleukin (IL)-1β and IL-6, have been shown to cross the blood–brain barrier (BBB) (Banks, 2005) and may act in concert with proinflammatory factors produced locally by microglial cells to foster brain inflammation in AD (De Felice and Ferreira, 2014). Importantly, such cytokines has been shown to modulate synaptic plasticity and cognition both in health and disease states (Nelson et al., 2012; Gruol, 2015; Rizzo et al., 2018). Moreover, as occurs in peripheral tissues in obesity and type 2 diabetes, proinflammatory cytokines, in particular TNFα, mediate the development of neuronal insulin resistance (Bomfim et al., 2012; Lourenco et al., 2013). Since both insulin and cytokine signaling in the brain regulate synaptic plasticity, learning and memory, neuroinflammation and neuronal insulin resistance may be key mediators of obesity-associated cognitive decline (reviewed in De Felice and Ferreira, 2014).

The increased risk for obese individuals to develop CNS pathology reflects the capacity of adipose tissue to communicate

with the brain and impact brain function. It is not clear yet how this cross-talk occurs, but growing evidence indicate that adipokines are involved. Adipokines may impact brain physiology through different mechanisms. Some adipokines such as leptin and TNFα can cross the BBB and act directly in the brain while other adipokines would act on endothelial brain cells, regulating BBB permeability and the access of other circulating mediators into the brain. Importantly, in pathological states such as inflammation, the BBB integrity is compromised allowing the penetration of adipokines and other substances to which the brain is normally inaccessible. Finally, local expression of adipokines such as leptin and adiponectin have been reported in the mammalian brain (Denver et al., 2011; Thundyil et al., 2012). Advances on adipokine research have been providing information to understand how obesity affect brain function to cause brain atrophy, cognitive dysfunction, mood disorders and increase the risk for neurological diseases.

In the following sections, we will examine key aspects of leptin and adiponectin – the two most abundant and well studied adipokines – regarding their roles in brain physiology and their involvement in obesity-related cognitive dysfunction, dementia and AD (**Figure 1**).

LEPTIN

Leptin in the Brain

The discovery of leptin in 1994 is considered the cornerstone for adipokine research (Zhang et al., 1994). Leptin is classically related to the central control of food intake and energy homeostasis (Friedman, 2016). However, other neurophysiological functions have been attributed to leptin, including brain development (Ahima et al., 1998; Bouret, 2010) neurogenesis (Garza et al., 2008), neuronal protection (Doherty et al., 2013), mood and stress regulation (Lu et al., 2006; Paz-Filho et al., 2010), reproduction and reproductive behaviors (Caprio et al., 2001; Lu et al., 2006; Chehab, 2014).

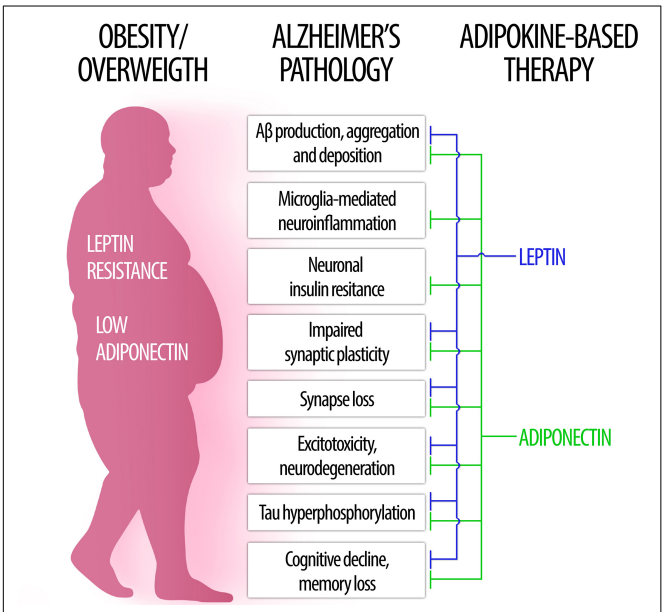

FIGURE 1 | The role of leptin and adiponectin in Alzheimer's disease physiopathology. (Left) In obesity, low levels of circulating adiponectin as well as central leptin resistance may contribute to brain pathology and increased risk for Alzheimer's disease. (Right) Conversely, therapeutic approaches based on leptin (blue) and adiponectin (green) signaling may counteract a wide range of pathological processes associated to AD (center).

Leptin is mainly produced by adipose tissue, more specifically by visceral white adipocytes in rodent and by subcutaneous adipose tissue in human (Trayhurn et al., 1995; Hube et al., 1996). However, local expression of leptin mRNA and protein in CNS has been reported (Morash et al., 1999; Scott et al., 2009; Patterson et al., 2011). Circulating levels of leptin are dynamic and susceptible to different regulatory factors such as metabolism, body fat mass, circadian cycle and sexual dimorphisms (Trayhurn et al., 1995; Licinio et al., 1997; Saad et al., 1997; Park and Ahima, 2015). Leptin crosses the BBB by binding to specific receptors or interacting with BBB. The partially saturate influx of leptin through BBB indicates that membrane proteins facilitate leptin uptake where the entrance is limited by transport dynamics (Banks et al., 1996). There is evidence that leptin transport into the brain can be mediated by megalin at the choroid plexus epithelium (Dietrich et al., 2008; Bartolome et al., 2017) and by short LepR isoforms in tanycytes (Hileman et al., 2002; Balland et al., 2014; Di Spiezio et al., 2018). Therefore, levels of leptin in the brain are subject to regulation by local leptin production, circulating leptin levels and leptin transport across BBB through different mechanisms.

Leptin receptors (LepR or ObR) were first detected in 1995 at the mouse choroid plexus (Tartaglia et al., 1995). Posterior studies revealed that alternative splicing during LepR-coding *db* gene expression result in six different LepR isoforms (LepRa-f) (Lee et al., 1996; Wang et al., 1996). Long leptin receptor (LepRb) is the isoform which best bind to leptin and activate intracellular pathways (Allison and Myers, 2014). LepRs are tyrosine-kinase receptors that undergo autophosphorylation upon leptin binding to activate different signaling cascades, including JAK/STAT, ERK/MAPK and IRS/PI3K pathways. STAT (signal transducer and activator of transcription) is a family of transcription factor which, upon phosphorylation, migrate to the nucleus and regulate transcription of target genes such as the suppressor of cytokine signaling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B). STAT3 is the major signaling pathway recruited by leptin and, due to its responsive property, STAT3 phosphorylation became an alternative way to identify leptin-responsive cells and infer leptin sensitivity (Ramos-Lobo and Donato, 2017; Pan and Myers, 2018).

LepRb is the main isoform of leptin receptor expressed in the brain. Robust expression comprehends the arcuate nucleus (ARC), the paraventricular nucleus and the dorsomedial, lateral and ventromedial regions of the hypothalamus (Schwartz et al., 1996; Wauman and Tavernier, 2011; Mercer et al., 2018). Extrahypothalamic sites also express leptin receptors including hippocampus, cortex, midbrain and hindbrain (Elmquist et al., 2005; Patterson et al., 2011). A transgenic mouse expressing LepRb-cre in combination with cre-inducible enhanced green

fluorescent protein (EGFP) and farnesylated EGFP (EGFPf) was used to reveal a detailed distribution of LepRb expressing neurons as well as their projection sites throughout the brain (Patterson et al., 2011). Regions found to contain LepRb expressing neurons were consistent with those previously determined by examination of LepRb mRNA expression via *in situ* hybridization (Elmquist et al., 1998), but this study further identified projections sites of LepRb neurons in the brain. LepRb neurons and projection were observed throughout the prefrontal cortex, while more discrete, circumscribed regions containing both LepRb neurons and projections were found in insular, temporal, auditory, somatosensory and visual cortices (Patterson et al., 2011). The hippocampus displayed few LepRb neurons along with projections from LepRb neurons mostly concentrated in CA1–3 regions and, to a lower extent, in the dentate (Elmquist et al., 2005; Mercer et al., 2018).

The wide variety of leptin effects in the CNS likely reflects the peculiar distribution of leptin receptors through different brain structures as well as the particular signaling cascades activated by these receptors in distinct regions (Bjørbaek and Kahn, 2004).

In the following sections, we review key aspects of brain leptin signaling that are relevant for its role in cognitive decline and AD (**Table 1**).

Leptin in Synaptic Function and Memory

Synaptic plasticity is a crucial event for learning and memory process. Long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission are pivotal mechanisms in hippocampal memory formation and consolidation (Bliss and Collingridge, 1993). At the molecular level, LTP and LTD are regulated by insertion or removal of NMDA- and AMPA-type glutamate receptors from post-synaptic terminals, thereby modulating synaptic transmission strength (Malenka and Bear, 2004).

There is substantial evidence indicating that leptin regulates hippocampal synaptic plasticity and memory. LepRs in hippocampal neurons are closely associated with somato-dendritic and synaptic regions, indicating the potential for leptin to modulate synaptic function (Harvey, 2015). Recent studies demonstrate that leptin activation of JAK2/STAT3 signaling pathway induces transcription of genes related to LTD (McGregor et al., 2017). Moreover, learning and memory formation are modulated by JAK2/STAT5 signaling (Furigo et al., 2018), a pathway also subject to regulation by leptin receptor signaling in the hippocampus (Gong et al., 2007). Two different LepR-deficient rodent strains (Zucker rats and db/db mice) show impaired LTP in the CA1 region of the hippocampus, which is accompanied by poor performance in the water maze spatial memory test (Li et al., 2002). Conversely, leptin treatment has been shown to improve learning and memory performances in different models (Shanley et al., 2001; Oomura et al., 2006; reviewed in Irving and Harvey, 2013).

Leptin was shown to regulated synaptic plasticity by targeting NMDA- and AMPA-receptors trafficking, particularly in the CA1 region of the hippocampus. Schaffer collaterals (SCs) and temporoammonic (TA) projections to CA1 pyramidal neurons exhibit an age-dependent response to leptin. Interestingly, these functionally distinct circuits appear to respond differently to leptin exposure. In SC-CA1 synapses, leptin was shown to induce depression of synaptic transmission in young rodents

TABLE 1 | Summary of the main findings associating leptin signaling to physiopathological processes relevant to AD.

Finding	Model(s)	Reference
Leptin signaling modulates synaptic function and plasticity	Hippocampal slices from adult rats	Shanley et al., 2001; Xu et al., 2008; Moult et al., 2009, 2010; Luo et al., 2015; McGregor and Harvey, 2017; McGregor et al., 2018
LepR-deficiency causes impairment of LTP and spatial memory	Zucker rats and db/db mice	Li et al., 2002
Leptin treatment improves learning and memory	Hippocampal slices from adult rats; rats ICV-infused with Aβ; AD mouse models (SAMP-8 mice; CRND8 transgenic mice (TgCRND8) and APP/PS1	Shanley et al., 2001; Farr et al., 2006; Oomura et al., 2006; Greco et al., 2010; Pérez-González et al., 2014; Tong et al., 2015
Deficient leptin transport across BBB	Aged mice, APP/PS1 mice	Carro et al., 2005; Dietrich et al., 2008
PTP1B upregulation	APP/PS1 mice; Human AD patients	Pei et al., 1994; Mody et al., 2011; King et al., 2018
SOCS3 upregulation	APP/PS1 mice; human AD patients	Walker et al., 2015; Iwahara et al., 2017; King et al., 2018
Impaired leptin signaling	Human AD patients; Tg 2576 mice; APP/PS1 mice	Bonda et al., 2014; Maioli et al., 2015
Decreased leptin levels in CSF and plasma	Human AD patients	Holden et al., 2009; Lieb et al., 2009; Bigalke et al., 2011; Chakrabarti, 2014; Johnston et al., 2014; Khemka et al., 2014b; Baranowska-Bik et al., 2015
Increased leptin levels in CSF and brain	Human AD patients	Bonda et al., 2014; Yin et al., 2018
Unchanged leptin levels in CSF and brain	Human AD patients	Maioli et al., 2015; Oania and McEvoy, 2015
Aβ disrupts leptin signaling	Human AD patients; rabbit hippocampal slices	Marwarha et al., 2010; Bonda et al., 2014
Leptin reduce Aβ levels	SH-SY5Y cells; Neuro2a cells; primary neuronal cultures from rat embryos; APP/PS1 mice; adult rats	Fewlass et al., 2004; Pérez-González et al., 2014; Tong et al., 2015
Leptin reduce tau hyperphosphorylation	SH-SY5Y cells; NT2 cells; primary rat cortical neurons	Greco et al., 2008

hippocampus (Xu et al., 2008; Moult et al., 2009) whereas, in adult animals, leptin promote synaptic strengthening and LTP (Moult et al., 2010; Moult and Harvey, 2011). Different effects were observed in TA-CA1 synapses, where leptin induces LTP in young (Luo et al., 2015), promote LTD in the adult but had no effect on synaptic transmission in aged rat hippocampus (McGregor et al., 2018). Importantly, the age- and regional-variability of leptin effects on hippocampal synaptic plasticity appears to be driven by the composition of NMDARs subtypes, and to be achieved by regulating the traffic of different subsets of AMPARs toward and away from synapses. Leptin regulation of synaptic plasticity involves several downstream targets, including JAK2-STAT3, PTEN, PI3K, ERK, and CaMKII (for more comprehensive reviews, see Moult and Harvey, 2009; McGregor and Harvey, 2017).

Leptin Resistance

Several studies demonstrated that leptin responsiveness decreases with obesity, aging and neurodegenerative diseases, a phenomenon called leptin resistance. Leptin resistance affects a range of processes such as food intake, insulin sensitivity, inflammation and cognition. In obesity, leptin resistance leads to increased production of leptin by adipocytes and hyperleptinemia, in an attempt of the organism to compensate for low leptin responsiveness. Decreased leptin signaling in the CNS may be related to defective leptin transport across BBB, LepR downregulation and/or deficient leptin signaling downstream LepRs (Myers et al., 2008, 2012; de Git and Adan, 2015; Bluher, 2016; Banks et al., 2018).

Triglycerides can impair BBB leptin transport causing central leptin deficiency (Banks et al., 2004). Furthermore, it was recently demonstrated that triglycerides can cross the BBB to directly induce hypothalamic leptin and insulin receptor resistance, leading to decreased satiety and cognitive impairment in mice (Banks et al., 2018). Interestingly, triglycerides increased leptin binding in different brain regions, suggesting an allosteric or post-receptor rather than a competitive mechanism of inhibition of LepR signaling by triglycerides (Banks et al., 2018). In light of longitudinal studies linking increased mid-life triglyceride levels to the risk for AD (Vemuri et al., 2017; Nägga et al., 2018), the above results suggest that triglycerides may contribute to AD pathogenesis and progression by suppressing leptin signaling in the brain. Deficient leptin transport across BBB by megalin leading to reduced leptin entry into the brain has also been described in aged mice and in mouse models of AD (Carro et al., 2005; Dietrich et al., 2008).

At the intracellular level, leptin signaling is negatively regulated by the suppressor of SOCS3 and by the PTP1B. SOCS3 binds to LepR and JAK2 to inhibit their activities, whereas PTP1B dephosphorylates tyrosine residues deactivating LepR and JAK2. PTP1B have been linked to central leptin resistance in humans (Myers et al., 2010) as well as in a variety of animal models of obesity (Cheng et al., 2002; Zabolotny et al., 2002; White et al., 2009) and aging (Morrison et al., 2007). SOCS3 and PTP1B were also found upregulated in the brains of AD mouse models (Mody et al., 2011; Iwahara et al., 2017; King et al., 2018) and AD patients (Pei et al., 1994; Walker et al., 2015). Therefore, targeting PTP1B and SOCS3 may prove valuable to overcome central leptin resistance in obesity, aging, and AD (Engin, 2017; Vieira et al., 2017).

Central leptin resistance can also be mediated by downregulation of LepRs expression. In this regard, LepR levels were found to be decreased in the hippocampus of AD patients, whereas leptin levels were upregulated both in CSF and locally in the hippocampus, possibly due to a compensatory mechanism for receptor dysfunction (Bonda et al., 2014; Maioli et al., 2015). Age-dependent changes in LepR expression levels were also reported in Tg2576 and APP/PS1 mouse models of AD (Maioli et al., 2015; King et al., 2018). Although the results from these studies are hard to interpret, they are consistent with an impact of AD phenotypes in LepR expression in brain areas relevant to cognition and memory.

Taken together, the results described above indicate that impaired brain leptin signaling may play a role in AD pathophysiology, and that restoring leptin signaling may constitute a valid approach to restore synaptic function and cognition in AD.

Leptin Signaling, Obesity, and Alzheimer's Disease

Several studies indicate that over-weight and obesity during mid-age increases risk to develop AD (Whitmer et al., 2008; Hassing et al., 2009; Xu et al., 2011; Gustafson et al., 2012; Emmerzaal et al., 2014; Alhurani et al., 2016). In contrast, high weight in late-life was shown to be protective against AD and cognitive decline (Hughes and Ganguli, 2009; Gustafson et al., 2012; Emmerzaal et al., 2014; Bell et al., 2017). Surprisingly, weight loss in late age is related to higher risk for dementia and AD (Barrett-Connor et al., 1996; Buchman et al., 2005; Stewart et al., 2005; Johnson et al., 2006; Hughes and Ganguli, 2009; Gao et al., 2011; Joo et al., 2018). This apparently paradoxical influence of body weight on dementia and AD risk is far from being understood. Interestingly, a similarly complex relation is observed between overweight/obesity and other neurodegenerative diseases. For instance, in amyotrophic lateral sclerosis (ALS) patients, mild obesity is associated with increased survival rates, whereas morbid obesity increases mortality rates (Paganoni et al., 2011; Gallo et al., 2013). The influence of body weight on the clinical outcomes of dementia, AD and other neurodegenerative diseases suggest that adipose tissue dysfunction and adipokines dysregulation may play a broad role across the spectrum of neurodegenerative diseases. Moreover, the explanation for this puzzling and somehow paradoxical influence of adiposity on brain diseases may lie in how weight changes impact production and action of adipokines such as leptin and adiponectin.

Evidence regarding how leptin levels are affected in AD are controversial. Several studies show decreased leptin levels in CSF and plasma in AD patients (Holden et al., 2009; Lieb et al., 2009; Bigalke et al., 2011; Chakrabarti, 2014; Johnston et al., 2014; Khemka et al., 2014b; Baranowska-Bik et al., 2015) whereas increased leptin levels (Bonda et al., 2014; Yin et al., 2018) and unaffected levels (Maioli et al., 2015; Oania and McEvoy, 2015) in CSF and cerebral tissue were also reported. Conflicting findings

can be associate to variations in dementia score, post-mortem neuropathological analyses to confirm dementia, sample size, stratification in sex, age, and others (Kiliaan et al., 2014; Mcguire and Ishii, 2016).

Leptin signaling was found to interact with several mechanisms associated with AD physiopathology. Aβ disrupt leptin signaling leading to down-regulation of hippocampal leptin and LepR expression (Marwarha et al., 2010; Bonda et al., 2014). Conversely, leptin was reported to be neuroprotective in AD models by suppressing Aβ accumulation and toxicity and attenuating tau pathology. Leptin administration reduce Aβ levels in the Tg2576 mouse model of AD (Fewlass et al., 2004). The anti-amyloidogenic effect of leptin involve inhibition of APP processing by down regulating β-amyloid precursor protein (APP) cleaving enzyme (BACE1) and increase in APOE-dependent Aβ uptake, and seems to be mediated by activation of AMP-activated protein kinase (AMPK) (Fewlass et al., 2004; Marwarha et al., 2010; Pérez-González et al., 2014; Tong et al., 2015).

Tau phosphorylation can be suppressed by leptin modulation of GSK3β activity (Greco et al., 2008). Importantly, leptin treatment also improve memory performance in different mouse models of AD (Farr et al., 2006; Greco et al., 2010; Pérez-González et al., 2014). In a rat model of intracerebroventricular Aβ injection, chronic leptin administration rescued Aβ-induced impairment of spatial memory and late-phase LTP (Tong et al., 2015). Leptin was further shown to enhance hippocampal neurogenesis in AD mice (Pérez-González et al., 2011). Collectively, the above studies provide initial validation for the potential therapeutic applications of leptin signaling enhancement in AD brains.

ADIPONECTIN

Adiponectin is a 30 KDa adipokine encoded by the AdipoQ gene, mainly produced and secreted by adipocytes and highly abundant in human plasma. Adiponectin is known to increase insulin sensitivity of target organs such as liver and muscle, ultimately regulating peripheral glucose and fatty acid metabolism (Hotta et al., 2001; Yamauchi et al., 2001, 2002). Besides being a metabolic regulator, adiponectin is also known for its anti-inflammatory and anti-oxidant activity (Takemura et al., 2007; Liu Y. et al., 2015). These characteristics make adiponectin a protective factor in conditions such as obesity, type 2 diabetes and cardiovascular diseases (Yamauchi et al., 2003a; Spranger et al., 2006; Antoniades et al., 2009). Levels of circulating adiponectin are decreased in obesity and metabolic syndrome, likely contributing to the development of insulin resistance (Arita et al., 1999; Hotta et al., 2001; Yang et al., 2001). Low levels of adiponectin have also been linked to several types of cancer (Miyoshi et al., 2003; Bao et al., 2013; Ma et al., 2016) and cardiovascular diseases (Komatsu et al., 2004).

Adiponectin naturally self-associate to form different types of aggregates. In plasma, adiponectin exists as trimers, hexamers or high molecular weight (HMW) multimers. Adiponectin trimers are mainly stabilized by non-covalent interactions, whereas larger aggregates require crosslinking between subunits by disulfide bonds (Waki et al., 2003). In addition, adiponectin also circulates as biologically active globular fragment generated through proteolysis of full-length adiponectin (Fruebis et al., 2001). Importantly, these post-translational modifications affect biological activity, as distinct adiponectin complexes present tissue-specificity and may activate different signaling pathways (for reviews, see Wang et al., 2008; Liu and Liu, 2014).

Adiponectin acts through binding to three different receptors: adiponectin receptor 1 (AdipoR1), adiponectin receptor 2 (AdipoR2) and T-cadherin. AdipoR1 and AdipoR2 are the most abundant sites for adiponectin binding and mediate most of adiponectin actions through the organism (Yamauchi et al., 2003b, 2007). Activation of AdipoRs by adiponectin leads to the recruitment of the adaptor protein APPL1 (adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1) (Mao et al., 2006) which, in turn, mediates downstream adiponectin signaling through a number of different pathways including AMPK, PI3K-Akt, MAPK-Erk1/2, PPARα, p38-MAPK, PTEN and JNK (Cheng et al., 2007; Chandrasekar et al., 2008; Coope et al., 2008; Lee et al., 2008). APPL1 also plays a crucial role in the insulin sensitization effect of adiponectin by interacting with and facilitating the binding of IRS1/2 to the insulin receptor (Ryu et al., 2014).

Adiponectin in the Brain

Data from epidemiological studies demonstrate that diabetes, obesity, and metabolic syndrome increase the risk of developing cognitive problems and dementia (Stewart and Liolitsa, 1999; Baker et al., 2011; Kerwin et al., 2011; McCrimmon et al., 2012; Lehtisalo et al., 2016; Espeland et al., 2017; Pal et al., 2018; Palta et al., 2018). More recently, evidence indicate that insulin signaling dysfunction and chronic neuroinflammation are key factors in cognitive decline, MCI and AD (Steen et al., 2005; Arnold et al., 2018, reviewed in De Felice, 2013; Ferreira et al., 2014; Heneka et al., 2015). In face of these observations, adiponectin has gained attention in the context of such CNS disorders due to its potentially protective actions as an anti-inflammatory and insulin sensitizing hormone.

Adiponectin is present in human and mouse CSF, albeit in much lower concentrations than in blood (Kubota et al., 2007; Kusminski et al., 2007; Neumeier et al., 2007). However, the source(s) for adiponectin in CNS are not completely clear. Initial studies suggested that adiponectin expression was limited to adipocytes and, to a lower extent, other peripheral tissues such as liver, muscle, placenta, and epithelium. More recently, however, some studies reported adiponectin expression at both mRNA and protein levels in the chicken and mammalian brain (Maddineni et al., 2005; Hoyda et al., 2007; Kaminski et al., 2014; Shen et al., 2014). Whether peripheral adiponectin crosses the BBB to reach the brain is a matter of debate. While some studies conclude that adiponectin does not cross the BBB (Pan et al., 2006; Spranger et al., 2006), other studies found evidence suggesting that peripheral adiponectin does reach the brain through the BBB (Qi et al., 2004; Yau et al., 2014). This is an important open question with physiological and potential translational relevance, since adiponectin-based therapies to treat brain conditions

would possibly consist of peripheral administration and require adiponectin to reach the CNS. Therefore, further studies are required to clarify whether or not peripheral adiponectin can reach the brain tissue through BBB.

Adiponectin receptors are also expressed in different structures of the brain. The hypothalamus is the best documented site for AdipoRs expression in the mammalian brain, but there is also evidence for expression in endothelial brain cells, cerebral cortex, brain stem and hippocampus (Yamauchi et al., 2003b, 2007) reviewed in (Thundyil et al., 2012). Interestingly, a study in post mortem human brains also revealed intense immunostaining for AdipoR1 in the nucleus basalis magnocellularis (NBM) (Psilopanagioti et al., 2009), a small structure rich in cholinergic neurons projecting diffusely to the whole neocortex and other brain areas, and that is severely affected in AD (Liu A.K. et al., 2015).

Adiponectin functions in the brain are highly diversified. It has been shown to act locally in the brain to control key processes of brain physiology including neuronal excitability and synaptic plasticity, neuroprotection, neurogenesis and regulation of glial cells activation (Yau et al., 2014; Chabry et al., 2015; Song et al., 2015; Nicolas et al., 2017; Shah et al., 2017; Pousti et al., 2018). More recently, adiponectin was found to modulate glucose metabolism in hippocampal neurons, increasing glucose uptake, glycolysis and ATP production rates (Cisternas et al., 2018). Adiponectin also acts on brain to regulate peripheral and systemic processes such as thermogenesis, energy expenditure (Qi et al., 2004) and reproduction (Angelidis et al., 2012). Finally, central adiponectin has been shown to regulate behaviors like food intake (Suyama et al., 2016), locomotor activity (Miyatake et al., 2015) as well as cognition, anxiety and mood (Jeong et al., 2012; Zhang et al., 2017; Cao et al., 2018; Cezaretto et al., 2018; Nicolas et al., 2018; Platzer et al., 2018; Sun et al., 2018). This variety of central effects of adiponectin likely reflects the wide distribution of different adiponectin receptors throughout the brain. For the purpose of this review, the following sections will focus on roles of adiponectin that are most likely relevant to cognitive dysfunction and AD, namely synaptic regulation, insulin sensitivity, neuroinflammation, neuroprotection and neurogenesis (**Table 2**).

Adiponectin in Synaptic Function and Memory

Synapse loss is the best pathological correlate for clinical manifestations of cognitive dysfunction in AD (DeKosky and Scheff, 1990; Terry et al., 1991). Compelling evidence indicate that Aβ oligomers, toxins that accumulate in AD brains, target synapses impairing synaptic function and plasticity and causing synapse loss (reviewed in Lambert et al., 1998; Ferreira and Klein, 2011; Forny-Germano et al., 2014) Aβ oligomers inhibit LTP and promote LTD of hippocampal synapses *in vitro* and *in vivo* (Wang et al., 2002; Shankar et al., 2008; Li et al., 2009; Jürgensen et al., 2011).

Interestingly, recent data indicate that adiponectin signaling directly regulates synaptic function and plasticity, preserving and enhancing cognitive functions in a number of different models. Intracerebroventricular administration of adiponectin in anesthetized rats potentiates high frequency stimulation (HFS)-induced LTP and suppresses low-frequency stimulation (LFS)-induced LTD. Furthermore, adiponectin administration alone induced a chemical LTP, independent of presynaptic stimulus (Pousti et al., 2018). Osmotin, a plant-derived homolog of adiponectin capable of activate AdipoRs, improves LTP impairment and ameliorates memory deficits in a mouse model of AD (Shah et al., 2017). This effect appears to be mediated by AdipoR1 and the Nogo66 receptor 1 (NgR1) and involve promotion of neurite outgrowth and increasing of dendritic spine and synapse density in the hippocampus (Zhang et al., 2016; Yoon et al., 2018). Adiponectin-deficient mice displays increased excitability of hippocampal dentate gyrus (DG) granule neurons, associated with impaired extinction of contextual fear memory. Adiponectin and its mimetic drug AdipoRon restored fear memory extinction via AdipoR2 activation and inhibition of DG neuron excitability (Zhang et al., 2017). Aged adiponectin-deficient mice have reduced levels of synaptic proteins suggesting synapse loss, and also performs poorly in spatial memory and contextual fear conditioning tests (Ng et al., 2016). Importantly, both adiponectin and osmotin treatments ameliorate learning and memory deficits in AD animal models (Ali et al., 2015; Shah et al., 2017). Caloric restriction increases circulating adiponectin levels and improve cognition in mice probably via regulation of the AMPK signaling pathway in mouse hippocampus (Ma et al., 2018). Finally, a recent clinical study found that individuals with higher adiponectin levels tend to perform better in a delayed word recall test, supporting the notion that adiponectin is a protective factor against cognitive decline and represents a promising therapeutic strategy in cognitive disorders (Cezaretto et al., 2018).

Collectively, these recent studies consistently established a previously ignored ability of adiponectin to regulate hippocampal synaptic function and plasticity and to improve cognitive function, learning and memory. This further support a protective role for adiponectin in AD, suggesting that diminished brain adiponectin signaling in obesity may favor AD onset and progression and that adiponectin signaling may be an interesting target in AD therapy.

Adiponectin in Central Insulin Signaling

Neuronal insulin signaling is important for synaptic plasticity and memory, mainly by regulating glutamate receptors trafficking (Beattie et al., 2000; Man et al., 2000; Skeberdis et al., 2001; Zhao et al., 2004). Impaired insulin signaling is well documented both in human AD patients and in a variety of AD animal models (Steen et al., 2005; Bomfim et al., 2012; Talbot et al., 2012) and is considered an important mechanism for neuronal dysfunction and cognitive impairment in AD (Ferreira et al., 2014; Vieira et al., 2018) Synaptotoxicity of Aβ oligomers is accompanied by insulin receptor dysfunction *in vitro* and

TABLE 2 | Summary of the main findings associating adiponectin signaling to physiopathological processes relevant to AD.

Finding	Model(s)	Reference
Adiponectin and AdipoRs signaling modulates synaptic function and plasticity	APP/PS1 mice; NSE-APPsw mice; adiponectin-deficient mice; rats	Zhang et al., 2016, 2017; Shah et al., 2017; Pousti et al., 2018; Yoon et al., 2018
Adiponectin deficiency causes AD-like synapse loss and memory impairment	Adiponectin-deficient mice	Ng et al., 2016
Adiponectin signaling improves memory	Adiponectin-deficient mice; APP/PS1 mice; NSE-APPsw mice; Aβ i.c.v.-infused mice	Ali et al., 2015; Shah et al., 2017; Zhang et al., 2017
High adiponectin levels is associated with better cognitive performance	Middle-aged non-diabetic humans	Cezaretto et al., 2018
Adiponectin signaling deficiency leads to impaired brain insulin signaling	Adiponectin knockout mice; AdipoR1 deficient mice	Ng et al., 2016; Kim et al., 2017
Adiponectin improve neuronal insulin sensitivity	Insulin resistant SH-SY5Y human neuroblastoma cells	Ng et al., 2016
Adiponectin deficiency promote neuroinflammation	Adiponectin knockout mice	Ng et al., 2016
Adiponectin signaling attenuates microglia-mediated neuroinflammation	Environment-enriched mice; adiponectin deficient mice; mouse model of intracerebral hemorrhage; brain sorted microglia; primary microglial cells; BV2 microglial cells exposed to Aβ	Chabry et al., 2015; Nicolas et al., 2015, 2017; Song et al., 2017; Zhao et al., 2018
Adiponectin and AdipoRs agonists are neuroprotective	Primary rat hippocampal neurons; SH-SY5Y cells; rodent models of hemorrhagic and ischemic stroke	Jung et al., 2006; Chen et al., 2009; Jeon et al., 2009; Qiu et al., 2011; Chan et al., 2012; Song et al., 2013, 2015; Guo et al., 2015; Wang et al., 2016; Yang et al., 2017; Ma et al., 2018
Adiponectin and AdipoRs agonists promotes neurogenesis	Exercised mice; adiponectin deficient mice; corticosterone-induced anxiety/depressive-like mice	Yau et al., 2014; Chan et al., 2017; Nicolas et al., 2018
Adiponectin and AdipoRs agonists has antidepressive properties	Exercised mice; corticosterone-induced anxiety/depressive-like mice	Yau et al., 2014; Zhang et al., 2016; Chan et al., 2017; Nicolas et al., 2018
Adiponectin signaling deficiency produce AD-like phenotypes	Adiponectin knockout mice; AdipoR1 deficient mice	Ng et al., 2016; Kim et al., 2017
Adiponectin signaling impairment in AD models	APP/PS1 mice	Várhelyi et al., 2017
Adiponectin reduce Aβ production and aggregation	APP/PS1 mice; SH-SY5Y cells overexpressing APP	Shah et al., 2017
Adiponectin attenuates tau hyperphosphorylation	Streptozotocin injected rats	Xu et al., 2018
Altered levels of adiponectin in AD (conflicting results have been reported, see the Section "Adiponectin in Alzheimer's Disease" for details)	Human subjects	Kamogawa et al., 2010; Une et al., 2010; van Himbergen et al., 2012; Teixeira et al., 2013; Khemka et al., 2014a; García-Casares et al., 2016; Ma et al., 2016

in vivo and can be prevented by treatment with insulin sensitizing drugs and by insulin itself (De Felice et al., 2009; Bomfim et al., 2012; Batista et al., 2018). These discoveries encouraged several groups to evaluate the efficacy of different classes of anti-diabetic drugs in AD models, and positive preclinical results paved the way for human clinical trials (for comprehensive reviews, see De Felice, 2013; Yarchoan and Arnold, 2014; de la Monte, 2017). In this context, interest has recently been directed to the insulin-sensitizing actions of adiponectin to correct aberrant insulin signaling in AD. Impaired brain insulin signaling was observed, along with several other AD-like pathological features, in adiponectin knockout mice and in AdipoR1 deficient mice (Ng et al., 2016; Kim et al., 2017). Conversely, adiponectin increases insulin sensitivity in SH-SY5Y neuronal cell line modeling insulin resistance, through AdipoR1 receptor activation of AMPK (Ng et al., 2016). These data indicate that adiponectin has the potential to restore neuronal insulin signaling, with possible therapeutic implications for AD and other neurodegenerative diseases. However, further translational studies using proper animal models of AD are required to validate adiponectin signaling as a therapeutic approach to overcome brain insulin resistance in AD.

Adiponectin in Neuroinflammation

Adiponectin is well known for its anti-inflammatory activity in peripheral tissues, which include suppression of macrophage activation and secretion of pro-inflammatory cytokines (Yokota et al., 2000; Elfeky et al., 2016, 2018). For that, adiponectin is considered a protective factor against pathological processes such as peripheral insulin resistance and cardiovascular diseases, whereas low levels of adiponectin in obesity contributes to chronic inflammation and obesity-associated risk for related diseases (For recent reviews, see Ohashi et al., 2015; Liu et al., 2016).

Alzheimer's disease is also characterized by a chronic state of low-grade inflammation in the brain. This response is mediated by microglial cells activation and secretion of pro-inflammatory cytokines TNFα, IL-6, and IL-1β (Bamberger et al., 2003; Lourenco et al., 2013; Heneka et al., 2015; reviewed in De Felice and Lourenco, 2015; Sarlus and Heneka, 2017). Proinflammatory cytokines trigger a series of detrimental events in the AD brain,

including neuronal insulin resistance, endoplasmic reticulum stress, synaptotoxicity and neurodegeneration (Bomfim et al., 2012; Lourenco et al., 2013; Rizzo et al., 2018).

Adiponectin-knockout mice develop a series of pathological features in the brain resembling AD, including insulin resistance, reduced levels of synaptic proteins and the presence of neuroinflammatory markers such as microgliosis, astrogliosis and elevated levels of the pro-inflammatory cytokines TNFα and IL-1β (Ng et al., 2016). Environmental enrichment, housing conditions that promote physical activity, cognitive engagement and social interactions, has been shown to improve cognitive functions and be protective in AD mouse models. These effects are at least in part due to modulation of microglial response to the insult of Aβ oligomers (Xu et al., 2016; Vieira and Beckman, 2017). Interestingly, it was recently shown that beneficial effects of environmental enrichment to the brain are mediated by adiponectin, and involves the promotion of an anti-inflammatory activation state of microglia with decreased production of pro-inflammatory cytokines (Chabry et al., 2015; Nicolas et al., 2015). The same group also showed that globular adiponectin directly inhibits microglia pro-inflammatory profile *in vivo* and *in vitro* (Nicolas et al., 2017) in a mechanism involving AdipoR1 and NF-κB. Adiponectin also modulates microglial activation profile under Aβ toxicity *in vitro*, via PPARγ activation (Song et al., 2017). Further evidence for the anti-inflammatory actions of adiponectin in the CNS come from a study showing that CTRP9, an AdipoR1 agonist, attenuates neuroinflammation in a mouse model of intracerebral hemorrhage through a AdipoR1/AMPK/NFκB signaling mechanism (Zhao et al., 2018). Moreover, AdipoRon treatment suppresses macrophage recruitment in a model of spinal cord injury (Zhou et al., 2018). Finally, it has been proposed that adiponectin can also modulate neuroinflammation by reducing expression of pro-inflammatory cytokines by brain endothelial cells (Spranger et al., 2006). These evidences support a role for adiponectin in mitigating brain inflammation, and suggest that adiponectin deficiency in obesity may trigger neuroinflammatory events leading to AD and other related CNS disorders.

Adiponectin in Neuroprotection and Neurogenesis

Neurodegeneration in AD is mediated by overactivation of glutamate receptors and excessive neuronal calcium influx, a process called excitotoxicity (Dong et al., 2009; Wang and Reddy, 2017; Arshad et al., 2018). Excitotoxicity can be triggered by toxic amyloid-β aggregates *in vitro* and *in vivo*, and represents an important pathological mechanism in synaptic failure and neuronal death in AD (reviewed in Paula-Lima et al., 2013). Excitotoxicity is commonly associated with mitochondrial dysfunction and oxidative stress (Johnston et al., 2014; Bhat et al., 2015), and also occurs in other pathological conditions such as stroke and brain and spinal cord injuries (Li and Stys, 2000; Ahuja et al., 2017).

In the past few years, a growing number of studies demonstrated the neuroprotective properties of adiponectin and other AdipoRs agonists against a variety of neuronal toxic insults *in vitro* and *in vivo*. *In vitro*, adiponectin was shown to protect SH-SY5Y human neuroblastoma cells against oxidative stress and cytotoxicity induced by Aβ and MPP+, an inhibitor of mitochondrial complex I (Jung et al., 2006; Chan et al., 2012). Adiponectin neuroprotection was also observed in a model of kainic acid (KA)-induced excitotoxicity in primary cultures of rat hippocampal neurons (Jeon et al., 2009; Qiu et al., 2011). A series of studies also reported neuroprotective roles of adiponectin and AdipoRs in different models of hemorrhagic and ischemic stroke. Cellular mechanisms reported to underlie adiponectin and AdipoRs neuroprotection include suppression of oxidative stress, apoptosis and inflammation, and involve a remarkable variety of intracellular targets, including antioxidant enzymes, AMPK, JNK/PI3K/Akt, PKA, GSK3β, NFkappaB, Bax/Bcl-2 and caspase 3 (Chen et al., 2009; Song et al., 2013, 2015; Guo et al., 2015; Wang et al., 2016; Yang et al., 2017; Ma et al., 2018). These neuroprotective actions of adiponectin may be therapeutically applicable in neurodegenerative diseases.

The antidepressant effects of physical exercise are well known and widely used in the clinic as a non-pharmacological treatment for depression. Recently, adiponectin was shown to play a crucial role in antidepressant effects of exercise, by mediating exercise-induced hippocampal neurogenesis (Yau et al., 2014; Chan et al., 2017). The neurogenic and neurotrophic effects of adiponectin signaling were further demonstrated by intracerebroventricular injection of adiponectin in adiponectin-deficient mice (Zhang et al., 2016). Remarkably, chronic intraperitoneal administration of the AdipoRs agonist AdipoRon in an anxiety/depression mouse model reversed depression-like state by modulating several CNS processes, including neurogenesis (Nicolas et al., 2018). This study is of particular translational relevance since AdipoRon, a small-molecule adiponectin-mimetic drug, showed central activity while administered peripherally. It has been suggested that, for its neurogenic and antidepressant actions, adiponectin can be explored as a pharmacological surrogate for physical exercise to treat depression and, possibly, other brain disorders (Li et al., 2015).

Adiponectin in Alzheimer's Disease

Recent studies indicate that adiponectin signaling deficiency is sufficient to induce an AD-like phenotype in mice. Aged adiponectin-knockout mice recapitulate several aspects of AD pathology, including increased Aβ levels and deposition, tau hyperphosporylation, neuroinflammation, synapse loss, neuronal apoptosis and impaired insulin signaling. Importantly, aged adiponectin knockout mice also performed poorly in spatial memory and fear conditioning behavioral tests (Ng et al., 2016). These observations were further corroborated by another recent study showing that gene-therapy induced suppression of AdipoR1 also produces an AD-like phenotype, which includes impaired spatial memory and learning, increased levels of Aβ aggregates and hyperphosphorylated tau, insulin signaling dysfunction, neuroinflammation and neurodegeneration markers (Kim et al., 2017). Collectively, these studies make strong case for a role for adiponectin deficiency in AD pathogenesis. The NBM is a cholinergic nucleus in the basal forebrain which

is severely affected in AD and other neurodegenerative diseases. It is well documented that neuronal loss of NBM cholinergic neurons contributes to cholinergic dysfunction and, most importantly, it correlates with clinical measures of dementia (Arendt et al., 1983; Whitehouse et al., 1986; Iraizoz et al., 1999; reviewed in Liu A.K. et al., 2015). Interestingly, NBM was found to be a prominent site of expression of AdipoR1 (Psilopanagioti et al., 2009). Therefore, it is possible that adiponectin deficiency contributes to the onset and progression of AD by promoting NBM dysfunction and degeneration. This hypothesis, however, remain to be tested.

Conversely, impairment in adiponectin function is also observed in amyloid-based AD models, whereas activating adiponectin signaling reduces AD-like pathology. In APP/PS1 mice, changes in AdipoRs expression levels were less responsive to a stress-inducing paradigm as compared to wild-type mice (Várhelyi et al., 2017). In the same model, the adiponectin-homolog osmotin ameliorated AD-like neuropathological features such as Aβ production and aggregation, synaptic dysfunction and impaired LTP, memory and cognitive deficits. AdipoR1 silencing abolished osmotin beneficial effects and further aggravated brain pathology in AD-mice (Shah et al., 2017). Osmotin was further shown to reduce Aβ deposition in cultured SH-SY5Y human neuroblastoma cells overexpressing APP. Osmotin effects were mediated by activation of AMPK, an enzyme downregulated by Aβ oligomers in hippocampal neurons (Seixas da Silva et al., 2017). In a rat model of streptozotocin-induced brain pathology, intracerebroventricular injection of adiponectin rescued cognitive deficits and attenuated GSK3β-mediated tau hyperphosphorylation in AD-relevant sites (Xu et al., 2018). These results suggest boosting adiponectin signaling, particularly through AdipoR1, as a potential therapeutic approach in AD (Ng and Chan, 2017). In this regard, chronic treatment with donepezil, an acetylcholinesterase inhibitor widely use to treat AD, was recently shown to increase serum levels of adiponectin (Pákáski et al., 2013). Moreover, thiazolidinediones (TZDs) such as rosiglitazone and pioglitazone, PPARγ agonists used for decades to treat type 2 diabetes due to its insulin sensitization activity (Saltiel and Olefsky, 1996; Malinowski and Bolesta, 2000; Hong et al., 2018) were recently repurposed to treat AD. The insulin-sensitizing effect of TZDs is in part mediated by induction of peripheral adiponectin and AdipoRs expression (Yu et al., 2002; Tsuchida et al., 2005; Nie and Li, 2017). Therefore, it is possible that adiponectin mediate part of the observed beneficial effects of donepezil and TZDs.

AdipoRon, an orally bioavailable small-molecule agonist of adiponectin receptors (Okada-Iwabu et al., 2013), was shown to modulate hippocampal synaptic transmission and to facilitate fear memory extinction in rodents (Zhang et al., 2017). AdipoRon was further shown to regulate activity of dopaminergic neurons in the ventral-tegmental area (Sun et al., 2018) and to act as an antidepressant and metabolic regulator in a mouse model of depression (Nicolas et al., 2018). Importantly, central AdipoRon effects were obtained by peripheral administration, and it was shown that it crosses the BBB to activate AdipoRs in the brain. However, to our knowledge, there are no available data on AdipoRon effects in AD models. It should be interesting to investigate the efficacy of this adiponectin-mimetic drug with translational potential in AD models.

Studies relating adiponectin levels to AD in humans are controversial. Increased baseline adiponectin levels in plasma have been associated with a higher risk of women, but not men, to develop AD and other types of dementia (van Himbergen et al., 2012). Furthermore, elevated adiponectin levels were reported in the plasma and CSF of subjects with MCI and sporadic AD (Une et al., 2010; Khemka et al., 2014a), whereas plasma levels of adiponectin positively correlated with the degree of dementia. The correlation between high blood adiponectin and AD has been replicated and supported by meta-analysis study (Ma et al., 2016). However, while the studies described above suggest increased adiponectin levels to be associated with AD and dementia, opposite results have also been reported. One study found lower levels of circulating adiponectin in MCI and AD subjects. Moreover, adiponectin levels failed to predict progression of cognitive dysfunction from normal to MCI and from MCI to AD (Teixeira et al., 2013). One larger study also reported low levels of plasma adiponectin to be associated with MCI, even though this association was observed in men, but not in women (Kamogawa et al., 2010). In line with these observations, it has been shown that, in diabetic patients, low plasma levels of adiponectin correlate with lower gray-matter volume and reduced glucose utilization in temporal regions of the brain, similarly to what is observed in AD (García-Casares et al., 2016). Finally, a recent study reports that levels of adiponectin are higher in blood but lower in CSF of AD and MCI patients.

Available data regarding a possible association between adiponectin levels in blood and CSF to MCI and AD are conflicting and inconclusive. Therefore, further studies are warranted to reveal the potential use of adiponectin measurement for diagnostic purposes and its clinical relevance for the physiopathology of these neurological conditions. Worth mentioning, some studies found that the association between adiponectin levels and dementia can be sex-specific (Kamogawa et al., 2010). This may be of particular relevance in the case of AD, where a considerable sexual dichotomy is observed with women being at a significantly higher risk to develop the disease.

CONCLUDING REMARKS

Obesity is pandemic in present days. Beyond its intrinsic complications, and the obvious social and psychological impact, obesity harms extend to a wide range of associated health conditions to which it represents a major risk factor. More recently, growing attention is being given to the impact of obesity on CNS function, as accumulating evidence indicate higher incidence of neurological disorders in the obese population. The mechanisms by which fat accumulation and adipose tissue dysfunction in obesity result in CNS pathology are poorly understood. It is widely accepted that dissection of such mechanisms will greatly improve our knowledge on the cross-talk between peripheral metabolism and brain physiology, and may

provide novel targets for therapeutic intervention for prevention and/or treatment of neurological dysfunctions associated with obesity.

Adipokines are secreted factors which carry regulatory signals from adipose tissue through systemic circulation to control a wide range of physiological functions throughout the human body. Not surprisingly, adipokine dysregulation in obesity results in the disruption of homeostasis in a variety of organs and systems, and underlie obesity complications and risk for associated chronic conditions. In this context, adipokines emerged as strong candidates to represent the mediators of pathological signals from adipose tissue to CNS in metabolic disorders.

The studies reviewed here provide evidence supporting a role of leptin and adiponectin, two highly abundant and well characterized adipokines, as key mediators of obesity-related CNS dysfunctions (**Figure 1**). We found consistent evidence that leptin and adiponectin, as well as their receptors, are present in the brain and function as important regulators of different aspects of brain physiology. Importantly, leptin (**Table 1**) and adiponectin (**Table 2**) signaling have been shown to interfere with a range of neuropathological events covering those most commonly present in neurodegenerative diseases and, in particular, in AD. These include amyloidogenesis, tau hyperphosphorylation, neuroinflammation, oxidative stress, endoplasmic reticulum stress, insulin resistance, synaptic dysfunction and cognitive impairment. Remarkably, the phenotype of adiponectin- or adiponectin receptor-deficient mice recapitulates the majority of AD neuropathological hallmarks. Therefore, dysregulated adiponectin and leptin signaling may mediate the detrimental impact of obesity on CNS and raise the risk for cognitive decline and AD. Importantly, restoring proper leptin and adiponectin signaling in the brain may constitute beneficial, disease-modifying therapeutic interventions in such neurological conditions (**Figure 1**).

AUTHOR CONTRIBUTIONS

LF-G and MV wrote the manuscript. MV designed the figure. LF-G, FDF, and MV planned the scope and reviewed the final manuscript.

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REVIEW
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Neurons and Microglia; A Sickly-Sweet Duo in Diabetic Pain Neuropathy

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Diabetes is a common condition characterized by persistent hyperglycemia. High blood sugar primarily affects cells that have a limited capacity to regulate their glucose intake. These cells include capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, Schwann cells, and neurons of the peripheral and central nervous systems. As a result, hyperglycemia leads to largely intractable complications such as retinopathy, nephropathy, hypertension, and neuropathy. Diabetic pain neuropathy is a complex and multifactorial disease that has been associated with poor glycemic control, longer diabetes duration, hypertension, advanced age, smoking status, hypoinsulinemia, and dyslipidemia. While many of the driving factors involved in diabetic pain are still being investigated, they can be broadly classified as either neuron -intrinsic or -extrinsic. In neurons, hyperglycemia impairs the polyol pathway, leading to an overproduction of reactive oxygen species and reactive nitrogen species, an enhanced formation of advanced glycation end products, and a disruption in Na^+/K^+ ATPase pump function. In terms of the extrinsic pathway, hyperglycemia leads to the generation of both overactive microglia and microangiopathy. The former incites a feed-forward inflammatory loop that hypersensitizes nociceptor neurons, as observed at the onset of diabetic pain neuropathy. The latter reduces neurons' access to oxygen, glucose and nutrients, prompting reductions in nociceptor terminal expression and losses in sensation, as observed in the later stages of diabetic pain neuropathy. Overall, microglia can be seen as potent and long-lasting amplifiers of nociceptor neuron activity, and may therefore constitute a potential therapeutic target in the treatment of diabetic pain neuropathy.

Keywords: pain, diabetes, neuropathy, neurons, microglia, oxidative stress, hyperglycemia

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INTRODUCTION

Pain is defined as an unpleasant sensation triggered by noxious stimuli, inflammation, or damage to the nervous system. It is an evolutionarily-conserved defensive mechanism that prevents excessive tissue damage and preserves homeostasis by generating defensive withdrawal reflexes (Scholz and Woolf, 2002). Nociception is initiated by the detection of mechanical, chemical, or thermal noxious stimuli by specialized ion channel receptors present on sensory neurons (Scholz and Woolf, 2002).

The activation of these ion channels triggers an influx of various cations, depolarizing the neurons' membrane potentials, which, in turn, activate voltage-gated sodium channels (Na_Vs). This leads to an influx of sodium, and the subsequent firing of action potentials. In the peripheral nervous system (first order fiber), painful sensations are relayed by small, myelinated Aδ-fibers (fast pain transmission) and unmyelinated C-fibers (slow pain transmission) to the spinal cord (second order fiber) (Tesfaye and Kempler, 2005). These action potentials then trigger defensive reflexes, and travel up to the brain (third order fiber) where pain information is integrated and its emotional perception occurs.

Chronic Pain

Chronic pain is a highly debilitating condition and it is the most common reasons for visits to health care providers (Scholz and Woolf, 2002). The most incapacitating type of chronic pain is peripheral neuropathic pain. This pain is unique in regard to its constancy, the severity of its symptoms, and its resistance to current pharmacological treatment (Zimmermann, 2001; Woolf, 2004). Neuropathic pain is usually generated by peripheral nerve damage resulting from neuronal or spinal cord injuries, surgery, cancer, infection, or diabetes (Scholz and Woolf, 2002; Woolf, 2004; Tsuda et al., 2005). In pathological states, this pain often persists after the disappearance of its causal stimulus. In some cases, pain can be perceived more severely, a phenomenon known as hyperalgesia, or can be generated by normally innocuous stimuli, a condition known as allodynia. Tactile allodynia originates from afferent Aβ fibers (light touch/pressure transmission) that gain the ability to release pro-inflammatory neuropeptides (SP and CGRP) in the synaptic cleft, and/or the sprouting of these fibers to the dorsal superficial laminae IIb, a zone normally restricted to the projection of C fibers (Woolf et al., 1992; Miki et al., 1998). In the CNS, thalamic higher-order neurons often become hyperexcitable and act as pain generators or amplifiers (Fischer and Waxman, 2010). For example, increasing N-Methyl-D-Aspartate receptor (NMDAR) phosphorylation reduces its endogenous blockade by magnesium, thereby enhancing calcium (Ca^{+2}) and sodium (Na^{+}) influx. This ultimately promotes the establishment of spinal windup, which is an increase in the excitability of spinal neurons (Haigh and Blake, 2001).

Sensitization of Sensory Neurons

Most drugs targeted to alleviate neuropathic pain are designed to block neurotransmission, and as such, only bring temporary relief (Ji and Suter, 2007). Neuropathic pain is often accompanied by persistent inflammation, as evidenced by the high levels of oxidative substances (Pabreja et al., 2011), inflammatory cytokines (Pabreja et al., 2011), and mediators (Tsuda et al., 2005) present in the neuronal micro-environment. Unlike classical neurotransmitters, these inflammatory molecules are mainly produced by peripheral immunocytes and central glial cells (Marchand et al., 2005; Tsuda et al., 2005). The transcriptomic data of nociceptor neurons notably revealed the expression of specific receptors for immunoglobulins, cytokines and chemokines (Chiu et al., 2014). This evidences the role of nociceptors in directly detecting and responding to interleukins (IL)-1β (Samad et al., 2001; Binshtok et al., 2008) and IL-6 (Opree and Kress, 2000), activin (TGFβ member) (Zhu et al., 2007), TNF-α (Wagner and Myers, 1996), CCL3 (Zhang et al., 2005), GDNF (Malin et al., 2006), histamine (Shim et al., 2007), kinin (Talbot et al., 2012; De Brito Gariepy et al., 2013), and PGE_2 (Samad et al., 2001, 2002) released in the context of pain. Nociceptors can also sense IL-5 produced during allergic airway inflammation (Talbot et al., 2015), IL-31 produced during lymphoma-associated itch (Cevikbas et al., 2014), thymic stromal lymphopoietin (TSLP) and IL-4 produced during atopic dermatitis (Wilson et al., 2013; Oetjen et al., 2017), and IL-33 derived from contact with poison ivy (Liu et al., 2016). Additionally, nociceptors can drive IL-23 production during psoriasis (Riol-Blanco et al., 2014). Intracellular kinases and transcription factors downstream of these tyrosine kinase receptors, include PI3K (Pereira et al., 2015), MAP kinases (Ji et al., 2002a), p38 (Ji et al., 2002b), JAK1 (Ludbrook et al., 2016; Oetjen et al., 2017), and STAT3 (Mori et al., 2011) and their activation can lead to pain. Thus, these kinases lead to the post-translational modifications of ion channel transducers or voltage gated sodium channels (Julius, 2013). Nociceptor sensitization is thereby largely due to a decrease in the activation threshold of transient receptor potential vanniloid-1 (TRPV1) or transient receptor potential ankyrin-1 (TRPA1) (Davis et al., 2000; Bautista et al., 2006), and $Na_V1.7$, $Na_V1.8$, and $Na_V1.9$ (Kerr et al., 2001; Nassar et al., 2004). In short, lowering the activation threshold of nociceptors results in pain hypersensitivity. For example, prostaglandin PGE_2 is a well-known neuron sensitizer (Samad et al., 2001), which partly explains why non-steroidal anti-inflammatory drugs exhibit analgesic effects in inflammatory conditions (Vardeh et al., 2009). Nerve growth factor (NGF) has also been recognized as a major neuronal sensitizer (Ji et al., 2002b), which has led to the development of neutralizing monoclonal anti-NGF antibodies as a treatment for chronic inflammatory pain conditions (Hefti et al., 2006). These changes in hypersensitivity are limited to sites where sensitizing mediators are produced, which are known as zones of primary hyperalgesia. Outside of these zones, pain hypersensitivity usually results from central sensitization, which involves changes in the CNS (Woolf et al., 1992; Woolf, 2007; von Hehn et al., 2012). During inflammation, many sensitizing mediators are likely to be released simultaneously; therefore, the targeted pharmacological blockade of only one of these agents will have a limited effect. Conversely, targeting the sensitized nerve or convergent signaling mediators or enzymes may have broader and more durable effects as to treating inflammatory pain by stopping it at its source (Khoutorsky and Price, 2017).

DIABETES

Diabetes, derived from the Greek word *diabanein*, means "to pass through," in reference to the symptomatic excessive urine production observed in patients (Kumar et al., 2005). The term diabetes, without qualification, usually refers to *diabetes mellitus*, which roughly translates to "excessive production of sweet urine," known clinically as glycosuria (Kumar et al., 2005). According to the World Health Organization, at least 422 million people

worldwide suffered from diabetes in 2014, representing 8.5% of the world's adult population (WHO, 2016). In 2012, diabetes was responsible for 1.5 million deaths, and its incidence is increasing by more than 8% per year (WHO, 2016). In some regions, such as in Eastern Mediterranean countries, prevalence is increasing by nearly 14%. The National Diabetes Information Clearinghouse estimates the yearly costs of diabetes to more than $132 billion in the United States. In terms of pathology, diabetes is the result of chronic high blood sugar stemming from either low insulin production, as observed in type 1 diabetes; or to a severe reduction in the response of insulin receptors (IR) to insulin, as observed in type 2 diabetes (Kumar et al., 2005). Chronic hyperglycemia causes the classical symptoms of diabetes, including polyuria (frequent urination), polydipsia and polyphagia (Kumar et al., 2005). While both types of diabetes share similar symptoms, they can be distinguished by measuring endogenous insulin production (Kumar et al., 2005).

Type 1 Diabetes

Formerly known as juvenile diabetes, type 1 diabetes (T1D) represents approximately 10% of diabetes cases in North America and Europe. There is currently no known preventive measure against type 1 diabetes, which is considered immune-mediated or idiopathic (Kumar et al., 2005). Insulin-dependent diabetes mellitus is characterized by the auto-immune, T-cell mediated (Rother, 2007) destruction of insulin-producing beta cells of the pancreatic islets of Langerhans. The destruction of β cells triggers insulin deficiency, which leads to increases of glucose in the patient's blood and urine. Evidence indicates that type I diabetes is induced by a combination of genetic susceptibility [mutation(s) to *iddm1*, *drb*1, *dqa,* and *dqb*1 gene locus], environmental factors [diet, vitamin D deficiency (Mathieu et al., 2005)], or exposure to a driving antigen (exposure to wheat protein (Knip and Siljander, 2008), antibody from cow's milk protein (Virtanen et al., 1994). There is no current preventive measure against T1D, which can be highly pathogenic, or even fatal, if left untreated. Emerging treatments such as pancreas (Noguchi, 2010) and islet transplants (Noguchi, 2009) have shown relatively positive outcomes in pre-clinical models, and are currently being studied in clinical trials. However, drawbacks to implantation include the necessity for immunosuppressant administration, which increased susceptibility to infection and cancer, graft rejection of the implanted pancreas/islets, hypoglycemia, and a current lack of suitable donors (Balamurugan et al., 2014).

Type 2 Diabetes

Type 2 diabetes (T2D), also known as non-insulin dependent diabetes mellitus, is a metabolic disorder characterized by chronic high blood glycemia and insulin receptor resistance, sometimes in combination with relative insulin deficiency (Kumar et al., 2005). This type of diabetes can be initially managed by increasing exercise and dietary modification. It represents almost 90% of Western countries' diabetic populations (Kumar et al., 2005). The onset of T2D is related to genetic and environmental factors. The environmental detrimental factors can include smoking, obesity, diet, alcoholism, low physical activity, high cholesterol, hypertension, metabolic syndrome, and Cushing syndrome (Kumar et al., 2005). In recent years, compelling research and efforts have been made to genetically identify mutant or polymorphic genes that predispose individuals to develop type 2 diabetes. These have been found to include *tcf7l2, pparγ, fto, kcnj11, notch2, wfs1, cdkal1, igf2bp2, slc30a8, jazf1, hhex* (Groop and Lyssenko, 2008; Lyssenko, 2008) and *mody* genes, which themselves can account for up to 5% of T2D cases (Billings and Florez, 2010). Mutations in both human leptin production and the human leptin receptor gene can cause severe obesity and pituitary dysfunction, which can in turn engender T2D (Clement et al., 1998; Wabitsch et al., 2015).

Complications of Diabetes

The chronic impairment of glucose metabolism associated with both types of diabetes has been associated with severe macrovascular (cardiovascular) disease and microvascular complications including retinopathy, nephropathy and sensory poly-neuropathy (Schemmel et al., 2009). Neuropathy is the most common complication seen in ambulatory care of type 2 diabetes patients (Schemmel et al., 2009). Overall, the aforementioned complications can result in debilitating and/or life-threatening conditions such as renal failure, erectile dysfunction, blindness, macular edema, impaired wound healing, hypertension, obesity, coronary artery disease, cerebrovascular accidents, heart failure, allodynia, hyperalgesia, nerve degeneration, insensitivity, and limb amputation.

Diabetic Pain Neuropathy

Diabetic pain neuropathy (DPN) is defined as the presence of signs and symptoms of peripheral nerve dysfunction in people with diabetes after having excluded other potential causes (Crofford, 1995). DPN is considered the principal cause of mortality, morbidity (Ziegler, 2008), and amputation (Molines et al., 2010) in diabetic patients, as well as the most common cause of neuropathy (Obrosova, 2009). The prevalence of DPN is thought to be proportional to disease duration and seems to be potentiated by an improper control of blood glycemia (Kumar et al., 2005). Ten percentage of 1-year diabetes patients suffer from neuropathy; this number increases to 50% amongst 25-year diabetes patients. Overall, 30% of diabetic patients suffer from DPN (Guastella and Mick, 2009). Interestingly, 39% of diabetic patients either receive no treatment for their symptoms or remain unmanaged (Daousi et al., 2004). While the prevalence of poorly-managed blood glycemia makes a significant proportion of diabetic patients highly susceptible to developing DPN, glycemic management in clinical care is slowly improving (Aschner et al., 2018). There is emerging evidence that genetic factors may play an important role in DPN pathogenesis (Prabodha et al., 2018).

DPN symptoms include paresthesia, numbness, and burning (Schemmel et al., 2009), which vary in nature and severity depending on the particular subpopulation of neurons being affected (Kumar et al., 2005). Certain patients with DPN do not present any symptoms; however, most report pain and/or loss of function in distal regions such as in their toes, feet, fingers, hands, or arms (Ziegler, 2008). Thus, at the onset of DPN, peripheral nerves often act as pulse generators, maintaining distal terminals of sensory nerve fibers in a state

of hyperexcitability (Obrosova, 2009). When these fibers undergo active degeneration or impaired regeneration, they can begin to generate ectopic discharges, which induce positive pain symptoms. Later stages of DPN are characterized by a progressive loss of neuronal fibers, which is associated with a loss of sensation, and can ultimately cause diabetic foot syndrome (Yagihashi et al., 2007). The specific clinical diagnosis of DPN involves both electrophysiological and electromyography testing, respectively, assessing nerve conduction and muscular responses to electric stimulation (Kumar et al., 2005; Guastella and Mick, 2009). The metrics of blood glycemia, arterial pressure, heart rate, muscle force, reflex quality, and sensitivity to spatiotemporal changes can be used to indirectly help diagnose diabetic neuropathy in a more general sense (Guastella and Mick, 2009).

A FOCUS ON THE MOLECULAR DRIVERS OF DIABETIC PAIN NEUROPATHY

The origins of DPN are multifactorial (**Figure 1**), and result from neuron intrinsic (**Figure 2**) and extrinsic factors (**Figure 3**). This review will examine pre-clinical evidence supporting how chronic hyperglycemia dysregulate neurons' biochemical pathways, activates glia and how such impairments trigger DPN. Current theories (Brownlee, 2001, 2005) regarding neurons intrinsic factor driving the development of DPN include: uncontrolled oxidative stress (section Reactive Oxygen Species) (Nishikawa et al., 2000; Pop-Busui et al., 2006), the formation of reactive nitrogen species (section Reactive Nitrogen Species) (Zochodne and Levy, 2005), the formation of advanced glycation end products (section Advanced Glycation End Products) (Brownlee, 2005; Sugimoto et al., 2008), impaired Na^+/K^+ ATPase activity (section Ion Imbalance) (Vague et al., 1997; Gerbi et al., 1998; Raccah, 1998), an imbalance in the polyol pathway (section Polyol Pathway) and/or to the activity of the aldol reductase (section Aldose Reductase) (Oates, 2002). Extrinsically, it is believed that, at the spinal synapse, the neuro-immune interplay occurring between activated microglia and pain-sensing neurons maintains DPN (section Painful Glia to Microglia, an Emerging Target in DPN). The neuronal loss of energy supply occurring through microangiopathy (see Microangiopathy section) appears to be responsible for the loss of sensation observed in later stages of DPN. Finally, future therapeutic avenues will be discussed in the Conclusion and Future Therapeutic Directions section.

NEURON INTRINSIC FACTORS DRIVING DIABETIC PAIN NEUROPATHY

Reactive Oxygen Species

Cellular aerobic respiration generates the majority of intracellular free radicals (Kumar et al., 2005; Marieb et al., 2009), which are implicated in normal aging processes (Kumar et al., 2005). Cells are said to be in an oxidative stress state when their levels of reactive oxygen species (ROS) exceed their antioxidant capacity (Kumar et al., 2005). ROS are characterized by their high reactivity stemming from their unpaired valance electrons. These electrons can damage or modify the function of RNA, DNA and proteins. Given that neurons are unable to limit their glucose uptake (Brownlee, 2001), possess numerous mitochondria and long axons make them highly sensitive to oxidative damage. Excessive glucose metabolism by the mitochondrial respiratory chains increases the generation of superoxide anions (Nishikawa et al., 2000).

As seen in **Figure 2**, mitochondrial superoxide anions reduce glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, which in turn reduces cells' anti-oxidative capacity (Du et al., 2000). The exposure of mitochondria to ROS progressively induces mitochondrial dysfunction, which in turn promotes energy deficiency, axonal degeneration and DPN. Mitochondria are key regulators of cell survival and apoptosis. Damaged mitochondria trigger axon degeneration through caspase activation and cycles of fusion and fission (Green and Reed, 1998). The fission of mitochondria is partly regulated by dynamin related protein 1 (DRP1) (Twig et al., 2008). Increased DRP1 levels have been associated with mitochondria dysfunction, reduced ATP production, and axonal degeneration (Leinninger et al., 2006). DRP1 is notably upregulated in the axons of diabetic patients (Leinninger et al., 2006). Overall, an excess of ROS generation along with the inability of neurons to metabolize free radicals can promote the progressive loss of organelles and dysfunction in nuclear cell membranes (Figueroa-Romero et al., 2008).

ROS also reduce axon neurotrophic factor (IGF-1, IGF-II, NGF, and NT-3) production levels, thereby impairing neurons' ability to regenerate (Ishii, 1995; Tomlinson et al., 1997). High glucose levels stimulate the generation of pro-oxidant and highly reactive advanced glycation end products (AGE; section Advanced Glycation End Products) (Baynes and Thorpe, 1999). AGEs and ROS appear to be interdependent (Metz et al., 2003; Monnier, 2003), and central to the etiology of neurovascular dysfunction (Cameron et al., 2001). AGE generation is enhanced by oxygen and ROS; AGE formation can trigger ROS generation and oxidative damage (Monnier, 2003). Finally, hyperglycemia promotes the over-activation of polyol pathways (section Polyol Pathway), reducing cells' NADPH/$NADP^+$ ratios and neurons' antioxidant capacities (Figueroa-Romero et al., 2008). Chronic hyperglycemia also enhances PKC activity, either through PLC-DAG pathways, or by reducing DAG-kinase activity (Xia et al., 1994) (King and Loeken, 2004). Enhanced PKC activity also increases mitochondrial NADPH oxidase activity (Inoguchi et al., 2000), further enhancing ROS levels (Balbi et al., 2018). A visual summary of ROS effects on neurons and glia can be found in **Figures 2**, **3**.

ROS are normally metabolized by endogenous antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and by certain vitamins such as A, C (ascorbic acid) and E (tocopherol) (Figueroa-Romero et al., 2008; Midaoui et al., 2015; Talbot et al., 2016a). Nutritional supplementation with antioxidants was shown to reduce DPN in rodents (Pop-Busui et al., 2006). While alpha-lipoic acid and superoxide dismutase improved symptoms and electroneurographic parameters among subjects with diabetic neuropathy (Bertolotto and Massone, 2012), clinical studies generally have shown mixed results in terms of antioxidant efficacy (Oyenihi et al., 2015). Overall, it is

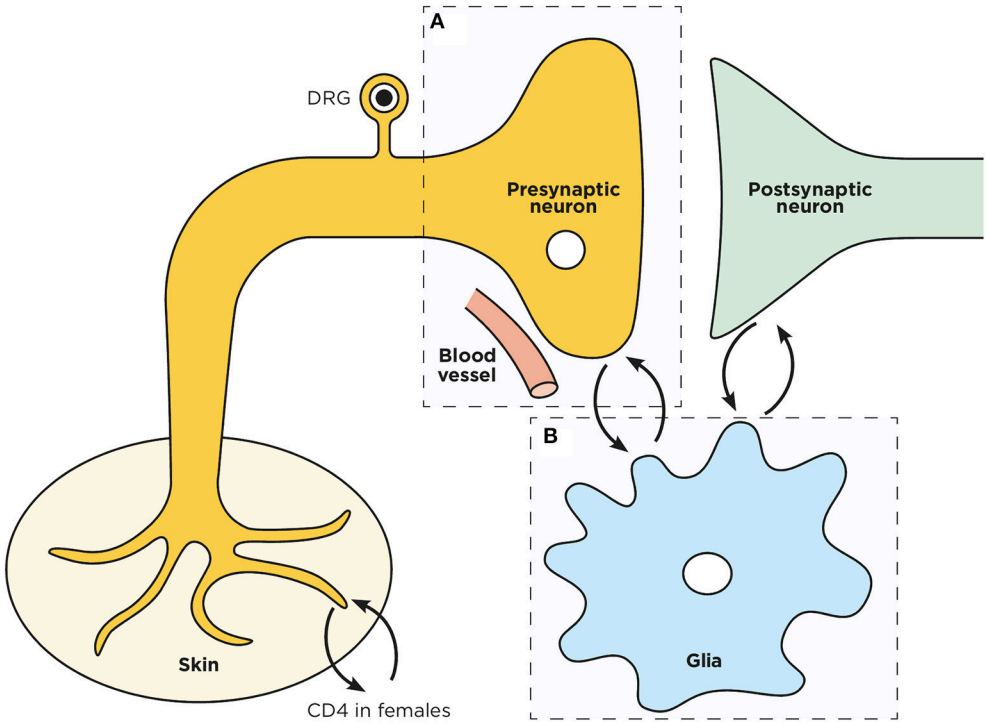

FIGURE 1 | Schematic representation of a spinal dorsal horn tripartite synapse. Overview of the pre **(A)** and post- synaptic neurons interplay with microglia **(B)** in the spinal cord dorsal horn.

believed that increasing the bioavailability of antioxidant, as well as associated reductive stress, have limited impact on the patients' health outcome.

Reactive Nitrogen Species

Nitric oxide (NO) is a potent vasoactive gas formed by three nitric oxide synthase (NOS) isoenzymes: neuronal (nNOS), endothelial (eNOS), and inducible (iNOS) (Kumar et al., 2005). In physiology, eNOS releases NO, which dilates vascular endothelial cells (Kumar et al., 2005) and reduces platelet aggregation (Riddell and Owen, 1999). In the context of inflammation, as is seen in diabetes, iNOS is overexpressed/activated, producing large amounts of NO (∼100-fold than other NOS) (Vareniuk et al., 2008; Haddad and Couture, 2016). In DPN, iNOS hyperactivation is found in keratinocytes, macrophages, leukocytes, sensory neurons, and microglia (Zochodne et al., 2000). Excess NO from nNOS acts as a pro-nociceptive mediator in sensory C fibers (Matsui et al., 2010). It can also overactivate neuronal NADPH oxidase and mitochondrial xanthine oxidase. These effects reduce the antioxidant capacity of cells and contribute to ROS overproduction. Hyperglycemia-induced ROS react with cellular and/or circulating NO to form reactive nitrogen species (RNS) and peroxynitrite (Zochodne et al., 2000). RNS react with the thiol groups of SNAP proteins (Di Stasi et al., 2002), impairing the formation of neuronal regeneration cones. Consequently, RNS severely impact the capacity of neurons to repair themselves after oxidative damage (Kennedy and Zochodne, 2005). Currently, no therapy aims to reduce RNS generation, as it is still considered to be a contributing factor to DPN rather than an inducer. Please refer to **Figures 2**, **3** for visual summaries of NO interactions with neurons and glia.

Advanced Glycation end Products

Advanced glycation end products (AGE) comprise a heterogeneous group of molecules formed by the non-enzymatic reaction of a sugar with an amino acid, a protein, a lipid, or a nucleic acid (Marieb et al., 2009). AGE precursors pass through several dehydration and redox reactions and molecular rearrangements to form AGEs (Sugimoto et al., 2008). The initial reaction leading to AGE formation is reversible, and depends on the available quantity of substrate (glucose) (Brownlee, 2005). However, in cases of chronic hyperglycemia (diabetes), AGE precursors are not degraded, but rather build in numbers, thereby generating AGEs (Sugimoto et al., 2008). As seen in **Figure 2**, AGEs are highly reactive, and can affect any type of protein, including matrix, basal membrane and structural proteins (Sugimoto et al., 2008). For example, AGEs bind to and modify the myelin of nervous fibers, prompting their phagocytosis by circulating macrophages or microglia (Bruck and Friede, 1990). This process contributes to classical DPN segmental demyelination (Said, 2007). AGEs can also directly interact with tubulin and actin neurofilaments found in neurons' axonal cytoskeletons (Sugimoto et al., 2008). AGE-directed modification of these proteins impairs axonal transport, and promotes axonal atrophy and/or degeneration (Sugimoto et al., 2008). The glycation of extracellular matrix (laminin)

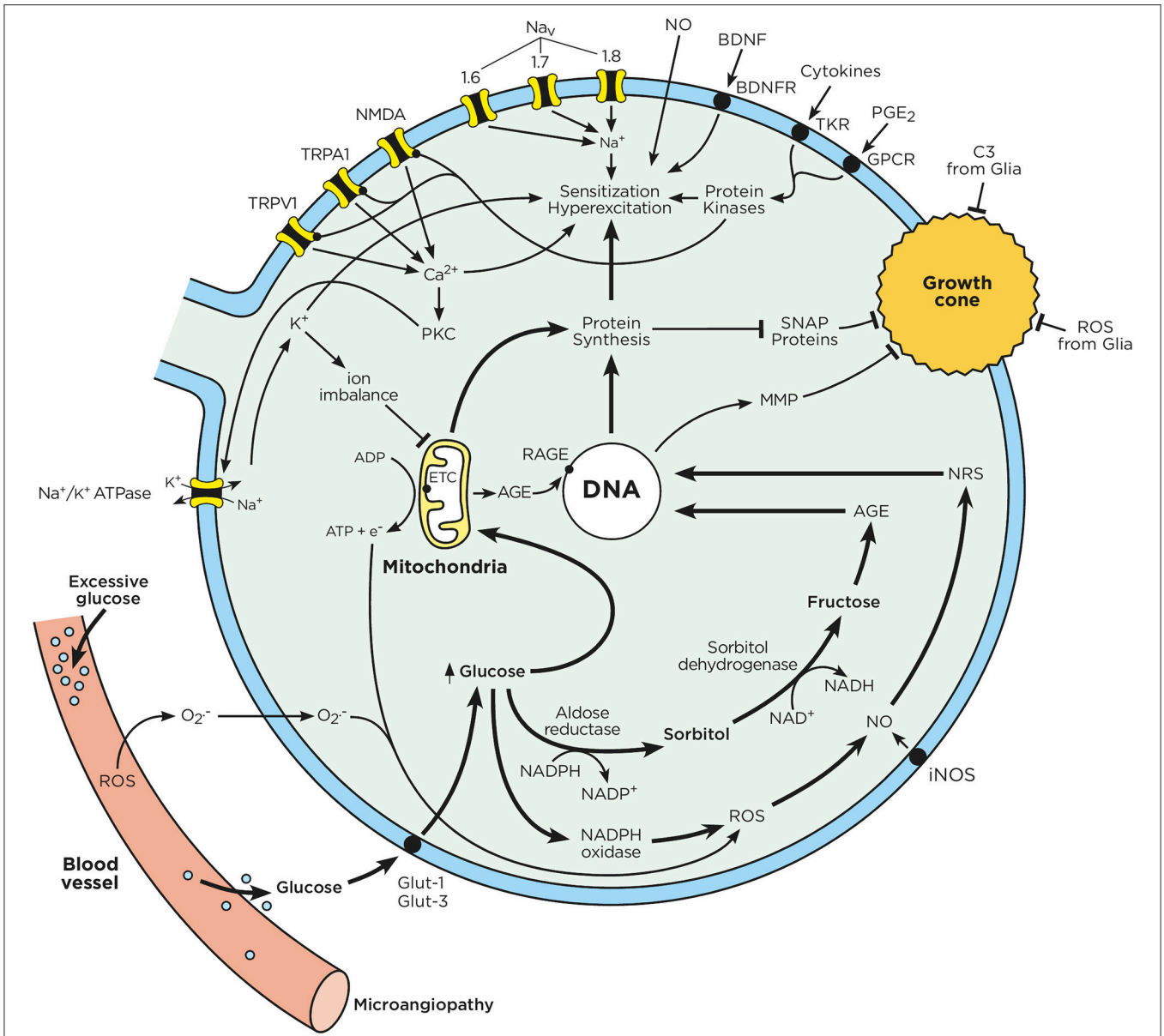

FIGURE 2 | Chronic hyperglycemia impairs neuron function. Sensory neurons have a limited capacity to regulate their uptake of glucose. In the context of chronic hyperglycemia, such as in diabetes, high glucose concentrations drive mitochondria to produce ATP and transfer electrons. Excess glucose is also metabolized through the polyol pathway, leading to the production of advanced glycation end products. The electrons from the mitochondrial respiratory chain combine with intracellular oxygen and nitric oxide to produce ROS and RNS. Consequently, RNS, ROS, and AGE activate nuclear transcription factors, which enhance the expression of ion channel transducers (TRP and Na_V channels) in addition to impairing neurons' capacity to self-repair. At the same time, microglia-released mediators (cytokines, ATP, BDNF, NO) stimulate GPCR and tyrosine kinase receptors, triggering downstream signaling cascades, which lead to the phosphorylation of TRP and Na_V channels. A decrease in the activation threshold of these ion channel transducers can augment the influx of cations, which ultimately results in action potential firing and ectopic discharges. These effects enhance pain perception and signaling to the CNS. Chronic hyperglycemia also increases oxidative stress in the blood vessels that supply oxygen and nutrients to neuron terminals. This oxidative stress can cause microangiopathy, a phenomenon characterized by the loss of capillaries, which starves neuronal energy supplies. These phenomena are responsible for the loss of neuron terminals and pain insensitivity, as typically observed in later stage of DPN.

membranes is AGE-mediated and can counteract the innate ability of neurons to self-repair (Duran-Jimenez et al., 2009; Singh et al., 2014). AGE additionally binds to specific membrane receptors known as RAGE (Haslbeck et al., 2005), driving the transcription of pro-inflammatory mediators (Brownlee, 2005). RAGE stimulation increases matrix metalloproteinase production, which can further exacerbate nerve fiber damage (King, 2001). AGEs demonstrably accumulate in hyperglycemic patients experiencing retinopathy, nephropathy, hypertension and neuropathy (Brownlee, 2005). Environmental pollutants,

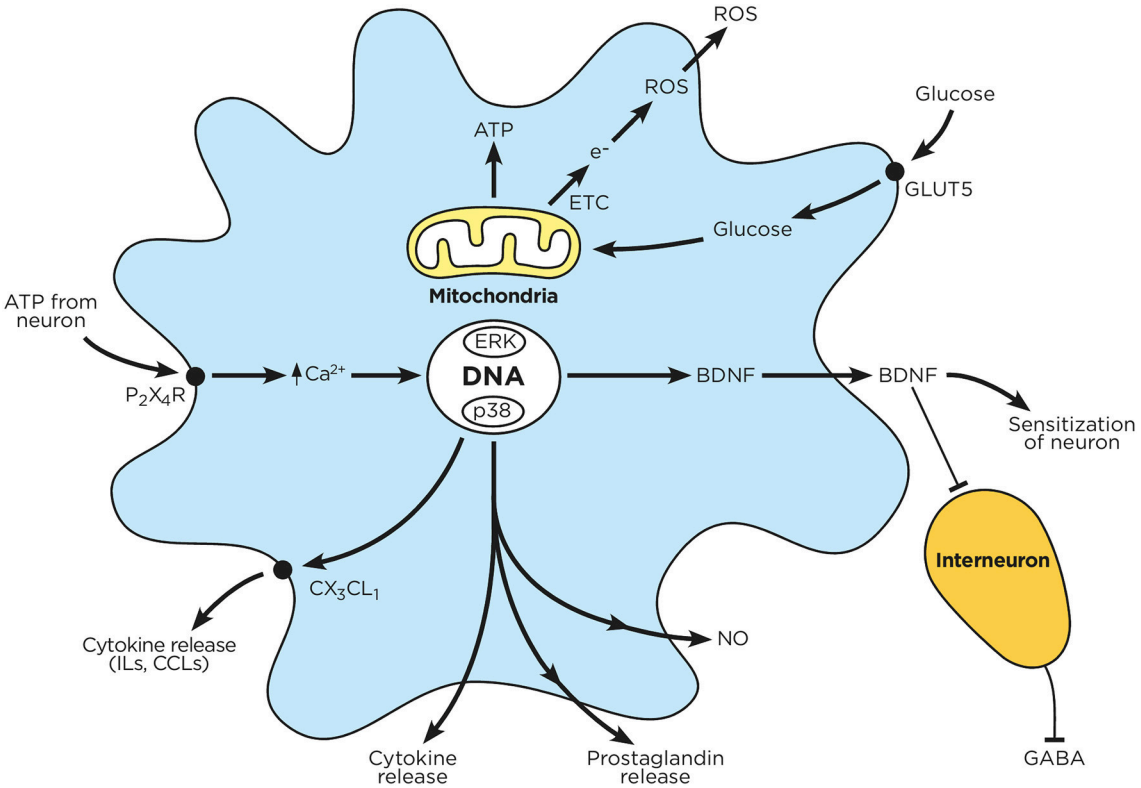

FIGURE 3 | Chronic hyperglycemia impairs microglial function. Circulating glucose is taken up by microglia, which enhances mitochondrial ATP production and electron transfer. The released electrons combine with intracellular oxygen to produce reactive oxygen species. Sensory neurons release ATP, which in turn activates microglial P_2X_4R; this drives microglial calcium influx, MAPK activation, receptor phosphorylation and protein transduction (cytokines, prostaglandins, BDNF) as well as NO production. These mediators are subsequently released by microglia, and either block inhibitory interneurons or enhance neuronal activation.

smoking, and poor nutrition also enhance AGE formation (Sugimoto et al., 2008). Avoiding these factors can help control AGE formation and its associated damage (Sugimoto et al., 2008; Singh et al., 2014). Specific inhibitors such as aminoguanidine improve patients' nerve conduction velocity and neuronal blood flow, in addition to mitigating apoptosis and oxidative stress (Sugimoto et al., 2008; Orman et al., 2015).

Ion Imbalance

Na^+/K^+ ATPase Pump

The Na^+/K^+ ATPase pump is a ubiquitous, energy-dependent enzyme implicated in the cellular membrane transport of ions. Using ATP, it transfers three sodium ions outside the cell in exchange for two potassium ions transported into the cell (Kumar et al., 2005). In doing so, it maintains the membrane's electric potential and nerve conductance (Creange et al., 2006). During hyperglycemia, impairments to polyol pathways (section Polyol Pathway) and PKC activity alter the function of the Na^+/K^+ ATPase pump leading to faulty nerve conduction (Creange et al., 2006). Reduced intracellular potassium levels curbed nodal potassium conductance, thereby affecting axonal excitability (Misawa et al., 2005). Hypokalemia can also alter Ca^{2+}/K^+ pump function, leading to neuronal hypocalcaemia.

Calcium

In early stages of DPN, the elevated levels of intracellular calcium disrupt nerve conductance (Kostyuk et al., 2001) and can, through calcium cytotoxicity, cause irreversible damage to the nerve fibers (Creange et al., 2006). Conversely, the hypocalcaemia observed in later stages of DPN mediates axonal degeneration, as seen in **Figure 2** (Gispen and Hamers, 1994). Overall, impaired neurotrophic factor levels in DPN-afflicted neurons drive mitochondrial depolarization and Ca^{2+} concentration impairment, which in turn negatively impacts the TCA cycle and ATP production (Fernyhough and Calcutt, 2010). ATP dysregulation impairs cellular calcium homeostasis, reducing levels of endoplasmic reticulum (ER) and plasma membrane Ca^{2+} pumps (PMCA), as evidenced in streptozotocin (STZ)-treated rats. The impairment of ER calcium homeostasis disrupts protein synthesis, post-translational modification, and trafficking, all of which may contribute to distal axonal degeneration (Fernyhough and Calcutt, 2010). T-type calcium channel blockers notably improve thermal and mechanical hypersensitivity in T2D mice (Misawa et al., 2009).

Sodium

The DRG neurons of STZ-treated rats show an increased expression of sodium channels ($Na_V1.3$, $Na_V1.6$, and $Na_V1.9$) (Craner et al., 2002; Hong et al., 2004), which contributes

to ectopic impulse generation and neuronal hypersensitivity (Fischer and Waxman, 2010). The groups of Howe (Howe et al., 1977) and Wall (Wall and Gutnick, 1974) demonstrated that thermal, mechanical, and chemical stimuli thresholds are reduced following spontaneous electrical activity. Uninjured axons proximal to the affected neurons also exhibit ectopic discharge. Both of these phenomena result in increased electrical impulses in the spinal dorsal horn. The association between ectopic discharges and increased sodium channel expression can partly account for the therapeutic efficacy of anticonvulsant and tricyclic antidepressants in the treatment of DPN (Spruce et al., 2003).

Polyol Pathway

Cellular glucose is converted into pyruvate by the actions of diverse enzymes implicated in glycolysis. In hyperglycemic conditions, excess glucose is not oxidized, but rather directed to the polyol pathway (Oates, 2002). Firstly, aldose reductase (AR) metabolizes glucose into sorbitol, which is later transformed into fructose by sorbitol dehydrogenase. Fructose is, notably, ten times more potent than glucose in generating AGE (Oka and Kato, 2001) (section Advanced Glycation End Products). Aldose reductase and sorbitol dehydrogenase are characterized by their lowered substrate affinity (elevated Km); the concentration of available substrate is therefore the limiting factor of this reaction (Oates, 2002).

Elevated sorbitol levels have been associated with cellular and organ damage (Oyama et al., 2006). It is believed that sorbitol directly depletes bioavailable myoinositol (MI) and increases its expulsion from the cell (Oka and Kato, 2001; Oates, 2002). Elevated blood sugar also prevents sorbitol's cellular reuptake by saturating its membrane transporter. A deficit in MI alters the metabolism of phospho-inositides, reducing diacylglycerol (DAG) and inositol triphosphate (IP_3) production. This results in a lesser activation of PKC, which is itself a key activator of the Na^+/K^+ ATPase pump (Oka and Kato, 2001). A reduction in Na^+/K^+ ATPase pump activity triggers an intracellular reduction in K^+, combined with increases in the concentration of Na^+. In neurons, imbalances in ionic charges directly contribute to DPN by generating conductance anomalies (Oka and Kato, 2001). In this context, decreases in intracellular sodium concentrations affect sodium-dependent membrane transport. This transport is implicated in the reuptake of several amino acids and MI, contributing to a retro-positive feedback loop (Das Evcimen and King, 2007; Oates, 2008). Disruptions in the polyol pathway also increase ROS levels (section Reactive Oxygen Species) by reducing the production of glutathione, in addition to intracellular levels of antioxidants (NADPH). Polyol pathway pathologies also reduce the production of NO, which can in turn enhance vessel constriction. This impairs endothelial cell function can lead to the onset of microangiopathy (section Microangiopathy) (Oka and Kato, 2001); the effects of polyol pathways in DPN can be seen in **Figure 1**.

Aldose Reductase

In patients with T1D, polymorphisms in the genes coding for aldose reductase (AR) can impair thermal nociceptive thresholds (Thamotharampillai et al., 2006). Higher AR levels also correlate with a higher severity of intra-epidermal nerve fiber loss (Hirai et al., 2000). AR specific inhibitors (ARi) have been shown to reverse or delay the onset of DPN in diabetic animals (Schemmel et al., 2009). While ARis are unavailable on the US market, they are currently being used clinically in Japan (Hotta et al., 1996). Epalrestat is currently the only commercially available inhibitor (Singh Grewal et al., 2016); and prevented the progression of diabetic neuropathy and retinopathy/nephropathy in neuropathic patients as compared to a control group (Hotta et al., 2012).

NEURON EXTRINSIC FACTORS PROMOTING DIABETIC PAIN NEUROPATHY

Several neuron-extrinsic factors contribute to the onset and maintenance of neuropathic pain. Recent data has highlighted the key contribution of immune cells, acting as extrinsic factors, in driving DPN. Normally, the immune and sensory nervous systems work in concert to preserve homeostasis. They do so via interactions and exchanges between receptors, cytokines and neuropeptides (Talbot et al., 2016b; Veiga-Fernandes and Mucida, 2016). While this bidirectional communication helps to protect humans from danger, it can also contribute to disease pathophysiology (Chiu et al., 2013; Wilson et al., 2013; Talbot et al., 2015, 2016b; Foster et al., 2017). In fact, the somatosensory nervous system is anatomically positioned within primary and secondary lymphoid tissues and mucosa so as to interact with the cells of the immune system (Downing and Miyan, 2000; Rosas-Ballina et al., 2011; McMahon et al., 2015; Talbot et al., 2015; Veiga-Fernandes and Mucida, 2016; HD iPSC Consortium., 2017). Nociceptors, when sensing immunocyte-released cytokines, lower their firing thresholds; in doing so, they incite pain hypersensitivity (Wilson et al., 2013). While various immunocytes contribute to this phenomenon, this review will focus on the crucial role of microglia. Finally, we will review the role of blood vessels, which supply oxygen and glucose to the nerve, in generating DPN.

Microglia

The central nervous system (CNS) is primarily composed of afferent and efferent neuron fibers that transport electrical signals to and from the periphery, oligodendrocytes that form and repair myelin, and astrocytes and microglia that support and protect neurons (Marieb et al., 2009). Microglial cells represent 10–15% of all cells found within the human brain (Foster et al., 2015). Glia act as resident macrophage-related cells of the CNS, comprising the first line of defense against pathogen invasion, generating innate immune responses by recognizing, sequestering and processing antigens (Lawson et al., 1992). While there are two major types of microglia: resident, and perivascular (Gosselin et al., 2010), which express receptors for most inflammatory neurotransmitters (Hickey and Kimura, 1988), it seems that glia exists in nine distinct subtypes with different functions, appearance, and presence. Resident microglial cells

are bone marrow-derived hematopoietic cells that invade the CNS during embryonic development (Pocock and Kettenmann, 2007). These are very rarely replaced, and rapidly proliferate while activated (Milligan and Watkins, 2009). Conversely, perivascular microglial cells are continuously replenished by bone marrow-derived hematopoietic precursors (Gosselin et al., 2010), particularly during CNS inflammation (Romero-Sandoval et al., 2008). Perivascular microglia can alter the blood-brain barrier's permeability, and exert anti-inflammatory effects, while resident microglial cells exert both pro- and anti-inflammatory effects (Milligan and Watkins, 2009).

While on a polarization continuum, microglia activation can be classified as either resting or activated. A resting microglial cell possesses a small soma with thin and ramified processes (Tsuda et al., 2005), express immunoreceptors (Lawson et al., 1992), and perform immune roles to maintain CNS homeostasis (Tsuda et al., 2005). Upon activation due to trauma, inflammation, or infection, microglia undergo several stereotypic changes in morphology, gene expression, function, and number (Tsuda et al., 2005). They upregulate various transmitters and receptors, including the complement receptor 3 (CR3) (Eriksson et al., 1993; Lassmann et al., 1993), major histocompatibility complex 2 (MHC2) (Shi et al., 2017), TLR4 (Sweitzer et al., 2002), and CD14 (Sweitzer et al., 2002). The intracellular events promoting glial cell activation remain unclear, but are known to involve the activation of cannabinoid CB2 receptors (Tanga et al., 2004), kinin B1 receptors (Li and Kim, 2017), P_2X_4R (Noda et al., 2007), NK-1R (Inoue, 2008), CX3CR-1 (Abbadie et al., 2009; Gao and Ji, 2010; Zhou et al., 2010), CCR-2 (Zhang and De Koninck, 2006; Milligan et al., 2008), MMP9 (Thacker et al., 2009), BDNF-R (Kawasaki et al., 2008), TLR3 (Tender et al., 2010) and TLR4 (Kim et al., 2007); leading to the phosphorylation of p38 MAPK (Jin et al., 2003; Tsuda et al., 2004; Tanga et al., 2005; Daulhac et al., 2006; Ji et al., 2009). For more information on microglia intracellular signaling refer to Popiolek-Barczyk and Mika (Chang et al., 2010). Furthermore, microglial activation can occur via endogenous pro-inflammatory signals (IL-1β, TNFα, IL-6, and NO), opioids (Popiolek-Barczyk and Mika, 2016) or heat shock protein (Hutchinson et al., 2008). Activated microglial soma increase in size and their long and thin ramifications withdraw, ultimately resulting in an amoeboid shape with few ramifications (Tsuda et al., 2005). They have implicated microglia in reward behavior (Costigan et al., 1998) as well as in the onset of chronic neurodegenerative diseases (Taylor et al., 2015; Hammond et al., 2018), such as lupus erythematosus (Salter and Stevens, 2017), Huntington's chorea (Nestor et al., 2018), and Alzheimer's disease (Eriksson et al., 1993).

Painful Glia

Microglial cells produce and release various excitatory peptides, including PG, SP, EAA, NO, and ATP, and express selective receptors for immunomodulatory neurotransmitters. Both peptides and receptors allow microglia to detect, and respond to, neuronal signals, thereby generating autocrine or paracrine feed forward inflammatory loops (McMahon et al., 2005). For example, TNFα and MMPs activate microglial p38 MAPK in the spinal cord dorsal horn during peripheral neuropathic pain (Svensson et al., 2005). MMP9-induced pro-IL-1β cleavage leads to p38 MAPK phosphorylation in microglia during the onset and early stages of neuropathic pain. MMP2-induced pro-IL-1β cleavage leads to astrocyte activation in later disease stages (Kawasaki et al., 2008). ATP-stimulated microglial P_2X_4R enhances the levels of intracellular Ca^{2+}. Such influx activates various transcription factors, including NF-κB, p38, and ERK-MAPK (Tsuda et al., 2005), leading to the synthesis of pro-inflammatory cytokines (IL-1β, TNFα, and IL-6) (Watkins et al., 2001a; Marchand et al., 2005) or neuroexcitatory substances such as D-serine (Petrenko et al., 2003). This transcriptomic profile can initiate and maintain neuropathic pain by facilitating neuron-glial interactions (Hickey and Kimura, 1988; Abbadie et al., 2009). The interplay between neurons and glia can therefore sustain neuronal stimulation and sensitization by increasing glutamatergic stimulation and by reducing GABAergic inhibitory signals (Tsuda et al., 2005; Inoue, 2006; Scholz and Woolf, 2007; Biggs et al., 2010); the process can be seen in **Figure 3**.

In current pre-clinical literature, activated microglia have emerged as key drivers of pathological pain in chemotherapy-induced neuropathy and peripheral nerve and spinal cord injuries (Watkins et al., 2001b; Marchand et al., 2005; Tsuda et al., 2005; Daulhac et al., 2006; Pocock and Kettenmann, 2007; Gadani et al., 2015; McMahon et al., 2015). For example, CB2 receptor agonists dramatically attenuate iNOS induction and ROS generation in LPS-activated microglia (Ribeiro et al., 2013). The incitement of inflammation signals microglia to migrate, proliferate, synthesize and release pro-inflammatory mediators that maintain neuron activation. In keeping with the fact that an intrathecal injection of activated microglia induces both thermal hyperalgesia and tactile allodynia (Tsuda et al., 2003; Narita et al., 2006), while resting microglia or activated astrocytes are without effect (Narita et al., 2006). Blockades of p38 MAPK (Tanga et al., 2005; Ji et al., 2009), CX3CR-1 (Milligan et al., 2004; Verge et al., 2004; Sun et al., 2007) or P_2X_4R (Tsuda et al., 2003) have been shown to alleviate chronic pain in rodents. Other chemokines, such as RANTES, IP-10, and SDF1 are also implicated in enhanced microglia migration, infiltration, phagocytosis; they are therefore contributors to microglia-induced neuropathic pain (White et al., 2007).

Salter and Beggs have made several discoveries linking neuronal hypersensitivity to overactive microglia (Beggs and Salter, 2016). Firstly, they were able to show that nerve injury activates microglia and causes them to express P_2X_4R. They demonstrated that the IRF-8/IRF-5 transcriptional cascade clearly regulates the expression of P_2X_4R gene. Additionally, they showed that external stimulation (CCL2 and LPS) leads to the translocation of P_2X_4R protein from lysosome to cell surface (Tsuda and Inoue, 2016). A targeted silencing of P_2X_4R suppressed injury-induced tactile allodynia, while an intraspinal administration of P_2X_4R-expressing glia had the opposite effect (Tsuda et al., 2003). They also identified spinal dorsal horn neurons as a source of ATP (Masuda et al., 2016), and that ATP-stimulated microglia release brain derived neurotrophic factor (BDNF). BDNF limits GABAergic inhibitory signals sent to afferent nociceptor neurons (Torsney and MacDermott, 2005);

this enhances their activity by uncoupling the transmembrane anion gradient (Coull et al., 2005). A disruption of chloride transport changed spinal lamina I neurons' phenotype, causing them to (i) increase nociceptive responsiveness, (ii) relay innocuous mechanical inputs, and (iii) generate spontaneous bursts of activity; respectively, accounting for (a) hyperalgesia, (b) mechanical allodynia, and (c) spontaneous pain (Keller et al., 2007). The microglia-to-neuron P_2X_4R -BDNF-KCC2 axis was also found to drive opioid-induced thermal hyperalgesia. Interestingly, Salter and Beggs found that this hyperalgesia mechanistically differs from opioid-induced tolerance (Beggs and Salter, 2016). Microglia-derived BDNF also emerged in their research as a negative regulator of reward in opioid-dependent states (Taylor et al., 2016), while the Panx1-mediated release of microglial ATP controls morphine withdrawal without affecting opiate-induced analgesia (Burma et al., 2017).

Peripheral tissue injury was found to increase the intensity, spatial distribution, and persistence of Iba-1^+ microglial activity within the spinal dorsal horn, resulting in a long-lasting priming of withdrawal reflex sensitivity and microglial responsiveness (Beggs et al., 2012). MCP-1^+ neurons drive the bone marrow-derived microglial infiltration of injured spinal cords. These neurons also facilitate glial activation and drive mechanical allodynia (Zhang et al., 2007). CCL21, a microglial activator, is also found to be upregulated in the cell bodies of spinothalamic tract neurons following nerve injury. This molecule is transported rostrally to the thalamus, where it activates microglia and drives neuron hyperexcitability (Zhao et al., 2007b). Spinal microglia consequently remain activated for more than 3 months following nerve injury in rodents, thereby maintaining chronic pain (Echeverry et al., 2017). Newly-generated microglia also appear to be coded with an inherent "memory" of previous injuries, contributing to long-lasting neuropathic pain (Yao et al., 2016).

Following a spinal cord injury, microglial ERK kinases are activated, prompting the intraspinal release of PGE_2, which results in spinal cord dorsal horn neuron hyperresponsiveness (Zhao et al., 2007a). Following the spinal release of IL-1β and IL-6, fractalkine triggers pain by activating microglia expressing CX3CR1 (Milligan et al., 2004, 2005). IL-1β upregulates both neuronal NMDAR phosphorylation and expression. These effects enhance NMDAR conductivity (Zhang et al., 2008) and calcium influx (Viviani et al., 2003), increasing neuronal excitability and synaptic strength (Beattie et al., 2002; Stellwagen and Malenka, 2006). Microglial NO and PGE_2 can also increase the excitability of pain-projecting neurons (Besson, 1999). Overall, a modulation of microglial polarization was shown to alleviate neuropathic pain (Chang et al., 2010; Piotrowska et al., 2016; Xu et al., 2018). Intriguingly, microglia only induce mechanical pain hypersensitivity in male rodents (Mapplebeck et al., 2018), as T-lymphocytes appear to be responsible for this mechanism in females (Sorge et al., 2015).

Microglia, an Emerging Target in DPN

Given that db/db mice and T2D patients show increased levels of activated microglia (Arroba and Valverde, 2017), and that the pharmacological inhibition of spinal resident microglia reverses painful neuropathy (Wodarski et al., 2009; Sun et al., 2015; Lin, 2017; Zhang et al., 2018), we take the overall view that microglia may drive DPN (**Figure 1**). Activated microglia levels have been found to correlate with thalamus hyperresponsiveness (Fischer and Waxman, 2010), and the brain thalami of DPN patients demonstrate increases in blood flow (Paulson et al., 2007), spontaneous neuronal activity (Fischer et al., 2009), receptive field size enhancement (Fischer et al., 2009), and alterations in neuronal connectivity (Cauda et al., 2010). Additionally, activated microglia drive DPN in STZ-treated rats (Wodarski et al., 2009; Talbot et al., 2010); this process can successfully be reversed by gabapentin (Wodarski et al., 2009) or minocycline treatment (Talbot et al., 2010).

Additionally, diabetes-induced hyperglycemia enhances microglial NADPH oxidase and iNOS activation (**Figure 3**), promoting the production of ROS (Quan et al., 2007, 2011) and peroxynitrite (Li et al., 2005). As a result of the iNOS-NO-NRS axis, activated microglia are a major source of free radicals in the spinal cords of animals with DPN (Li et al., 2005; Candelario-Jalil et al., 2007). Glial-released NO inhibits neuronal cytochrome oxidase, blocking mitochondrial respiration, which in turn depletes the production of ATP (Brown and Bal-Price, 2003). Moreover, ROS and RNS deplete endothelial cell NO levels, contributing to the generation of microangiopathy (section Microangiopathy). A reduction in neuronal blood supply leads to hypoxia and mitochondrial dysfunction; this results in cellular energy deficits, and ultimately, neuron death. Finally, elevated ROS levels also impair axonal transport (Larsen and Sidenius, 1989) and the capacity of the nerve to repair itself (Longo et al., 1986), further worsening nerve health in DPN patients.

Microglia exposed to high glucose levels show increases in mRNA expression, and secrete TNFα and MCP-1, leading to neuronal activation (Quan et al., 2011). The spinal microglia's activation of P2Y12 and P2Y13 receptors triggers the production of IL-1β and IL-6 in a rat model of DPN (Liu et al., 2017; Zhou et al., 2018). The activation of microglial RAGE (Thornalley, 1998) leads to the release of chemokines CCL3, CCL5, and CXCL12, which activate microglia (Bianchi et al., 2011). The resulting activated microglia actively phagocyte neuronal myelin, thereby promoting DPN (Mosley and Cuzner, 1996).

Microangiopathy

Microangiopathy is characterized by the shrinking and weakening of small blood vessels. This pathology leads to a reduction in blood flow, protein leakage, and bleeding. In diabetic states, endothelial cells take up excessively large quantities of circulating glucose, raising the production of AGE (section Advanced Glycation End Products). These AGEs enhance the proliferation of endothelial cells, which leads to a thickening of basal membranes and progressive vessel occlusion, which are associated with reduced neuronal blood flow (Tuck et al., 1984; Cameron et al., 1991) and ischemia. In turn, the ischemia reduces neuronal oxygen and nutrient supplies, triggering nerve fiber loss (Yagihashi et al., 2007). Microangiopathy in neuron-irrigating vessels also impairs their capacity to regenerate and repair (Kennedy and Zochodne, 2005). A thickening of basement membranes and endothelial cell swelling thereby positively correlates with reduced nerve

fiber myelin densities (Yagihashi et al., 2007). The occlusion of neuronal capillaries promotes axonal degeneration and a dysfunctional loss of sensory perception, as typically observed in the later stages of DPN.

Multifocal nerve lesions and alterations in endoneurial capillaries indicate a role for circulatory factors in the symmetrical form of DPN (Dyck et al., 1986; King et al., 1989). Peripheral nerve fiber loss in DPN is well-associated with the increased migration and infiltration of inflammatory cytokines released by T-lymphocytes and macrophages (Said et al., 2003; Said, 2007). These cytokines increase vessel damage and promote microangiopathy (Said, 2007). Considering that microglia and macrophages share similar roles and inflammatory characteristics, it is probable that microglia may also promote microangiopathy within the CNS (**Figure 3**).

CONCLUSION AND FUTURE THERAPEUTIC DIRECTIONS

Multiple molecular mechanisms generate and amplify hyperglycemia-induced neuropathic pain. This collaboration involves voltage-gated ion channels, ligand-gated channels, cytokine receptors, direct myelin damage (Edwards et al., 2008; Vincent et al., 2008), neuronal depolarization (Abbadie et al., 2009), conduction impairment (Kramer et al., 2004), a loss of interneuron inhibitory input (Wood, 2008), Aβ-fiber sprouting (Yasuda et al., 2003), and neuronal death (Inoue, 2006). This multicentric view accounts for why hyperglycemia-induced DPN remains highly refractory to treatment paradigms. Given such potential heterogeneity amongst patients, personalizing of DPN management may prove useful. This will necessitate improved diagnostic methods and personalized medicine tailored to the specific pathology in question.

While design, safety and drug efficacy often vary between rodent and clinical models of disease, calcium channel α2-delta ligands (pregabalin, gabapentin), antidepressants (TCA and SSRI), and opioid-like drugs as well as topical agents including capsaicin, lidocaine, or botulinum toxin A can help alleviate patients' DPN (Finnerup et al., 2015). The long-term efficacies of these approaches have yet to be demonstrated. Overall, the high plasticity of microglial phenotypic and transcriptomic changes persists even after glycemic control, inciting and maintaining neuron sensitization (Milligan et al., 2008). Therefore, the microglia, which potentially act upstream of pain neurons, should rightly be considered as a crucial therapeutic target in the treatment of diabetic pain neuropathy. Future therapies may therefore involve targeting specific receptors and signaling cascades which engage such deleterious neuro-immune crosstalk.

AUTHOR CONTRIBUTIONS

TR, RC, AC, and ST conceived the study and wrote the manuscript while SCT, J-CW, MA, MB, TC, and JD contributed to its design.

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Pituitary Gland Functional Connectivity and BMI

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The pituitary gland (PG) influences body weight through hormonal releases; however, the relation between body weight and PG's co-activities with other brain regions remains unclear. Here, we aimed to identify (1) the functional connectivity of the PG and (2) PG functional connectivity associated with body mass index by examining resting state functional magnetic resonance imaging data. Using enhanced Nathan Kline Institute-Rockland Sample, PG functional connectivity of 494 individuals was analyzed to assess in voxel-wise fashion. A negative association was found between BMI and PG functional connectivity with the orbitofrontal cortex, hippocampus, putamen, and temporal lobe. Our results show PG dysconnectivity to these regions is associated with higher BMI and implicate that the connectivity between these dopaminergic regions and PG may be associated with body weight maintenance through feeding behavior and growth.

Keywords: pituitary gland, functional connectivity, resting state fMRI, gustatory cortex, caudate head

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INTRODUCTION

The hypothalamic-pituitary-adrenal (HPA) axis including the pituitary gland (PG) has been shown to be associated with obesity (Pasquali et al., 2006). In obesity, responses to growth hormone releasing factor are impaired (Williams et al., 1984). Chronic stress has been implicated to promote obesity through the HPA axis (Bose et al., 2009). Genetic variation in leptin receptors also shows association with obesity (Clement et al., 1998). The PG synthesizes and secretes hormones that regulate body weight, including somatotropins and oxytocin; however, the brain regions responsible for associating the PG activity and obesity are not clearly known.

Functional connectivity analysis using resting state fMRI (Fox and Raichle, 2007; Biswal et al., 2010) has provided newer insights into brain coactivity and obesity. The default mode network (DMN) and temporal lobe network have been found to be associated with obesity (Kullmann et al., 2012). In obesity, the precuneus shows an increased functional connectivity in the DMN while that of the anterior cingulate cortex is decreased. The insular cortex shows reduced connectivity in the temporal lobe network. Dysconnectivity between the hypothalamus and left insula has been found in obesity (Wijngaarden et al., 2015). After 48 h of fasting, connectivity of the hypothalamus to the dorsal anterior cingulate cortex increases in lean populations and decreases in the obese populations, suggesting a differential influence of obesity to functional connectivity of the hypothalamus (Wijngaarden et al., 2015). The putamen has been found to have elevated functional connectivity in obesity, while cognitive processing speed was negatively associated with the connectivity of the putamen to the salience network, suggesting an altered processing of salience detection (García-García et al., 2013). Obesity-preventive eating tendency has been found to be associated with caudate-precuneus functional connectivity (Nakamura and Ikuta, 2017). Caudate-precuneus connectivity inversely predicts the personal characteristics of avoiding

obesity-inducing behaviors, suggesting the functional connectivity signature of avoiding causes of obesity independent of current body weight status. Surgical and behavioral weight loss has been found to differentially influence functional connectivity (Lepping et al., 2015). Despite its strong relationships to obesity, the influence of functional connectivity of the PG to body weight has not been yet studied.

Resting state functional connectivity of the PG is minimally studied, although functional connectivity is associated with adrenocorticotropic hormone (ACTH) levels. Cortisol concentrations have been found to predict interhemispheric connectivity, and ACTH concentrations were shown to be associated with the subcallosal anterior cingulate cortex (Kiem et al., 2013). Nonetheless, the resting state PG connectivity remains unclear. In this study, we (1) examined the resting state functional connectivity of the PG and (2) aimed to isolate resting state functional connectivity of the PG associated with BMI.

MATERIALS AND METHODS

Data Acquisition

The MRI images, the clinical data, and the demographic data of the enhanced Nathan Kline Institute-Rockland Sample (Nooner et al., 2012) were obtained from Collaborative Informatics and Neuroimaging Suite (Biswal et al., 2010). This data subset consisted of 494 individuals without known neurological preconditions (such as stroke, tumor, and traumatic brain damage) and MRI counter indications (43.46 ± 20.81 years old), 310 females and 184 males, six Native Americans, 25 Asians, 102 Black or African Americans, two Native Hawaiians, 346 Caucasians, and 13 other races, with the mean BMI of 27.32 ± 6.35 (between 15.29 and 56.28), for whom resting state and structural data were both available. Participants in the sample were recruited from Rockland County, NY, whose demographics represent the United States (Nooner et al., 2012). The subjects with known pituitary conditions (such as pituitary tumor) were not included in the analysis data.

Resting state echo planar image (EPI) volumes had 64 slices of 2 mm 112 × 112 matrix with 2 mm thickness (voxel size = 2 mm × 2 mm × 2 mm), FOV = 224 mm, with repetition time (TR) of 1400 ms and echo time (TE) of 30 ms. A total of 404 volumes (∼10 min) were used in the analysis. High-resolution structural T1 volume was acquired as 176 sagittal slices of with 1 mm thickness (voxel size = 1 mm × 1 mm ×1 mm, TR = 1900 ms and TE = 2.52 ms, FOV = 256).

Data Processing

Data processing followed previous publication (Kiparizoska and Ikuta, 2017). Data preprocessing and statistical analyses were conducted using FMRIB Software Library (FSL,) as well as Analysis of Functional NeuroImages (AFNI). The anatomical volume for each subject was skull stripped, segmented (gray matter, white matter, and CSF), and registered to the MNI 2 mm standard brain. First four EPI volumes were removed. Transient signal spikes were removed by de-spiking interpolation. To correct head motion, the volumes were linearly registered to the then first volume, through which six motion parameters and displacement distance between two consecutive volumes were estimated. The first volume is registered to the standard MNI152 2 mm brain. Through this registration, 12 affine parameters were created between rs-fMRI volume and MNI152 2 mm space, so that the processed EPI volume can later be registered to the MNI space. Each of the resting state volumes was regressed by white matter and cerebrospinal fluid signal fluctuations as well as the six motion parameters. After smoothing with a 6 mm FWHM Gaussian kernel, the volumes were resampled, spatially transformed and aligned to the MNI 2 mm standard brain space. To perform scrubbing where the volumes with excess motion are removed, as a displacement distance between two EPI volumes, the root mean square deviation was calculated from motion correction parameters, at an $r = 40$ mm spherical surface using FSL's rmsdiff tool (Power et al., 2012, 2015). Volumes whose displacement distance exceeded the threshold (0.3 mm) were removed (*scrubbed*) from further statistical analyses (Siegel et al., 2014).

The PG ROI was manually defined in the MNI 2 mm space centered approximately at [MNI: 0, 2, −32] (**Figure 2**: Red), following previous MRI literature (Klomp et al., 2012). Data were excluded if the PG was located outside of the acquisition. Voxel-wise connectivity analysis was conducted in each individual brain. The time course was spatially averaged within the PG ROI that was registered to the EPI space so that correlations could be tested between the ROI and each individual voxel across the brain. The *Z*-scores representing the correlations between the ROI and a voxel were used for group level analysis after registration to the MNI 2 mm brain space.

In order to elucidate the regions which showed functional connectivity and dysconnectivity, one-sample *t* test was conducted assessing correlation (positive connectivity) and anti-correlation (negative connectivity) to the PG. Using *randomize* script in FSL, contrast images were estimated with cluster threshold of $Z > 3.72$. The association between BMI and whole brain functional connectivity to the PG was tested in a voxel-wise fashion using *randomize* script in FSL, taking age as a covariate. Contrast images were estimated with voxel-wise threshold of $p < 0.05$ (family wise error corrected), and minimum cluster size of 10 voxels.

RESULTS

In one-sample *t* test, in addition to the hypothalamus, the ventral and medial prefrontal cortex, inferior temporal gyrus, postcentral gyrus, insular cortex, parahippocampal gyrus, putamen, caudate head, and midbrain (periaqueductal gray), bilaterally showed positive connectivity with the PG (yellow/red in **Figure 1**). The dorsolateral prefrontal, parietal, occipital, and anterior cingulate cortices, hippocampus, caudate body, thalamus, pons medulla, and cerebellum showed bilateral negative connectivity (blue in **Figure 1**).

No regions showed positive association between the PG functional connectivity and BMI. The left orbitofrontal cortex,

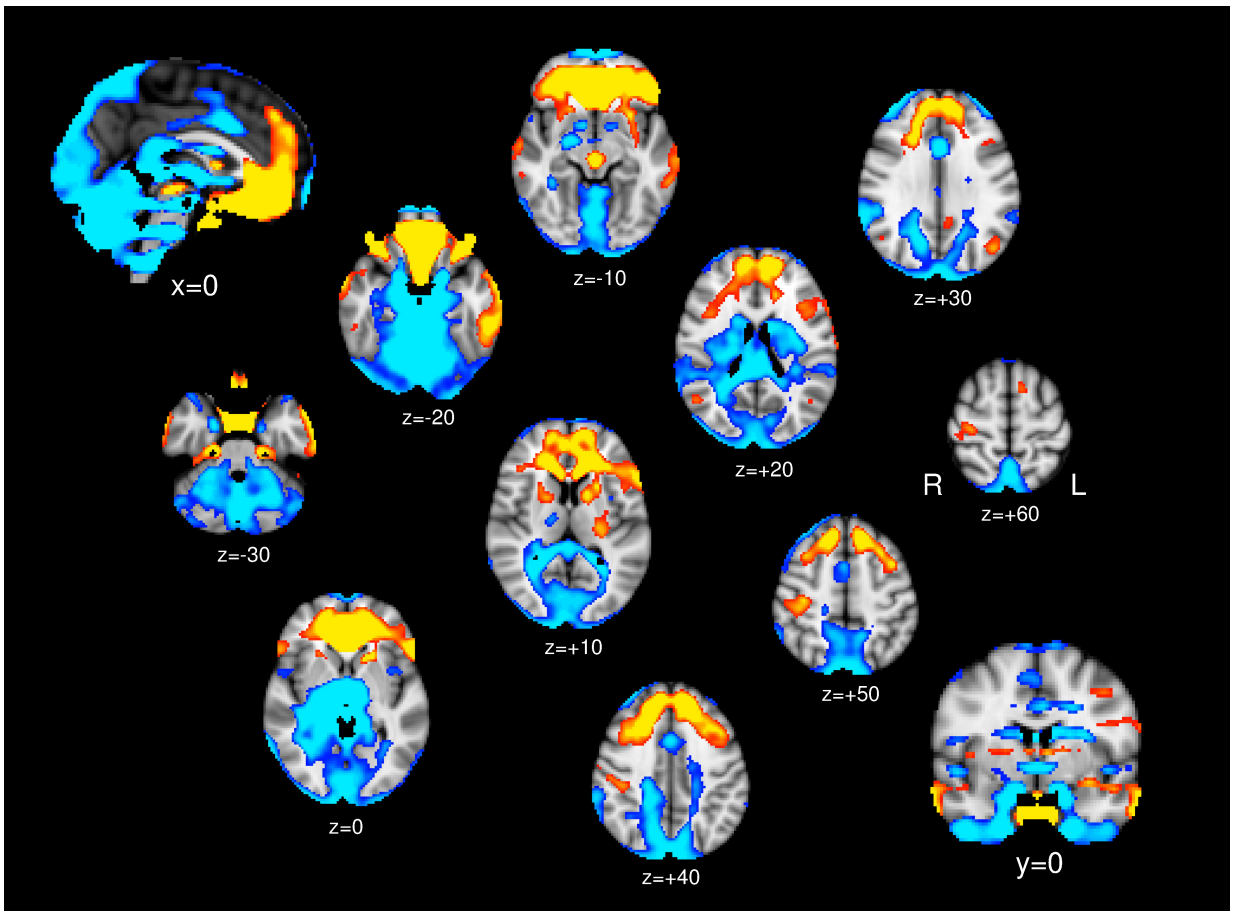

FIGURE 1 | One-sample *t* test; Brain regions show significant positive connectivity (yellow/red) and negative connectivity (blue) to the PG.

TABLE 1 | Regions whose PG connectivity showed negative association with BMI.

	Voxels	Peak *p* (corrected)	Cluster *p* (corrected)	MNI coordinates			Cluster Region
				x	y	z	
1	45	0.0002	0.015	−26	12	−20	Left Orbitofrontal Cortex
2	41	0.0012	0.019	−24	−12	−20	Left Hippocampus
3	33	0.0064	0.046	40	−26	−4	Right Superior Temporal Gyrus
4	23	0.0024	0.019	16	10	−4	Right Putamen
5	22	0.006	0.022	26	−2	−4	Right Putamen/Pallidum
6	21	0.013	0.030	−44	16	−30	Left Temporal Pole
7	14	0.009	0.024	16	−36	0	Right Hippocampus
8	10	0.023	0.035	−14	8	−10	Left Putamen

bilateral hippocampus, bilateral putamen, and right superior temporal gyrus showed functional connectivity with the PG inversely associated with BMI (**Table 1** and **Figure 2**).

DISCUSSION

This study aimed to identify (1) the functional connectivity of the PG and (2) brain regions whose functional connectivity to the PG shows associations with Body Mass Index. In one-sample *T*-test, the hypothalamus showed positive associations with the PG. This conforms to their close relationship classically know as a part of the HPA axis, which is known to be associated with obesity (Chalew et al., 1995). In this current study, however, the hypothalamus-PG functional connectivity did not show significant association with BMI.

Given their close relationship, we conducted an ROI-ROI connectivity analysis between the hypothalamus and PG. To

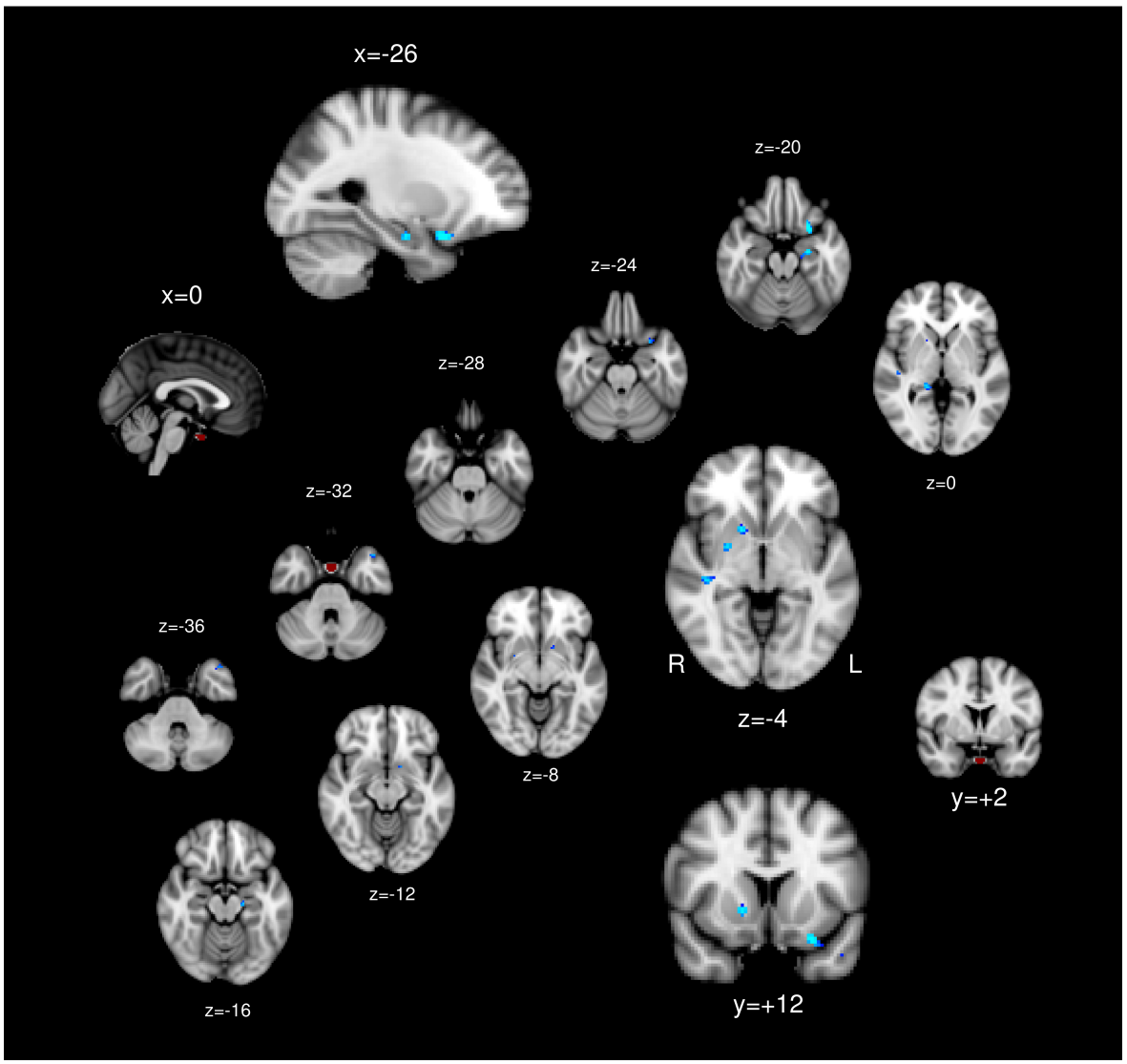

FIGURE 2 | The pituitary ROI (red) and regions whose pituitary connectivity showed negative association with BMI (blue).

assess the association between the BMI and hypothalamus-PG connectivity, a multiple linear regression was calculated to predict the hypothalamus-PG connectivity based on BMI, age, and sex. The regression was not significant, suggesting that the connectivity between hypothalamus-PG connectivity is constant across BMI, while PG and hypothalamus connectivity to the other parts of the brain identified in our voxel-wise analysis are associated with BMI. That is, the PG and hypothalamus are, similarly, influenced by the rest of the brain, resulting in the hypothalamus-PG connectivity remaining constant across BMI. Since the hypothalamus and PG are strongly positively connected, it is expected that the functional connectivity of the hypothalamus would show similar pattern with the PG in its association with BMI.

The orbitofrontal cortex (OFC) showed positive functional connectivity with the PG and inverse association with the BMI in its connectivity to the PG. It is implicated that lesser connectivity between the PG and OFC is associated with BMI. The OFC has been shown to be associated with obesity, as well as being responsible for taste and flavor processing (Small et al., 2007) and food choice (Cohen et al., 2011). Reduced OFC gray matter volume has been found in both adolescent (Ross et al., 2015) and midlife obesity (Driscoll et al., 2011). The OFC volume has been found to predict the risk for obesity (Smucny et al., 2012). More specifically, the OFC has been implicated in its role in preventing overeating. The OFC has been shown to be responsible for disinhibition of eating (Maayan et al., 2012), implicating its importance in preventing obesity. OFC volume has also been found to be associated with reward response in obesity (Shott et al., 2015). Reduced connectivity between the PG and OFC in a more obese population may suggest downregulated control of the OFC over PG.

The putamen showed inverse association between PG connectivity and BMI. That is, lesser PG-putamen connectivity is implicated in higher BMI. The association between pituitary-putamen dysconnectivity and BMI may be also accounted for by its role in the reward processing since the putamen and PG are both rich in dopamine D2 receptors. Striatal D2 receptors were shown to be reduced in individuals with obesity (Wang et al., 2004) and deficits of D2 receptor availability predicts future weight gain (Michaelides et al., 2012). Antipsychotics, whose pharmacological mechanism is represented by D2 antagonism, is widely known to induce weight gain (Correll et al., 2011). A D2 agonist bromocriptine has been found to counteract obesity (Kok et al., 2006). On the genetic level, polymorphisms in the D2 receptor gene (*DRD2*) have been implicated in its association to obesity (Nisoli et al., 2007; Ariza et al., 2012), as well as the weight gain response to antipsychotics (Lencz et al., 2010). The D2R profiles in the PG also show association with obesity. Disrupting D2 receptors in the pituitary lactotropes results in weight gain (Perez Millan et al., 2014). Dopaminergic modulations in the PG and striatum are both implicated in their association with BMI.

The PG receives dopaminergic projections from the hypothalamic arcuate nucleus as the terminal of the tuberoinfundibular pathway. Although this may partially account for their lack of BMI-differentiated association, it does not account for the associations with the striatal regions which are independently dopaminergic as the terminal of mesolimbic dopaminergic pathway originating in the substantia niagra. While the striatum and PG are independently dopaminergic in their primary source of their dopaminergic afferents, prolactin has been shown to upregulate both tuberoinfundibular and striatal dopamine neurons. While dopamine is inhibitory against secretion of prolactin (Ben-Jonathan, 1985; Liang et al., 2014), prolactin has been found to promote dopamine discharge in the tuberoinfundibular pathway (Lyons et al., 2012), forming a loop to regulate serum prolactin. In the striatum, rat prolactin increases dopamine turnover in the striatum (Fuxe et al., 1977). Prolonged intake of excessive sucrose has been found to upregulate striatal prolactin, thus showing implication to induce compulsive eating behavior (Ahmed et al., 2014). The prolactin-mediated dopaminergic response may be the underlying factor in the association between pituitary-caudate/NAcc functional dysconnectivity and BMI, whereby dopaminergic neurons in the PG and striatum are downregulated by prolactin.

It has to be noted that prolactin would not be the only mechanism that could both interact with the PG and striatum in the complex HPA axis. A voxel-based morphometric study showed a reduced volume of the putamen in obesity and a negative correlation between fasting plasma leptin concentrations and obesity (Pannacciulli et al., 2006), suggesting leptin mediated role of the putamen in regulating food intake. Dysconnectivity between the putamen and PG in obesity could be associated through the mechanism for leptin regulation.

The hippocampus also showed inverse association in its connectivity with the PG, while the hippocampi showed anti-correlation with the PG in the one-sample *T*-test. It is implicated that the anti-correlation is positively associated with BMI. Whilst the hippocampi also receive dopaminergic afferent from the ventral tegmental area, the association between hippocampus and food intake manipulation as well as physical exercise has been well documented. Physical exercise, which is shown to prevent excessive weight gain, acutely increases hippocampal volume, while hippocampal reduction is found after a series of cafeteria-diet in mice (Sack et al., 2017). Calorie restriction has been shown to improve cognition by upregulating brain-derived neurotrophic factor (BDNF) and downregulating oxidative stress in the hippocampus (Kishi et al., 2015). The hippocampus has been shown to be activated when images of high-calorie food are presented and this activation has been found to be a function of fasting plasma levels of insulin (Wallner-Liebmann et al., 2012), suggesting that the hippocampi respond to energy stimuli under the influence of hunger status. Our PG-hippocampus dysconnectivity finding may implicate that the PG is regulated by the hippocampus based on the hunger status and food availability.

Several limitations of the current study need to be addressed. First, as this study is based on functional connectivity, where functional connectivity is estimated by co-activations of two regions, causal relationships are not illuminated, although it could be inferred through known endocrinological properties of the PG. Second, despite the known subdivisions within the PG, such as the anterior and posterior pituitary, we avoided to make distinctions due to the imaging resolution of 2 mm^3 voxels and smoothing employed in processing. These two regions that showed functional dysconnectivity associated with BMI may arise from two distinct sub-regions of the PG.

It needs to be also addressed that the current study does not make distinction whether the PG connectivity influences BMI or BMI influences PG connectivity. While brain connectivity has been shown to influence body weight specifically in the context of the reward system, body weight status induced by surgical procedures has also been shown to influence functional brain connectivity (Lepping et al., 2015). It remains unclear whether our connectivity findings are the cause or results of the body weight.

In this study, we found functional dysconnectivity between the PG and dopaminergic regions including the putamen, hippocampus, and OFC. The results implicate dopaminergic modulation between the PG and these regions that influences body weight.

ETHICS STATEMENT

This study was approved by the University of Mississippi Institutional Review Board. This study had no direct involvement of human or animal subjects. All human subjects gave written informed consent.

AUTHOR CONTRIBUTIONS

PR and TI designed the study and drafted the manuscript. TI analyzed the data.

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Conflict of Interest Statement: TI has been a consultant for Sumitomo Dainippon and received speaker's honoraria from Eli Lilly and Daiichi Sankyo, and Dainippon Sumitomo.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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The Role of PGC-1α/UCP2 Signaling in the Beneficial Effects of Physical Exercise on the Brain

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In understanding the pathology of neurological diseases, the role played by brain energy metabolism is gaining prominence. Animal models have demonstrated that regular physical exercise improves brain energy metabolism while also providing antidepressant, anxiolytic, antioxidant and neuroprotective functions. This review summarizes the latest evidence on the roles played by peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator 1-alpha (PGC-1α) and mitochondrial uncoupling protein (UCP) in this scenario. The beneficial effects of exercise seem to depend on crosstalk between muscles and nervous tissue through the increased release of muscle irisin during exercise.

Keywords: physical exercise, mitochondria, irisin, FNDC5, PGC-1α, UCP2

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INTRODUCTION

A physically inactive lifestyle is associated with the development of non-communicable diseases (NCD), such as cardiovascular diseases, type 2 diabetes, some cancers, and an overall increased mortality rate (Booth et al., 2012; Koster et al., 2012; Biswas et al., 2015; Same et al., 2016; Patterson et al., 2018). Physical inactivity is also considered a risk factor for abdominal obesity, high serum triglyceride levels, low-density lipoprotein, cholesterol, hypertension, and hyperglycemia, which together characterize metabolic syndrome (Bankoski et al., 2011). Physical exercise has several benefits for physical and mental health (Aguiar et al., 2008; Garber et al., 2011; Esteban-Cornejo et al., 2015; Loprinzi, 2015), including increased physical and cardiorespiratory capacity (or fitness), improved body composition and balance (or fatness), and greater muscle strength and flexibility (Garber et al., 2011; Geneen et al., 2017). Physical exercise also improves the serum lipid profile, decreases glucose intolerance, and attenuates insulin resistance (Lin et al., 2015; Brymer and Davids, 2016; Qiu et al., 2018). The literature supports the fitness-fatness hypothesis, which suggests that a higher level of cardiorespiratory fitness will reduce the adverse effects of obesity on morbidity and mortality, making obesity a much less important factor for health than is generally believed (Hainer et al., 2009; Fogelholm, 2010; Barry et al., 2014). The data are mixed, but for many authors, fitness is more important than fatness for early mortality (Blair et al., 1989; Wei et al., 1999; McAuley et al., 2009; Barry et al., 2014). This is important for individuals who are unable to lose weight but are able to engage in a regular physical activity program.

The American College of Sports Medicine (ACSM) recommends increasing total energy expenditure (TEE; kcal/day) for health, with a minimum of 30 min of moderate physical exercise 5 days/week or 20 min of vigorous exercise 3 days/week (Haskell et al., 2007). The ACSM also recommends combining moderate (3–6 MET) and vigorous activities (>6 MET) (Haskell et al., 2007). The World Health Organization (2000) recommends that individuals participate in at least 150 or 75 min/week of moderate or vigorous physical activities, respectively (World Health Organization, 2010). For overweight and obesity, the ACSM recommends increased physical activity, between 150 and 250 min/week to prevent weight gain or provide modest weight loss (Donnelly et al., 2009). Larger amounts of exercise (>250 min/week) are needed for clinically significant weight loss (Donnelly et al., 2009). However, physical activity for weight loss is controversial; the amount of weight lost due to an exercise intervention is often less than what is predicted to be lost based on the exercise workload, suggesting a smaller increase in TEE (and smaller energy imbalance) than expected (Thomas et al., 2012; Melanson et al., 2013; Flack et al., 2018). This reduced energy imbalance occurs through metabolic and behavioral modifications in humans (Pontzer, 2018) and reinforces the hypothesis that fitness is more important than fatness for health. In general, the 150 min/week of moderate physical activity or 60–75 min/week of vigorous activity recommendations of ACSM and WHO is effective for overall health.

Even single exercise sessions, which increases the production of endogenous opioids (Geneen et al., 2017), angiogenesis factors (such as vascular endothelial growth factor [VEGF], hypoxia-induced 1 alpha factor [HIF-1α] and erythropoietin [EPO]) (Ribeiro et al., 2017) appear to be healthy, and they protect against hyperglycemia peaks (Lang Lehrskov et al., 2018) in humans. A single exercise session increases the plasma endocannabinoid levels in mice (Fuss et al., 2015), which is a possible mechanism for the euphoric state (runner's high) that occurs after long runs (Boecker et al., 2008).

The central nervous system (CNS) was the last physiological system approached by the exercise sciences. Lack of exercise is a major cause of chronic diseases (Booth et al., 2012), including brain diseases, such as depression (Farmer et al., 1988; Aguiar et al., 2014), and neurodegenerative diseases (Radak et al., 2010; Xu et al., 2010; Aguiar et al., 2016). However, physical exercise is a neuroprotective agent against depression (Schuch et al., 2017), anxiety disorders (Jayakody et al., 2014), cognitive decline/dementia in elderly people (Aguiar et al., 2011; Shen et al., 2016), Parkinson's disease (Chen et al., 2018), and Alzheimer's disease (Aguiar et al., 2016; Law et al., 2018). Animal studies have shown that physical exercise increases neuronal survival, cerebral vascularization, neurogenesis, and mitochondrial metabolism, while it decreases the effects of neurotoxins on the CNS (Aguiar et al., 2014; Zhang and Zhang, 2016). Iris and uncoupling proteins (U) are candidate mechanisms for these exercise-induced changes.

In mammals, transcriptional peroxisome proliferator-activated receptor gamma (PPAR-γ) coactivator 1-alpha (PGC-1α)/fibronectin type III domain-containing protein 5 (FNDC5, the precursor of irisin), which is secreted during exercise, promotes the browning of beige fat cells in white adipose tissue (**Figure 1**), resulting in enhanced thermogenesis and increased energy expenditure (Hofmann et al., 2014). In the CNS (**Figure 2**), FCDN5/irisin regulates central mechanisms that mediate adaptive responses by (a) improving neuronal mitochondrial decoupling and (b) increasing the expression of neurotrophins and neuroprotective proteins such as neuronal PAS domain protein 4 (NPAS4), cFOS, activity-regulated cytoskeleton-associated protein (ARC), and zinc finger protein 268 (ZIF268) (**Figure 1**; Wrann et al., 2013; Wrann, 2015). In brown adipose tissue, mitochondrial uncoupling is effected by a specific protein, referred to as uncoupling protein-1 (UCP1), in the inner mitochondrial membrane (Ricquier and Bouillaud, 2000). The cloning of UCP2 and UCP3, two homologs of UCP1, has boosted research into the importance of respiration control in metabolic processes, metabolic diseases and energy balance (Ricquier and Bouillaud, 2000). PPAR-γ/PGC-1α expression also improves mitochondrial decoupling, which reduces mitochondrial membrane potential and reactive oxygen species (ROS) production, oxidative damage, mitochondrial calcium overload and potential apoptotic events through the induction of uncoupling protein 2 (UCP2) (Andrews et al., 2005). Therefore, FND5/irisin is essential for processes involving neurotrophins and synaptic plasticity, mitochondrial biogenesis, and resistance to neuronal stress (Wrann et al., 2013; Marosi and Mattson, 2014; Raefsky and Mattson, 2017).

The purpose of this manuscript is to review the roles played by FND5/irisin and UCP2, which are important for energy metabolism, in the neuroprotective and antioxidant effects of physical activity in the CNS.

THE EXERCISE-INDUCED RELEASE OF IRISIN AND ITS NEUROPROTECTIVE EFFECTS

Because irisin is an exercise-induced hormone (or myokine), it is unclear whether the physical exercise-related CNS benefits are attributable to irisin. Irisin is a 112 amino acid peptide that is cleaved (by an unknown protease) from the glycosylated type I membrane protein FNDC5 and released into the bloodstream in a PGC-1α-dependent manner through a muscle contraction-mediated transcription mechanism (Bostrom et al., 2012). PGC-1α is a transcriptional coactivator and does not bind to DNA directly; it needs to interact with another transcription factor to induce neuronal FNDC5 gene expression (Xu, 2013). Several clues indicate that the PGC-1α binding partner is orphan nuclear estrogen-related receptor alpha (ERRα) (Kamei et al., 2003; Xu, 2013). Moreover, irisin enhances PGC-1α expression in the hippocampus and prefrontal cortex of mice in a positive feedback loop (Siteneski et al., 2018). The irisin released from muscles is a myokine that acts preferentially on the subcutaneous 'beige' fat and causes it to 'brown' by increasing the expression of UCP1 and other thermogenic genes (Bostrom et al., 2012). Irisin is involved in human biological adaptations such as increased muscle strength, decreased obesity and insulin resistance, and also has physical and psychological benefits (Bostrom et al., 2012;

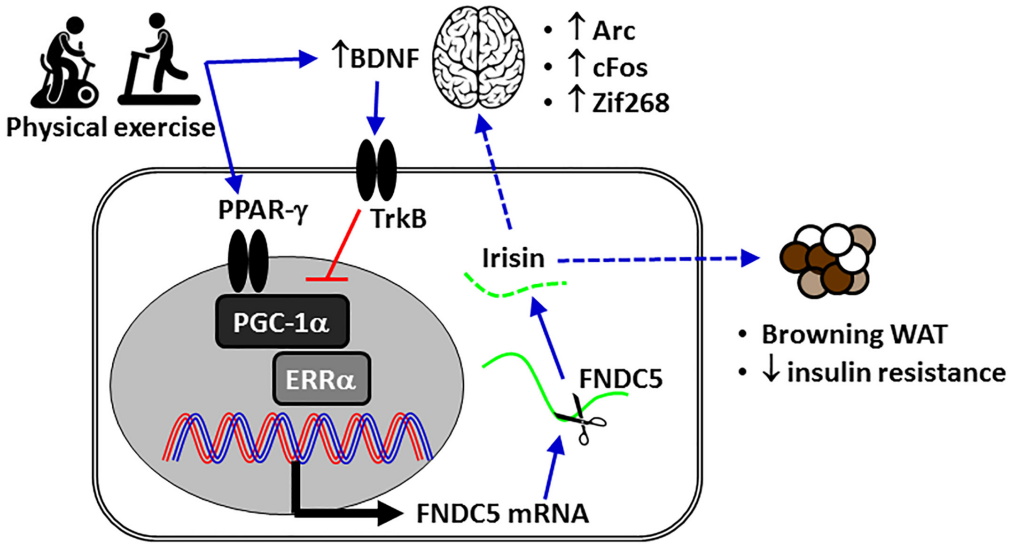

FIGURE 1 | The mechanism of action of irisin in metabolism-associated health issues or metabolic diseases. The expression of Arc, cFos, and Zif268 is induced by neuronal activity. BDNF, brain-derived neurotrophic factor; ERRα, estrogen-related receptor alpha; FNDC5, fibronectin domain-containing protein 5; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1-alpha; TrkB, tyrosine receptor kinase B; WAT, white adipose tissue.

Ghahrizjani et al., 2015). Currently, it is hypothesized that irisin circulates in the blood in vesicles containing other molecules, such as proteins, miRNA and nucleic acids, until reaching the target tissues, which include adipose tissue and the brain (Safdar et al., 2016). The exercise-induced release of peptides and nucleic acids from skeletal muscle (collectively termed 'exerkines') has been implicated in mediating systemic biological adaptations (Safdar et al., 2016).

The contraction of large muscle groups increases the muscle-specific expression of PGC1-α and FNDC5 and consequent release of irisin (Bostrom et al., 2012). In humans, blood irisin levels reach approximately 3.6 ng/ml in sedentary individuals and 4.3 ng/ml in active individuals after 12 weeks of regular aerobic exercise (Jedrychowski et al., 2015). Irisin contributes to exercise-induced physiological adaptations in the cardiovascular, immune, digestive, and adipose systems (Bostrom et al., 2012; Zhang et al., 2015; Mazur-Bialy et al., 2017). In obese adult humans, exercise combined with caloric restriction improves health (Mozaffarian et al., 2011) and increases concentrations of circulating irisin (Huang et al., 2017). Although skeletal muscle is the major source of exercise-induced irisin that is released into the plasma (Roca-Rivada et al., 2013), it remains unclear whether neuronal irisin is derived from muscles or is produced in neurons. In neurons, PGC-1α interacts with estrogen-related receptor alpha (ERRα) to regulate the expression of FNDC5 (**Figure 1**; Wrann et al., 2013). Moreover, the increased expression of FNDC5 promotes neuronal development and differentiation (Forouzanfar et al., 2015; Ghahrizjani et al., 2015). FNDC5 can be found in the cerebrospinal fluid, cortical neurons, paraventricular neurons in the hippocampus, Purkinje cells in the cerebellum, hypothalamus, multipolar neurons in the anterior nerve of the spinal cord, and in astrocytes and microglia in the cerebral tissue (Dun et al., 2013; Moon et al., 2013; Piya et al., 2014; Albayrak et al., 2015). In the rat H19-7HN cell line, irisin (50–100 nmol/l) increased the proliferation of hippocampal cells, thus reinforcing its role in neurogenesis (Moon et al., 2013).

Wrann et al. (2013) demonstrated that the expression of PGC-1α and FNDC5 in the hippocampal neurons was enhanced after the mice spent 2 weeks running in exercise wheels. Higher FNDC5 expression also increased the expression of the genes BDNF, Arc, cFos, and Zif268, which is induced by neuronal activity. FNDC5 expression is counterbalanced by BDNF expression in a negative feedback mechanism (**Figure 1**; Wrann et al., 2013). It is possible that this feedback loop is a CNS detraining mechanism that requires regular exercise to maintain its neurological benefits. This evidence suggests that the induction of FNDC5 is part of the transcriptional response to exercise, including neuroplasticity and neuroprotection, in the CNS. Exercise-induced PGC-1α and irisin reduced ischemia-induced neuronal injury (Zhang et al., 2012) via activation of the Akt and ERK1/2 signaling pathways in mice (Li et al., 2017). Exercise-induced irisin also reduced the brain infarct volume, neurological deficits, brain edema and the body weight decline of mice subjected to middle cerebral artery occlusion (MCAO) (Li et al., 2017). Since BDNF is a critical regulator of neural plasticity, irisin may act as a key regulator of neuronal survival following cerebral ischemia. Physical activity (running wheel, 12 weeks) increases levels of circulating irisin and BDNF even in 20-month-old female rats (Belviranli and Okudan, 2018), increases the expression of BDNF and decreases neuroinflammation in the hippocampus of aged rats and mice, and has motor and cognitive benefits (Aguiar et al., 2011; Dallagnol et al., 2017).

Exercise is an antidepressant (Blumenthal et al., 1999; Cunha et al., 2013), and irisin has been linked to the antidepressant effects of exercise. Reduced irisin levels are associated with

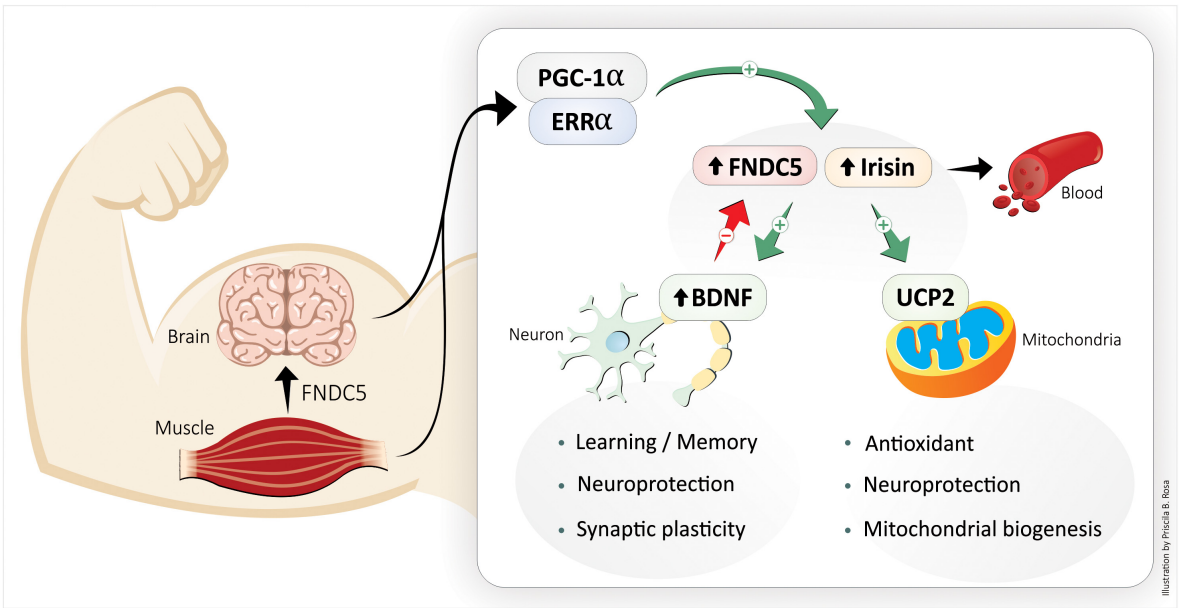

FIGURE 2 | The exercise-increased circulating protein irisin links structural and functional modifications in muscle and brain. BDNF, brain-derived neurotrophic factor; FNDC5, fibronectin type III domain-containing protein 5; UCP2, uncoupling protein 2.

mood impairment and reduced BDNF levels in humans (Papp et al., 2017; Szilasi et al., 2017), and increased circulating concentrations of irisin have been shown to have antidepressant effects in mice (Siteneski et al., 2018). A possible mechanism for the antidepressant effect is the activation of the PGC-1α/BDNF pathway by irisin after exercise (Wrann et al., 2013). As previously mentioned, BDNF is a critical neurotrophin involved in the differentiation, survival, maintenance, and function of neurons; it is also involved in learning and memory processes (Wrann et al., 2013). Torma et al. (2014) demonstrated that the neurotrophic role of BDNF is dependent on PGC-1α.

In humans, the increased plasma levels of FNDC-5, irisin and BDNF seem to depend on exercising large muscle groups, as can be achieved with regular Nordic walking training (Gmiat et al., 2018), aquarobics (16 weeks) (Kim and Kim, 2018), and CrossFit training (12 weeks) (Murawska-Cialowicz et al., 2015). The electrical stimulation of small muscle groups increases BDNF but not irisin in the hippocampus of rats (Maekawa et al., 2018). In healthy elderly women, Nordic walking training improved body composition, anaerobic capacity and cardiovascular fitness (Gmiat et al., 2018), and CrossFit training improved psychological (Quality-of-Life Assessment and The Beck Depression Inventory-2) and cognitive functions (D2 test of attention and Trail Making Test A&B).

NEURONAL UCP2 –AN ANTIOXIDANT MECHANISM OF EXERCISE

The expression of neuronal uncoupling proteins (UCP) is induced by metabolic and oxidative challenges such as physical exercise and caloric restriction (Liu et al., 2015). UCP facilitates proton flux through the internal mitochondrial membrane, thereby dissociating the oxidative phosphorylation of ATP synthesis (Wei et al., 2009). The enhanced proton flux process reduces the mitochondrial membrane potential, increases mitochondrial respiration, decreases the ATP/ADP ratio, and dissipates chemical energy in the form of heat (Chu et al., 2009). Acute mitochondrial decoupling reduces mitochondrial ATP production; however, chronic mitochondrial decoupling promotes an increase in the number of mitochondria and an increased level of ATP production (Coppola et al., 2007).

Initially, UCP1, which functions in heat production, was identified in brown adipose tissue (Geisler et al., 2017). UCP2 is found in organs and tissues such as the liver, kidney, pancreas, endothelium, immune cells, and the CNS (Pecqueur et al., 2001; Chu et al., 2009). UCP2, UCP4, and UCP5 are expressed in the CNS, are referred to as neuronal U, and are involved in the adaptation to cellular stress (Chu et al., 2009). The distribution of neuronal UCPs demonstrates the relevance of mitochondrial decoupling in the CNS to the control of neuronal, neuroendocrine, and autonomic responses (Richard et al., 1998). UCP2 is expressed in the hypothalamus, especially in the arcuate nucleus, limbic system, cerebellum, choroid plexus, and encephalic trunk (Richard et al., 1998; Arsenijevic et al., 2000). UCP4 is detected in most brain tissues, but it is expressed at lower levels in the spinal cord and *Substantia nigra* (Mao et al., 1999). UCP5 is expressed in the cerebral cortex, hippocampus, thalamus, hypothalamus, amygdala, basal ganglia, and spinal cord (Kwok et al., 2010).

Neuronal UCPs influence the regulation of mitochondrial biogenesis, calcium flux, ROS production, and local temperature (Teshima et al., 2003). Neuronal UCPs play an important role in the reduction of ROS production and consequent reduction in oxidative stress without compromising the production of ATP (Arsenijevic et al., 2000). Exposure of cultured neurons to decoupling agents, such as carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) or 2,4-dinitrophenol (2,4-DNP or simply DNP), reduces the mitochondrial membrane potential and inhibits mitochondrial calcium absorption, and thus prevents cell death (Stout et al., 1998). Neuronal UCPs also influence the temperature of neuronal microenvironments and thus contribute to the dynamics of neuronal activity through greater synaptic plasticity and neuronal transmission (Arsenijevic et al., 2000). Some studies have suggested that mitochondrial decoupling is linked to neuroprotection against physiological processes and pathological mechanisms including aging, Alzheimer's and Parkinson's diseases, neuronal hypoxia and ischemia, and epilepsy (Bechmann et al., 2002; Dietrich et al., 2008).

Among the neuronal UCPs, UCP2 is involved in central autonomic, endocrine, and metabolic regulation and is thus associated with cognition, mood, and behavior (Diano et al., 2000; Wang et al., 2014). UCP2 in the ventromedial nucleus restores glucose tolerance and regulates insulin sensitivity mediated by glucose-excited neurons, which is important for the physiological control of systemic glucose metabolism (Toda et al., 2016). In the arcuate nucleus, UCP2 is associated with mitochondrial fission, increased mitochondrial density and diminished mitochondrial size (Toda et al., 2016). UCP2 shows increased expression after neuronal injury (Bechmann et al., 2002). UCP2 induces mitochondrial decoupling in nigral neurons of the substantia nigra pars compacta (SNpc) and can prevent the loss of dopaminergic cells after 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP)-induced toxicity, which is an essential effect to delay Parkinson's disease pathophysiology (Echtay et al., 2001; Horvath et al., 2003). The relation of UCP2 to exercise occurs through the PGC-1α/PPARα pathway, which can regulate neuronal UCP2 (Wu et al., 2014) and BDNF (Gomez-Pinilla et al., 2008). Physical activity (running wheel, 4 weeks) increased UCP2 expression and mitochondrial oxygen consumption in coupled and uncoupled mitochondria in the hippocampus of mice (Dietrich et al., 2008). Moreover, physical activity (running wheel, 1 week) and exercise (treadmill, 12 weeks) increased UCP2 levels in the hippocampus, cerebellum and brain cortex mitochondria of adult rats (Gomez-Pinilla et al., 2008; Marques-Aleixo et al., 2015). The exercise-induced (running wheel, 1 week) increase in UCP2 correlated with increased BDNF in the hippocampus of rats (Gomez-Pinilla et al., 2008). These changes in BDNF content and mitochondrial metabolism (Vaynman et al., 2006; Aguiar et al., 2007, 2014) coincided with an increase in the number of mitochondria and dendritic spine synapses in the granule cells of the dentate gyrus and the stratum radiatum of the CA1 region and were dependent on UCP2 expression because such changes were not observed in UCP2 knockout mice (Dietrich et al., 2008). The absence of proper mitochondrial decoupling reduced the number of synapses in hippocampal neurons due to the increase in free radical production in response to exercise, thus demonstrating the characteristic protective effect of UCP2 in this knockout mouse model (Dietrich et al., 2008). For example, doxorubicin is an effective antineoplastic agent that is limited by mitochondrial toxicity in non-target tissues, including the brain (Marques-Aleixo et al., 2016; Flanigan et al., 2018). Doxorubicin (2 mg/kg, i.p.) impaired spatial learning/memory and decreased UCP2 protein content in cerebellum and brain cortex mitochondria of adult rats, both of which were prevented by physical activity (treadmill, 12 weeks) (Marques-Aleixo et al., 2016). The UCP2-related nuclear respiration factor 1 (NRF1) and mitochondrial transcription factor A (TFAM) genes, which are involved in mitochondrial biogenesis, are associated with synaptic plasticity and decreased neuronal vulnerability to cellular stress (Simon-Areces et al., 2012). In an animal model of Parkinson's disease, 8 weeks of treadmill exercise stimulated mitochondrial biogenesis and increased NRF2 and TFAM expression in the striatum of mice, which protected against neuronal death caused by the neurotoxin 6-OHDA (Aguiar et al., 2016). The mitochondrial mechanism related to UCP2 function is essential for the appropriate bioenergetic adaptation of neurons to increased neuronal activity and synaptic plasticity in response to physical activity.

CONCLUSION

Exercise improves the PGC-1α/BDNF pathway (muscle/brain) through the signaling of circulating irisin, which strengthens synapses and exhibits neuroprotective and antidepressant effects. These neuroprotective effects of exercise are enhanced by the antioxidant effects of UCP2, which is expressed at increased levels in neurons in response to exercise. Therefore, the evidence suggests a role for irisin/UCP2 in the mechanism underlying the benefits of physical exercise on the CNS. Consequently, irisin/UCP2 might be a potential therapeutic target to improve brain function and prevent or treat neurological and neurodegenerative diseases.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Cholinergic Control of Inflammation, Metabolic Dysfunction, and Cognitive Impairment in Obesity-Associated Disorders: Mechanisms and Novel Therapeutic Opportunities

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Obesity and obesity-associated disorders have become world-wide epidemics, substantially increasing the risk of debilitating morbidity and mortality. A characteristic feature of these disorders, which include the metabolic syndrome (MetS) and type 2 diabetes, is chronic low-grade inflammation stemming from metabolic and immune dysregulation. Inflammation in the CNS (neuroinflammation) and cognitive impairment have also been associated with obesity-driven disorders. The nervous system has a documented role in the regulation of metabolic homeostasis and immune function, and recent studies have indicated the important role of vagus nerve and brain cholinergic signaling in this context. In this review, we outline relevant aspects of this regulation with a specific focus on obesity-associated conditions. We outline accumulating preclinical evidence for the therapeutic efficacy of cholinergic stimulation in alleviating obesity-associated inflammation, neuroinflammation, and metabolic derangements. Recently demonstrated beneficial effects of galantamine, a centrally acting cholinergic drug and cognitive enhancer, in patients with MetS are also summarized. These studies provide a rationale for further therapeutic developments using pharmacological and bioelectronic cholinergic modulation for clinical benefit in obesity-associated disorders.

Keywords: cholinergic, brain, vagus nerve, obesity, metabolic syndrome, inflammation, neuroinflammation, cognition

INTRODUCTION

Obesity and obesity-related disorders have become prevalent conditions in our modern society, impacting over 1 billion people worldwide (Zimmet et al., 2001; Grundy, 2008; Aguilar et al., 2015; Kim et al., 2019). Obesity and the closely related metabolic syndrome (MetS) generate a substantial risk of developing type 2 diabetes, cardiovascular disease, and other debilitating and life-threatening diseases (Eckel et al., 2005; Grundy, 2008; van Dieren et al., 2010). Therefore, treating these conditions is of primary importance. Diet and exercise are key approaches, but in many cases

these lifestyle modifications are either not sustainable or difficult to implement. Clearly, there is a considerable need for better understanding of the complex pathology underlying obesity-driven disorders and for strategizing novel therapeutic approaches. Obesity-related chronic low-grade inflammation provides an important link to metabolic derangements including insulin resistance in these disorders (Shoelson et al., 2007). In addition to peripheral inflammation, inflammation in the CNS (neuroinflammation), affecting the hypothalamus and other brain regions, has also been described and associated with cognitive impairment in the context of obesity (Guillemot-Legris and Muccioli, 2017). Multiple links between peripheral and brain alterations, involving inflammatory, metabolic, and neural components, have been identified in obesity-associated conditions (Schwartz and Porte, 2005; Pavlov and Tracey, 2012). Of note, the role of the nervous system in this relationship has been a specific focus of ongoing research. The nervous system and the brain regulate feeding behavior, energy intake and expenditure, and metabolic homeostasis (Pavlov and Tracey, 2012; Kaelberer et al., 2018). The vagus nerve (the tenth cranial nerve), which contains fibers carrying ascending sensory signals to the brain and descending motor signals to the visceral organs, is importantly involved in these regulatory processes (Pavlov et al., 2018).

The nervous system also communicates with the immune system (Dantzer, 2018; Pavlov et al., 2018), and research during the last 20 years has revealed the important role of the vagus nerve in this communication. A body of preclinical evidence has demonstrated that the vagus nerve regulates inflammatory responses within a physiological mechanism termed *the inflammatory reflex* (Tracey, 2002; Pavlov et al., 2003). Accordingly, electrical vagus nerve stimulation (VNS) has been shown to control the release of pro-inflammatory cytokines and aberrant inflammation in many conditions (Chavan et al., 2017; Pavlov and Tracey, 2017). The mechanisms of the inflammatory reflex, which will be discussed in more detail later, involve alpha7 nicotinic acetylcholine receptor (α7nAChR)-mediated signaling in its efferent arm. Cholinergic compounds, including α7nAChR agonists and centrally acting acetylcholinesterase inhibitors (AChE), have also been shown to alleviate inflammation and metabolic derangements in obesity and MetS (Pavlov and Tracey, 2012). One of these drugs, the centrally acting AChE inhibitor galantamine is in clinical use for counteracting cognitive impairment in Alzheimer's disease (Hampel et al., 2018). A recent clinical trial revealed the anti-inflammatory and beneficial metabolic effects of galantamine in patients with MetS (Consolim-Colombo et al., 2017). Recent clinical studies also demonstrated the utility of bioelectronic VNS in rheumatoid arthritis and inflammatory bowel disease (IBD) – conditions characterized by immune and metabolic dysregulation (Bonaz et al., 2016; Koopman et al., 2016). Future applications of VNS in the arena of MetS and other obesity-driven disorders are feasible and of significant interest.

In this review, we briefly summarize the role of brain and the vagus nerve cholinergic signaling in the regulation of metabolic homeostasis and the role of the vagus nerve-based inflammatory reflex in controlling inflammation. We further point to important aspects of the relationship between inflammation, metabolic deterioration, neuroinflammation, and cognitive impairment in obesity-driven disorders. In this context, we elaborate on accumulating pre-clinical and clinical evidence for therapeutic benefit of stimulating brain and vagus nerve cholinergic signaling. We also consider the potential benefit of enhancing cholinergic signaling by centrally acting AChE inhibitors and VNS for counteracting cognitive deterioration in obesity-driven conditions.

THE BRAIN AND VAGUS NERVE IN METABOLIC REGULATION AND A ROLE FOR CHOLINERGIC SIGNALING

One of the first indications that the brain regulates body metabolism came from studies performed by the 19th century French physiologist Claude Bernard. He reported that electrically stimulating the floor of the fourth ventricle in the brain increased circulating blood glucose and induced a type of transient diabetes, thus connecting the brain with glucose and diabetes (Bernard, 1855). This intriguing brain-to-body metabolism and diabetes link was not intensely studied as the discovery of insulin in 1923 subsequently dominated the diabetes field. However, recent studies have provided important new insights into the role of the brain in the control of peripheral metabolic function and in the context of obesity-driven disorders, including type 2 diabetes. Accumulating evidence indicates that the brain closely monitors peripheral metabolic processes and plays a key role in regulating energy intake and metabolic homeostasis (Morton et al., 2006). The mechanisms involved in this regulation are complex, involving cholecystokinin, leptin, and insulin signaling, and several adiposity-related feedback loops (Morton et al., 2006; van Dijk et al., 2011).

Extensive research has characterized the hypothalamus as an important forebrain region in the regulation of metabolic homeostasis (van Dijk et al., 2011). Neuronal circuitry in the arcuate nucleus within the mediobasal hypothalamus plays a major regulatory role in food intake and metabolism mediating brain effects of leptin, insulin, melanocortins, and other metabolic molecules (Morton et al., 2006; Andermann and Lowell, 2017). For instance, the complex action of leptin on feeding behavior importantly involves activation of an appetite-suppressing population of pro-opiomelanocortin neurons and inhibition of neurons that express agouti-related protein and neuropeptide Y in the arcuate nucleus. Through a circuitry involving other proximal hypothalamic nuclei, such as the paraventricular nucleus and lateral hypothalamic area, these neurons communicate with the brainstem nucleus tractus solitarius (NTS) and promote afferent vagus nerve-mediated satiety and meal termination (Morton et al., 2006). Insulin also stimulates pro-opiomelanocortin neurons, which in turn causes suppression of appetite-promoting population of agouti-related protein neurons (Guilherme et al., 2019). In addition, insulin action in the arcuate nucleus is importantly implicated in the regulation of

hepatic glucose production and adipose lipolysis (Konner et al., 2007; Shin et al., 2017). Specific insulin signaling in agouti-related peptide-expressing neurons mediates the suppression of hepatic glucose production by this molecule (Konner et al., 2007) while insulin action on pro-opiomelanocortin neurons is linked to restraining adipose tissue lipolysis (Shin et al., 2017).

The pro-opiomelanocortin neuronal circuit in the hypothalamic arcuate nucleus was also identified as a major mediator of the appetite-suppressing effect of nicotine, thus providing a mechanistic insight into the apparent link between smoking and suppressed appetite (Mineur et al., 2011). α3β4nAChRs on these neurons have an essential role in mediating the suppressive effects of nicotine and other more selective agonists on food intake and weight gain in mice (Andermann and Lowell, 2017). These and other studies implicated brain α3β4 and other nAChRs, and the brain cholinergic system in the regulation of feeding behavior (Jo et al., 2002; Picciotto et al., 2012). As shown in **Figure 1**, major components of this system are the basal forebrain cholinergic system, comprised by several nuclei and the mesopontine/brainstem cholinergic system represented by the pedunculopontine and laterodorsal tegmental nuclei (Woolf and Butcher, 2011; Ballinger et al., 2016; Hampel et al., 2018). Basal forebrain cholinergic neurons innervate different cortex areas, the hippocampus, amygdala, and other regions (**Figure 1**). Among others, major projections of the cholinergic neurons in the mesopontine nuclei include the thalamus and hypothalamus (**Figure 1**). Cholinergic neurons residing in the brainstem dorsal motor nucleus of the vagus (DMN) and nucleus ambiguus (NA) provide peripheral axonal projections within the vagus nerve (**Figure 1**). Recently, basal forebrain cholinergic signaling was importantly implicated in the regulation of feeding behavior using selective optogenetic modulation (Herman et al., 2016; **Figure 1**). Both acute and chronic inactivation of cholinergic neurons in the basal forebrain diagonal band of Broca increases food intake while their stimulation results in decreased food intake (Herman et al., 2016). It was proposed that an extended brain network that regulates targets in the hypothalamic arcuate nucleus may mediate these cholinergic effects (Herman et al., 2016). In addition to nAChRs, muscarinic acetylcholine receptors (mAChRs) importantly mediate brain cholinergic neurotransmission and have also been associated with peripheral metabolic regulation. For instance, cholinergic mAChR-mediated activation in the hypothalamus results in increased hepatic glycogen synthesis and this effect is vagus nerve mediated (Shimazu et al., 1976; Matsushita et al., 1979). In addition, cholinergic M1 mAChR-mediated hypothalamic activation has also been shown to result in increased pancreatic exocrine secretion through the vagus nerve (Li et al., 2003; **Figure 1**).

The vagus nerve is a major conduit linking the brain and periphery in the regulation of metabolism (Berthoud, 2008; Andermann and Lowell, 2017; Metz and Pavlov, 2018). Sensory (afferent) fibers within the vagus nerve with cell bodies in the nodose ganglia communicate signals for alterations in nutrients and metabolic molecules, including cholecystokinin, leptin, and glucose from the gastrointestinal tract and the hepatic portal system to the brainstem (Pavlov and Tracey, 2012; Kaelberer et al., 2018; **Figure 2**). This communication involves neural synaptic transmission that occurs on the timescale of milliseconds, and slower humoral communication on the order of minutes. These signals arrive at NTS in the brainstem medulla oblongata, which is anatomically and functionally linked to DMN. Efferent (motor) vagus nerve cholinergic neurons originating from DMN and NA provide preganglionic innervations to visceral organs and regulate a range of vital cardiovascular, respiratory, and gastrointestinal functions, mediated through mAChRs on the effector cardiac myocytes, smooth muscle cells, and glandular cells (**Figure 1**). The

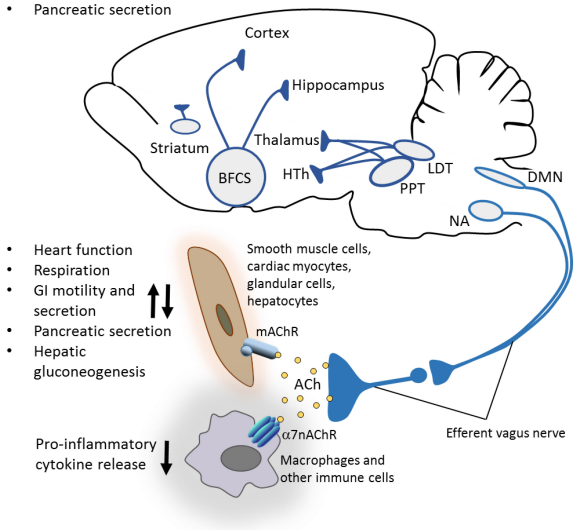

FIGURE 1 | Brain cholinergic system: anatomy and functional control. Important constituents of the brain cholinergic system are the basal forebrain cholinergic system (BFCS) comprised of several nuclei, the mesopontine pedunculopontine and laterodorsal tegmental nuclei (PPT and LDT), and intraneurons in the striatum. Cholinergic neurons provide innervations to different cortex areas, hippocampus, amygdala, olfactory bulb, hypothalamus (HTh), thalamus, and other regions. In addition to its well-documented role in the regulation of cognition, brain cholinergic signaling is implicated in the regulation of appetite and feeding behavior, local brain inflammatory responses (neuroinflammation), hepatic glycogen synthesis, pancreatic secretion, and control of peripheral inflammation (through vagus nerve-mediated mechanisms). Cholinergic neurons in the brainstem dorsal motor nucleus of the vagus (DMN) and nucleus ambiguus (NA) provide axonal projections within preganglionic efferent vagus nerve fibers. These long fibers interact with short postganglionic neurons in proximity or within the innervated organs, including the heart, lungs, gastrointestinal tract, liver, and pancreas. Acetylcholine (ACh) released from these neurons interacts with muscarinic acetylcholine receptors (mAChRs) on targeted cells and regulates several metabolic functions. ACh also regulates (inhibits) the release of pro-inflammatory cytokines and inflammation via alpha 7 nicotinic acetylcholine receptors (α7nAChRs) on immune cells (see the text for more details). (A rodent brain is shown as a substantial part of the information summarized is based on preclinical studies.)

efferent vagus nerve also regulates hepatic gluconeogenesis and pancreatic exocrine and endocrine secretion (Schwartz, 1983; Pocai et al., 2005; Pavlov and Tracey, 2012). For instance, efferent vagus nerve cholinergic signaling stimulates insulin release in the pancreas through M3 mAChR-mediated mechanism (Ruiz de Azua et al., 2011). In addition, mice with selective pancreatic β cells deficiency of the M3 mAChR have lower insulin secretion and impaired glucose tolerance (Gautam et al., 2006). NTS, DMN, and the closely located area postrema (a circumventricular organ) form the dorsal vagal complex, with reciprocal neuronal connectivity with hypothalamic nuclei and other forebrain regions, thus providing an extended brain network in the control of metabolic homeostasis (Pavlov and Tracey, 2012). The vagus nerve through its afferent fibers is a major mediator of satiety and a regulator of feeding behavior (Smith et al., 1981; Berthoud, 2008; Owyang and Heldsinger, 2011; **Figure 2**). Accordingly, a recent study demonstrated the substantial efficacy of implanted battery-free device-generated stimulation of afferent vagus nerve fibers (associated with stomach peristalsis) in achieving and maintaining weight loss in rats (Yao et al., 2018). This finding suggests the possibility of specifically targeting abdominal afferent vagus nerve fibers by bioelectronic devices as a therapeutic approach in obesity.

VAGUS NERVE AND BRAIN CHOLINERGIC SIGNALING IN CONTROLLING INFLAMMATION

Inflammation is a vital physiological response to harmful stimuli, including pathogen invasion and tissue injury through a number of processes and pathways, including the activation of specific immune cell types (e.g., neutrophils and macrophages) and the release of inflammatory mediators (e.g., cytokines and chemokines) (Chen and Nunez, 2010; Olofsson et al., 2017). Inflammation is generally a localized event, which resolves and then the body returns to homeostasis (Chen and Nunez, 2010; Serhan and Levy, 2018). However, different forms of non-resolved, exacerbated, or chronic inflammation cause secondary tissue injury and mediate pathogenesis in sepsis, IBD, rheumatoid arthritis, and many other diseases (Firestein, 2003; Tracey, 2007; Chavan and Tracey, 2017). Therefore, controlling inflammation is critically important in disease prevention and a useful therapeutic strategy in disease treatment. In addition to immune and hormonal regulation, research during the last 20 years has demonstrated an important role of vagus nerve-mediated neural mechanisms in controlling inflammation (Chavan and Tracey, 2017). Several studies have shown that sensory vagus neurons can be activated by cytokines, including IL-1β, TNF, and other inflammatory molecules (Goehler et al., 2000; Steinberg et al., 2016; Zanos et al., 2018). These peripheral inflammatory alterations are communicated to the brainstem and in a reflex arc, vagus nerve cholinergic anti-inflammatory output is generated (Tracey, 2002).

These studies led to the concept of a physiological immunoregulatory mechanism termed the inflammatory reflex (Tracey, 2002). The efferent arm of this mechanism was termed *the cholinergic anti-inflammatory pathway* (Borovikova et al., 2000; Pavlov et al., 2003). Electrical VNS has been instrumental in revealing the anti-inflammatory role of the vagus nerve innervating the liver, gastrointestinal tract, pancreas, and other organs in animal models (Borovikova et al., 2000; de Jonge et al., 2005; Bonaz et al., 2018; Metz and Pavlov, 2018). Cholinergic signaling is translated into suppression of pro-inflammatory cytokine release via α7nAChR-mediated signaling (Wang et al., 2003; Olofsson et al., 2012) and intracellular mechanisms, including suppression of NF-κB nuclear translocation, and JAK2/STAT3 activation (**Figure 3**) (Guarini et al., 2004; de Jonge et al., 2005; Parrish et al., 2008). In addition, recent studies have shown a mediating role for inflammasome inhibition and cAMP signaling (Tarnawski et al., 2018). Substantial advance in our understanding of the inflammatory reflex was achieved by revealing the functional cooperation between the vagus nerve and the splenic nerve and identifying a subset of splenic T cells, containing the enzyme choline acetyltransferase, as a source of acetylcholine in this circuit (Rosas-Ballina et al., 2011; **Figure 3**). Identifying the mediating role of the α7nAChR in the inflammatory reflex generated a line of research demonstrating the anti-inflammatory and disease-alleviating efficacy of α7nAChR agonists in numerous murine models of inflammatory diseases (Pavlov et al., 2007; Parrish et al., 2008; Pavlov and Tracey, 2015). Several studies have also shown that the inflammatory reflex and its efferent arm – the cholinergic anti-inflammatory pathway – can be activated through brain mAChR signaling. The anti-inflammatory and beneficial metabolic effects of centrally acting mAChR ligands and the AChE inhibitor galantamine have been demonstrated in murine models of endotoxemia, IBD, hemorrhagic shock, lupus, and other disorders and linked to the inflammatory reflex (Pavlov et al., 2006, 2009; Lee et al., 2010; Ji et al., 2014; Munyaka et al., 2014; Rosas-Ballina et al., 2015; Pham et al., 2018). In addition to galantamine, the anti-inflammatory effects of other AChE inhibitors and cholinergic drugs clinically approved for the treatment of Alzheimer's disease, such as donepezil and rivastigmine, have also been demonstrated (Lataro et al., 2015; Pavlov and Tracey, 2015; Zhang et al., 2016).

In addition to inflammation in the periphery, inflammation in the CNS and specifically in the brain also occurs in response to tissue damage and pathogens. Persistent neuroinflammation is a characteristic feature of traumatic brain injury, sepsis, multiple sclerosis, other neurodegenerative diseases, and other disorders (Amor et al., 2014; Borst et al., 2018; Pavlov et al., 2018). A link between inflammation, neuroinflammation, and cognitive deterioration has also been identified (Nizri et al., 2008; Terrando et al., 2011; Miller and Spencer, 2014; McManus and Heneka, 2017; Borst et al., 2018). Of note, galantamine, rivastigmine, and donepezil have been shown to alleviate neuroinflammation and improve cognition in preclinical studies (Nizri et al., 2008; Dasuri et al., 2016; Wang et al., 2018; **Figure 2**). In addition, a very recent study demonstrated that in addition to suppressing peripheral inflammation, VNS also alleviates neuroinflammation and cognitive dysfunction in murine endotoxemia (Huffman et al., 2019).

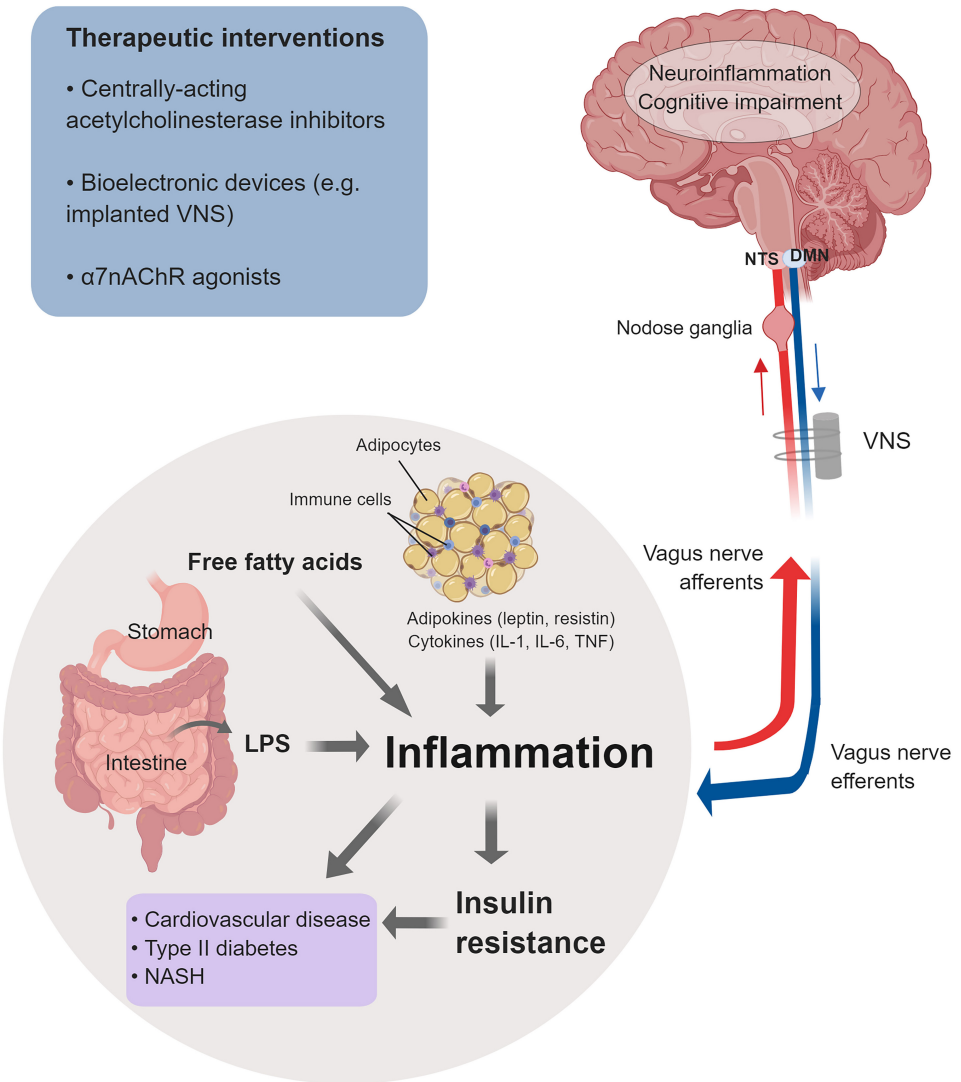

FIGURE 2 | Cholinergic control of inflammation and metabolic derangements in obesity-driven disorders. Inflammation in obesity is a major driver of insulin resistance and other metabolic derangements linked to an increased risk of cardiovascular disease, type 2 diabetes, NASH, and other disorders. Main factors that contribute to this low-grade chronic inflammation are: adipokines and cytokines released from enlarged adipocytes and immune cells infiltrating the expanded white adipose tissue; LPS as a result of microbiota alterations and increased intestinal permeability; and increased levels of free fatty acids. Inflammation and neuroinflammation in obesity are also linked to cognitive impairment. The vagus nerve provides a major conduit for communication between the brain and the periphery. Afferent (sensory) neurons residing in the nodose ganglia and terminating in the NTS detect alterations in peripheral inflammatory and metabolic molecules and communicate this information to the brain. Signaling through efferent cholinergic fibers that originate in the dorsal motor nucleus (DMN) plays an important role in controlling inflammation and metabolic derangements. Brain cholinergic signaling also is implicated in the regulation of cognition and controlling neuroinflammation. Brain and peripheral (vagus nerve) cholinergic signaling can be explored for therapeutic benefit in obesity and obesity-driven conditions. Preclinical and clinical studies have shown the efficacy of galantamine and other centrally acting acetylcholinesterase inhibitors, bioelectronic VNS, and α7nAChRs agonists in alleviating inflammation, metabolic derangements, neuroinflammation, and in improving cognition (See text for details). (This figure was created with BioRender.)

CHOLINERGIC CONTROL OF OBESITY-ASSOCIATED INFLAMMATION AND METABOLIC DERANGEMENTS

Chronic low-grade inflammation is a characteristic pathological feature of obesity and MetS (Eckel et al., 2005; Tilg and Moschen, 2006; Nathan, 2008; Gregor and Hotamisligil, 2011; Lumeng and Saltiel, 2011; Pavlov and Tracey, 2012). Inflammation in obesity is manifested by increased circulating levels of classical pro-inflammatory cytokines, such as TNF and altered levels of adipokines, including leptin, resistin, and adiponectin (Tilg and Moschen, 2006; Lumeng and Saltiel, 2011; Pavlov and Tracey, 2012). Expanded abdominal white adipose tissue in obesity and the crosstalk between metabolically active adipocytes and immune cells infiltrating the adipose tissue, including macrophages, neutrophils, and T lymphocytes, have

been identified as a major source of cytokines and adipokines and contributors to this characteristic inflammation (Tilg and Moschen, 2006; Nishimura et al., 2009; Pavlov and Tracey, 2012; Engin, 2017; **Figure 2**). Both enlarged adipocytes and infiltrating immune cells release pro-inflammatory cytokines such as TNF, interleukin-1 (IL-1β), and IL-6 (Pavlov and Tracey, 2012; Engin, 2017). Increased circulating levels of lipopolysaccharide (LPS, endotoxin) have been also detected in obesity (Cani et al., 2007). Microbiota alterations in the gut (increased LPS-containing microbiota) as a result of high-fat diet intake and increased body weight, and the consequent increased intestinal permeability have been associated with this "metabolic endotoxemia," which is another major contributor to inflammation in obesity (Cani et al., 2007; Cani and Delzenne, 2009; Delzenne et al., 2011). LPS, acting through a toll-like receptor 4 (TLR4)-mediated mechanism, triggers the release of TNF and other pro-inflammatory cytokines, mediating pro-inflammatory signals in liver, skeletal muscle, and adipose tissue (Cani et al., 2007; Castanon et al., 2014). Another important contributor to inflammation and other metabolic derangements in obesity is the high levels of free fatty acids (Lumeng and Saltiel, 2011). Acting through TLR4-mediated mechanisms on adipocytes, macrophages, and hepatocytes, free fatty acids trigger intracellular signaling resulting in NF-κB activation and increased TNF and other pro-inflammatory cytokine release (Shi et al., 2006; Baker et al., 2011; Lumeng and Saltiel, 2011; Pavlov and Tracey, 2012). Inflammation in obesity is linked to insulin resistance (Cani et al., 2007; Shoelson et al., 2007; Olefsky and Glass, 2010; Vandanmagsar et al., 2011). For instance, TNF has been shown to directly induce insulin resistance (Hotamisligil et al., 1993; Hotamisligil et al., 1996). In addition, obesity-related inflammation and insulin resistance are linked to fatty liver disease and the development of non-alcoholic steatohepatitis (NASH) (Shoelson et al., 2007; Carter-Kent et al., 2008; Lumeng and Saltiel, 2011; Schuppan and Schattenberg, 2013; **Figure 2**).

Approaches based on electrical VNS have been successfully explored in the treatment of obesity (Val-Laillet et al., 2010; Malbert et al., 2017; Masi et al., 2018). Several studies have also indicated the efficacy of targeting the inflammatory reflex using pharmacological modalities in alleviating inflammation and metabolic derangements interrelated in obesity-driven conditions (Pavlov and Tracey, 2012; **Figure 2**). Administration of nicotine (an α7nAChR agonist) to genetically obese *db/db* mice lacking the leptin receptor and mice with high-fat diet-induced obesity suppresses adipose tissue and systemic TNF levels (Wang et al., 2011). Nicotine also decreases adipose tissue expression of *CCL2* and the macrophage marker *F4/80*, pointing to alleviation of adipose tissue macrophage infiltration. In addition, α7nAChR KO mice on a high-fat diet have increased M1 macrophage infiltration and upregulated expression of TNF and CCL2 in adipose tissue compared to WT controls (Wang et al., 2011). Oral administration of the selective α7nAChR agonist TC-7020 to *db/db* mice significantly lowers systemic TNF and this effect is abrogated by co-administering methyllycaconitine – a selective α7nAChR antagonist (Marrero et al., 2010). TC-7020 administration to *db/db* mice also reduces weight gain, food intake and blood glucose, HbA1c, and triglyceride levels and

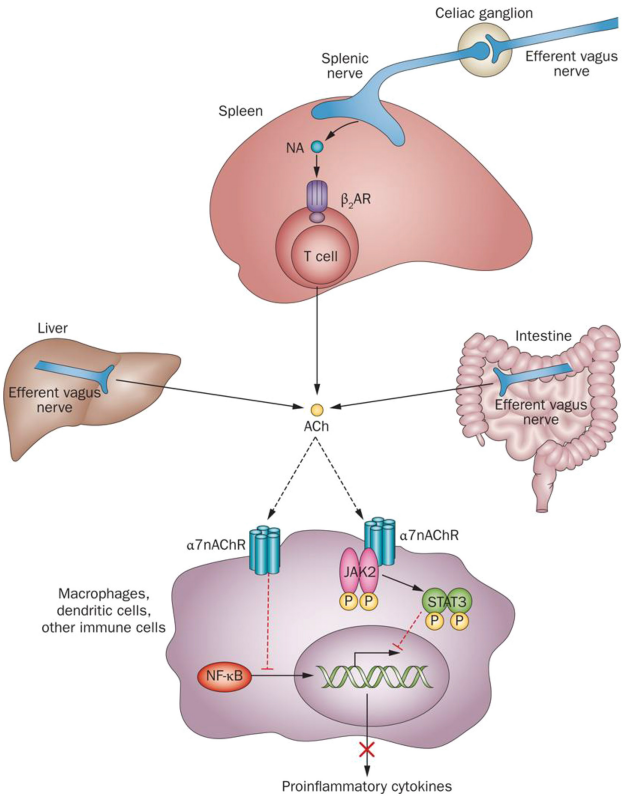

FIGURE 3 | Molecular mechanisms of cholinergic control of inflammation. Efferent vagus nerve activity is translated into catecholamine-mediated activation of T-cell-derived ACh release in the spleen and into direct ACh release from efferent vagus nerve endings in other organs. Inhibition of NF-κB nuclear translocation and activation of a JAK2-STAT3-mediated signaling cascade in macrophages and other immune cells are implicated in cholinergic α7nAChR-mediated control of pro-inflammatory cytokine production. ACh, acetylcholine; β_2AR, β_2 adrenergic receptor; JAK2, Janus kinase 2; α7nAChR, α 7 nicotinic acetylcholine receptor; NA, noradrenaline; NF-κB, nuclear factor κB; STAT3, signal transducer and activator of transcription 3. (This figure was originally published in Nature Reviews Endocrinology. 2012; 8: 743–754 and is used here in agreement with Springer Nature copyright regulations for reuse of author's own work.)

these beneficial effects are also abrogated by methyllycaconitine (Marrero et al., 2010). Importantly, administration of a JAK2 inhibitor significantly diminishes the TC-7020 effects on body weight, food intake, and blood glucose levels, a finding that suggests a link between α7nAChR- and JAK2-mediated signaling (Marrero et al., 2010). An important role for vagus nerve α7nAChR-mediated cholinergic signaling in experimental NASH was also demonstrated (Nishio et al., 2017). In a murine model of diet-induced NASH, α7nAChR KO chimeric mice (produced by transplanting α7nAChR bone marrow cells into γ-irradiated and Kupffer cell-depleted wild-type recipients) develop an accelerated form of disease (Nishio et al., 2017). These mice have significantly upregulated pro-inflammatory cytokine expression and altered/abnormal lipid metabolic pattern. Selective hepatic vagotomy in this model also results in increased TNF, IL-12, and CCL2 (MCP-1) levels indicative for increased

hepatic inflammation (Nishio et al., 2017). Using α7nAChR KO mice, another recent study also demonstrated the tonic anti-inflammatory and anti-fibrotic role of this receptor in models of atherogenic high-fat diet- and methionine/choline-deficient diet-induced NASH (Kimura et al., 2018). In these models, α7nAChR deficiency resulted in exacerbated hepatic fibrosis, higher plasma transaminase levels, and significantly increased Col1a1 gene-encoding alpha-1 type I collagen (mediating liver fibrosis) *Ccl2* and *Tnf* gene expression (Kimura et al., 2018).

As noted above, the inflammatory reflex can be activated by the centrally acting cholinergic drug, the AChE inhibitor galantamine (Pavlov et al., 2009; Ji et al., 2014; Pham et al., 2018). Galantamine alleviates inflammation and metabolic derangements in a high-fat diet-induced model of obesity and MetS (Satapathy et al., 2011). Galantamine treatment of mice with established obesity (after 8 weeks on a high-fat diet) significantly lowers plasma IL-6, CCL2, leptin, and resistin levels, and reduces body weight, food intake, and abdominal white adipose depots (Satapathy et al., 2011). Galantamine also decreases blood glucose, plasma insulin, and cholesterol levels, and alleviates insulin resistance and fatty liver disease in these mice (Satapathy et al., 2011). Recent work has also shown that galantamine has anti-diabetic effects in murine models (Ali et al., 2015; Hanes et al., 2015). Of note, the anti-diabetic effects of galantamine treatment of rats with established n5-STZ diabetes are greater than the effects of the anti-diabetic drug vildagliptin also used in the study (Ali et al., 2015). These and other preclinical studies indicate that neural cholinergic modulation, either pharmacologically or through bioelectronic VNS, can be further explored to treat obesity-related inflammatory and metabolic derangements.

NEUROINFLAMMATION AND COGNITIVE IMPAIRMENT IN OBESITY-DRIVEN DISORDERS: A CHOLINERGIC LINK TO TREATMENT

In addition to inflammation in the periphery, obesity is associated with neuroinflammation (Miller and Spencer, 2014; Guillemot-Legris and Muccioli, 2017; Lainez et al., 2018; **Figure 2**). This neuroinflammation occurs in multiple brain structures, including the hypothalamus, hippocampus, amygdala, neocortex, and cerebellum, and there is evidence that it is sex-specific (Miller and Spencer, 2014; Guillemot-Legris and Muccioli, 2017; Lainez et al., 2018). In these brain regions, diet-induced obesity is associated with increased levels of pro-inflammatory cytokines along with higher expression of NF-κB and TLR4, two important molecular mediators of innate immune responses (Biessels et al., 2014). This type of neuroinflammation may involve recruitment of peripheral immune cells (Miller and Spencer, 2014; Lainez et al., 2018) and there is evidence that peripheral inflammation precipitates brain inflammation (Miller and Spencer, 2014; Guillemot-Legris and Muccioli, 2017). Studies have linked obesity with cognitive impairment and both inflammation and neuroinflammation may play a mediating role in this context (Pistell et al., 2010; Sellbom and Gunstad, 2012; Miller and Spencer, 2014). Association between obesity and diabetes derangements, and increased risk of developing dementia has also been indicated (Whitmer et al., 2005; Strachan et al., 2011; Biessels et al., 2014).

The brain cholinergic system (**Figure 1**) plays a major role in the regulation of memory, attention, and learning (Ballinger et al., 2016; Hampel et al., 2018). Cholinergic neurodegeneration predominantly affecting the basal forebrain neurons and neuroinflammation are hallmarks of brain pathology in Alzheimer's disease (Heneka et al., 2015; Hampel et al., 2018). As mentioned earlier, centrally acting AChE inhibitors, including galantamine, donepezil, and rivastigmine, are clinically approved cholinergic drugs for treating cognitive impairment in Alzheimer's disease (Hampel et al., 2018). There is accumulating experimental evidence that brain cholinergic dysfunction is implicated in cognitive impairment in obesity as recently reviewed (Martinelli et al., 2017). High-fat diet-induced obesity in mice results in increased brain AChE expression and neuroinflammation manifested by microglial activation (Dasuri et al., 2016). Of note, donepezil treatment suppresses brain microglial activation and neuroinflammation in this model (Dasuri et al., 2016). There is also experimental evidence that enhancing cholinergic signaling by galantamine and rivastigmine also results in anti-inflammatory effects in the brain and improved cognition (Nizri et al., 2008; Wang et al., 2018). It is interesting and important that many brain regions affected by neuroinflammation in obesity, including the hypothalamus, hippocampus, amygdala, and cortex receive cholinergic innervations (**Figure 1**). Whether cholinergic dysfunction facilitates neuroinflammation in obesity is a question that remains to be further addressed.

Together these findings paint a picture of peripheral inflammation and neuroinflammation interrelated with cognitive impairment and cholinergic dysfunction in obesity. An improved understanding of the relationship between these elements of pathology in obesity would provide a solid rationale for designing new therapeutic approaches.

CLINICAL TRANSLATION OF CHOLINERGIC MODULATION IN OBESITY-DRIVEN DISORDERS

Obesity and obesity-related conditions, including MetS, type 2 diabetes, NASH, and cardiovascular disease, present a substantial health burden. Current treatment options are limited to lifestyle modifications, non-specific drugs, or insulin injections for those with type 2 diabetes. Unfortunately, lifestyle changes and dietary modifications are only temporarily effective for durable weight loss. For instance, a study with obese women subjected to caloric restrictions and behavioral therapy showed that nearly 50% of lost weight is regained within 1 year and almost all of it is regained within 5 years (Wadden et al., 1989). Non-specific weight loss medications have a blemished track record

and are also often ineffective as long-term solutions. Obesity-related type 2 diabetes is a progressive disorder and most patients eventually require treatment with insulin to control blood glucose levels as β cells in the pancreas lose their ability to produce insulin (Kahn and Hull, 2006). While generally effective, injectable insulin can produce side effects such as hypoglycemia, hunger, weakness, irritability, and requires frequent monitoring of blood glucose levels. The current studies on closed-loop control systems in diabetes known as "the artificial pancreas" may provide a significant advance in overcoming these disadvantages (Kovatchev, 2018). Other treatment options for obesity-driven disorders include surgical interventions with bariatric surgery which can result in long-term weight loss, but surgical treatments come with significant risks and are only recommended for the morbidly obese (Chang et al., 2014). Thus, the limitations of currently available treatments for obesity-driven disorders invite an exploration of new targets and pathways. In this context, targeting inflammation remains an attractive area for further exploration (Goldfine et al., 2011; Esser et al., 2015).

Based on preclinical evidence for beneficial anti-inflammatory and metabolic efficacy, cholinergic modulation in treating obesity-driven disorders is of specific interest (**Figure 2**). An additional advantage is that cholinergic drugs such as centrally acting AChE inhibitors and bioelectronics, including implanted devices for VNS are already clinically approved for other indications. Galantamine is a centrally acting AChE inhibitor that has been FDA approved to treat the cognitive impairment in patients with Alzheimer's disease in the United States for more than a decade (Hampel et al., 2018). There is a large amount of safety information available, making galantamine an attractive candidate to be clinically repurposed. A major obesity-driven disorder is MetS, a cluster of conditions that includes high blood pressure, high blood glucose levels, abdominal obesity, and dyslipidemia (Eckel et al., 2005; Grundy, 2008). The combination of these conditions within MetS generates a significantly higher risk of developing type 2 diabetes, cardiovascular disease, cancer, and other life-threatening and debilitating diseases, compared to the individual condition-related risk (Eckel et al., 2005). Apart from lifestyle modifications, treating MetS as a whole presents a significant challenge, and usually several medications targeting the separate risk factors are prescribed. Inflammation as a driver of insulin resistance and other pathogenesis in MetS provides an attractive therapeutic target (Esser et al., 2015). Based on preclinical findings, the efficacy of galantamine in alleviating inflammation and insulin resistance alongside other metabolic indices in people with MetS was recently studied in a randomized, placebo-controlled, double blind trial (Consolim-Colombo et al., 2017). Relatively short treatment (for 12 weeks) with galantamine in doses clinically approved for Alzheimer's disease, significantly decreased plasma TNF and leptin levels and increased IL-10 and adiponectin levels (Consolim-Colombo et al., 2017). Galantamine treatment vs. placebo also modulated the autonomic neural regulation, as determined by heart rate variability analysis, toward parasympathetic (vagal) predominance (Consolim-Colombo et al., 2017).

Another approach of cholinergic neuromodulation to explore in treating obesity-driven diseases is VNS. The feasibility of this approach for treating immune and metabolic derangements in humans was recently demonstrated in clinical trials with patients with IBD and rheumatoid arthritis (Bonaz et al., 2016; Koopman et al., 2016). Interestingly, humans with obesity have reduced vagal tone (Karason et al., 1999; Carnethon et al., 2003), coexisting with inflammation and metabolic deterioration. Since activation of vagal efferents attenuates immune and metabolic dysfunction, it is possible that reduced vagal cholinergic output plays a causative role in immune and metabolic dysregulation. VNS is clinically approved for epilepsy and depression (Ben-Menachem, 2001; Bonaz, 2018). Of note, patients receiving VNS for medication-refractory epilepsy or depression have also reported weight loss, in as high as 60% of the patients tested (Burneo et al., 2002; Pardo et al., 2007). While these patients were receiving VNS for other non-metabolic indications, these weight loss effects suggest the possible therapeutic utility of VNS in obesity-associated conditions. VNS implants for epilepsy and depression have been routinely used and the therapy is well-tolerated (Ben-Menachem, 2001). The risks associated with VNS implantation surgery are low and side effects of the stimulation itself are minor, typically limited to cough and hoarseness (Sackeim et al., 2001; O'Reardon et al., 2006). A recent study demonstrated that specific vagus nerve signals in response to alterations in cytokine levels can be recorded (Zanos et al., 2018). Therefore, in the future, an advanced version of bioelectronic vagus nerve modulation could potentially provide the additional advantage of enabling "closed-loop" control of immune and metabolic dysfunctions. Preclinical research has also indicated the possibility to use α7nAChR agonists in treating inflammatory and metabolic derangements in human obesity. This is also supported by demonstrating α7nAChR expression in human adipocytes and its role in controlling pro-inflammatory gene expression (Cancello et al., 2012). Furthermore, obesity is associated with significant decrease in human adipocyte α7nAChR and weight loss partially restores its expression (Cancello et al., 2012).

Cognitive deterioration and an increased risk of developing dementia are documented in obesity, MetS, and type 2 diabetes (Strachan et al., 2011; Biessels et al., 2014). This cognitive dysfunction is interrelated with inflammatory and metabolic derangements and treating these conditions is challenging (Strachan et al., 2011; Guillemot-Legris and Muccioli, 2017). An important point is that while designing new approaches for treating obesity and related conditions, one should account for their effects on the brain in relation to cognitive impairment (Strachan et al., 2011). The cholinergic system in the brain has a key role in the regulation of cognition (Picciotto et al., 2012; Ballinger et al., 2016). Numerous studies have evaluated galantamine, donepezil, and rivastigmine as cognitive enhancers and these drugs are in current clinical use for the symptomatic treatment of Alzheimer's disease. As recently demonstrated, galantamine has beneficial anti-inflammatory and metabolic effects in patients with MetS (Consolim-Colombo et al., 2017).

Therefore, future developments with galantamine and other centrally acting AChE inhibitors in the treatment of patients with obesity-associated disorders may include assessing the potential benefit of these drugs on cognition. VNS has been also associated with pro-cognitive effects especially in the domains of verbal recognition, memory, and executive function (Groves and Brown, 2005; Vonck et al., 2014). The precise mechanisms of these VNS pro-cognitive effects are largely unknown and one possibility that remains to be explored is a mediating role of brain cholinergic signaling. Recent findings have indicated that NTS afferent vagus nerve signaling reaches basal forebrain cholinergic nuclei innervating the hippocampus and cortex and implicated in cognitive regulation (Suarez et al., 2018). These studies and other clinical trials currently underway (ClinicalTrials.gov Identifier: NCT02365285) generate a growing platform for further studies on the therapeutic utility of cholinergic modulation in obesity-associated disorders.

CONCLUDING REMARKS

In obesity and obesity-associated conditions, immune and metabolic dysregulation result in chronic systemic inflammation, neuroinflammation, exacerbated insulin resistance, fatty liver disease, cognitive impairment, and other pathological manifestations. An improved understanding of this complex pathology requires providing new insight into the regulatory role of the nervous system. Neural circuitry, including vagus nerve cholinergic signaling, plays a major role in controlling metabolic and immune homeostasis (**Figures 1–3**). Vagus nerve cholinergic signaling within the inflammatory reflex has an important regulatory role in the crosstalk between immune and metabolic alterations in obesity-driven disorders. Activation of cholinergic signaling by VNS, α7nAChR agonists, and centrally acting drugs such as galantamine results in anti-inflammatory effects, alleviation of insulin resistance and hepatic steatosis, and other beneficial effects in murine models of obesity, MetS, NASH, and type 2 diabetes. This large body of preclinical evidence and the fact that both centrally acting AChE inhibitors and VNS are already in clinical use provide a rationale for expanding these approaches into clinical settings of obesity. A recent clinical trial with galantamine in MetS demonstrated the translational applicability of this research and the anti-inflammatory and beneficial metabolic effects of AChE inhibitors (Consolim-Colombo et al., 2017). Enhancing brain cholinergic signaling by these drugs and the use of VNS to alleviate obesity-associated brain pathology, including neuroinflammation and cognitive deterioration in humans, are feasible approaches that remain to be studied. Recent discoveries of gut–brain neural circuits that involve the vagus nerve (Han et al., 2018; Kaelberer et al., 2018) and future preclinical research using advances in molecular genetics within the growing field of bioelectronic medicine (Olofsson and Tracey, 2017; Pavlov and Tracey, 2019) will improve our understanding of neural regulation of immunity and metabolism and its implications in obesity. This research holds the potential to identify new therapeutic avenues for alleviation of the obesity disease burden.

AUTHOR CONTRIBUTIONS

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REVIEW
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Deep Brain Stimulation for Obesity: A Review and Future Directions

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The global prevalence of obesity has been steadily increasing. Although pharmacotherapy and bariatric surgeries can be useful adjuvants in the treatment of morbid obesity, they may lose long-term effectiveness. Obesity result largely from unbalanced energy homeostasis. Palatable and densely caloric foods may affect the brain overlapped circuits involved with homeostatic hypothalamus and hedonic feeding. Deep brain stimulation (DBS) consists of delivering electrical impulses to specific brain targets to modulate a disturbed neuronal network. In selected patients, DBS has been shown to be safe and effective for movement disorders. We review all the cases reports and series of patients treated with DBS for obesity using a PubMed search and will address the following obesity-related issues: (i) the hypothalamic regulation of homeostatic feeding; (ii) the reward mesolimbic circuit and hedonic feeding; (iii) basic concepts of DBS as well as the rationale for obesity treatment; (iv) perspectives and challenges in obesity DBS. The small number of cases provides preliminary evidence for the safety and the tolerability of a potential DBS approach. The ventromedial (n = 2) and lateral (n = 8) hypothalamic nuclei targets have shown mixed and disappointing outcomes. Although nucleus accumbens (n = 7) targets were more encouraging for the outcomes of body weight reduction and behavioral control for eating, there was one suicide reported after 27 months of follow-up. The authors did not attribute the suicide to DBS therapy. The identification of optimal brain targets, appropriate programming strategies and the development of novel technologies will be important as next steps to move DBS closer to a clinical application. The identification of electrical control signals may provide an opportunity for closed-loop adaptive DBS systems to address obesity. Metabolic and hormonal sensors such as glycemic levels, leptin, and ghrelin levels are candidate control signals for DBS. Focused excitation or alternatively inhibition of regions of the hypothalamus may provide better outcomes compared to non-selective DBS. Utilization of the NA delta oscillation or other physiological markers from one or multiple regions in obesity-related brain network is a promising approach. Experienced multidisciplinary team will be critical to improve the risk-benefit ratio for this approach.

Keywords: obesity, deep brain stimulation, hypothalamus, nucleus accumbens, metabolic disorders, neuroinflammation

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INTRODUCTION

According to the World Health Organization (WHO), obesity is defined as an abnormal and excessive fat accumulation. The increase in fat mass is a major health problem and is a risk factor for the development of several metabolic complications. Obesity decreases the quality of life and decreases life expectancy. The incidence of obesity has continued to rise rapidly during the last several decades. Similarly, the prevalence of obesity has been rising in most countries worldwide, and its prevalence has doubled in more than seventy countries over the last four decades. It was estimated that in 2015 there were a total of 603.7 million obese adults (body mass index [BMI] $>$ 30 kg/m^2) and 107.7 million obese children (defined as a BMI at or above the 95th percentile). Approximately 1.9 billion adults are by definition overweight (BMI $>$ 25 kg/m^2) (Gonzalez-Muniesa et al., 2017). The rising prevalence of obesity has been associated with a higher incidence of chronic metabolic disorders including type 2 diabetes. Both diseases share similar features including early cognitive dysfunction and a substantial socio-economic impact (Gaspar et al., 2016; Remor et al., 2018).

The etiology of obesity results from a complex interaction of several factors, including socioeconomic status, genetics, epigenetics, cultural features, and lifestyle. The obesity problem is even more complex with a link to compulsive eating behaviors and to depression (Val-Laillet et al., 2015; Ivezaj et al., 2016). Although no cause-and-effect relationship has been conclusively established, a systematic review revealed that obesity and depression had a significant and bidirectional association. The association with anxiety was modest but potentially important (Rajan and Menon, 2017). Despite its multifactorial etiology, a general consensus has emerged that the majority of obesity cases result from the combination of an increase in food intake along with a decrease in energy expenditure which together results in excessive fat accumulation. In most cases, obesity is a preventable disease and is largely dependent on lifestyle. Living a healthy lifestyle has recently become more of a challenge with elevated levels of stress, reduction of physical activity and the availability of palatable foods that are high in sugar, fat, and calories. The increased availability of an energy-dense diet, high in saturated fat and sugar, has been strongly associated with weight gain and increased adiposity, precipitating a strong change in energy balance not only resulting from a simple increase in energy intake (McClelland et al., 2013).

The palatable and densely caloric foods affect brain circuits involved with the control of energy metabolism in the hypothalamus and those involved with reward and humor perception within the limbic system. In predisposed, individuals this combination may result in overeating patterns and manifest as a "food addiction" or "food abuse." This phenomenon can increase the difficulty in achieving a long-term successful reduction of caloric intake by non-pharmacological and pharmacological approaches. This phenomenon has also increased the challenges of bariatric surgery for individuals with morbid obesity (Stice et al., 2013). Additionally, genetic factors might contribute to vulnerability to weight gain that, in combination with the environment precipitants result in brain network changes. This may reinforce the desire for food intake even if this desire is incongruent with the aspiration to reduce weight (Horstmann, 2017). Neuromodulation for the regulation of homeostatic and hedonic feeding has been suggested as a potential approach.

We review all the cases reports and series of patients treated with DBS for obesity using a PubMed search and will address the following obesity-related issues: (i) the hypothalamic regulation of homeostatic feeding; (ii) the reward mesolimbic circuit and hedonic feeding; (iii) basic concepts of DBS as well as the rationale for obesity treatment; (iv) perspectives and challenges in obesity DBS.

HYPOTHALAMUS, HOMEOSTATIC FEEDING, AND OBESITY

Homeostatic feeding is necessary for basic metabolic processes and survival (Rossi and Stuber, 2018). Energy homeostasis which is defined as a balance between food intake and energy expenditure is regulated primarily by the hypothalamus. The hypothalamus regulates food intake and energy expenditure but also regulates peripheral glucose and lipid homeostasis as well as metabolism (Morton et al., 2014; Timper and Bruning, 2017).

The discovery of the hypothalamus as a key center for energy homeostasis had its genesis with the studies of Hetherington and Ranson (1940) which revealed that adiposity increased in rats with hypothalamic lesions. Additionally, the discovery of leptin in 1996, by Zhang et al. (1994) opened new pathways for the molecular understanding of the role of the hypothalamus in the regulation of food intake and energy expenditure.

The **Figure 1** shows the interconnected hypothalamic nuclei, including the arcuate nucleus (ARC), the paraventricular nucleus (PVN), the ventromedial nucleus (VMN), the dorsomedial nucleus, and the lateral hypothalamic area (LHA) in both the healthy and the obesity state. Due to its privileged anatomical location, ARC is the principal region to receive and to sense afferent signals (nutrients and hormones) from the gut and the brainstem, as well as the region that processes efferent signals capable of modulating both food intake and energy expenditure. Two well-defined neuronal populations within the ARC have been described and each has been characterized by the expression of specific neuropeptides. These neuronal populations have potent effects on energy homeostasis namely, proopiomelanocortin (POMC) and agouti-related protein/neuropeptide Y (AgRP/NPY) (Morton et al., 2014; Sohn, 2015; Timper and Bruning, 2017). However, more recently it was identified that there was a third neuronal population which expressed tyrosine hydroxylase and had orexigenic characteristics (Zhang and van den Pol, 2016). All of these neuronal populations express high levels of hormone receptors (e.g., insulin, leptin, ghrelin, GLP-1, among others) and these receptors seem to facilitate a response to metabolic signals and to reflect the energy state of the organism as well as to control energy homeostasis (**Figure 1A**).

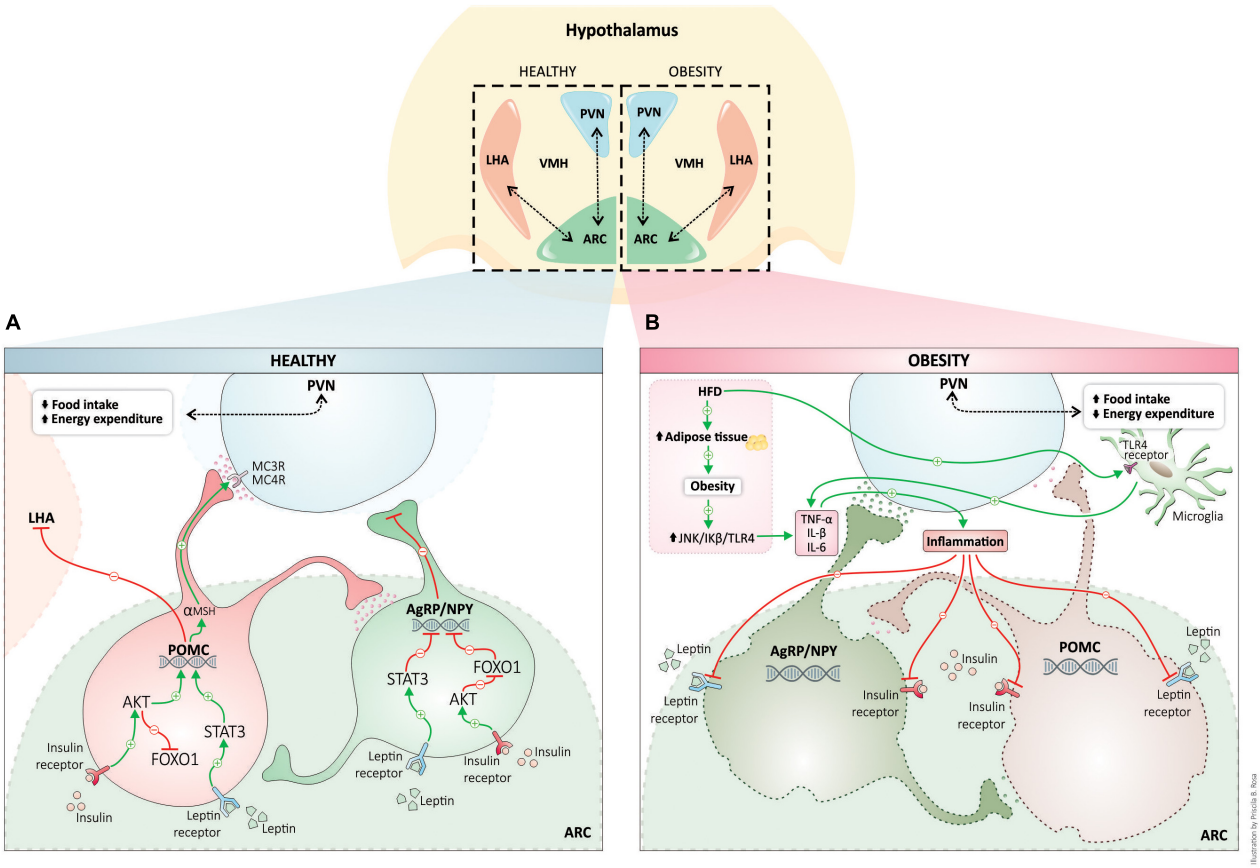

FIGURE 1 | Hypothalamic regulation of energy homeostasis in healthy (blue) and obesity (pink). The neuronal populations in the arcuate nucleus of the hypothalamus, POMC, and AgRP, senses and integrates peripheral adipostatic hormones (insulin and leptin), that circulate in levels proportionate to nutritional status and adipose tissue stores. Under the physiological postprandial state, insulin and leptin bind to respective receptors, both in POMC and AgRP neurons regulating the transcription of neuropeptides. Leptin and insulin signaling pathway stimulates POMC peptide transcription (activation of POMC neurons) and inhibits the transcription of AgRP neuropeptide (leading to AgRP neuronal inhibition). POMC neuronal activation involves the processing of POMC with the formation of α-MSH that is an agonist of MC4R in PVN neurons. PVN activation culminates in satiety (decreased food intake) and stimulation of energy expenditure **(A)**. High-fat diet consumption and obesity induces a whole-body chronic inflammatory state. Proinflammatory cytokines produced during inflammation are responsible for hypothalamic insulin and leptin resistance, and consequently, the neuronal control of energy homeostasis is disrupted inducing an increase in food intake and a decrease in energy homeostasis **(B)**. AgRP, agouti-related protein; Akt, protein kinase B; ARC, arcuate nucleus; FOXO1, forkhead box protein O1; IL-1β, interleukin-1β; IL-6, interleukin-6; JNK, c-Jun N-terminal kinases; LHA, lateral hypothalamus; MC4R, melanocortin 4 receptor; NPY, neuropeptide Y; POMC, proopiomelanocortin; α-MSH, alpha-melanocyte-stimulating hormone; PVN, paraventricular nucleus; STAT3, Signal transducer and activator of transcription 3; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; VMH, ventromedial hypothalamus.

The anorexigenic neurons, the POMC, are activated by adiposity signals, such as leptin, insulin, and some nutrients. The binding of leptin to its receptor induces the phosphorylation of the transcription factor STAT3 and this reaction upregulates POMC expression (Hakansson and Meister, 1998; Lee et al., 2018). Neuronal insulin signaling induces phosphorylation of forkhead box protein O1 (FOXO1), which is also a transcription factor that increases the expression of POMC (Belgardt et al., 2008). POMC is a neuropeptide that is post-translationally processed in several active peptides, including α and β melanocyte-stimulating hormone (MSH) and β-endorphins (Liotta et al., 1980; Wardlaw, 2011). After activation, neuronal projections of POMC neurons activate second-order neurons in the PVN, acting on the melanocortin receptors (MC3R/MC4R) leading to a decrease in food intake and to an activation of energy expenditure. The activation of POMC neurons has been shown to decrease body weight (Cheung et al., 1997; Wardlaw, 2011; Mountjoy, 2015). By contrast, the second population of AgRP/NPY neurons, inhibited by leptin and activated by fasting and ghrelin, has been shown to have a potent orexigenic effect, increasing food intake and decreasing energy expenditure (Wu et al., 2014). Ghrelin is primarily produced by stomach cells under fasting conditions. Ghrelin modulates feeding behavior and metabolism through the ghrelin receptor. The ghrelin receptor is abundantly expressed in the hypothalamus, mainly in AgRP/NPY neurons. AgRPq/NPY neurons are depolarized and activated by ghrelin. Concomitantly, ghrelin hyperpolarizes and inhibits POMC neurons (Kamegai et al., 2000; Al Massadi et al., 2017). In the fed state, AgRP expression is suppressed by leptin. Under fasting conditions (no leptin release) there is an

increase in AgRP and consequent increases in food behavior and adiposity. Activation of these neuronal populations leads to a release of AgRP, which is an antagonist of the MC4 receptor, and it inhibits PVN neurons. This process blocks the satiety feeling and stimulates feeding behavior through activation of lateral hypothalamic neurons (LHA) (**Figure 1A**) (Hahn et al., 1998; Luquet et al., 2005; Sohn, 2015).

Obesity is considered by many experts to be a low-grade chronic inflammatory condition. For several years, it was believed that the link between peripheral insulin resistance and obesity was a consequence of several variable factors including feeding patterns, consumption of high-fat diets, sedentary lifestyle and also that obesity occurred from stress. All of these factors are involved in the induction of systemic inflammation which can result in changes in metabolic and endocrine signaling (Hotamisligil, 2017). The expansion of white adipose tissue which occurs during the development of obesity produces proinflammatory cytokines (TNF-α, IL-1β, and IL-6) through the activation of intracellular serine kinases, such as JNK and IkB kinase. These kinases participate in the induction of insulin resistance (see review, Hotamisligil, 2017). Inflammation is also a hallmark in the brain of rodents under peripheral metabolic dysregulation (Remor et al., 2018), and in the hypothalamus of diet-induced obesity models (De Souza et al., 2005; Wang et al., 2012) (**Figure 1B**). Besides neuroinflammation, hypothalamic dysregulation induced by high-fat diets can result in an increase in markers of oxidative stress, endoplasmic reticulum stress, autophagy defects and changes in the rate of apoptosis and neuronal regeneration (Cavadas et al., 2016).

Unlike inflammation in peripheral tissues, which develops as a consequence of obesity, hypothalamic inflammatory signaling, microglia activation, and gliosis is observed during the first week of high fat diet consumption. They can also be observed prior to weight gain and in ARC (Thaler et al., 2012). A growing body of studies has demonstrated that saturated fatty acids are the trigger for early hypothalamic inflammation and are mediated mainly by microglial cells and astrocytes (Kleinridders et al., 2009; Gupta et al., 2012; Valdearcos et al., 2014, 2017; Mendes et al., 2018). Toll-like receptor 4 (TLR4) can be activated by saturated fatty acids, inducing the activity of NF-κB which transactivates gene expression of various proinflammatory cytokines and oxidative stress (Zhang et al., 2008; Kleinridders et al., 2009; Milanski et al., 2009). NF-κB also activates the expression of the suppressor of cytokine signaling 3 (SOCS3) which promotes negative feedback in the insulin and leptin intracellular signaling pathways, potentially linking hypothalamic inflammation with central leptin and insulin resistance (Valdearcos et al., 2015). Blocking the inflammatory process in the hypothalamus can prevent diet-induced obesity and insulin/leptin resistance (Kleinridders et al., 2009; Valdearcos et al., 2014, 2017; Mendes et al., 2018) (**Figure 1B**).

Neuroinflammatory damage to ARC neurons might disrupt integrated circuitry, including the communication with the downstream effector nucleus (such as PVN and LHN), and may generate a pathologic activation pattern that disturbs energy homeostasis. Targeting hypothalamic structures with DBS could thus possibly lead to weight loss and reduction in binge eating behavior if the appropriate circuits can be selectively activated or inactivated (Whiting et al., 2013).

REWARD CIRCUITS, HEDONIC FEEDING, AND OBESITY

The reward circuit structures have been implicated in motivation and desire, associative learning, and in emotions with a pleasure component related to reward. 'Liking' mechanisms include hedonic circuits that connect forebrain limbic structures such as NA and ventral pallidum (where opioid/endocannabinoid/orexin signals can amplify sensory pleasure). 'Wanting' mechanisms include larger opioid networks in NA, striatum, and amygdala that extend beyond the hedonic hotspots, as well as mesolimbic dopamine systems, and corticolimbic glutamate signals that interact with those systems (Berridge et al., 2010). Hedonic feeding is driven by sensory perception or pleasure involving reward structures and these also show a close anatomic and functional relationship with the hypothalamus and the homeostatic feeding (Rossi and Stuber, 2018). Hyperpalatable foods may be potentially "addictive" and have been compared to drugs of abuse. The cues that predict drug and food reward activate similar regions that have been implicated in reward and reward learning. The circuits involved include the mesolimbic dopamine system, which projects from the ventral tegmental area (VTA) to the NA (Stice et al., 2008). Functional MRI has shown that blood oxygen dependent signal (BOLD) in the NA is selectively increased during the perception of pleasant, emotionally arousing pictures and during mental imagery of pleasant, emotional scenes (Sabatinelli et al., 2007; Costa et al., 2010). In comparison to lean individuals, obese individuals experience greater activation in the gustatory cortex and somatosensory regions in response to anticipation and consumption of food (consummatory and anticipatory food reward) and experience a weaker activation in the striatum during food intake (Stice et al., 2008). The findings of reward responsivity in obese people can be inherited (genetic predisposition) or can result from repeated food intake or from both scenarios (Berridge et al., 2010).

On the other hand, the incidental finding of increased weight gain after discontinuation of pharmacological dopaminergic in rats suggested that hypofunctioning of dopamine circuits related to overstimulation by high palatable function could contribute to obesity (Reinholz et al., 2008). The Taq1A minor (A1) allele of the gene codifying dopamine receptors 2 and 3 (DRD2/3 gene) is associated with lower DRD2/3 density and has been found to exist in higher frequencies in obese subjects (Dang et al., 2016). In rodents, reduced DA transmission has been well documented in the striatum of obese rats (Johnson and Kenny, 2010; Rada et al., 2010). The results were in agreement with the earlier report showing lower striatal levels of D2 receptors of obese subjects in comparison to controls in 2001 (Wang et al., 2001). However, a large sample of adult subjects (n = 130, age between 18 and 81 years), Dang et al. (2016) show no association between o relation between DRD2/3 and BMI (range from underweight to extreme obesity) after controlling

for age distribution. Besides that, a randomized pilot study with a dopamine agonist (cabergoline) treatment for 16 weeks did not affect significantly the weight loss, but improve the glucose tolerance (Gibson et al., 2012). Also, genetic studies in humans have suggested that striatal D2Rs for obesity etiology have been considered somewhat controversial. Experts have questioned the reward deficiency theory of "food addiction" (Benton and Young, 2016). Recently Labouesse et al. (2018) outlined a causal relationship between striatal D2Rs and obesity in mice. These authors showed that high striatal D2R during development increased the risk for obesity in the mouse and that obesogenic diets were necessary to reveal the full effects of the D2R on obesity in the mouse. This data suggested that diet was an important cofactor along with the genetic predisposition.

Although the genetic and neuroimaging studies in humans and the experimental findings in rodents suggest a complex role for the reward-related dopaminergic system in obesity, the results support the use of this circuit as a target for "stimulation" or "inhibition" by DBS.

DEEP BRAIN STIMULATION

Based partially on the notion that high-frequency stimulation could suppress extrapyramidal tremor during functional ablative neurosurgery (Hassler et al., 1960; Alberts et al., 1965, 1966), Benabid et al. (1987) moved the field toward chronic stimulation with high-frequency continuous stimulation (130 Hz) of the thalamic nucleus ventralis intermedius for essential tremor and for parkinsonian tremor. He showed that DBS could be applied bilaterally without pseudobulbar and cognitive side-effects, an important observation as this was the major shortcoming of bilateral lesion therapy. Collectively, these observations consolidated the concept that high-frequency DBS could provide a "functional" lesion or alternatively modulation of a relevant brain network (Grill et al., 2004). We do not completely understand the mechanism of action of DBS, however, there seem to be important associated neurophysiological, neurochemical, neurovascular, neurogenic, neurochemical (e.g., glial cells), and neuro-oscillatory changes. The application of DBS should always be focused to selectively influence a specific and even multiple brain targets to modulate a relevant neural network. It should only be applied to alleviate human suffering (Benabid et al., 1987; Miocinovic et al., 2013; Williams and Okun, 2013; Okun, 2014).

The target size and the anatomical relationship to adjacent structures should be carefully considered during surgical planning. Presently, most DBS leads contain four platinum/iridium cone shape electrodes around the lead with a 1.27 mm diameter and 1.5 mm length. The spacing between each electrode can be 1.5 mm or 0.5 mm providing, respectively, a total of 10.5 mm or 7.5 mm length distance between the deepest and the most superficial electrode borders. There are newer models with eight contacts and segmented leads with variable spacing. The DBS leads are inserted by stereotactic surgery into the targeted brain structure, and an extension wire is subcutaneously tunneled to connect the implanted pulse generator (IPG) to the DBS lead (Oluigbo et al., 2012; Okun, 2014; Klooster et al., 2016). The IPG delivers adjustable pulses through the quadripolar electrodes and can be programmed to many settings based on voltage (or current in newer devices) amplitude, pulse width, and frequency (Hassler et al., 1960; Alberts et al., 1966; Benabid et al., 1991; Kuncel and Grill, 2004; Kringelbach et al., 2007; Brocker and Grill, 2013; Udupa and Chen, 2015). The DBS can be switched ON or OFF on demand (Oluigbo et al., 2012) in some paradigms. When the monopolar configuration is utilized a large sphere of current is generated and when bipolar is used the current is more restricted and elliptical. Newer devices may provide multiple independent current sources as well as segmented leads which can be used to steer or shape the current.

Deep brain stimulation has steadily become a safer procedure especially when an experienced multidisciplinary approach is applied. Severe adverse events are usually less than 5% in well-selected cases (Shukla et al., 2015). DBS is FDA approved in the United States for the treatment of advanced Parkinson's disease (Odekerken et al., 2013), dystonia (Meoni et al., 2017), and essential tremor (Barbe et al., 2018) and also has a humanitarian device exemption for dystonia and obsessive-compulsive disorder. DBS has been utilized in several refractory neuropsychiatric disorders including Tourette syndrome, depression (Schlaepfer et al., 2013), post-traumatic stress disorder (Koek et al., 2014), epilepsy (Salanova, 2018), obsessive-compulsive disorder (Goodman et al., 2010), pain (Farrell et al., 2018), Alzheimer's disease (Leoutsakos et al., 2018); drug addiction (Wang et al., 2018) and appetite disorders (Whiting et al., 2018).

Several observations have described a "feeling of happiness" accompanied by smile that progressed to natural laughter with DBS applied to the internal capsule/NA region for obsessive-compulsive disorder (Okun et al., 2004, 2007; Springer et al., 2006; Haq et al., 2011). Mood elevation during stimulation-induced smiles and laughter could be transient and was found to habituate with chronic NA stimulation (Springer et al., 2006; Haq et al., 2011). Rarely DBS induced mood elevation or "manic-like" symptoms in PD which were rapidly reversed with IPG reprogramming. Also, non-mood-related symptoms may also occur. Responses such as taste, smell, and smile were strongly associated with the most ventral lead positions. Similarly, physiological responses – for example, autonomic changes, increased breathing rate, sweating, nausea, cold sensation, heat sensation, fear, panic and panic episodes were significantly associated with ventral stimulation (Okun et al., 2007). Some authors showed that resistant depression treated with medial forebrain bundle DBS may improve over weeks to months (Schlaepfer et al., 2013; Fenoy et al., 2016). Perhaps most compelling was a paper by Halpern et al. (2011) which showed a delta oscillation in NA that was associated with feeding behavior in the mouse. These investigators also explored the use of neuromodulation applied to the NA in the animal and in one human case (an OCD case) as a method to modulate this "feeding" oscillation.

Taken together, clinical observations have shown that modulation can affect different limbic symptoms and this response can be exhibit variable latency. Neuromodulation for obesity treatment will need to consider the overlapping circuits

involved with hedonic and homeostatic feeding. Selectivity of a DBS treatment to specific cells using optogenetics may also be a viable approach (Creed et al., 2015; Lüscher et al., 2015; Mastro et al., 2017).

HYPOTHALAMIC DBS AS APPLIED TO OBESITY

The hypothalamic nuclei have become targets for DBS therapy in obesity because of the known association with vegetative functions, social behavior, food behavior and control of metabolism. Human hypothalamus is a 0.7 cm^3 volume and it is functionally heterogeneous and complex. It is located below the thalamus and it forms the lateral walls and floor of the third ventricle (Saper, 2004). The four hypothalamic nuclei directly involved with appetite control and food behavior are the VMH, LHA, DMH, PVN, and ARC (Lemaire et al., 2013; Barbosa et al., 2017). These are the nuclei of interest for neuromodulation.

Classical ablative studies have established VMN as the "satiety center," and LHA as the "feeding center." This dichotomy has, however, been replaced by a more complex hypothalamic neural network coupled to hormonal, metabolic and energy regulation signals (Lacan et al., 2008) as shown in **Figure 1**. In both rodent models and in humans obesity has been associated with hypothalamic gliosis and neuronal injury and this has been raised by some experts as a concern for future neuromodulation therapy (Thaler et al., 2012). Variations in the pattern of gliosis and also of neuronal injury distribution may impact local and distal neuroplasticity and may have implications for clinical outcome. The two nuclei require different stimulation frequencies to produce their therapeutic effects (Hamani et al., 2008; Wilent et al., 2010). Moreover, as previously described, systemic metabolic signals under fasting or non-fasting conditions (such as leptin and ghrelin) have antagonistic effects over ARC neurons (namely the POMC and AgRP) and result in the selective activation of PVN or LHA, which either favors weight loss or stimulates food behavior (Morton et al., 2014; Timper and Bruning, 2017).

As shown in **Figure 1**, in healthy conditions the PVN activation culminates in satiety (decreased food intake) and stimulation of energy expenditure. This nucleus is modulated by the anorexigenic POMC and orexigenic AgRP/NPY neurons from ARC nucleus. It has been suggested that to be effective for obesity, the PVN should be activated by a device directly implanted into the nucleus itself or in the ARC nucleus that projects to the PVN. A DBS neuromodulation approach of the ARC to treat obesity would preferably activate selectively the anorexigenic POMC and/or inactivate the orexigenic AgRP/NPY neurons. Because obesity is associated with complex changes in the neurophysiology of the POMC and AgRP/NPY neurons (see **Figure 1**), the result of ARC neuromodulation by DBS on the PVN in obese patients is tricky and an optogenetic manipulation may be a preferred approach. Also, because the PVN and ARC nuclei are relatively small, their selective modulation by DBS remains a barrier.

The DMH has been implicated in day-time feeding, in emotional responses to stress and when lesioned in male and female patients has been shown to be associated with persistent impotence and loss of libido (Meyers, 1962; Barbosa et al., 2017). Implications for DMH as a target for DBS in obesity remain to be explored.

The series and case reports about the VMH, LHA, and NA as DBS targets for obesity are discussed below and also summarized in **Table 1**.

Ventral Medial Hypothalamus DBS

Hamani et al. (2008) performed the first DBS trial to treat human obesity. The trial was inspired by the positive results obtained from preclinical (Halpern et al., 2011; Prinz and Stengel, 2018) and clinical studies, and it revealed that inhibition of the hypothalamus resulted in decreased food intake and weight loss (De Salles et al., 2018). Hamani et al. (2008) implanted DBS leads bilaterally in the ventral hypothalamus of one 50-year-old man with a life-long history of obesity (190.5 kg; BMI, 55.1 kg/m^2). The first stimulation parameter settings (130 Hz, 60 μs, and 2.8 V) did not reveal a significant effect (e.g., weight loss) during the first 6 months following surgery. Interesting, after a slight adjustment (50 Hz, 210 μs, and 3–4 V) the patient had a consequent weight loss (12 kg) without significant dietary changes and a reduction in food craving (Hamani et al., 2008). Because the patient reported difficulties in sleeping, the stimulation was turned off at night. The patient then began to eat at night and had increased weight (Wilent et al., 2010; Whiting et al., 2013). Wilent et al. (2010) used the same target as Hamani et al. (2008), however, in their case the stimulation (135 Hz, 60 μs, and 1–7 V) was associated with an unexpected panic event, and resulted in the interruption of the treatment.

An open-label clinical trial of hypothalamic DBS for morbid obesity is currently in progress and six patients will be included (De Salles et al., 2018). The subjects are required to have a BMI greater than 40 kg/m^2 or to have undergone bariatric surgery with therapeutic failure, as defined by BMI $\geq$ 35 kg/m^2 (must be 5 years after the procedure). Subjects must have no obesity comorbidities such as diabetes or cardiopulmonary abnormalities. The trial aims to assess the safety, identify possible side effects, and to optimize stimulation parameters in a paradigm of continuous VMH-DBS. Additionally, the study aims to determine if continuous VMH-DBS will lead to weight loss associated with changes in body composition, basal metabolism or food intake control (De Salles et al., 2018).

Lateral Hypothalamic Area (LHA) DBS

Weight gain has been observed in patients with PD treated with the subthalamic nucleus (STN) DBS and globus pallidus internus (GPi) DBS (Novakova et al., 2007). The weight gain could be an influence of spreading electrical currents into hypothalamic pathways though this remains speculative. There are also other factors including impulse control and dyskinesia reduction which could confound the finding. Nevertheless, it is interesting that STN and GPi DBS for Parkinson's disease have both been associated with weight gain.

TABLE 1 | Series and cases reports of patients treated with DBS for obesity.

References	Target	Stimulations parameters	*N*	Efficacy assessments	Follow-up (months)	Main results	Side effects
Hamani et al., 2008	Ventromedial hypothalamus (bilateral)	50 Hz, 210 μs and 3–4 V	1 (♂)	Body weight Neuropsychological assessment Binge eating	5 months	↓ Body weight ↑ Memory	Warming sensation (>4 V), flashes of light
Wilent et al., 2010	Ventral medial hypothalamus (bilateral)	135 Hz, 60 μs and 1–7 V	1 (♀)	–	–	–	Panic attack (increase in heart rate and blood pressure and anxiety feelings)
Whiting et al., 2013	Lateral hypothalamic (bilateral)	185 Hz, 90 μs and 1–7 V	3 (2♀; 1♂)	Body weight Psychological testing Binge Eating Scale Body shape Quality of life	35 months	↓ Body weight (2/3) ↓ Binge eating behaviors (1/3) No changes in Psychological Scores and life quality (3/3) ↑ Body image	Nausea, anxiety or "hot of flushed" sensations during programming changes
Talakoub et al., 2017	Lateral hypothalamic (bilateral)	8 Hz, 90 μs, and 3 V	1 (♂) a Prader–Willi	Local field potentials (LFPs) from the DBS contacts during acute evaluation during hunger and satiety.		Exposure to food-related cues during hunger induced an increase in beta/low-gamma activity. Satiety was marked by alpha rhythms (8 Hz). Alpha frequency DBS delivered prior to and during food intake resulted in sensation of fullness without effects on crave for food. Body weight was not reported.	None
Franco et al., 2018	Lateral hypothalamic (bilateral)	Off (2 months) 40 Hz (1 month) 15 days washout 130 Hz (1 month)	4 (2♀; 2♂) all were Prader–Willi	Calorimetry, bioimpedanciometry, neuropsychological assessments, hormonal levels, blood workup, and sleep studies	6 months	No major effects on anthropometric and calorimetric variables, hormonal levels, blood workup, sleep and neuropsychological evaluation	Two had stimulation-induced manic symptoms, one improves with discontinuation of DBS and one required topiramate increase. One removes the system because infection and one successfully treated with antibiotics
Mantione et al., 2010	Nucleus accumbens (bilateral)	185 Hz, 90 μs and 3.5 V	1 (♀)	Psychological assessment (anxiety and depression) Body weight	24 months	↓Obsessive–compulsive symptoms ↓Body weight ↓ Anxiety ↓ Depression	None reported
Harat et al., 2016	Nucleus accumbens (bilateral)	130 Hz, 208 μs and 2–3, 75 mA	1 (♀)	Body weight, body mass index, and neuropsychological assessment	14 months	↓ Weight ↓ BMI Without neuropsychological impairment	None
Tronnier et al., 2018	Nucleus accumbens (bilateral)	130 Hz, 90 μs and 3–4 V	1 (♀)	Body weight, Binge Eating Scale, Weight Efficacy Life-Style, Depression Scales, SF36 mood, simples mood	14 months	↓ Body weight ↓ Resist desire to eat and binge eating behaviors ↓ Depressed symptoms ↑ Mood and quality of life	Difficulties to falling sleep
Rezai et al., 2018	Nucleus accumbens (bilateral)	Not reported (high-frequency)	3 (♀)	Body weight, body mass index	36 months	One patient finished the follow-up period with ↓ weight and ↓ BMI Two patients did not complete the trial	One patient withdrew from the study with 13 months; one patient completed suicide after 27 months in the study, non-attributed to DBS (see the main text)

A right-side LHA hypothalamotomy lesion in two patients and bilateral in one (staged over 3 months) resulted in a transient decrease in caloric intake. This finding was compared to two patients with transient stimulation but no lesion(s) (Quaade et al., 1974).

In 2013, the first DBS treatment with electrodes implanted in the LHA was performed in three patients, (two females). All patients were previously treated with gastric bypass between 2001 and 2003 (Whiting et al., 2013). During the intraoperative microelectrode recording isolated but not discernible firing patterns were recorded. Microstimulation within the LHA produced sensations of nausea and thermal responses while more ventromedial microstimulation (presumably within the VMH) resulted in an anxiety or panic response. DBS was initiated 2 months after surgery. No significant weight loss trends were observed when DBS was programmed utilizing standard settings similar to the high-frequency parameters used for movement disorder surgery. Weight loss trends were observed when monopolar DBS stimulation was applied via specific contacts found to be associated with an increase in the resting metabolic rate as measured in a respiratory chamber. After 35 months of follow-up, two patients lost weight and one had a decrease in binge eating behaviors. There were no significant neuropsychological changes across patients and no significant side effects were reported.

Hyperphagia in Prader–Willi syndrome (PWS) is an important cause of genetic obesity. Recently, Talakoub et al. (2017) recorded the local field potentials (LFPs) from the DBS contacts implanted in the LHA of a 19-year-old Prader–Willi male patient undergoing DBS for obesity. During hunger exposure to food-related cues, there was an induced increase in beta/low-gamma activity. During satiety, the recordings were marked by alpha rhythms. An alpha frequency DBS was delivered prior to and during food intake. The patient reported a sensation of fullness but had a persistent food craving. The long-term effects have yet to be reported (Talakoub et al., 2017).

Franco et al. (2018) described four patients (two males) with Prader–Willi syndrome, treated with lateral hypothalamus DBS. The cohorts age ranged from 18 to 28 and their mean (SD) baseline BMI was 39.6 (11.1). Two patients had previous bariatric surgery. All patients had psychiatric comorbidities, including skin picking, nail biting, aggressive behavior, hypersexuality, episodes of hypomania, psychosis, and impulsiveness. These behaviors were reported as adequately controlled with medications. After DBS implantation, the treatment included the following phases: titration (1–2 months), stimulation off (2 months), low-frequency DBS (40 Hz; 1 month), washout (15 days), high-frequency DBS (130 Hz; 1 month), and long-term follow-up (6 months). Six months after receiving DBS at the "best" settings, the patients had a mean 9.6% increase in weight, a 5.8% increase in BMI, an 8.4% increase in abdominal circumference, a 4.2% increase in neck circumference, a 5.3% increase in the percentage of body fat, and a 0% change in calorimetry (as compared to baseline). The hormonal levels and results of a blood workup, sleep studies, and neuropsychological evaluations were unchanged by DBS therapy. Two patients developed manic symptoms during the titration phase, one improved with DBS programming and one required an increase in topiramate. One patient was receiving preoperative testosterone injections for hypogonadism, which infrequently resulted in priapism. One episode of priapism during the titration phase required drainage. One patient developed an infection over the connector site thought to be associated with skin picking 7 months following treatment. The family requested explantation since the device was ineffective for obesity. Another patient developed a superficial infection over IPG 1 month after the implant and this issue was successfully addressed with antibiotics.

NUCLEUS ACCUMBENS DBS

The first clinical report on NA DBS was published by Mantione et al. (2010) who treated refractory obsessive-compulsive disorder with associated nicotine dependence and also obesity (Mantione et al., 2010). In this study, a 47-year-old female patient weighed 107 kg with a height of 170 cm, corresponding to a BMI of 37 was implanted with a monopolar DBS (185 Hz, 90 μs, and 3.5 V). The clinical team utilized the two deepest contacts (0 and 1) for initial programming (accumbens region) and adjusted it using the more dorsal 1 and 2 contacts 3 weeks later, when she gradually started to improve her obsessive-compulsive symptoms. Within 5 months the Yale-Brown Obsessive-Compulsive Scale scores decreased from 38 to 2 and she reported reducing the time spent on obsessions and compulsions from 20 h a day to less than 1 h a day. The HAM-A (subclinical anxiety) and the HAM-D (subclinical depression) gradually decreased from 17 to 2 and 11 to 3, respectively. Seven months after surgery she realized she was no longer dependent on her compulsions, but that she was still dependent on her cigarettes. She stopped smoking and stated she did not crave cigarettes and did not experience withdrawal symptoms. The 2-year follow-up evaluation showed her weight was 71 kg, she was not smoking and she had no desire to start smoking. She scored 1 on the Yale-Brown Obsessive-Compulsive Scale, 3 on the HAM-A, and 3 on the HAM-D.

More recently in 2016, Harat et al. (2016) described a 19-year-old woman with obesity associated with a hypothalamic region that was damaged by a craniopharyngioma (neurosurgery was in 2004). She was approved for bariatric surgery but preferred DBS surgery. She was treated with bilateral NA DBS (130 Hz, 208 μs, and 2–3.75 mA). After 14 months her weight was reduced from 151.4 kg to 138 kg and her BMI from 53 to 48. No neuropsychological or other side effects were observed as a result of the surgery (Harat et al., 2016).

Tronnier et al. (2018) reported that bilateral NA DBS (130 Hz, 90 μs, and 4 V) decreased body weight, BMI and binge behavior without significant psychological impairment in a woman who was followed for 14-months. She had depression resistant to electroconvulsive therapy (ECT). Her peak weight in November 2013 was 183.6 kg which at a height of 167 cm translated to a BMI of 66. In March 2015, she underwent gastric bypass surgery after a course of behavioral therapy for her obesity and it was reported to have limited success. Because the temporary improvement of depression was less than 2 weeks and further antidepressant medication was not successful, the decision was

made for treatment with NA DBS in November 2015. Morbid obesity of the patient was considered a secondary target of the DBS procedure. After DBS surgery, her weight loss accelerated to 2.85 kg/month. At the time of writing of this report (12/2016) her body weight has continuously decreased to 106 kg, corresponding to a BMI of 38. Her depressive symptoms have also been reported to be significantly reduced. Interestingly, the weight loss was accompanied with heightened feelings of "self-efficacy of ingestive behavior" that was not felt following bariatric surgery. This case raised the issue as to whether obesity associated with depression could be addressed with NA DBS.

Rezai et al. (2018) described their experience with NA DBS for obesity in three female patients followed by their multidisciplinary team. The inclusion criteria were age between 22 and 60 years, "extreme obesity" defined as BMI > 40, and patients had to fail Rou-en-Y gastric bypass surgery. Although all patients lost weight with DBS, only one participant successfully completed the 3-year trial, losing 100 pounds with a 30% BMI reduction (55.7–39.3). After 13 months in the study, one subject requested explantation. One patient died as a result of a completed suicide after 27 months into the study. All patients had significant psychiatric illness and during the trial reported major psychosocial stressors including family violence, divorce, job loss, family illness, and pet death. The study team concluded that DBS itself was not responsible for the suicide, patient withdrawal or explantation. Regarding the association between obesity and suicide, a recent systematic review and meta-analysis of 15 prospective studies showed an inverse association between obesity with suicide mortality and attempted suicide (Amiri and Behnezhad, 2018). The mechanisms related to an association between DBS and suicide are not understood but the recent Veterans Affairs study with a long term follow-up did not show an association with STN and GPi DBS and suicide (Weintraub et al., 2013). One confounding factor has been the early reports of suicide led most major groups to implement more careful pre-operative screening and post-operative monitoring.

Rezai et al. (2018) has suggested the following strategies to reduce suicide risk (i) Requirement of close psychiatric monitoring by clinicians trained in bariatric psychiatry and mandatory psychological therapy during the study period; (ii) Requirement of subject commitment to compliance with the study requirements and close involvement with a social support system and a study partner (a family member or close friend committed to the trial with the subject) from the subject's environment; (iii) Upfront consideration should be given to the long-term follow-up of the DBS system after the trial conclusion and should be discussed with the subjects and family support system in advance of implantation.

Finally, because of its assumed role in reward-related behavior, the ventral anterior limb of the internal capsule (vALIC) could be a potential target for obesity DBS. However, Linssen et al. (2017) reported no significant change in body weight on a group level after vALIC DBS (mean follow-up was 3.8 years, range 10 months to 8.7 years) for either obsessive-compulsive disorder (n = 30) or major depressive disorder (n = 16). The average baseline BMI in their sample of 46 patients was 28.0 (SD 7.3), with 26 (57%) being overweight (BMI 25–30, n = 11), obese (BMI 30–40, n = 12), or morbidly obese (BMI $\geq$ 40, n = 3).

CONCLUSION AND FINAL REMARKS

Prevention of obesity is a worldwide issue. It will required broad changes in the worldwide pattern of food ingestion and physical activity. The cost-effectiveness of innovations must be taken into account in epidemiological terms. However, in selected cases of morbid obesity, DBS, similar to gastric surgery, may in the future be refined into a therapeutic modality. Hypothalamic DBS for obesity has been shown to be reasonably safe in well-selected patients. The effectiveness has, however, not been shown to be robust or reproducible. Based on both biological plausibility and on observational studies, the NA has emerged as an alternative obesity DBS target.

Future studies should also focus on better understanding the patient characteristics most likely to benefit from obesity DBS (Tronnier et al., 2018). Specific DBS targets may be optimal for specific clinical phenotypes. The therapy could thus become more personalized (Famm et al., 2013).

The identification of electrical control signals may provide an opportunity for closed-loop adaptive DBS systems to address obesity (Carron et al., 2013; Kuhn and Volkmann, 2017). Metabolic and hormonal sensors such as glycemic levels (Christiansen et al., 2018), leptin and ghrelin levels are candidate control signals for DBS.

Newer approaches for obesity DBS should be explored. Focused excitation or alternatively inhibition of regions of the hypothalamus may provide better outcomes compared to non-selective DBS. Utilization of the delta oscillation (Halpern et al., 2011) or other physiological markers from one or multiple regions in the obesity network is a promising approach.

Finally, it will be important to implement expert multidisciplinary screening teams as well as post-operative monitoring to lessen the occurrence of neuropsychiatric adverse events.

AUTHOR CONTRIBUTIONS

DF, JG, HM, TE, RZ, AL, and RW wrote the manuscript. TE, JG, and DF elaborated the tables and figures. AL, JG, MO, and RW participated in the conception of the idea and revised the manuscript.

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Brain Innate Immune Response in Diet-Induced Obesity as a Paradigm for Metabolic Influence on Inflammatory Signaling

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Obesity is a predisposing factor for numerous morbidities, including those affecting the central nervous system. Hypothalamic inflammation is a hallmark of obesity and is believed to participate in the onset and progression of the obese phenotype, by promoting changes in neuronal functions involved in the control of metabolism. The activation of brain immune cells in the hypothalamus, which are represented by microglia and brain macrophages, is associated with obesity and has been the focus of intense research. Despite the significant body of knowledge gathered on this topic, obesity-induced metabolic changes in brain cells involved in innate immune responses are still poorly characterized due, at least in part, to limitations in the existing experimental methods. Since the metabolic state influences immune responses of microglia and other myeloid cells, the understanding and characterization of the effects of cellular metabolism on the functions of these cells, and their impact on brain integrity, are crucial for the development of efficient therapeutic interventions for individuals exposed to a long-term high fat diet (HFD). Here we review and speculate on the cellular basis that may underlie the observed changes in the reactivity and metabolism of the innate immune cells of the brain in diet-induced obesity (DIO), and discuss important points that deserve further investigation.

Keywords: hypothalamus, inflammation, metabolism, microglia, obesity, saturated fatty acids

INTRODUCTION

According to the World Health Organization, obesity has almost tripled since 1975 and is now considered a worldwide epidemic, affecting more than 650 million people[1]. This rapid increase in the number of obese individuals raises profound concerns in the health community, since obesity is a predisposing factor for a wealth of morbidities, many of which are life-threatening (Guh et al., 2009). In fact, obese people have been shown to be more prone to developing central pathologies, including: stroke, depression, and Alzheimer's disease (Bruce-Keller et al., 2009).

[1]http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight

One of the hallmarks of obesity is the presence of metaflammation, an atypical systemic and sterile inflammation. This inflammatory state involves the participation of the innate and adaptive immune systems, resulting in the production of cytokines, chemokines and inflammatory lipids (Iyer et al., 2010; Cooke et al., 2016; Ralston et al., 2017). Metaflammation has been detected in a variety of different tissues, including different areas of the brain (Beilharz et al., 2016; Guillemot-Legris et al., 2016).

Inflammation in brain areas, other than the hypothalamus, might certainly contribute to the onset and progression of some of the central pathologies associated with obesity, however, hypothalamic inflammation was previously shown to play a central role in disease processes, affecting the control of energy intake and energy expenditure (Velloso and Schwartz, 2011). Moreover, these inflammation-induced alterations in the mediobasal hypothalamus (MBH) directly impact the integrity of homeostatic processes, such as hepatic glucose storage and delivery into the blood (Mravec et al., 2018). Interestingly, some brain-related obesity effects are modulated by sex (reviewed in Morselli et al., 2016 and Ávalos et al., 2018). Since *in vivo* experiments tend to employ male animals, this must be taken into consideration when interpreting data obtained from animal studies.

Here we review a variety of factors that contribute to modifications of brain innate immune cell metabolism and reactivity in diet-induced obesity (DIO). In this particular context, it was found that very little is known about the metabolism of microglia and brain macrophages. Thus, since a high fat diet (HFD) impacts pro-inflammatory gene expression, activity and morphology of hypothalamic immune cells, future studies should focus on and investigate which metabolic pathways in these cells are modulated by DIO, and determine how these changes contribute to the progression of obesity.

DIET-INDUCED OBESITY (DIO) LEADS TO HYPOTHALAMIC INFLAMMATION

In 2005, the first report showing evidence demonstrating an association between DIO and hypothalamic inflammation was published (De Souza et al., 2005). Since then, numerous groups have confirmed and extended this finding in both rodents and humans (Thaler et al., 2012; Schur et al., 2015; Valdearcos et al., 2015). It is also well established that a HFD rapidly induces hypothalamic inflammation, with an associated increase in inflammatory gene expression and gliosis, that subsides and returns if the HFD is not interrupted (Thaler et al., 2012; Berkseth et al., 2014). Interestingly, early hypothalamic inflammation can be observed weeks before adipose tissue (AT) expansion and inflammation (Thaler et al., 2012; Gao et al., 2014), suggesting that hypothalamic inflammatory signaling contributes to the genesis of the overt obese phenotype, and is not simply a consequence of peripheral inflammation. The central role hypothalamic inflammation plays in obesity was further supported by two studies reporting that the detrimental HDF-related effects could be alleviated through genetic ablation or pharmacological inhibition of hypothalamic inhibitor of NF-κB[2] kinase subunit β (IKKβ) (Zhang et al., 2008; Posey et al., 2009). Although pro-inflammatory signaling in the hypothalamus is a key event in the onset of DIO, the widespread inflammation and metabolic changes promoted by a HFD may further impact the hypothalamus.

A Different Inflammatory Profile in Obesity

Obesity is characterized by a distinct level of systemic innate immune response, often referred to as "chronic low grade inflammation." Metabolic dysfunction is accompanied by increased levels of non-esterified fatty acids and systemic inflammatory mediators, such as plasma pro-inflammatory cytokines (Iyer et al., 2010). While a discussion on the accurate use of the term inflammation, for a systemic and chronic response, is beyond the scope of this review, the notion of a generalized response from tissues and blood immune cells, as well vascular endothelial cells, is quite commonsense these days. Interestingly, the presence of inflammatory molecules in the blood and CNS induces sickness behavior, as characterized by decreased food intake (reviewed in Thaler et al., 2010). The paradox on how DIO-mediated inflammation results in a different outcome has been discussed (Thaler et al., 2010). According to the authors, it is plausible that the net impact, resulting from the complex cellular interplay between hypothalamic cells, along with factors such as stimulus quality, duration and intensity, could account for these differences. However, it is clear that HFD-induced hypothalamic inflammation diverges from other pro-inflammatory stimuli that promote sickness behavior. Understanding how this diet triggers inflammation and identifying why innate immune cells in the hypothalamus respond differently is essential to combating obesity.

The Unknown Pathway That Triggers Inflammation in the MBH

It is important to note that the typical neurovascular unit that forms the blood-brain barrier (BBB) (Obermeier et al., 2013) is not the only example of vascular organization in the hypothalamus. Structures devoid of blood-barriers, such as fenestrated median eminence (ME) capillaries, or conversely some of the more tightly sealed endothelia in the arcuate nucleus (ARC), are actually surrounded by cellular processes projected from the third ventricle tanycytes (Langlet, 2014). This atypical cellular disposition contributes to the peculiar properties of the MBH in terms of collecting metabolic information carried by the blood stream (**Figure 1**). As an example, circulating pro-inflammatory mediators and biomolecules characterized as pathogen- and damage/danger- associated molecular patterns (PAMPs and DAMPs, respectively) were shown to be readily detected by brain immune cells associated with structures that lack the BBB (Rivest et al., 2000; Mallard, 2012). PAMPs and DAMPS represent an important concept in immunology, since

[2]Nuclear factor kappa-B, a master activator of pro-inflammatory gene expression.

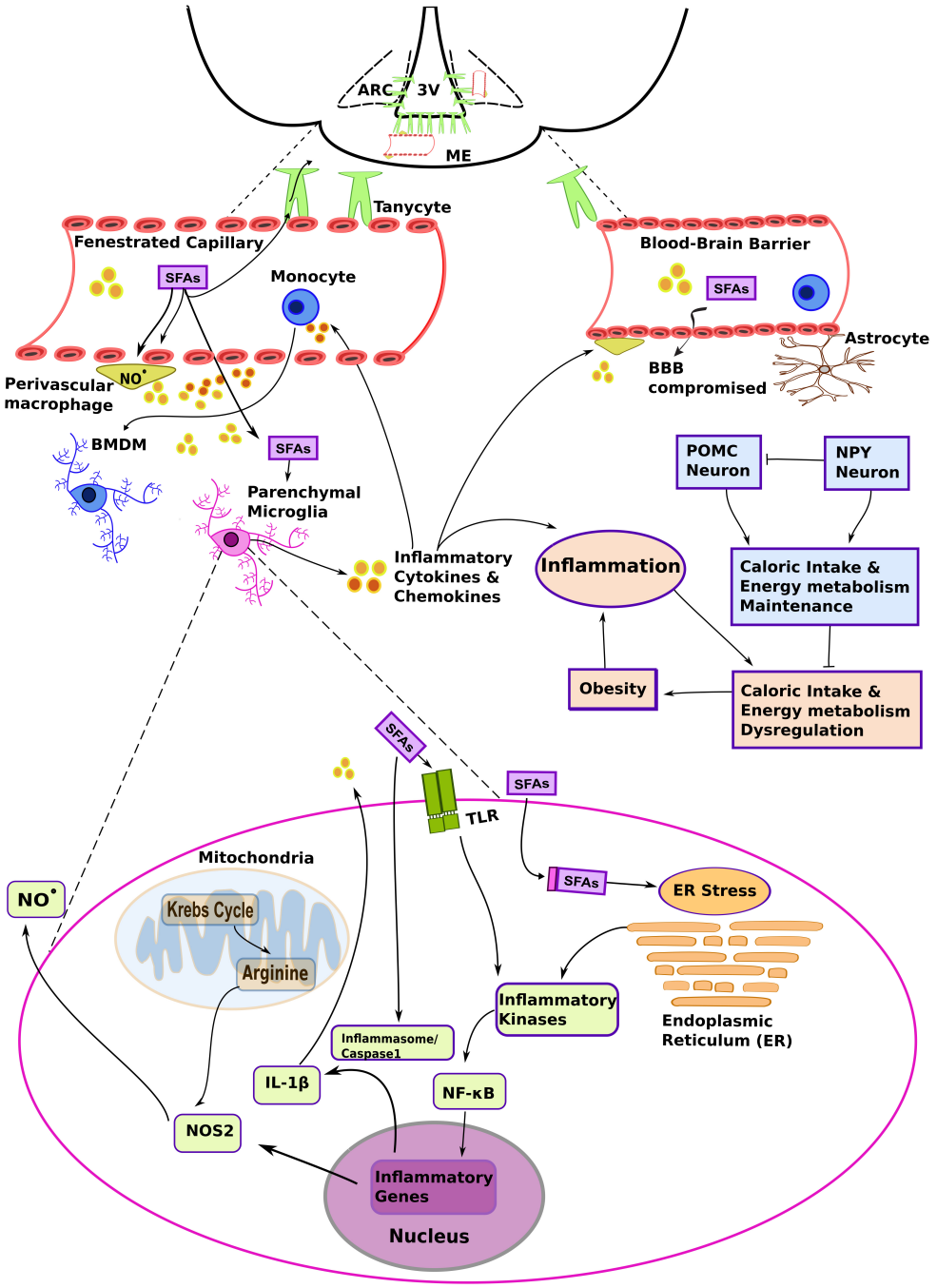

FIGURE 1 | The complex interplay among different myeloid cells and metabolic substrates promotes hypothalamic inflammation in diet-induced obesity (DIO). A high fat diet (HFD) increases the levels of non-esterified saturated fatty acids in the blood and systemic inflammatory mediators, such as plasma pro-inflammatory cytokines. These molecules primarily target fenestrated capillaries in the median eminence (ME), and also influence the mediobasal hypothalamus (MBH) region, including the arcuate nucleus (ARC). Saturated fatty acids (SFAs) and inflammatory molecules activate brain endothelia and its associated immune cells, such as the perivascular macrophages. Changes in vascular permeability due to hypothalamic inflammation and signaling molecules such as nitric oxide ($NO^{\cdot}$), act in concert to promote lipid load accumulation in the MBH. Brain myeloid cells are heterogeneous and present particular phenotypes that result from the microenvironment and ontogeny. Resident parenchymal microglia are activated in DIO. Although several mechanisms still remain to be determined, it has been suggested that chronic low-level microglial activation perturbs metabolic control, and that this contributes to the development of the obese phenotype. At the same time, SFAs may influence the metabolic program and inflammatory signaling in microglia. The figure depicts hypothetical metabolic and inflammatory signaling changes in microglia based on data collected from metabolic macrophages (MMe) (see main text for details). Other abbreviations: 3V, third ventricle; BBB, blood brain barrier; BMDM, bone marrow-derived microglia; IL-1β, interleukin 1β. NF-κB, nuclear factor κB; NOS2, nitric oxide synthase 2 (inducible form; iNOS); TLR, *Toll*-like receptor.

these molecules are recognized by germline-encoded non-clonal receptors, and are the primary triggers of innate immune cell responses. However, it remains to be firmly established how an HFD could initiate hypothalamic inflammation.

Studies aiming at the characterization of the inflammatory molecules responsible for HFD-induced hypothalamic inflammation identified fatty acids as determinant factors. Indeed, a key characteristic of obesity is increased systemic free fatty acids, which accumulate in diverse tissues, including the hypothalamus (Boden, 2008; Posey et al., 2009; Valdearcos et al., 2014). In fact, the hypothalamus is particularly prone to the accumulation of long chain saturated fatty acids (SFAs), such as palmitoyl-CoA, thus promoting hypothalamic inflammation in animals fed a HFD. Moreover, while there has been no evidence showing that increased caloric or unsaturated fatty acid intake influences hypothalamic inflammation (Huang et al., 2004; Posey et al., 2009; Valdearcos et al., 2014), the amount of evidence implementing SFAs in this response is growing. With regards to the effects associated with other lipids, including unsaturated fatty acids, the reader is referred to dedicated reviews (White and Marette, 2014; Hammad and Jones, 2017; Rogero and Calder, 2018). A plausible rationale for how SFAs and HFD promote pro-inflammatory signaling in microglia cells—key players in DIO progression—will be presented in the next sections.

MICROGLIA AS PRIMARY TARGETS IN DIO

Of the various cell types that populate the MHB, microglia have been the most studied in terms of DIO-induced hypothalamic inflammation, perhaps due to the fact that they are considered the resident brain macrophages. Indeed, microglial reactivity and inflammatory molecule production were found to be increased in the hypothalamus of animals fed an HFD and obese humans (Thaler et al., 2012; Gao et al., 2014; Valdearcos et al., 2014).

Microglia Cells Respond to HFD and Play an Important Role in DIO

The fact that manipulation of microglia interferes with DIO indirectly shows that an HFD provokes profound effects on these brain myeloid cells, resulting in drastic metabolic consequences. Experimental approaches employing alternative strategies have demonstrated that microglia play a fundamental role in DIO. For example, interventions that impair microglia response (i.e., depleting these cells, restraining their responsivity or inhibiting microglia proliferation) alleviate the DIO phenotype and blunt the HFD-induced inflammation *in vivo*. A similar beneficial effect of microglial inhibition was observed in cultured brain slices stimulated with palmitate (Valdearcos et al., 2014, 2017; André et al., 2017). In contrast, forcing microglial activation by cell-specific deletion of tumor necrosis factor α-induced protein 3 (TNFAIP3), an anti-inflammatory molecule and negative regulator of NF-κB, rapidly induced obesity in mice, even when animals were fed control diets (Valdearcos et al., 2017). While this latter study does not explain how an HFD activates microglia, it certainly implicates hypothalamic microglia as a key player in DIO. Interestingly, persistent secretion of TNF-α by microglia in the MHB of mice fed a high fat/high carbohydrate (HFHC) diet was reported to cause dysfunction in anorexigenic neurons that produce proopiomelanocortin (POMC) (Yi et al., 2017), a characteristic feature of obesity (Pinto et al., 2004; Horvath et al., 2010). Assuming that lipids participate in microglial pro-inflammatory signaling, we direct the reader to **Table 1**, which summarizes the findings supporting a pro-inflammatory effect of SFAs on the hypothalamus and microglia.

Experimental Limitations and Thoughtful Interpretations

While the above-mentioned set of data suggests a prominent role for microglia in the detrimental effects of DIO, it is appropriate to introduce some notes of caution. At first glance, brain myeloid cells are thought to be either pathogen eradicators, or instigators of neuronal dysfuntion and cell death in various neurodegenerative diseases. However, microglia are important for the proper shaping of the brain, and function as a neuroprotective component of the innate immune response, a concept strongly supported by experimental data (Wyss-Coray and Mucke, 2002; Glezer et al., 2007; ElAli and Rivest, 2016). Thus, while experimental results indicate that preventing hypothalamic myeloid cell responses improves the outcome of HFD-treated animals, the consequences of DIO on the unprotected brain for a long period of time are not known. Secondly, due to limitations in experimental tools, "microglia" was frequently used as a generic term for all brain myeloid cells (myeloid as lineage term, not to be confused with bone marrow origin) in the past. Now it is known that in addition to the prototypical parenchymal microglia, other myeloid cells are present in the brain, including the non-parenchymal meningeal, perivascular and choroid-plexus macrophages, and disease-associated monocytes (Prinz et al., 2017). In this sense, it is likely that inflammatory signaling in the hypothalamus is coordinated by a complex interplay between microglia/macrophages, endothelial cells and macroglia (astrocytes, oligodendrocytes and their progenitors). Thus, efforts to target specific pathways and/or microglia/brain macrophage subpopulations are valuable and necessary, but the interpretation of the results should be performed carefully and objectively.

Lastly, it is important to keep in mind that brain myeloid cells do not necessarily influence metabolism through stereotyped responses to noxious stimuli. Indeed, myeloid cell specific *Cx3cr1*-driven ablation of receptors for the "satiety" hormone leptin was shown to result in increased food intake and body weight, dystrophic and less phagocytic microglia, and altered neuronal circuitry in the MBH (Gao et al., 2018). However, this genetic strategy cannot provide a conclusion about the specific role for leptin signaling in microglia/brain macrophages, since this approach may affect myeloid cells from multiple tissues, a commonly encountered experimental

TABLE 1 | Microglia activation induced by high fat diet or fatty acid treatment.

Experimental model	Treatment	Duration of treatment	Results	References
Hypothalamus	Normal diet (ND) HFD	13 and 16 weeks	↑ TNFα ↑ IL-1β ↑ IL-6	De Souza et al., 2005
		16 weeks	↑ TNFα mRNA ↑ IL-1β mRNA	
Hypothalamus	ND HFD	16 weeks	↑ TNFα ↑ IL-1β ↑ IL-6	Milanski et al., 2009
	Intracerebroventricular infusion of oleic acid	3 days	↑ IL-6 and IL-6 mRNA ↑ IL-10 and IL-10 mRNA	
	Intracerebroventricular infusion of stearic acid	3 days	↑ TNFα ↑ IL-1β ↑ IL-10	
	Intracerebroventricular infusion of linolenic acid	3 days	↑ IL-6 ↑ IL-10	
	Intracerebroventricular infusion of arachidic acid	3 days	↑ TNFα ↑ IL-1β ↑ IL-6 ↑ IL-10	
	Intracerebroventricular infusion of behenic acid	3 days	↑ TNFα ↑ IL-1β ↑ IL-6	
Hypothalamus	Intracerebroventricular infusion of palmitate	20 min	↑ pIKKβ ↓ IκBα	Posey et al., 2009
	LFD HFD	7 weeks	↑ pIKKβ	
Primary glial cells	Control serum DIO serum	48 h	↑ CD11b positive cells	Hsuchou et al., 2012
BV-2 microglial cells	Vehicle 0.125 mM Palmitate	24 h	↓ TNFα mRNA ↑ Chi3L3 mRNA ↑ Arg1 mRNA	Kappe et al., 2012
Hypothalamus	ND HFD	4 weeks	↑ TNFα mRNA ↑ IL-6 mRNA	Thaler et al., 2012
BV-2 microglial cells	Vehicle 0.125 mM Palmitate	24 h	↑ Arg1 mRNA	Tracy et al., 2013
Primary microglial cells	Control serum HFD serum	120 min	↑ Iba-1 ↑ TNFα ↑ IL-1β	Gao et al., 2014
Hypothalamus	ND HFD	4 and 16 weeks 4 weeks	↑ Iba-1 positive cells ↑ TNFα mRNA ↑ IL-6 mRNA ↑ IL-1β mRNA	Valdearcos et al., 2014

(Continued)

TABLE 1 | Continued

Experimental model	Treatment	Duration of treatment	Results	References
	Enteric gavage of lauric acid	3 days	↑ TNFα mRNA ↑ IL-6 mRNA	
	Enteric gavage of palmitic acid	3 days	↑ TNFα mRNA ↑ IL-6 mRNA ↑ IL-1β mRNA	
Primary microglial cells	Vehicle 0.1 mM lauric acid	24 h	↑ TNFα ↑ IL-6 ↑ MCP-1	
	Vehicle 0.1 mM myristic acid	24 h	↑ TNFα ↑ IL-6 ↑ MCP-1	
	Vehicle 0.1 mM palmitic acid	24 h	↑ TNFα ↑ IL-6	
	Vehicle 0.1 mM stearic acid	24 h	↑ TNFα ↑ IL-6 ↑ MCP-1	
BV-2 microglial cells	Vehicle 0.1 mM Palmitate	12 h	↑ iNOS mRNA	Duffy et al., 2017
Hypothalamus	ND HFD	4 weeks	↑ Iba-1 ↓ P2Y12	Valdearcos et al., 2017
BV-2 microglial cells	Vehicle 0.3 mM Palmitate	48 h	↑ IL-1β ↑ CD11b mRNA ↑ IL-6 ↑ MCP-1	Yang et al., 2017
Hypothalamus	Low fat diet (LFD) HFD	8 weeks	↑ TNFα mRNA ↑ IL-1β mRNA	

limitation associated with attempting to target, regulate and/or delete a specific gene in microglia (Han et al., 2017). The results, nonetheless, suggest that these cell types are responsive to a vast array of signals. Interestingly, recent data demonstrated that *Cx3cr1*-driven microglia and monocyte ablation in rats disrupts the gustatory circuitry at the paraventricular nucleus of the hypothalamus, leading to anorexia and weight loss (De Luca et al., 2019). Strikingly, it was reported that mice fed an HFD and treated with an intracerebroventricular injection of AraC, to block cell proliferation induced by HFD, presented reduced food intake, weight gain and adiposity, when compared with mice fed the same diet, but without being administered AraC (André et al., 2017). Thus, it is tempting to speculate that the reported benefits of microglia and monocyte ablation/inhibition in the DIO setting is secondary to decreased food intake, and not solely due to a reduction in inflammation.

Although many of the studies cited here indicate that there is a relationship among the HFD-induced increase in SFAs, brain myeloid cells pro-inflammatory response and hypothalamic energy metabolism control, the precise mechanisms linking these events are not definitively established. While improved experimental models and detailed molecular approaches are still necessary in order to unify these pieces of evidence into a compelling and complete model, it is clear that an HFD severely impacts the function of microglia.

LIPIDS ACTIVATE MICROGLIA/MACROPHAGES

Since long chain saturated fatty acids seem to be important for HFD-associated hypothalamic inflammation, *in vitro* studies have been employed, using palmitate as a stimulus, for elucidating the molecular pathways involved in the microglial response to HFD. However, mixed results were reported, ranging from anti-inflammatory responses (Tracy et al., 2013; Kim et al., 2018) to pro-inflammatory ones (Wang et al., 2012). This discrepancy may stem, at least in part, from different lipid loads, since they may be modulated not only by the concentration of lipid used in the assays, but

also by the ratio of lipid to the carrier protein albumin (Alsabeeh et al., 2018). Not surprisingly, the mechanisms through which parenchymal microglia respond to an HFD or SFAs are not clear yet, and much is still unknown. It is noteworthy that most of the research in the field has focused on typical macrophage readouts such as cytokine secretion, cellular recruitment, gene expression and cell morphology when exploring the effects of HFD or SFAs on microglia. Furthermore, data related to the mechanisms involved in the parenchymal microglia response to HFD or SFAs are extremely scarce. Therefore, we will summarize the findings related to macrophages, for which more information is available and discuss the extent to which these findings also apply to microglia.

Macrophages and Microglia Do Not Necessarily Respond to HFD in the Same Way

With regards to macrophages, the response to SFAs seems to involve canonical and non-canonical mechanisms. The innate immune signaling is largely dependent on pattern recognition receptors, such as the *toll*-like receptors (TLRs). This family of membrane proteins activates pro-inflammatory transcription upon the recognition of structurally distinct PAMPs, such as lipopolysaccharide (LPS) from gram-negative bacteria (Takeuchi and Akira, 2010). Additionally, this response is complemented by intracellular protein complexes called inflammasomes, which sense PAMPs/DAMPs or relay upstream signaling, and promote the secretion of cytokines such as interleukin (IL)-1β (Sharma and Kanneganti, 2016). While SFAs are far from being considered PAMPs or DAMPs, similar response mechanisms have been described. For example, palmitate, but not unsaturated oleate, has been shown to activate the NLRP3-ASC inflammasome (Wen et al., 2011). Additionally, other studies also point to engagement of TLR4, the same receptor for LPS, by SFAs (Lee et al., 2001; Shi et al., 2006; Huang et al., 2012), suggesting that SFAs present the natural capacity to trigger innate immune cells. However, the evolutionary basis for the controversial idea that the immune system would respond against important caloric nutrients remains to be clarified.

How do the findings reported in macrophages apply to microglia? While it is expected that signals triggered by PAMPs and DAMPs follow quite similar pathways in different myeloid cells, it is important to mention that resident brain cells present unique and remarkable features. Molecular tracing and fate mapping studies revealed that microglia originate from yolk sac erythro-myeloid progenitors (primitive macrophages). Additionally, unlike macrophages from other tissues (that are largely derived from fetal monocytes), microglia and brain macrophages preserve their primitive lineage by means of self-renewal of resident progenitors, which infiltrated the brain during embryo development (Ginhoux et al., 2010; ElAli and Rivest, 2016; Prinz et al., 2017; Hoeffel and Ginhoux, 2018). This is relevant because microglia and brain macrophages maintain distinct repertoires of signaling and function, and long-term brain exposure to macrophage activating molecules will follow different cellular dynamics when compared to other tissues that actively replenish macrophages with bone marrow-derived cells. Furthermore, it is important to point out that environmental factors clearly determine the transcriptional programs that define the identity of microglia. For example, specific transcripts from murine microglia are lost when these cells are removed from the brain and transferred to cell culture (Gosselin et al., 2017). In addition, microglia completely regain their identity when transferred from *in vitro* culture to the brain *in vivo*, an event specific for yolk-sac primitive ontogeny, that cannot be replicated by engrafted macrophages from other tissues (Bennett et al., 2018). The authors of this study identified that the microglia-like cells, and not microglia, have a few unique markers that are found in pathological states of the human brain. The impact of these findings is very clear in terms of how crucial it is to determine which specific brain myeloid cells respond to HFD and SFAs, and the extent to which *in vitro* generated data can be translated to the hypothalamic niche of animals subjected to DIO.

Very recently, perivascular macrophages of the ARC, whose ontogeny has yet to be defined, were shown to proliferate *in situ* and express high levels of inducible nitric oxide synthase (iNOS/NOS2) when mice were fed an HFD (Lee et al., 2018). The same study also showed that hypothalamic NOS2 inhibition diminished several deleterious effects associated with DIO, including BBB permeability and lipid efflux (Lee et al., 2018). The idea that inflammatory cells regulate lipid uptake into the brain is quite attractive, but it is not clear how lipid overload, that occurs with an HFD, upregulates the expression of iNOS and other genes typically involved in pathogen defense. This is of particular relevance, since "chronic low grade inflammation" is commonly observed in obesity.

Due to the emergent nature of the subject, detailed studies describing the ontogeny and neuroanatomical details of the microglia/macrophages that are involved in the *in vivo* response to HFD, as well as studies combining specific gene promoters to drive pro-inflammatory signaling interference without disrupting brain barriers, are awaited. A comprehensive understanding of the molecular and cellular mechanisms involved in the response of microglia/brain macrophage to DIO and SFAs will likely reveal novel therapeutic targets for the prevention and/or treatment of obesity.

DISTINCT METABOLIC PROGRAMS SUPPORT INFLAMMATORY PHENOTYPES IN MACROPHAGES AND (POSSIBLY) MICROGLIA

It is now clear that immune cells have a profound impact on metabolic homeostasis and, that metabolic pathways can also significantly influence immune cell function. Indeed, the transition of immune cells from one phenotype to another is

supported by and dependent on specific metabolic programs (reviewed in Van den Bossche et al., 2017). For example, macrophages stimulated with LPS(+ IFNγ) adopt a metabolic program that includes increased flux through glycolysis and the pentose phosphate pathway together with a reduction in oxidative phosphorylation (Rodríguez-Prados et al., 2010), due to a disruption of the citric acid cycle (Jha et al., 2015). This disruption repurposes the intermediary metabolites of the citric acid cycle for specific biosynthetic reactions, which sustain the inflammatory activities of LPS-stimulated macrophages (Tannahill et al., 2013; Littlewood-Evans et al., 2016; Mills et al., 2016; Williams and O'Neill, 2018). Interestingly, inhibition of specific mitochondrial membrane carriers impairs this repurposing and attenuates pro-inflammatory macrophage polarization by LPS, without affecting cell viability (Jha et al., 2015). Thus, indicating that metabolic pathways can influence the phenotype of immune cells and that modulating these pathways may represent a potent therapeutic strategy.

In obesity, macrophages acquire a specific phenotype that is strikingly different from the pro-inflammatory M1 phenotype, elicited by stimulation with LPS(+ IFNγ), and the anti-inflammatory M2 phenotype, induced by IL-4 (Lumeng et al., 2007; Zeyda et al., 2007; Kratz et al., 2014; Coats et al., 2017). In fact, macrophages stimulated with metabolic cues such as high SFAs, insulin and glucose, which are preponderant in obesity, are designated as metabolic macrophages (MMe). The MMe phenotype is associated with the production of cytokines, but presents a different set of surface molecules, when compared with macrophages stimulated with LPS. Additionally, these differences were also detected when macrophages isolated from the AT of obese patients were compared with macrophages isolated from the lungs of patients with cystic fibrosis, a disease in which the patients experience recurrent bacterial infections of the airway (Kratz et al., 2014). To date, little is known about the metabolic landscape of MMe macrophages, but different lipid metabolism programs seem to be important for MMe macrophage polarization and/or activity. In fact, SFAs seem to be important drivers of MMe activation, since macrophages stimulated with SFAs produce IL-1β and express surface markers characteristic of MMe isolated from obese mice (Kratz et al., 2014). The expression of many of the MMe surface markers are under the control of the lipid-engaged receptor PPARγ, reinforcing the idea that lipid metabolism is intimately linked to MMe activation and/or function. Indeed, it was shown that upon internalization, SFAs insert into phospholipid bilayer, changing the degree of membrane saturation. This altered lipid composition induces endoplasmic reticulum stress, a characteristic feature of MMe, which ultimately leads to the production of IL-1β (Robblee et al., 2016). Interestingly, the pharmacological inhibition of PPARγ or genetic inhibition of mitochondrial fatty acid oxidation in macrophages stimulated with SFAs exacerbate inflammation, and PPARγ deletion in macrophages aggravates insulin resistance in mice fed a HFD (Odegaard et al., 2007; Kratz et al., 2014; Namgaladze et al., 2014). Recently, gene expression data indicated that genes involved in glycolysis, oxidative phosphorylation and those related to lactate production were increased in AT macrophages isolated from obese mice, when compared to those isolated from lean animals. This metabolic program seems to be specific for AT macrophages as the expression of genes involved in energy metabolism was unaltered by an HDF in peritoneal macrophages (Boutens et al., 2018), highlighting the importance of the niche for macrophage response. Despite the data gathered so far, much more work is needed before we obtain a clear comprehensive understanding of the different metabolic programs that promote MMe polarization and activity (Namgaladze and Brüne, 2016), and how different niches (i.e., different AT depots) and the origin of the cell influence these responses.

With respect to microglia metabolism and its impact on cell phenotype and function, very little is known. Studies using LPS(+ INFγ) to stimulate microglia in culture reported increased pro-inflammatory cytokine and lactate production, which was accompanied by increased flux through glycolysis and the pentose phosphate pathway and decreased mitochondrial oxygen consumption (Voloboueva et al., 2013; Gimeno-Bayón et al., 2014). Thus, suggesting that there are parallels between macrophages and microglia in terms of their metabolic response to LPS(+INFγ), at least *in vitro*. However, these similarities seem to be limited, since there is still no clear evidence that microglia are responsive to IL-4, revealing the inadequacy of using the bimodal M1/M2 designation to describe the microglial response (Ransohoff, 2016). With regards to microglial metabolism in obesity, it was recently reported that impairing lipid uptake, through the silencing of lipoprotein lipase (LPL), specifically in microglia, exacerbates weight gain and glucose intolerance in mice fed an HFHC diet (Gao et al., 2017). The same study also reported that the absence of LPL in the microglia of mutant mice impaired the cellular responses (i.e., increase in number, changes in morphology and phagocytic capacity) observed in control mice fed an HFHC diet. In fact, this impairment was accompanied by an increased number of dysmorphic mitochondria in the microglia, possibly due to extensive alterations of the lipidome of those cells (Gao et al., 2017). While the work by Gao et al. indicated that lipid metabolism may play a central role in microglia phenotype and/or function in obesity, data related to microglia immunometabolism, especially in the context of obesity, are virtually non-existent. Thus, future studies investigating the modulation of microglia metabolism and, in a broader view, of myeloid cells are urgently needed, since these areas appear to be a promising avenue for treating obesity. It is important to note that although *in vitro* assays are important for providing mechanistic insights, *in vivo* work is crucial for accurately determining the metabolic response of microglia to different stimuli, as the niche exerts a profound influence on the cellular response. It is likely that changes in myeloid cell metabolism promote different outcomes in the MHB. In the context of obesity, more studies are needed to fully understand the impact of myeloid metabolism on cell function and reactivity; however, the origin of the cell and the niche must be taken into account when attempting to elucidate these response mechanisms.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Although much has been done, very little is known about how microglia cell metabolism affects the function of these brain cells. Additionally, it is unclear whether microglial metabolism is rewired, due to obesity-associated metabolic cues *in vivo*, or how this could occur. Due to the fact that microglia play a central role in obesity, and since obesity has devastating effects on human health, we believe that the metabolic program promoting obesity-induced microglial activation must be understood in detail. It will also be interesting to evaluate whether other brain myeloid cells present different metabolic programs, and to investigate the role each type of cell plays in hypothalamic inflammation. For example, if SFAs and myeloid cell metabolism induce an atypical inflammatory state, would it be possible to metabolically reprogram the microglia and restore normal hypothalamic function during DIO? Moreover, could the anti-inflammatory or deactivated microglia strategies employed in rodents be utilized in humans? Answering these questions, as well as others will pave the way for the development of therapeutic interventions aimed at preventing the onset and progression of obesity, as well as its associated co-morbidities.

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All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Insulin and Autophagy in Neurodegeneration

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Crosstalk in the pathophysiological processes underpinning metabolic diseases and neurodegenerative disorders have been the subject of extensive investigation, in which insulin signaling and autophagy impairment demonstrate to be a common factor in both conditions. Although it is still somewhat conflicting, pharmacological and genetic strategies that regulate these pathways may be a promising approach for aggregate protein clearancing and consequently the delaying of onset or progression of the disease. However, as the response due to this modulation seems to be time-dependent, finding the right regulation of autophagy may be a potential target for drug development for neurodegenerative diseases. In this way, this review focuses on the role of insulin signaling/resistance and autophagy in some neurodegenerative diseases, discussing pharmacological and non-pharmacological interventions in these diseases.

Keywords: obesity, type 2 diabetes, insulin, autophagy, neurodegenerative diseases

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INTRODUCTION

Metabolic disorders such as obesity and type 2 diabetes (T2D) are a worldwide epidemic challenge of the 21st century that, besides resulting in reduced life expectancy and increased medical comorbidities, have been said to be risk factors for the development of neurodegenerative diseases (El Sayed et al., 2012). According to literature, both obesity and T2D contribute to an increased prevalence of dementia and cognitive decline (Arvanitakis et al., 2004; Kopf and Frölich, 2009; Nameni et al., 2017; Zhang et al., 2017), as well as to the onset of insulin resistance (Schneeberger et al., 2015), which is proposed to play a critical role in the link between metabolic disorders and central nervous system (CNS) impairment (Schneeberger et al., 2015; Nameni et al., 2017). Nowadays, it is known that insulin receptor (IR) is widely expressed in the CNS, and, although the exact link between brain insulin and neurodegenerative diseases remains still unknown, a plethora of studies have demonstrated that an optimal insulin signaling homeostasis is important to the maintenance of brain health.

The maintenance of cellular homeostasis is a complex process, which includes an interesting crosstalk between autophagy and apoptosis, involving, among other processes, the regulation of the phosphatidylinositol (3,4,5)-trisphosphate kinase (PI3K)-protein kinase B (AKT)-mammalian target of rapamycin (mTOR) and AMP-dependent protein kinase (AMPK) pathways. The mTOR is a serine/threonine kinase that is ubiquitously expressed, modulating cell proliferation, protein synthesis, and death or survival signalings. Localized mainly in the cytoplasm, mTOR is composed of two different protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), which differ in many respects, including protein complex components, and

responsiveness to rapamycin, as well as activators or downstream signaling pathways. However, crosstalk between both complexes is mediated by AKT (Perluigi et al., 2015). The AKT signaling pathway can be triggered by insulin-like growth factor 1 (IGF-1) and insulin, which bind to transmembrane receptors and activate insulin receptor substrate 1 (IRS-1), leading to PI3K activation and subsequent phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) generation. AKT is responsible for the inhibition of negative regulators of mTOR, such as tuberous sclerosis complex (TSC1/2), whilst pyruvate dehydrogenase kinase 1(PDK1) induces the mTOR activator Rheb (Ras homolog enriched in brain), thereby releasing the mTOR complex to phosphorylate its targets (Perluigi et al., 2015). Interestingly, the chronically hyperactivation of mTOR by hyperinsulinemia is proposed to contribute to insulin resistance (Ueno et al., 2005).

Autophagy is an important cellular process, whereby cell debris, including proteins and organelles, are degraded and recycled in order to prevent the build up of protein clusters and aggregates (Klionsky and Emr, 2000; Rubinsztein et al., 2005). During this process, portions of cytoplasm or organelles are engulfed by double membrane structures called autophagosomes, which fuse with lysosomes or vacuoles allowing the degradation of their cargo material (organelles and macromolecules) and its subsequent release into the cytosol for recycling (Kundu and Thompson, 2005). This complex process is regulated by more than 30 autophagy-related proteins (ATGs), as well as by the PI3K, the Unc-51-like kinase 1 (ULK1) and the microtubule-associated protein 1 light chain 3 (LC3) pathways. Under physiological condition, PI3K type I is activated by growth factors resulting in mTOR activation and consequently autophagy downregulation by phosphorylating and inactivating ULK1, which is an initiator of mammalian autophagy (Axe et al., 2008). Besides mTOR, AMPK also suppresses autophagy through the phosphorylation of different amino acid residues of ULK1 (Nishida et al., 2009). However, under some stress condition such as nutrient starvation, ULK1 is dephosphorylated by protein phosphatase 2A (PP2A), resulting in phosphorylation of multiple substrates and initiation of the conventional autophagy (Axe et al., 2008; Nishida et al., 2009; Mizushima and Komatsu, 2011). After that, together with other components, the Beclin1-containing class-III PI3K complex (Beclin 1, VPS15 and ATG14, class III VPS34) induces the formation of phagophore, an initial membrane component of the isolation membrane, which then expands, curves and closes due to two conjugation pathways, the ATG5-ATG12 and the LC3, turning into a completed autophagosome (Mizushima and Komatsu, 2011; **Figure 1**).

In this way, this review summarizes the origin and the role of insulin in the CNS, and discusses the relationship between insulin and autophagy in some neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), as well as in spinocerebellar ataxia type 3 (SCA3) and Lafora disease (LD). Although there are at least three well-known distinct autophagic pathways, such as chaperone-mediated autophagy, microautophagy and macroautophagy, this review focuses on macroautophagy, hereafter referred to as autophagy.

INSULIN RESISTANCE IN THE CENTRAL NERVOUS SYSTEM AND NEURODEGENERATION

The sugar lowering properties of the endocrine secretion of the pancreas were described by Banting and Best (1922), and insulin itself was crystallized shortly after, in 1926 (Abel, 1926). The number of scientific publications describing insulin and its functions exploded in the 1940s and, until the 1960s, most of the scientific community considered the brain to be an organ insensitive to insulin. The first half of 60s, however, was punctuated with compelling work done by separate groups that forced this discussion to be reopened. Work published in 1961 by Rafaelsen showed glucose uptake modulation by insulin in isolated rat spinal cord (Rafaelsen, 1961a) as well as rat cortical brain slices and cerebellum (Rafaelsen, 1961b). Butterfield et al. (1966), after measuring brain glucose uptake in 5 healthy male volunteers with insulin infusions, suggested that the human brain could be, after all, sensitive to insulin (Butterfield et al., 1966).

Recent work has confirmed insulin effects in the brain, although these can be quite distinct from its effects in the periphery, although the idea that insulin has exclusively neurotrophic effects in the brain has been contested by a few recent publications. In 2016, García-Cáceres et al. (2016) showed that signaling through the IR in hypothalamic astrocytes may affect the activity of glucose-sensing pro-opiomelanocortin neurons, suggesting the importance of insulin in the regulation of brain glucose uptake (García-Cáceres et al., 2016). Insulin-like growth factor I (IGF-I) is another important endogenous ligand of the IR, and works alongside insulin binding to the astrocytic IR in order to activate a non-canonical pathway, the mitogen-activated protein kinases (MAPK)/protein kinase D (PKD) pathway, which ultimately leads to the membrane translocation of glucose transporter (GLUT)1, a glucose transporter previously known to be insulin-insensitive. The combined effects of insulin and IGF-1 therefore modulate brain glucose (Fernandez et al., 2017). The following sections focus on the neurotrophic effects of insulin.

ORIGINS OF BRAIN INSULIN

Insulin was first successfully identified in dog cerebrospinal fluid (CSF) in 1967 by Margolis and Altszuler (1967). Through a continuous intravenous insulin infusion and periodic CSF and blood sampling, Margolis & Altszuler showed, for the first time, that fasted levels of insulin in the CSF were, on average, 27% of the insulin concentration found in plasma. Furthermore, it was the first report proving that insulin could cross the brain barriers, albeit in a very limited way. The concept that insulin can cross the blood-brain and the blood-CSF through a saturable transport system, and not by passive diffusion, would then become a significant milestone that is still regarded as the most accepted hypothesis describing insulin within the brain.

Although pancreas is, undoubtedly, the body's primary source of insulin, the idea that insulin found in brain tissues and CSF is exclusively derived from the blood was first challenged in

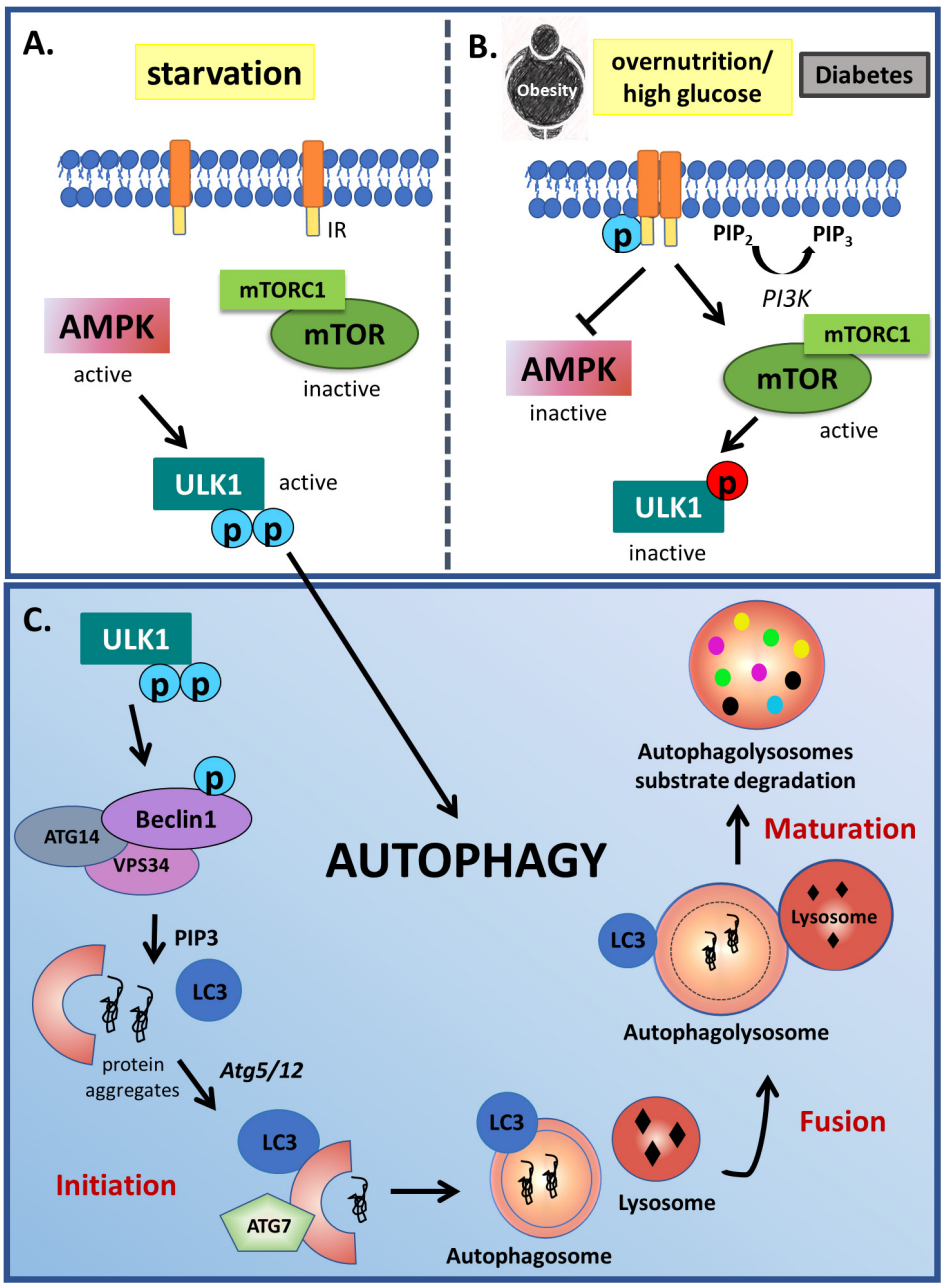

FIGURE 1 | Autophagy modulation by starvation and insulin signaling. **(A)** Autophagy is classically activated in starvation conditions through ULK1 activity. In low nutrient conditions, AMPK signaling is activated, phosphorylating ULK1 in two different activation sites. Opposite to starvation, **(B)** in overnutrition condition or diabetes, PI3K type I is activated by growth factor receptors resulting in mTOR activation and AMPK inactivation. mTOR complex TORC1 phosphorylates and inactivates ULK1 (red circles), which is an initiator of mammalian autophagy. **(C)** During this process, protein-prone aggregates or damaged organelles are engulfed by double membrane structures called autophagosomes, which fuse with lysosomes or vacuoles allowing the degradation of their cargo material in the autophagolysosomes. This complex process starts with ULK1 phosphorylation in 2 different activation residues (indicated by blue circles). Active ULK1 phosphorylates Beclin 1, that together with other components induces the formation of phagophore, an initial membrane component of the isolation membrane, which then expands, curves and closes due to two conjugation pathways dependent of ATG5-ATG12 action to LC3 lipidation, turning into a completed autophagosome.

1978 by Havrankova et al. (1978), in a paper showing high levels of insulin extracted from different brain regions, with the highest concentrations being found in the olfactory bulb and hypothalamus. Following this observation, the same group confirmed their findings in models of obese hyperinsulinemic and diabetic insulinopenic rats. In contrast to what was observed (and expected) in the liver, IR levels throughout the brain did not change in either experimental group, suggesting the

participation of the brain barriers in the protection of the CNS from dramatic peripheral insulin oscillations. Furthermore, central insulin levels also did not change in any of the tested models, suggesting *de novo* synthesis of the hormone in the brain (Havrankova et al., 1979).

Central insulin biosynthesis outside the hypothalamus is still controversial. Observations of insulin production in primary neuronal cell cultures were first reported in 1986 by Clarke et al. (1986). Analyzing the released media from whole-brain primary neuronal cultures, they showed by radioimmunoassay and HPLC analysis not only the presence of secreted insulin but also its positive regulation by depolarization, via K^+ and Ca^{2+}. As proof that these observations were specific to neuronal depolarization, Clarke showed that no such effect was possible in glial cells in culture. Preproinsulin mRNA and protein was reported in pyramidal neuronal cells of the hippocampus and olfactory bulb (Kuwabara et al., 2011), with further studies showing extensive distribution of insulin expression throughout the brain, with higher levels in the hippocampus, striatum, thalamus, entorhinal and prefrontal cortices (Mehran et al., 2012). Interestingly, recent reports show insulin expression and production also in primary cultured astrocytes, which was decreased by amyloid-β (Aβ) and lipopolysaccharide (LPS) (Takano et al., 2018). Another putative source of brain-derived insulin may be the choroid plexus (Lamotte et al., 2004; Yong et al., 2011).

Regardless of its origin, it is clear that insulin has different effects on brain function and may play a crucial role in some pathological conditions.

CENTRAL ACTIONS OF INSULIN

Insulin primarily plays a role in the regulation of glucose uptake of insulin-sensitive cells, with its effect on peripheral tissues such as muscle, adipose tissue, and liver, being very similar. Activation of its receptor leads to phosphorylation and activation of AKT and ERK pathways, culminating in the mobilization of glucose transporter 4 (GLUT4) to the cell membrane, allowing greater glucose uptake by these cells. The brain, however, behaves in a very different way, mainly expressing the insulin-insensitive glucose transporters GLUT1 (in astrocytes and blood-brain barrier endothelial cells) and GLUT3 (in neurons). Consequently, classical modeling of glucose uptake by cells in the brain considers this to be an insulin-insensitive process, although this is subject to some controversy, as indicated above. In contrast, central insulin effects are primarily regarded as neurotrophic, affecting synaptic physiology and, hence, memory and learning.

Insulin and its receptor have been implicated in neurite outgrowth and axon guidance, through activation of the PI3K/AKT pathway, as demonstrated in Drosophila (Song et al., 2003; Gu et al., 2014), murine (Grote et al., 2011) and human neuronal cells (Liu et al., 2013; Roloff et al., 2015). IRS p53 seems to play an essential role in dendritic arborization. IRSp53 is expressed in the post-synaptic membrane of neurons, where it co-localizes with the post-synaptic density and interacts with proteins that constitute the cytoskeleton (Abbott et al., 1999; Chiu and Cline, 2010). Overexpression of IRSp53 in neuronal cultures had been shown to correlate with higher levels of arborization (Govind et al., 2001), while its inhibition reduces the density and size of dendritic spines (Choi, 2005).

Insulin can modulate synaptic activity and plasticity by several different mechanisms, inducing the endocytosis of AMPA receptors for the generation of long-term depression in hippocampal cell cultures (Beattie et al., 2000) and the modulation of NMDA receptors in the post-synaptic membrane, linked to synaptic strengthening (Skeberdis et al., 2001). The modulation of these glutamatergic receptors allows insulin to participate in neuronal activity-dependent synaptic plasticity (Van Der Heide et al., 2005). Overall, such data clearly links central insulin effects to neuronal plasticity processes underpinning cognitive functioning.

INSULIN SIGNALLING AND AUTOPHAGY IN NEURODEGERATIVE DISEASES: AN INTRODUCTION

Although the literature data is still conflicting, as revised by Rotermund et al. (2018), the use of Metformin, one of the most famous anti-diabetic drugs, demonstrated to have some positive effects in, for example, PD and AD animal models (Lennox et al., 2014; Patil et al., 2014; Lu et al., 2016; Katila et al., 2017). According to the literature, both acute and chronic Metformin administration showed to increase the levels of glucagon-like peptide-1 (GLP-1), an incretin known as an inducer of insulin secretion (Maida et al., 2011), that may lead to the activation of PI3K/AKT signaling and higher brain ATG7 levels, thereby promoting autophagy (Candeias et al., 2017). Besides that, Metformin acts by regulating the AMPK signaling, which is already associated with insulin resistance in T2D (Xu et al., 2012) and is also related with the autophagy process (**Figure 2**).

As demonstrated by Yamamoto et al. (2018), transient autophagy inhibition can rescue glucose intolerance in $Becn1^{F121A}$ knocking mice fed with high-fat diet, which presents a constitutively active autophagy process, suggesting that finding the right regulation of autophagy may be a potential target for drug development for neurodegenerative diseases. Besides that, abnormal protein aggregates, which have been classically linked to neurodegenerative disorders (Lasagna-Reeves et al., 2011; Larson and Lesné, 2012; Lashuel et al., 2013) are known to be substrates of the ubiquitin-proteasome and macroautophagy systems (Filimonenko et al., 2010) that are less active along aging (Shibata et al., 2006). However, the relationship between these aggregates and cellular toxicity as well as between total plaque load and cognitive status remains a point of debate (Koss et al., 2016). Thus, this section summarizes and discusses the relationship between insulin signaling and autophagy in each of the neurodegenerative diseases.

ALZHEIMER'S DISEASE (AD)

Some research associating cerebral insulin resistance and neurodegeneration indicates that alterations in cerebral insulin

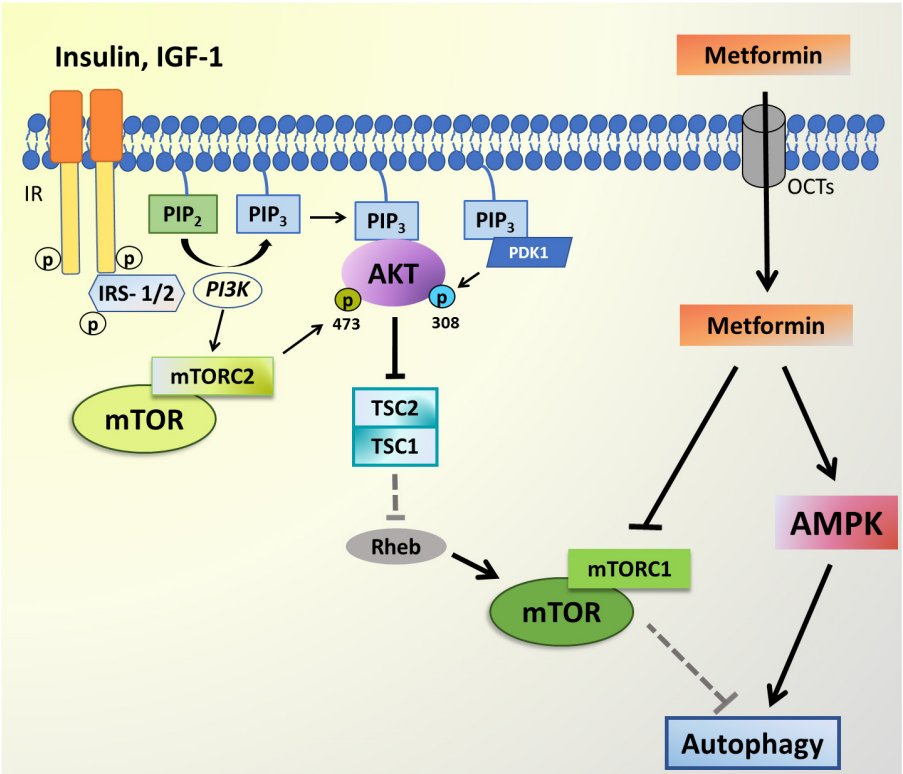

FIGURE 2 | PI3K-AKT-mTOR signaling and Metformin targets. The AKT signaling pathway can be triggered by insulin-like growth factor 1 (IGF-1) and insulin, which bind to transmembrane receptors tirosine kinase (RTK) leading to IRS-1/2 phosphorylation, PI3K activation and subsequent phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3) conversion. AKT and PDK1 bind to PIP_3 at the plasma membrane, and PDK1 phosphorylates the activation loop of AKT at T308. RTK signaling also activates mTOR complex 2 (mTORC2) through an unknown mechanism, and mTORC2 phosphorylates AKT on S473. Active AKT is responsible for the inhibition of negative regulators of mTOR, such as tuberous sclerosis complex (TSC1/2), which induces the mTOR activator Rheb, thereby releasing the mTOR complex to phosphorylate its targets. mTORC1 activity inhibits autophagy. The Metformin enters the cell through organic cation transporters (OCTs) and is able to inhibit mTORC1 complex, while also activating AMPK thus leading to autophagy activation.

signaling may be a different and more complex process than processes derived from classical type I and II diabetes pathophysiology (Rivera et al., 2005; Steen et al., 2005). As indicated above, both *in vitro* and *in vivo* studies show insulin effects in neuronal cells, specifically on synaptic plasticity as well as its positive regulation of memory and learning. The positive effects of insulin in learning were first shown in 1976 when insulin administration attenuated memory impairment in rats subjected to hippocampal lesions (de Castro and Balagura, 1976). This was followed by similar work showing insulin to positively regulate spatial learning and memory post-ischemia, while also reducing neuronal necrotic tissue in the hippocampal CA1 region (Voll et al., 1989). Insulin signaling through its receptor in the pyramidal neurons of the CA1 is also implicated in spatial learning in the water maze test (Zhao et al., 1999, 2004). Intracerebroventricular injection of insulin also improves memory, as tested by a passive-avoidance test (Park et al., 2000).

In recent decades, insulin effects on human cognition have been described, including effects on selective attention and verbal memory in healthy participants within hours of insulin infusion (Kern et al., 2001). Given that intravenous infusions of insulin produce a range of unwanted effects, such as hypoglycemia and blood ionic imbalance, different routes of administration had to be used in order to achieve a brain-specific action. It was known for more than a decade that peptides and small proteins could access the CSF from the nasal mucosa through the intercellular spaces in the olfactory nerve and olfactory bulb (Balin et al., 1986; Sakane et al., 1991; Illum, 2000). This prompted Benedict et al. (2004) to investigate the effects of intranasal insulin on the memory of 38 healthy participants over an 8-week period (Benedict et al., 2004), with results showing improved word-recall memory scores, mood assessments, and self-confidence. Importantly, these effects were achieved without any disturbances in blood glucose or insulin levels. Hence, intranasal insulin has become the preferred route of insulin administration for studies investigating insulin effects in the brain.

Data in recent years have suggested a direct relationship between autophagic processes and the development of type II diabetes. In 2013, He et al. (2013) showed that Beclin 2, like Beclin 1, is an important modulator of autophagy. Beclin 2 heterozygous-deficient mice (beclin2$^{+/-}$) were fed for 8 weeks with a regular or high-fat diet (60% fat) with results in either condition showing an increase in body weight, food intake and in insulin resistance (He et al., 2013). Another study performed

in diabetic and non-diabetic C57BL/6 mice observed an increase in autophagic vacuole formation in the presence of insulin resistance. The selectively inactivation of ATG7 in pancreatic β-cells showed that in the absence of autophagic process, ubiquitinated proteins and damaged organelles accumulate over time leading to pancreatic β-cell toxicity (Ebato et al., 2008).

Constitutively active autophagy can be achieved via a point mutation in Becn 1/Beclin 1 ($Becn1^{F121A/F121A}$). With a high-fat-diet challenge, such autophagy-hyperactive mice show improved insulin sensitivity dispite an increase in degradation of insulin granules in pancreatic β-cells (Yamamoto et al., 2018). The crossing of $Becn1^{F121A}$ mice with a transgenic murine model of AD, (5 × FAD), showed autophagy hyperactivation to significantly decrease Aβ accumulation, and to prevent cognitive decline (Rocchi et al., 2017). Autophagy hyperactivation in the 5 × FAD $Becn1^{F121A}$ murine model leads to lower levels of both soluble and insoluble Aβ42 in both the cortex and hippocampus, whilst no changes in amyloid precursor protein (APP) levels were observed. In a cellular model using HEK293 cells expressing $Becn1^{F121A}$ and APP, cells were transfected with siRNA to knockdown ATG7, leading to an increase in Aβ42, indicating the importance of autophagy in the regulation of Aβ formation (Rocchi et al., 2017). This suggests that strategies to hyperactivate the autophagic process, such as pharmacological treatment with ML246 or voluntary exercise, could decrease the risk of AD and cognitive decline (Rocchi et al., 2017).

In rodents, Caccamo et al. (2010) observed that 3 × Tg-AD mice fed for 10 weeks with rapamycin-containing food, versus a control diet, had a better performance in the Morris Water Maze task, suggesting a role for rapamycin in rescuing mice from early learning and memory deficits. 8 months of chronic rapamycin administration decreases mTOR activity in association with a decrease in hippocampal Aβ accumulation and tau hyperphosphorylation. Interestingly, this produced an autophagy gain of function, as indicated by raised levels of ATG7, ATG5/ATG12 complex and LC3-II (necessary of autophagy induction) in rapamycin-treated versus control 3 × Tg-AD mice. This effect was prevented by an autophagy inhibitor, 3-methyladenine (Caccamo et al., 2010).

Analyzing and comparing postmortem brain inferior parietal lobule tissue from patients diagnosed with mild cognitive impairment (MCI), AD, and healthy matched controls, Tramutola et al. (2015) showed that an increase in AKT and PI3K (p85 subunit) phosphorylation occurs in MCI and AD patients, coupled with mTOR hyperactivation. This increase in PI3K-AKT-mTOR signaling pathway was observed in early stage MCI as well as increased phosphorylation in the inhibitory site of IRS-1, coupled to a decrease in the autophagy markers, Beclin 1 and LC3, suggesting that such changes underpin the association of insulin resistance to early dementia. Importantly, the decrease in autophagy markers directly correlated with an increased Aβ levels. In a group of pre-clinical AD patients, with normal cognitive function ante-mortem coupled to AD neuropathology at autopsy, there was evidence of autophagy impairment although no changes in the PI3K-AKT-mTOR pathway were found, suggesting that disrupted autophagy may be an early event in Aβ deposition (Tramutola et al., 2015).

In a postmortem analysis of the midfrontal cortex gray matter, Beclin 1 levels were significantly decreased in severe AD (−30%) and mild-cognitive impairment (MCI) (−70%), compared to healthy controls. However, no significant decrease was observed in Huntington's disease (HD) or the LB variant of AD patients (Pickford et al., 2008). The same study also investigated cortical Beclin 1 levels in two different lines of aging APP transgenic mice expressing extensive Aβ deposits and high levels of mutant human APP, finding that Beclin 1 expression was the same as in nontransgenic, age-matched littermate control mice. Such data would suggest that autophagy impairment could happen in upstream events before APP pathology (Pickford et al., 2008). However, it should be noted that direct evidence of APP degradation by autophagic processes is the subject of some controversy (Boland et al., 2010; Jaeger et al., 2010; Tian et al., 2013; Rocchi et al., 2017).

Several processes and behaviors can regulate autophagy, including calorie restriction and exercise, which all improve insulin signaling and slow neurodegenerative processes. In rodents and primates maintained for several months on alternate day fasting, neurons are protected from neurodegenerative processes, in association with higher levels of brain derived neurotrophic factor (BDNF), CREB, and autophagy, as well as lower mTOR levels, improved mitochondrial function and decreased oxidative stress (reviewed in Mattson et al., 2017). Preclinical data indicate that intermittent fasting and alternate day fasting, which increase autophagy-promoting sirtuins, afford neuronal protection even in the presence of Aβ aggregates and tau accumulation, resulting in protection against cognitive impairment in 3 × TgAD mice (Halagappa et al., 2007). The mechanisms underlying the autophagy-promoting benefits of such calorie restriction diets require clarification, as these mechanisms are likely to afford some protection against neurodegenerative processes, especially in their early stages. Of note, a 24-hour fasting has been shown to increase autophagosomes and decrease mTOR signaling (Alirezaei et al., 2010). In the 5 × FAD transgenic mice that express mutant human APP770 and develop high levels of Aβ deposition at 3 months age, 48 hours of fasting was able to increase autophagy, although insufficient to clear the high levels of Aβ present in this model (Chen et al., 2015). As indicated previously, exercise is a significant positive regulator of autophagy, Aβ clearance, and improved cognition (Rocchi et al., 2017), with aerobic exercise, as well as food restriction, having a positive impact on cognitive performance of healthy participants (reviewed in Camandola and Mattson, 2017) and in animal models of AD (García-Mesa et al., 2011; **Figure 3**).

PARKINSON'S DISEASE

PD is a neurodegenerative disease with a slow progress that seems to begin many years before diagnosis with non-motor features such as olfactory and autonomic dysfunction, intestinal dysregulation, sleep disorders, fatigue, cognitive impairment and psychiatric symptoms. With disease progression classical motor symptoms appear, including bradykinesia, rest tremor, muscular

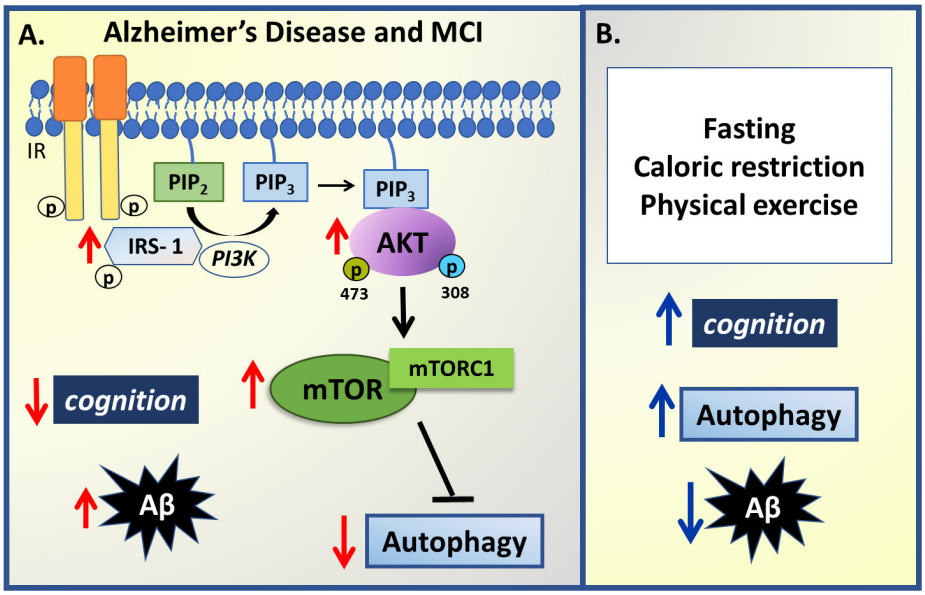

FIGURE 3 | Summary of insulin –autophagy signaling changes in Alzheimer's disease. **(A)** In mild cognitive impairment (MCI) and Alzheimer's Disease, an inverse correlation between cognition levels, insulin sensitivity, autophagy and amyloid-β (Aβ) aggregates is observed. An increase in IRS-1, PI3K and AKT phosphorylation coupled to mTORC1 hyperactivation downregulates autophagy activity, impairing Aβ degradation, which results in increased Aβ deposition and decreased cognition performance. **(B)** However, some non-pharmacological strategies have been presented to modulate some changes observed in AD and MCI such as fasting protocols, caloric restriction and physical exercise that can improve cognitive performance, increase autophagy and thus decrease Aβ aggregates. Red arrows represent changes in pathophysiology and blue arrows represent changes promoted by non-pharmacological strategies.

rigidity and postural impairment. Risk factors include genetic and environmental factors with classical symptomatology mediated by the loss of dopaminergic neurons within the substantia nigra pars compacta (Snpc), although other neuroanatomical regions can be also affected (Kalia and Lang, 2015).

In PD, as in many other neurodegenerative diseases, an increase in intracellular protein aggregates, LB, composed of abnormal aggregates αSynuclein protein (αSyn), is evident. Although the relationship between LB pathology and PD pathoetiology still requires clarification, there is epidemiological evidence to indicate that insulin signaling impairment is a relevant modulator (Driver et al., 2008; Schernhammer et al., 2011).

A study of 110 PD patients (53 with PD and dementia (PD/D) and 57 PD without dementia evaluated glucose and insulin levels after a 2 hours oral glucose tolerance-test (Bosco et al., 2012). These authors found that PD/D patients had higher prevalence of insulin resistance, with abnormal glucose metabolism (Bosco et al., 2012), although it should be noted that the association of diabetes with PD is complex and controversial (reviewed in Santiago and Potashkin, 2013). However, recent conceptualizations of type 2 diabetes have placed an emphasis on the importance of the gut-liver-pancreas axis, with alterations in the gut long appreciated as an early pathophysiological symptom in PD (Parkinson, 2002) as well as a possible site for the vagal transport of αSyn to the brain (Anderson et al., 2016). Clearly such systemic changes require further investigation.

In a postmortem study of four PD patients' brains homogenates from the substantia nigra and their age-matched controls, Sekar and Taghibiglou (2018) observed a significant decrease in PI3K p85, AKT, PIP_3, IRS-1 and IR levels, as well as an increase in glycogen synthase kinase (GSK)3β and nuclear translocation of tumor suppressor phosphatase and tensin homolog deleted on chromosome ten (PTEN), strongly suggesting an association between PD and insulin resistance (Sekar and Taghibiglou, 2018). Elevated levels of phosphorylated IRS-1 on serine residues block insulin/IGF-1 binding to the IR and so down-regulate downstream effectors such as AKT, which in turn blocks GSK3β phosphorylation. Once dephosphorylated, GSK3β becomes active and phosphorylates tau protein leading to hyperphosphorylation and neurofibrillary tangles deposition (Athauda and Foltynie, 2016). In advanced stages of AD and PD, protein aggregates of tau, Aβ and αSyn coexist (Yang et al., 2018).

In PD disruption of autophagy seems to be a process that is at least partially independent of alterations in the PI3K/AKT/mTOR pathway (Heras-Sandoval et al., 2014). Data from literature have been shown that overexpression of αSyn inhibits autophagy in cell culture and *in vivo* leading to accumulation of aggregate-prone proteins (Winslow et al., 2010). The αSyn aggregates can be alleviated by increased expression of Beclin 1, as observed in brain of transgenic mice model of αSyn overexpression, causing increased autophagy and neuronal protection (Spencer et al., 2010). Furthermore, work by Dehay et al. (2010) on a human neuroblastoma cell line as well as in the 1-methyl-4-phenyl-1, 2, 3, 6- tetrahydropyridine (MPTP) mouse model of PD led the authors to propose that the increased mitochondrial reactive oxygen species (ROS) evident in PD leads to abnormal permeabilization of lysosomal membranes, thereby causing

lysosomal depletion and the accumulation of autophagosomes, resulting in inefficient autophagy (Dehay et al., 2010). The role of oxidative stress-induced disruption in autophagy was also confirmed by other studies (Lin and Kuang, 2014; Navarro-Yepes et al., 2014; Filomeni et al., 2015; Teves et al., 2018). An increase in autophagic flux was also measured in lysates from a dopaminergic neuroblastoma cell line (SH-SY5Y) and mouse embryonic fibroblast cells treated with 6-hydroxydopamine (6-OHDA) for 3 hours, which was coupled to a reduction in glutathione content, indicating that treatment with 6-OHDA leads to an increase in ROS levels, and AMPK/ULK1 activating autophagy, in an mTOR independent pathway (Urano et al., 2018).

Furthermore, increasing evidence has implicated lysosomal dysfunction in PD as an important factor in autophagy disruption. It should be noted that a new type of nonfibrillar phosphorylated αSyn (pαSyn*), which is conformationally distinct, has been identified in PD patients' brains, which seem to be the result of an incomplete autophagic degradation of pαSyn. The pαSyn* associates with mitochondria and is highly mitotoxic, causing structural damage. According to the author, pαSyn* may be the missing link between LB and mitochondrial impairment (Grassi et al., 2018). Furthermore, Grassi et al. (2018) propose that preformed pαSyn fibrils activate autophagy but the incomplete and/or abnormal autophagolysosomal degradation observed in PD is responsible for generation of pαSyn* that should migrate away from the fibrils and associate with mitochondria. The incomplete autophagic degradation can also be caused by mutations in ATP13A2 gene and SYT11, both observed in PD (Bento et al., 2016). This is an area clearly requiring further research.

Regarding insulin signaling in humans, a recent PD trial tested the effect of Exenatide, the first synthetic agonist of glucagon like peptide 1 (GLP-1), indicated to restore insulin pathway in type II diabetes (Sandoval and Sisley, 2015), and previously known to preserve mitochondrial function in dopaminergic neurons, reducing apoptotic death and lysosomal depletion as well as decreasing tau hyperphosphorylation and plaque load (reviewed in Athauda and Foltynie, 2018). The Exanatide-PD trial analyzed 60 idiopathic PD patients (both genders) who received exenatide or placebo, once weekly for 48 weeks, followed by a withdrawal of 12 weeks for analysis. Extracellular vesicles isolated from blood samples were used to indicate the brain insulin signaling pathway. Results showed Exenatide-treated patients to have a sustained increase in IRS-1 phosphorylation levels, total AKT and pAKT S473 after 24 weeks treatment and an increase in total and p-mTOR (S2448-mTORC1) at 48 weeks of treatment, compared to placebo. Increased levels of total mTOR (mTORC1/2) were associated with sustained clinical improvement on motor scores at 60 weeks (Athauda et al., 2019).

On the other hand, effects of rapamycin, a specific inhibitor of mTOR, require further clarification in PD models. In the 6-OHDA rat model of PD, Jiang et al. (2013), observed that rapamycin treatment with three different doses was able to decrease oxidative stress and mitochondrial injury consequently avoiding apoptotic dopaminergic neuronal loss (Jiang et al., 2013). These effects involved modulation of the mTORC1 complex, as mTORC2 is unresponsive to acute rapamycin treatment (Perluigi et al., 2015). Another study using the 6-OHDA model showed mTORC1 inhibition by rapamycin, or the selective blockade of its downstream target, S6K (by PF-4708671), to improve the memory deficit evident in this model. Rapamycin treatment was also able to reduce the anxiety and depression-like behavior observed in the rat PD model (Masini et al., 2018).

Non-pharmacological strategies to improve PD symptoms indicate exercise to have beneficial effects. In the 6-OHDA model, 8 weeks of 4 days/week treadmill running protected animals from 6-OHDA-induced oxidative stress (Tuon et al., 2012), whilst in the MPTP murine model, 8 weeks of treadmill running, 5 days/week, reduced αSyn levels in the striatum, and dopaminergic neuronal loss, as well as improving motor deficits and recovery of Beclin-1 and LC3-II levels, indicating that exercise could up-regulate autophagy (Koo and Cho, 2017). It is important to note that exercise duration seems to be a critical factor for the benefits observed in animals, as shorter periods of exercise are not so effective (Aguiar et al., 2014).

However, in human studies the effects of exercise in PD patients are much less conclusive (Ebersbach, 2015), although a large cohort study published by National Parkinson Foundation Quality Improvement Initiative Database in 2014 showed that a higher frequency on exercise leads to lower levels of disease severity and slows cognitive decline. The study analyzed data from 4,866 PD patients who were at different disease stages, although all were receiving treatment in United States centers of excellence (Oguh et al., 2014). Whether any exercise benefits are evident at later stages of PD still requires determination (Petzinger et al., 2013).

Other factors known to modulate autophagy, including dietary restriction, may also slow PD progression, as indicated in the MPTP rhesus monkey model, as indicated by the numbers of nigro-striatal dopaminergic neurons (Maswood et al., 2004). Any strategy that inhibits the insulin-AKT-mTORC1 signaling pathway is associated with increased longevity via autophagy activation (Ntsapi and Loos, 2016), and therefore would be expected to positively modulate PD incidence and pathophysiology (reviewed in Mattson, 2010). The benefits of dietary restriction in PD patients remain to be determined.

In summary, correlations of insulin resistance with abnormal autophagy seem important processes in the pathogenesis of AD and PD. However, there are some important differences across these disorders that future research should clarify. Clearly, increasing autophagic processes is beneficial in AD, whilst this requires further studies in PD patients, including as to how concurrent dementia interacts with wider PD processes, given that PD patients with dementia are more prone to develop insulin resistance. PD patients also have lower levels of the IR and its downstream proteins, although the origin of autophagy abnormalities remains undetermined. The roles of autophagy and mTOR in PD are still highly controversial, which considerably complicates treatment aimed at such processes and pathways (**Figure 4**).

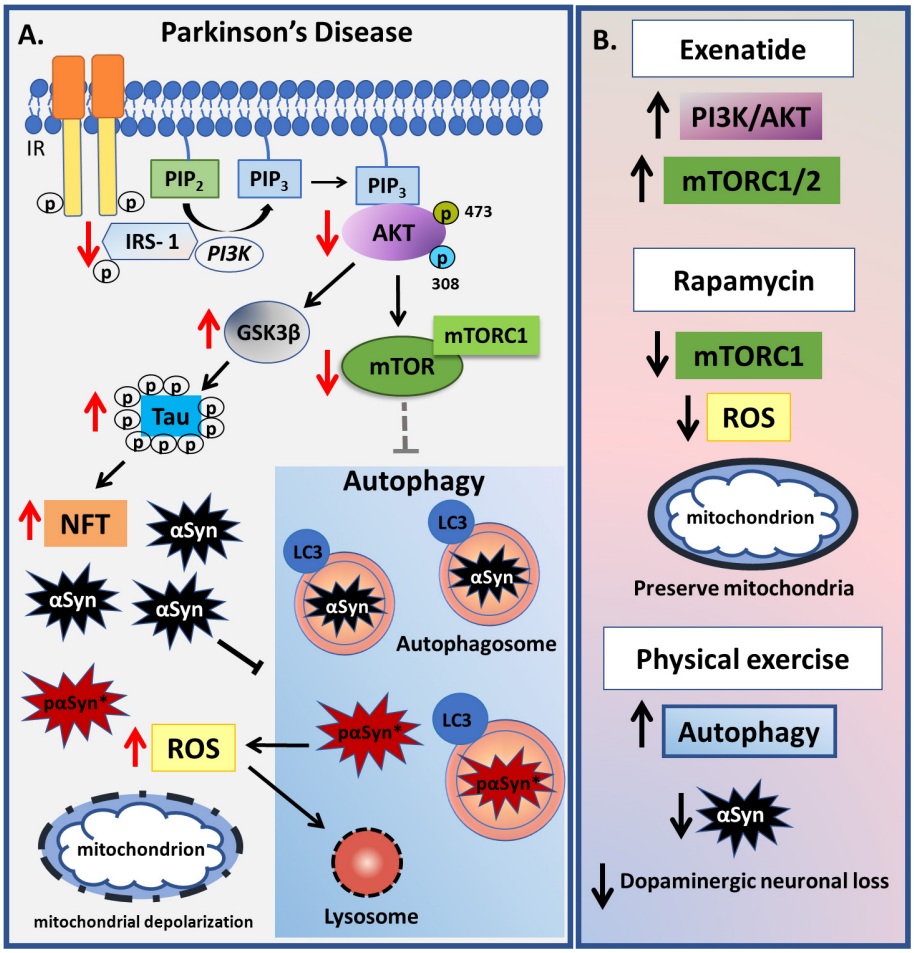

FIGURE 4 | Summary of changes related to insulin-autophagy pathway observed in Parkinson's Disease. **(A)** In Parkinson's disease, a decrease in PI3K-AKT-mTOR signaling is observed, which leads to GSK3β activation. GSK3β hyperphosphorylates tau protein leading to neurofibrillary tangles deposition. mTORC1 complex is inactive which causes autophagy to become activated. Otherwise, high levels of α-Synuclein (αSyn) impair the autophagic process together with increased mitochondrial reactive oxygen species (ROS). ROS leads to abnormal permeabilization of lysosomal membranes, thereby causing lysosomal depletion and the accumulation of autophagosomes, resulting in inefficient autophagy. As a result of an incomplete αSyn autophagic degradation, a new reactive specie called pαSyn* is generated inside autophagosomes and when released in cytosol it associates with mitochondrion being highly mitotoxic. Damaged mitochondrion is indicated by dashed line. **(B)** Some pharmacological and non-pharmacological strategies that act in PI3K-AKT-mTOR-autophagy signaling are presented. Exenatide-treated patients have a sustained increase in IRS-1 phosphorylation levels, total AKT and pAKT as well as increased levels of mTOR, reverting the impaired signaling observed in PD pathophysiology. Rapamycin, which specifically blocks mTORC1 activity, was able to decrease oxidative stress and mitochondrial injury in animal models, whereas physical exercise is associated with an increase in autophagy and a decrease in both αSyn aggregates and dopaminergic neuronal loss.

HUNTINGTON'S DISEASE

Huntington's disease is an autosomal dominant disorder characterized by severe motor and cognitive dysfunction, psychiatric disturbances and, ultimately, death. HD has long been appreciated to arise from a mutation in the Huntington (Htt) gene, which has a cytosine-adenine-guanine (CAG) expansion that encodes a polyQ repeat at the N-terminus in HD, resulting in impairments in protein functions and in protein aggregates (MacDonald et al., 1993). There is still no specific treatment for this condition, although studies in HD murine models show that the abolition of the expression of mutant Htt (mHtt) can rescue some of the symptoms and decrease protein aggregation in the brain (Yamamoto et al., 2000; Harper et al., 2005; Kordasiewicz et al., 2012). However, as functional Htt protein plays an important role in several physiological processes, such as mitochondrial dynamics, vesicle trafficking, axonal transport, synaptic function, and anti-apoptotic activity as well as autophagy, there are major problems in trying to suppress the activity of this gene (Choi et al., 2014; Ismailoglu et al., 2014; Wong and Holzbaur, 2014; Weiss and Littleton, 2016).

The loss of Htt functioning results in autophagy dysregulation, leading to protein accumulation and is likely to contribute to HD pathogenesis (Gelman et al., 2015). In contrast to other protein aggregation diseases, human and rodent HD samples show an upregulation of autophagosomes (Kegel et al., 2000; Petersen, 2001). However, these autophagosomes show some deficits in cargo recognition, resulting in inadequate degradation

of aggregate proteins and damaged organelles (Boland et al., 2008; Martinez-Vicente et al., 2010). Besides that, it is also known that the silencing of either Htt or its interactor, Htt-associated protein 1 (HAP1), impairs the transport of autophagosome along the axon (Wong and Holzbaur, 2014), indicating that mHtt also modulates endocytic trafficking.

According with the literature, HD patients present higher basal plasma levels of IGF-1, which have been linked to the cognitive decline seen in these patients, leading to the hypothesis that IGF-1 resistance may be one of the underlying mechanisms involved in this pathology (Saleh et al., 2010; Salem et al., 2016). IGF-1, an upstream activator of the AKT-mTOR pathway, can inhibit mHtt-induced neuronal death and decrease the formation of intranuclear mHtt inclusions, via AKT phosphorylation of Htt, thereby providing a neuroprotective effect in HD (Humbert et al., 2002). Besides that, data from HD animal model showed AKT to be up-regulated with the intranasal administration of recombinant human IGF-1, increasing phosphorylation of mutant Htt and leading to an improvement in motor activity, as well as in both peripheral and central metabolic abnormalities (Lopes et al., 2014). On the other hand, according to Yamamoto et al. (2006), the dose-dependent clearance of the protein aggregate after insulin and IGF-1 treatment involves IRS-2 activation, and not IRS-1 or AKT activity (Yamamoto et al., 2006). Interestingly, depletion of the IGF-1 receptor (IGF-1R) resulted in decreased autophagy and increased mHtt levels, which

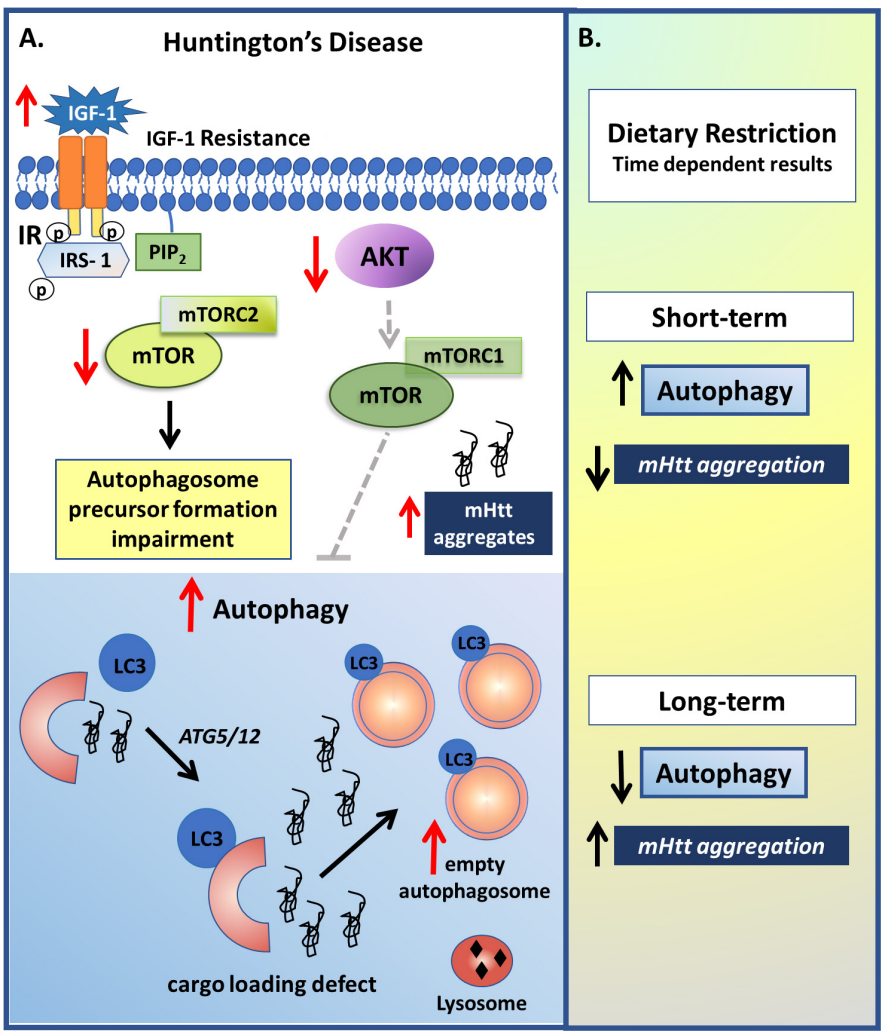

FIGURE 5 | Insulin and autophagy signaling modulation in Huntington's disease (HD). **(A)** Although the IGF-1 role on HD still seems paradoxical, it is clear that it develops an important role in this pathology. As observed in some studies, HD patients present higher basal plasma levels of IGF-1, which may be related with an IGF-1 resistance condition and a reduction in the insulin pathway. The reduction in IR activity results in reduced mTORC2 and AKT-mTORC1 activities, which leads to, respectively, a reduction in autophagosome precursor formation and an increase in the autophagy process, which may be related with the upregulation of autophagosomes with deficits in cargo recognition. However, long term IR down regulation, due to for example, receptor depletion or long term IR inhibition, results in reduced autophagy, which may be due to a lack of essential autophagosome precursor formation, and increased mHtt aggregates. **(B)** Non-pharmacological approaches such as dietary restriction also demonstrated to have a dual effect depending on the time. Short-term fasting resulted in increased autophagy and reduction on mHtt aggregation, while long-term serum deprivation resulted in reduced autophagosome formation.

was rescued only by prolonged (24 hours) IGF-1 treatment, in an AKT-independent pathway (Renna et al., 2013). Although another study demonstrated that IGF-1R deficiency affected only the size but not the mHtt levels in HD mice, it was observed that this deficiency had different consequences depending on the sex of the animal, causing some detrimental effects in the males, such as worsened rotarod performance and weight loss, but a delayed tremor onset in the females (Corrochano et al., 2014). Besides that, viral overexpression of the transcription factor EB (TFEB), a substrate of mTORC1 activity, resulted in increased autophagy and decreased levels of mHtt (Vodicka et al., 2016), indicating that the PI3K-AKT-mTOR pathway also plays an important role in this disease (Wu et al., 2009). As such, activation of the IGF-1/insulin pathway seems to have a dual and symptomatic utility, although the mechanisms involved require clarification.

Given that nutrient-rich or starvation diets can, respectively, suppress or increase autophagy via serine/threonine kinase phosphorylation of mTOR (Kim et al., 2008; Wong et al., 2015), a number of studies have investigated the utilization of dietary interventions in HD. Although the accumulation of mutant protein can lead to mTOR-independent autophagy with the degradation of accumulated proteins differing from the degradation under conditions of starvation (Yamamoto et al., 2006), dietary restriction in a HD mouse model resulted in an increase in autophagy and a reduction in mHtt aggregate formation and the normalization of blood glucose regulation, thereby contributing to a slower disease progression and an extended lifespan (Duan et al., 2003; Ehrnhoefer et al., 2018). However, although scheduled feeding demonstrates to increase autophagy, long-term serum deprivation, such as 8 to 24 hours, results in reduced autophagosome formation (Renna et al., 2013), suggesting that the autophagy regulation is influenced by the nutrient starvation time (**Figure 5**). Clearly, more studies are required on relevant processes in this area, including as to how dietary restriction may afford benefit in HD.

AMYOTROPHIC LATERAL SCLEROSIS AND FRONTOTEMPORAL DEMENTIA

Amyotrophic lateral sclerosis is a neurodegenerative disease that is characterized by the degeneration of upper and lower motor neurons of the primary motor cortex and spinal cord, resulting in muscle weakness and eventual paralysis, leading to death (Al-Chalabi and Hardiman, 2013). Although the pathoetiology of ALS is unknown, it has a not uncommon co-occurrence with FTD, which is marked by focal atrophy of both frontal and anterior temporal lobes (Van Langenhove et al., 2012). Moreover, as ALS and FTD share genetic risk factors, it has been proposed that they represent a continuum of neurodegenerative disorders (Van Langenhove et al., 2012).

ALS is associated with hypermetabolic and dyslipidemia states (Dupuis et al., 2008; Seelen et al., 2014), with an inverse risk factor association with obesity and type 2 diabetes (Gallo et al., 2013; Kioumourtzoglou et al., 2015). ALS patients with hyperlipidemia have an increased life expectancy, suggesting a neuroprotective role of lipids on ALS pathophysiology (Dupuis et al., 2008). Similar results are evident in the SOD1 mutant murine model of ALS, where high energy-diets prolong the lifespan (Dupuis et al., 2004). However, after adjusting for markers of disease severity, such as body mass index (BMI), forced vital capacity, and age, it was found that BMI, but not dyslipidemia, underlie this increased survival of ALS patients (Paganoni et al., 2011).

Although the role of lipids in ALS requires clarification, there is greater certainty as to the higher prevalence of hypermetabolism in ALS patients, which is linked with a faster rate of functional decline and shorter survival (Steyn et al., 2018).

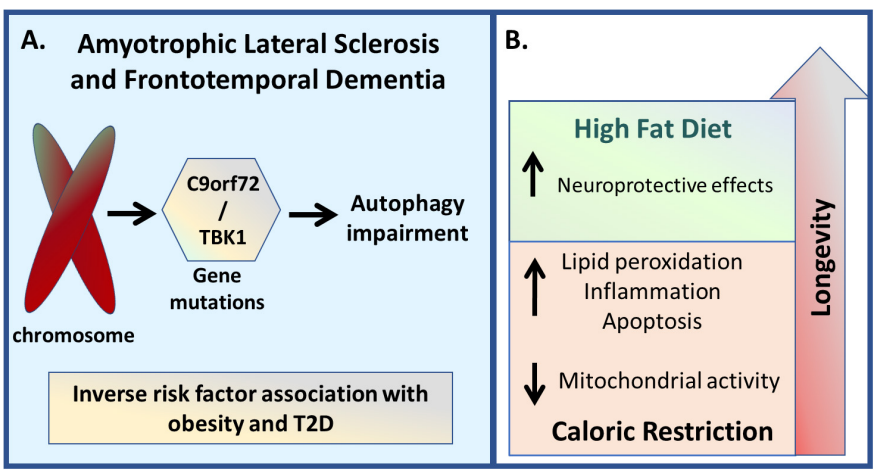

FIGURE 6 | Amyotrophic lateral sclerosis and Frontotemporal Dementia. **(A)** Genetic variations in genes such as C9orf72 and TBK1 are linked with both ALS and FTD disease development, which are associated with autophagy impairment. Besides that, since it is also associated with hypermetabolic and dyslipemia states, both conditions seem to present an inverse risk factor association with obesity and T2D. **(B)** Regarding the non-pharmacological inhibition, caloric restriction demonstrated to decrease lifespan due to an increase in lipid peroxidation, inflammation, and apoptosis, as well due to a reduction in mitochondrial activity, while high-energy diets resulted in increased lifespan.

Interestingly, hypermetabolism is associated with some genetic risk factors for ALS, although not for the C9ORf72 mutation (Steyn et al., 2018), which is the one most commonly associated with ALS-FTD (DeJesus-Hernandez et al., 2011). Remarkably, alterations in the C9ORf72 gene seem to have a dual function in autophagy regulation, with C9ORf72 positively regulating the initiation of autophagy by controlling the ULK1 complex, with its reduction resulting in autophagic dysfunction and protein aggregation (Webster et al., 2016). Other work shows the C9ORf72 gene to decrease autophagy through mTORC1 regulation (Ugolino et al., 2016). However, although Steyn et al. (2018) were not able to find any association between C9ORf72 and hypermetabolism, Liu et al. (2018) showed that loss of C9ORf72 gene function impairs lipid digestion and increases *de novo* fatty acid synthesis through dysregulated autophagic process under cell glucose starvation conditions (Liu et al., 2018). This could suggest that starvation or nutrient-rich conditions may play an important role in modulating C9ORf72 functions in autophagy.

Mutations leading to a loss-of-function in the TANK-binding kinase (TBK)1 phenotype also lead to an increased risk of developing ALS and FTD due to autophagy impairment (Cirulli et al., 2015; Freischmidt et al., 2015; Cui et al., 2018). Interestingly, a study performed on obese zucker rats provides evidence for the involvement of TBK1 in the mechanism of insulin resistance (Muñoz et al., 2009). This study shows that obese animals have increased hepatic TBK1 levels, leading to decreased IR activity due to Ser994 phosphorylation by TBK1 (Muñoz et al., 2009). Moreover, a high fat diet can positively modulate TBK1 gene expression in the liver and white adipose tissue (Chiang et al., 2009) whilst its accumulation in lipid rafts of hypothalamic neurons disrupts IR activation as well as AKT signaling, suggesting a potential role of TBK1 in brain insulin resistance (Delint-Ramirez et al., 2015). Overall, such data suggest that it is plausible that TBK1 may be one of the underlying mechanisms by which obesity is associated with a reduced risk of ALS and FTD.

An animal model of ALS under caloric restriction has a decreased lifespan due to an increase in lipid peroxidation, inflammation and apoptosis, as well a decrease in mitochondrial bioenergetic efficiency, when compared with mice fed *ad libitum* (Patel et al., 2010). Similarly, rapamycin treatment is detrimental, increasing motor neuron degeneration in a mouse model of ALS (Zhang et al., 2011). As autophagy and metabolism modulate ALS disease onset and progression, dietary interventions may prove of clinical utility in the management of this disorder, including the possibility of using a high-fat diet as treatment (**Figure 6**).

OTHER NEURODEGENERATIVE DISEASES

Spinocerebellar Ataxia Type 3

Spinocerebellar ataxia 3 (SCA3), a neurodegenerative disease characterized mainly by progressive ataxia affecting balance, gait and speech, is caused by a mutation in the C-terminus polyglutamine (PolyQ) region of the ataxin-3 protein, an ubiquitously expressed deubiquitinating enzyme with important functions in the proteasomal protein degradation pathway and regulation of transcription. According with the literature, the polyQ stretch in wild-type ataxin-3 induces autophagy by protecting Beclin-1 from proteasome-mediated degradation (Ashkenazi et al., 2017). As other neurodegenerative diseases, the only available treatments aim to reduce disease symptoms. However, although the underlying mechanisms are not completely understood, the regulation of autophagic process has demonstrated to be a potential target for ameliorating or stopping the progression of the disease (Menzies et al., 2010; Nascimento-Ferreira et al., 2011). As demonstrated by Menzies et al. (2010), the administration of rapamycin improved motor performance in an SCA3 animal model through mTOR inhibition and consequent autophagy upregulation. Besides that, according with Nascimento-Ferreira et al. (2011), Beclin-1 overexpression resulted in increased autophagic flux, mutant ataxin-3 clearance and other neuroprotective effects, rescuing the abnormal expression of endogenous autophagic markers, accumulation of autophagosomes and reduced levels of Beclin-1. In a similar way, another study observed decreased autophagosome production when comparing fibroblasts from SCA3 patients and healthy individuals. However, although Beclin-1 overexpression increased the autophagic flux, it did not result in higher levels of autophagosome production (Onofre et al., 2016). Taken together, this result supports the important role of autophagy in also regulating the SCA3 disease.

Lafora Disease

Lafora disease is a rare autosomal recessive, progressive and lethal neurodegenerative disease that is characterized by myoclonic epilepsy followed by continuous neurological decline. Reported in approximately 58% of the LD cases, mutation in the EPM2A gene, which encodes the glucan phosphatase laforin, is associated with the presence of intracellular inclusion bodies known as Lafora bodies (LB) in the brain, spinal cord and other peripheral tissues (Knecht et al., 2010). Although laforin demonstrated not to be absolutely necessary for autophagy, it was observed that it positively regulates this process (Knecht et al., 2010). According to this study, LD is associated with decreased levels and impaired formation of LC3-II, as well altered signaling in the AKT-mTOR pathway, which was rescued after laforin overexpression, reducing the amount of protein aggregates in an autophagy-dependent manner (Knecht et al., 2010). Moreover, besides autophagy, LD also seems to be associated with insulin resistance and hyperglycemia (Nicolescu et al., 2019), strengthening the crosstalk between insuling and autophagy signaling in neurodegenerative diseases.

CONCLUSION

Although it is clear that central insulin signaling and the regulation of autophagy are relevant to a host of diverse neurodegenerative disorders, which is supported by several studies including pharmacological inhibition, animal models, genetic strategies and patients, as well as by the

link between metabolic disorders and the development of some neurodegenerative diseases, it is still hard to define statements as data from literature is controversial and the underlying mechanisms as well as the possible benefits of autophagy modulation seem to be specific for each disorder. As many key cellular processes are intimately associated with insulin signaling and autophagic process, pharmacological inhibition and/or stimulation may have some detrimental effects, especially considering long-term treatments, which may partly be responsible for the conflicting results in the literature. Besides that, it is important to consider that the onset of the disease, the age and also the sex can have a strong influence on the development and also on the treatment outcomes. In this context, genetic strategies that modulate metabolism and autophagy regulation can contribute to having a better understanding of the underlying mechanisms among these pathways and the neurodegenerative diseases. Besides that, as it seems that outcomes may vary according to stimulus duration, the study of the effects of non-pharmacological interventions such as physical exercise and intermittent fasting in the onset and progression of neurodegenerative diseases can also bring light to new information for the literature, as well as possible new approaches that may contribute to the conventional treatments. Thus, the understanding of how such processes are integrated in different central and systemic cells should provide better targeted treatment for these still poorly managed conditions.

AUTHOR CONTRIBUTIONS

CS and EK conceived the review idea, and edited final version of the text and figures. CM, NdM, and AO contributed equally writing the text. NdM and AO contributed equally conceiving the figures ideas. GdM contributed by drafiting the work and drawing the figures.

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