Obesity, Neuroinflammation, and Reproductive Function

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The increasing occurrence of obesity has become a significant public health concern. Individuals with obesity have higher prevalence of heart disease, stroke, osteoarthritis, diabetes, and reproductive disorders. Reproductive problems include menstrual irregularities, pregnancy complications, and infertility due to anovulation, in women, and lower testosterone and diminished sperm count, in men. In particular, women with obesity have reduced levels of both gonadotropin hormones, and, in obese men, lower testosterone is accompanied by diminished LH. Taken together, these findings indicate central dysregulation of the hypothalamic–pituitary–gonadal axis, specifically at the level of the GnRH neuron function, which is the final brain output for the regulation of reproduction. Obesity is a state of hyperinsulinemia, hyperlipidemia, hyperleptinemia, and chronic inflammation. Herein, we review recent advances in our understanding of how these metabolic and immune changes affect hypothalamic function and regulation of GnRH neurons. In the latter part, we focus on neuroinflammation as a major consequence of obesity and discuss findings that reveal that GnRH neurons are uniquely positioned to respond to inflammatory changes. (***Endocrinology*** **160: 1–18, 2019)**

Hypothalamus Regulates Reproduction and Metabolism

Proper integration of metabolic stimuli with the hypothalamic–pituitary–gonadal (HPG) axis is critical for normal pubertal development and maintenance of reproductive function in adults. GnRH from the hypothalamus is the final brain signal that regulates reproduction (1). GnRH is secreted by a unique population of ~1000 to 2000 neurons that are scattered in the preoptic area, septum, and anterior hypothalamus in rodents, or periventricular area and mediobasal hypothalamus in primates (2, 3). They are unipolar or bipolar neurons that send long processes to the median eminence (ME). Because GnRH neurons are scattered, pubertal onset and synchronization of GnRH release in adulthood are regulated by upstream neurons, most notably those that produce kisspeptin (encoded by *Kiss1*) (4–7). GnRH is secreted in the pulsatile manner into the hypophyseal portal circulation in the ME. Upon binding to its receptor, GnRH stimulates gonadotrope cells in the anterior pituitary to synthesize and secrete LH and FSH (8, 9). LH and FSH regulate steroidogenesis and gametogenesis in the gonads, and gonadal steroids in turn provide feedback to the hypothalamus via kisspeptin.

Both extremes of body weight are not conducive for optimal HPG axis function. A minimum ratio of fat to lean mass is necessary for menarche and for the maintenance of female reproductive ability (10). Food intake and energy expenditure are regulated by several brain areas, primarily brain stem and hypothalamus, which receive short-term signals from the gastrointestinal tract and long-term signals from body energy stores, mainly adipose tissue (11). Hypothalamic neurons involved in

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Abbreviations: AgRP, agouti-related protein; ARC, arcuate nucleus; BBB, blood–brain barrier; BMI, body mass index; CNS, central nervous system; DIO, diet-induced obesity; GABA, γ-aminobutyric acid; GFP, green fluorescent protein; HFD, high-fat diet; HPG, hypothalamic–pituitary–gonadal; ICV, intracerebroventricular; LIF, leukemia inhibitory factor; LPS, lipopolysaccharide; ME, median eminence; NPY, neuropeptide Y; OVLT, organum vasculosum laminae terminalis; POMC, proopiomelanocortin; α-MSH, α-melanocyte-stimulating hormone; β-END, β-endorphin.

feeding and their interaction with reproductive circuitry are discussed below. Adipose tissue serves as an endocrine organ, and it is thought that increased secretion of leptin with increased adiposity is necessary for the initiation of puberty (12). Malnutrition or limited adipose tissue in athletes causes lower levels of both gonadotropin hormones and reduced frequency of LH secretion, implying central regulation of the reproductive axis by metabolic signals (13). Because metabolic influences on pubertal development have been extensively reviewed (14–19), in this review we concentrate on the negative effects of obesity on reproduction in adults.

Negative Effects of Obesity on Reproductive Function

During the past 30 years the prevalence of obesity has increased steadily worldwide (20). Currently in the United States, >30% of men and women are classified as obese, with a body mass index (BMI) of ≥30 kg/m^2 (21). In recent years, rates of obesity have disproportionally escalated in children and young individuals, which may lead to long-term consequences in a number of homeostatic processes, including reproductive function. According to the World Health Organization, obesity is linked to an increased risk of cardiovascular disease, cerebral ischemia, type 2 diabetes, and reproductive disorders for both men and women (22, 23).

Men with obesity exhibit reduced levels of LH (24), testosterone, and SHBG (25, 26). Obesity-associated reduction in testosterone is accompanied by reduced levels of LH, whereas age-related reduction in testosterone is correlated with increased LH (25), indicating central rather than gonadal dysregulation in obesity. Sperm number and quality are negatively impacted by increased adiposity as well (23, 27, 28). Moreover, increased BMI is associated with lower sperm concentration and fewer total spermatozoa (29). Meta-analysis of 21 reports and a total of 13,000 men associated obesity with increased prevalence of azoospermia and oligozoospermia (23). Fertilization rates during *in vitro* fertilization are reduced when the male partner is obese (30). Studies in animal models correlate with clinical findings in humans. Obese Zucker rats and C57BL/6 mice demonstrated decreased sperm production and increased sperm DNA fragmentation (28, 31). Our recent study demonstrated lower LH, testosterone, and sperm count in obese C57BL/6J mice (Fig. 1) (32). Decreased testosterone, accompanied by a reduction in LH, implicates central regulatory mechanisms at the neuroendocrine levels.

Similarly, women with obesity are more likely to have reduced fertility characterized by reduced levels of LH (22, 33–35). Obesity-related problems in women include early onset of puberty, menstrual irregularities, in particular a longer follicular phase indicating ovulatory problems, pregnancy complications, infertility, and spontaneous abortions (36–38). Studies in animal models also corroborate these findings. Female rhesus macaques placed on a high-fat diet (HFD) presented with reduced LH pulse amplitude (39). Female mice fed an HFD exhibit longer estrous cycles (32, 40, 41). Because women with obesity have lower levels of gonadotropin hormones (42), hypothalamus and pituitary are likely primary sites of obesity-mediated impairment of the reproductive axis.

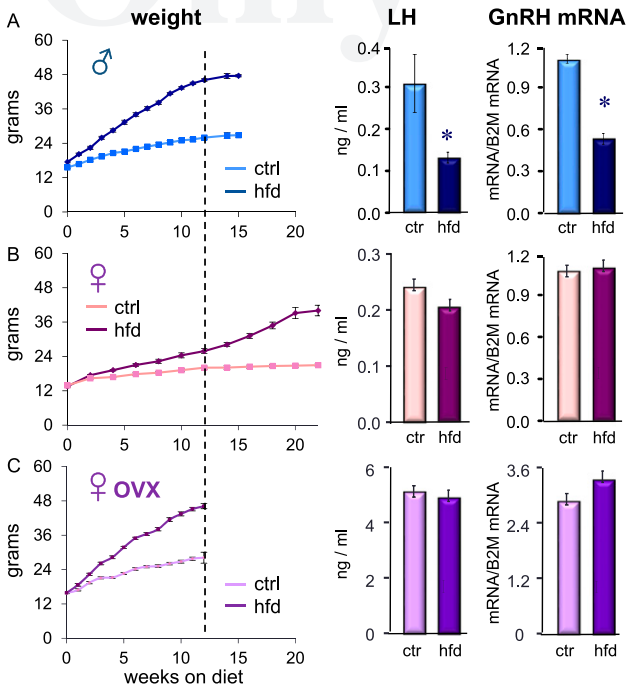

Figure 1. Ovarian hormones are protective from diet-induced obesity but not necessary for protection from hormonal changes in females. (A and B) Ten C57BL/6J mice per group were placed on control (ctr, 10 kcal % fat, Research Diet) or a high-fat diet (hfd, 60 kcal % fat, Research Diet) with the same sucrose levels, at 4 wk of age. Their weights were recorded twice a week (A, males; B, females). (C) Female mice were ovariectomized (OVX) at weaning and 1 wk later, at 4 wk of age, were placed on experimental diets. After 12 wk on their respective diets, males and OVX females were euthanized, whereas unmodified females were euthanized after 20 wk on diets, when the females on an hfd reached the same weight gain as males and OVX females, because the 12-wk diet showed lack of any differences. Serum LH was measured using ultrasensitive assay by UVA Ligand Core and GnRH mRNA in the hypothalami by quantitative RT-PCR. *$P < 0.05$, between control and hfd. Reported in Lainez *et al.* (32).

Differences in Animal Models and Sex

Significant strain differences in response to an HFD were observed in laboratory mice. A/J, FVB/NJ, and BALB/cJ strains are resistant to diet-induced obesity (DIO), whereas DBA/2J and C57BL/6J strains gain weight (43–45). The C57BL/6J mouse is a particularly faithful model of the human metabolic syndrome because it develops obesity,

hyperinsulinemia, hyperglycemia, and hypertension when allowed *ad libitum* access to an HFD (46, 47), and it is used most often for these studies. Owing to the mutation in the nicotinamide nucleotide transhydrogenase (*Nnt*) gene, the C57BL/6J mouse also exhibits slightly worse metabolic parameters in response to long HFD exposure than does the related C57BL/6N substrain (48–50). Similar differences, albeit less pronounced, exist between rat strains, with Wistar rats having a larger pathophysiological response to obesity than do Sprague-Dawley or Fischer 344 strains (51–54). Keeping these differences in mind is critical when evaluating and comparing studies in the literature. Owing to availability of genetic modifications, mice were used in a larger number of studies in recent years. It is also important to critically assess studies in mice containing single gene alterations, because obesity is a polygenic trait, with 244 genes cited so far for obesity-related phenotypes in mice and 253 quantitative trait loci in humans (55).

Sex differences in response to DIO are also profound (56). Based on the studies in animal models and observations in women who are menopausal, it was hypothesized that a lack of estrogen increased adiposity, whereas estrogen replacement diminished it. In accordance, estrogen receptor α knockout mice (57) and aromatase knockout mice (58) exhibit increased obesity. Women treated with aromatase inhibitors have higher adiposity (59). An increase in adiposity following ovariectomy and removal of ovarian estrogen was observed in rodents (60, 61) and in monkeys (62). However, in DBA and C57BL/6J mouse strains, both of which are prone to DIO, sex differences vary. Females of the DBA strain are prone to DIO, although they have estrogen (45). Alternatively, C57BL/6J females are resistant to DIO, whereas males are susceptible. A recent study from our laboratory concurred that ovarian estrogen is protective from DIO in C57BL/6J mice (Fig. 1). However, we further demonstrated that ovarian estrogen is not necessary for female protection from hormonal and immunological changes that obese males exhibit (32). Because it was assumed that hormonal changes would follow weight gain in females after ovariectomy, obesity-mediated endocrine changes had not been compared in unmodified and ovariectomized females before. We demonstrated that obese females are protected from hormonal and immune changes regardless of the gonadal status (32). Whether protection in females is provided by extraovarian estrogen (63, 64) or by other sex differences in neuroendocrine axes, metabolic rates, immune system, or fat deposition (61, 65, 66) remains to be determined.

Crosstalk Between Reproductive and Feeding Circuitry in the Hypothalamus

Metabolic cues from the periphery are integrated primarily by anorexigenic proopiomelanocortin (POMC) neurons and orexigenic neuropeptide Y (NPY) neurons located in the arcuate nucleus (ARC) of the mediobasal hypothalamus, and by neurons that synthesize orexin in the lateral hypothalamus (67). These neurons regulate food intake by sensing levels of leptin and insulin, and it is proposed that they convey metabolic status to neurons involved in reproduction, namely GnRH and/or kisspeptin neurons that regulate GnRH neuron pulsatility. Although studies have examined the involvement of neuropeptides produced by POMC, NPY, and orexin neurons in communicating metabolic status to the reproductive axis, results remain inconclusive. The role of these neurons have been reviewed in more detail elsewhere (68–73), and we will briefly summarize the findings.

POMC

POMC is an anorexigenic precursor expressed in neurons in the ARC that coexpress cocaine and amphetamine-regulated transcript (CART). Activation of POMC neurons by leptin reduces food intake and increases energy expenditure (67). Processing of POMC precursor creates anorexigenic α-melanocyte-stimulating hormone (α-MSH) melanocortin and orexigenic β-endorphin (β-END) opioid peptide. The receptors for melanocortins have been classified into five subtypes, MC1R, MC2R, MC3R, MC4R, and MC5R, whereas β-END acts on μ- and κ-receptors (74). Of the five melanocortin receptors, MC3R and MC4R are expressed in the brain, regulating energy expenditure and satiety, respectively (75). Although POMC neurons are considered primary brain targets of metabolic signals from the periphery via leptin, mice lacking leptin receptor specifically in POMC neurons are only mildly obese, compared with the whole-body leptin receptor knockouts (76). POMC neurons make direct contact with GnRH neurons in the rat brain (77), and POMC products, α-MSH, and β-END have differential effects on reproduction. Whereas α-MSH activates most GnRH neurons (78) and stimulates LH secretion (12), β-END inhibits a small percentage of GnRH neurons (78) and reduces LH secretion (79). Single-cell RT-PCR demonstrated that most GnRH neurons express MC4R and that treatment with an agonist, MTII, activates GnRH neurons (80). Because kisspeptin neurons express MC4R as well (81), kisspeptin neurons may convey an α-MSH response and activate GnRH neurons, in particular during initiation of puberty (12). However, regulation of either GnRH or kisspeptin neurons by POMC in adults remains to be explored in detail, and it is unknown whether normal interaction is altered in obesity.

Neuropeptide Y

Neuropeptide Y (NPY), an orexigenic neuropeptide that signals through G-protein–coupled receptors Y1 to Y6 is synthesized by neurons in the ARC that are activated during states of low energy, such as lactation or starvation. Ghrelin,

secreted by the stomach and intestine, activates NPY neurons to promote food intake. NPY neurons, which also synthesize γ-aminobutyric acid (GABA) (82), in turn inhibit POMC neurons (83, 84) through the Y1 receptor, as well as via GABA neurotransmission (83). Surprisingly, NPY knockout mice maintain body weight and exhibit similar food consumption as do control mice (85), as do genetically modified mice that overexpress NPY ($Npy^{tet/tet}$) (86). Agouti-related protein (AgRP) that is coexpressed in NPY neurons is also not required for the regulation of energy expenditure or body weight (87). Ablation of NPY/AgRP neurons, alternatively, causes rapid starvation, showing that the neurons, if not neuropeptides themselves, are necessary for weight maintenance and energy homeostasis (88). This may point to the necessary role for GABA neurotransmission. It is still not clear whether and how NPY neurons regulate reproductive circuitry. NPY/AgRP neurons from the ARC make contacts with most GnRH neurons (89–91). In a study using ovariectomized rhesus macaques, intracerebroventricular (ICV) administration of NPY inhibited LH pulses, whereas local administration of NPY in the ME stimulated the release of GnRH (92). In C57BL/6 mice and rats, chronic NPY treatment resulted in diminished LH and hypogonadism, whereas acute NPY administration into the ventricle stimulated LH release in steroid-primed ovariectomized rats (93, 94). In a recent elegant study using optogenetics and chemogenetics, acute stimulation of ARC GABA fibers, ~30% of which express NPY (82), in the proximity to GnRH neurons resulted in increased LH secretion in male and female mice, whereas chronic activation elevated LH pulse frequency, increased estrous cycle length, decreased corpora lutea number, and raised testosterone concentration in females (95). Central infusion of AgRP into the third ventricle resulted in reduction of LH pulse frequency in ovariectomized rhesus monkeys (96). Brain slices from adult female GnRH–green fluorescent protein (GFP) mice used in loose patch recording experiments and treated with AgRP resulted in reduced activity of 10% of GnRH neurons and stimulation of 25% of GnRH neurons (78). Use of NPY receptor agonists also had varying effects, including an increased firing rate of 50% GnRH neurons and a reduced firing of 46% GnRH neurons (78). Specifically, only receptors Y1 and Y5 are expressed on GnRH terminals and cell bodies, respectively (90, 91). However, in one study, a Y5 receptor agonist, hPP, had no effect on GnRH neuron excitability (78), although others have reported that infusions of a different Y5 agonist, $PYY_{3–36}$, in the lateral ventricle of male Sprague-Dawley rats and male C57BL/6J mice results in reduced levels of LH, testosterone, and reduced testicular weight (93, 97). Integration of the GABA signal, which is excitatory for GnRH neurons (98), with NPY neuropeptide in regulation of GnRH neuron excitability has also been reported (99). GABA transmission may have a critical role in conveying energy homeostasis to reproductive function, because knockdown of leptin receptors specifically in GABAergic neurons, but not glutamatergic neurons, delayed puberty onset and decreased fecundity in adults of both sexes (100). These variable effects of NPY and AgRP on GnRH are thought to be a result of the complex interplay of neuropeptides NPY and AgRP, GABA, and the variety of NPY receptors.

Orexin

The hypothalamic neuropeptide orexin plays a role in sleep and wakefulness and, as its name implies, in the stimulation of feeding behavior (101). Orexin neurons are Q:5 located in the lateral hypothalamus and send projections throughout the brain, including the preoptic area, where GnRH cell bodies are located, and to the mediobasal hypothalamus and ME, where GnRH terminals project (102). There are two forms of orexin, orexin A and orexin B, with orexin A being more physiologically potent due to its resistance to degradation (103). Rodent and primate orexin neurons express leptin receptors and NPY receptors Y1 and Y4 (104, 105). Orexin neurons were also shown to make synaptic contact with NPY neurons in the ARC and *vice versa* (106). Similar to NPY, orexin has both stimulating and inhibiting effects on GnRH/LH secretion. GnRH neurons express orexin 1 receptors, and orexin neurons appose to GnRH neurons (107). Both orexin A and orexin B stimulate LH secretion in steroid-primed ovariectomized rats, but they inhibit LH release in steroid-deficient rats (108). Tissue explants from male and female rats in proestrus show that orexin A stimulates GnRH release from the hypothalamus, although in diestrus or low-estrogen states orexin A is inhibitory (107). Similarly, orexin inhibited activity of GnRH neurons from ovariectomized mice (109). Expression of orexin receptor 1 was also observed in GT1-7 cells, an immortalized cell model of GnRH neurons, and orexin treatment resulted in increased GnRH mRNA expression and GnRH release (110). In summary, these studies describe a complex neuronal network linking metabolism and reproductive function, whereby POMC, NPY, and orexin neurons mediate effects of metabolic cues on GnRH neurons. However, none of these studies sufficiently explains the negative effects of obesity.

Metabolic Signals

Insulin

Insulin is the main anabolic hormone that regulates cell metabolism via glucose uptake. Brain-specific knockdown of insulin receptor using nestin-CRE, which is expressed early in brain development and affects most cell types, causes infertility due to low LH levels. Because pituitary responsiveness was intact, the effect on LH levels was

likely due to hypothalamic dysregulation (111). Infusion of insulin into the lateral ventricle increased LH pulsatility in insulin-deficient, diabetic sheep (112) and in diabetic rats (113). Although most GnRH neurons express insulin receptor, there is no evidence that insulin treatment activates them (114). Insulin receptor knockdown in GnRH neurons does not alter pubertal development, litter size, or estrous cyclicity (115). Only a limited number of kisspeptin neurons express insulin receptor and, consequently, kisspeptin neuron-specific knockdown of insulin receptor has a minor delay in vaginal opening, an external sign of puberty in one model (116) but not in the other (114), and no effect on estrous cyclicity or litter size in either model. Insulin may exert its function on pituitary gonadotrope by interacting with GnRH signaling pathway in a gonadotrope cell model (117, 118). Hyperinsulinemia in obesity, however, may affect reproductive function. Knockdown of insulin receptor specifically in pituitary gonadotrope or GnRH neurons partially improves fertility of obese, mixed background, mice fed an HFD (40, 119). Therefore, alterations in the normal insulin levels, or other metabolic signals, in obesity may elicit changes in the HPG axis.

Adipose tissue

Adipose tissue is an endocrine organ that regulates systemic nutrient and energy homeostasis via secretion of adipokines, most notably leptin and adiponectin. In addition to adipocytes, adipose tissue contains tissue macrophages, other immune cells, endothelial cells, preadipocytes, and fibroblasts. Fat is deposited in two main depots, visceral and subcutaneous. Subcutaneous fat accounts for 80% of all body fat. Although visceral fats account for 10% to 20% of total body fat in men and 5% to 8% in women, visceral deposits produce more adipokines than do subcutaneous deposits and their enlargement in obesity is more highly associated with negative outcomes, such as insulin resistance and metabolic syndrome (120, 121).

Leptin

Leptin is a protein product of the obese (*ob*) gene, secreted by adipocytes to function as a satiety factor and regulate food intake by signaling to the brain. Leptin also provides a link between metabolism and reproduction, because both male and female mice that lack leptin (*ob/ob* mice) are infertile, and leptin treatment restores reproduction (122–124). Leptin receptor null mice also experience delayed puberty and infertility in both sexes (125). Deletion of leptin receptors from forebrain neurons prevented the onset of puberty, resulted in infertility in males and females, and blocked estradiol-induced LH surge (126). In ovariectomized, fasted and fed Wistar female rats using the push–pull perfusion technique, leptin induced GnRH, LH, α-MSH, and prolactin secretion in a dose-dependent manner (127). GnRH neurons do not express leptin receptors themselves (126), and leptin may exert its effects on GnRH by acting through kisspeptin neurons (128). ICV leptin treatment of lean hypogonadotropic ewes with reduced levels of *KISS1* gene expression partially restored *KISS1* levels, whereas ICV treatment with kisspeptin peptide reduced POMC and increased NPY gene expression (129). However, deletion of leptin receptor specifically in kisspeptin neurons had no effect on puberty or fertility (130), and reexpression of leptin receptor solely in kisspeptin neurons did not alleviate lack of pubertal development or infertility (131). Therefore, kisspeptin neurons are not the direct target of leptin in the onset of puberty. Leptin signal is likely relayed by NO-synthesizing neurons in the ventral premammillary nucleus (130) and/or the organum vasculosum laminae terminalis (OVLT) (132). During obesity and altered nutritional status, increased levels of leptin are likely communicated via alternations of NPY and POMC neurons (133, 134). Alternatively, although leptin levels are elevated in obesity, the brain in particular exhibits cellular leptin resistance (135), and thus the biological effects of leptin in obesity may be limited.

Adiponectin

Adiponectin is secreted by adipose tissue and acts to increase insulin sensitivity, fatty acid oxidation, energy expenditure, and reduction of liver gluconeogenesis (136, 137). Unlike leptin, adiponectin levels are negatively correlated with BMI, specifically abdominal fat accumulation (138). Serum adiponectin in normal weight women and women with obesity is higher than serum adiponectin in men (139, 140), which may contribute to sex differences in the pathophysiology of obesity. Female adiponectin null mice displayed impaired fertility, reduced retrieval of oocytes, disrupted estrous cycle, elevated number of atretic follicles, and impaired late folliculogenesis. They also have lower estradiol and FSH, but elevated LH and testosterone at proestrus (141). Twenty percent of the total GnRH population responded to adiponectin, exhibiting hyperpolarization or decreased calcium oscillations (142, 143). Adiponectin also suppressed GnRH secretion in the immortalized GnRH neuron cell line, GT1-7 (144). In the female mouse, adiponectin decreased GnRH neuron activity (142), whereas in male rats, adiponectin inhibited testosterone secretion (145). Adiponectin treatment of pituitary model cell lines inhibited LH release (146). However, it is not known how a change in adiponectin due to increased obesity influences the HPG axis.

Obesity as a Chronic Inflammatory State

Adipose tissue macrophages

Chronic systemic inflammation is a consequence of increased adiposity and exposure to an HFD (147, 148). Obese adipose tissue is characterized by progressive infiltration by macrophages causing inflammation (149–151). Although other immune cells may also be present in the adipose tissue, macrophages are functionally the most significant (150). The number of macrophages in white adipose tissue correlates with adiposity in both humans and mice (149). Furthermore, functional activity is proportional to the degree of obesity, as macrophages become activated with increased adiposity and upregulate the cytokine production (149, 152). Visceral deposits contain a higher density of macrophages than do subcutaneous deposits (56, 153). Following increase in their size, adipocytes produce monocyte chemoattractant protein-1 (MCP-1, or CCL2 chemokine), a ligand for CCR2, which recruits monocytes and leads to macrophage activation (154, 155). Increased secretion of leptin may also contribute to macrophage accumulation by stimulating transport of macrophages to adipose tissue and promoting adhesion of macrophages to endothelial cells (152). In lean individuals, macrophages resemble an M2 phenotype and secrete anti-inflammatory cytokines (156). Development of obesity causes a change in adipose tissue macrophage phenotype and elevated secretion of proinflammatory cytokines, TNF-α, IL-6, and IL-1β, which increases their concentration in the circulation (152). Macrophage recruitment to the liver via CCR2 in obese mice contributes to insulin resistance (157), indicating that macrophages activated by increased adiposity infiltrate parenchyma of other tissues. Although the brain is considered an immune-privileged site, recruitment of peripheral immune cells to the brain in obesity has been observed as well and may contribute to the local inflammatory response (158, 159). Our study also demonstrated that macrophages are recruited to the hypothalamus of obese male mice (32). Others failed to detect infiltration of peripheral immune cells (160). The difference may be due to the approach, because a limited number of macrophages that infiltrate the hypothalamus may be insufficient for microscopic detection, whereas the flow cytometry that we used may be more sensitive. Macrophages, which we observed in the hypothalami of male mice fed an HFD, but not in females, express CCR2 and are recruited to specific areas of the hypothalamus (32). That males deposit more fat in visceral depots (32, 56, 61), which recruit and activate more macrophages than do subcutaneous depots, combined with findings that activated macrophages infiltrate other tissues, including the hypothalamus, may explain some of the sex differences observed in obesity and why males have a higher propensity for obesity-mediated neuroinflammation.

Brain-resident immune cells

Systemic inflammation following exposure to an HFD is accompanied with hypothalamic neuroinflammation (147). Increased inflammation in the central nervous system (CNS) of individuals with obesity may contribute to neuropathologies, such as cerebral ischemia and dementia (161, 162). Microglia are resident immune cells that survey the parenchyma and maintain normal circuit function and plasticity by pruning synapses during development and neurogenesis (163). Neurons communicate with microglia via expression of fractalkine, which binds fractalkine receptor, CX3CR1, expressed specifically by microglia (163). In neuroinflammation, in response to injury, infection, or disease, microglia engulf damaged synapses and cause activity-dependent structural remodeling (164). Microglia also secrete cytokines, which exert numerous functions, including recruitment of peripheral immune cells (165). In obesity, hypothalamic microglia change morphology and become activated (166, 167). Depletion of microglia or prevention of their activation via targeted gene knockdown partially reduces body weight, food intake, and macrophage infiltration (159). Initial reports identified morphological changes specifically in the ARC (159, 168) and postulated that activation of microglia occurs in response to the stress and injury of POMC or NPY neurons in the feeding circuit. Given that both fractalkine and CX3CR1 expression is altered in obesity (169, 170), this is one possibility. Our study detected morphological changes in microglia and an increase in their numbers around OVLT and ME, in addition to the changes previously observed in the ARC (32). In agreement with previous studies, microglia in the cortex did not show any change. We therefore posit that the activation of microglia in these specific areas occurs due to their proximity to the leaky blood–brain barrier (BBB; see below). Microglia in obesity may be activated by an increase in saturated fatty acids in the circulation (171), because an HFD rather than obesity *per se* leads to inflammatory changes (172). Sex differences in microglia activation in response to obesity are dependent on CX3CR1, but independent of ovarian estrogen, indicating that sex differences may arise due to differences in the innate immune cells or in neuron–glia communication (169). Our studies similarly detected morphological changes specifically in the male mice. As stated above, we postulate that this is due to higher inflammation in males due to larger visceral fat accumulation.

BBB and GnRH neurons

The CNS has long been regarded as an immune-privileged site due to the BBB, composed of the endothelial cells that express tight junction proteins, interspersed pericytes embedded in the basement membrane, and perivascular space bordered by astrocytic endfeet of the glia limitans (Fig. 2). Properties of the BBB have been recently discussed (173–175). Some of the CNS pathologies associated with obesity may occur due to alteration in BBB, as several tight junction proteins are downregulated (176–178). Alternatively, the hypothalamus is bordered by several areas that contain fenestrated capillaries and a leaky BBB (179). These areas, but not other brain regions, were stained by IV injected dyes. Anteriorly, the preoptic area surrounds the OVLT, and basally, the ARC is juxtaposed to the ME where secretion of pituitary-regulating neuropeptides occurs from neuronal terminals. Both the OVLT and ME contain fenestrated capillaries (180, 181). For example, the OVLT and surrounding thermoregulatory neurons are involved in changes in body temperature and febrile response to systemic inflammation. Pyrogenic, proinflammatory cytokines were previously thought to infiltrate the hypothalamus from circulation via fenestrated capillaries in the OVLT and stimulate thermoregulatory neurons. However, for substances to reach the brain parenchyma, permeability of the OVLT is limited to low molecular mass, whereas tracers >10 kDa are retained in the perivascular space (182). Retention is likely accomplished by the secondary barrier provided by astrocytes or tanycytes (183). Recent studies indeed demonstrate that cytokines, which are ~20 kDa, bind receptors on endothelial cells in the OVLT, which synthesize prostaglandins and in turn increase local production of cytokines in the hypothalamus that affect thermoregulatory neurons (184–187). Because we identified microglia activation around the OVLT following an HFD, we envision that, similarly, systemic inflammation is conveyed via endothelial or glial cells to activate microglia in the vicinity to fenestrated capillaries. This hypothalamic area was recently implicated in energy homeostasis and food intake as well (188). This may not be surprising, because systemic inflammation, in addition to fever, causes sickness behavior, which reduces energy expenditure and limits food intake (189, 190). Our understanding of interactions between the OVLT area and ARC in regulation of food intake, however, is only beginning to emerge.

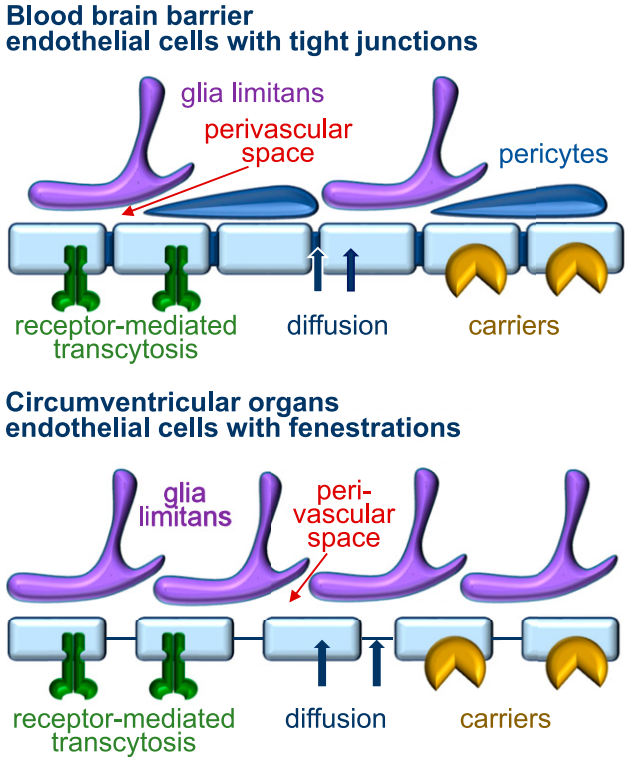

Figure 2. BBB and circumventricular organs. Most of the brain vasculature contains endothelial cells with tight junctions that establish the BBB. Endothelial cells are surrounded by basement membrane in which pericytes, vascular smooth muscle cells, are embedded, and by astrocytic endfeet or glia limitans, which form perivascular space and further regulate access to the brain parenchyma. Depending on the molecule mass and charge, molecules can access parenchyma by diffusion, via carrier transport or specific receptors. Several areas in the brain, called circumventricular organs, contain fenestrated capillaries and a leaky BBB. Although access of small molecules to brain parenchyma in these areas was demonstrated using dye or fluorescent labels, recent studies suggest that access is also regulated and primarily occurs via the above-mentioned mechanisms. Access into the brain parenchyma is controlled by the astrocyte endfeet or tanycytes that express tight junction proteins. Hypothalamus is bordered by several circumventricular organs: the OVLT in the rostral part, and the ME and posterior pituitary at the ventral side.

The ME is a target for hypothalamic neuron terminals that secrete hypophyseal-releasing hormones into the portal vasculature via fenestrations. Permeability of the ME is greater than that of OVLT (191, 192), and tanycytes play a major role providing a barrier and regulating transport to the parenchyma and third ventricle (181, 183, 193–196). However, at times, tanycyte endfeet retract and allow GnRH terminals to contact the perivascular space (197). Our studies demonstrate macrophage infiltration specifically in the areas surrounding the OVLT and ME following an HFD (32). Although surprising, because immune cell infiltration into the CNS occurs only after injury or infection, macrophage infiltration in obesity was demonstrated previously (159). It may be facilitated by the increased permeability of the BBB or elicited by active recruitment from activated microglia. Immune cell infiltration may impair neuronal function in these areas, because macrophages phagocytose damaged cells and engulf synapses (198, 199). We hypothesize that the impairment is more

highly associated with the location of the impacted neurons rather than their function. POMC and NPY neurons are located in the ARC, which is close to the fenestrated capillaries of the ME. A population of kisspeptin neurons is also located in the ARC (200, 201) and may be exposed to the metabolic changes as well. GnRH neurons are uniquely positioned to sense these changes in the circulation. A large number of GnRH neuron cells bodies are located around the OVLT, and a portion of these send processes beyond the BBB, where they may be able to directly respond to circulating molecules, or to cytokines and prostaglandins secreted by endothelial or glial cells (202). GnRH neurons have long projections that terminate in the ME and directly contact perivascular space during times of high secretory activity when tanycyte endfeet retract (197). Therefore, GnRH neurons on both ends can be exposed to metabolic changes during obesity. We recently demonstrated that obese male mice have increased cFOS expression specifically in GnRH neurons and in cells located close to the OVLT, but not in cells located farther dorsally [Fig. 3 (203)]. Others reported increased cFOS following HFD in specific areas and postulated that the location is specific for the function of the neuronal population (204, 205). cFOS is similarly induced following lipopolysaccharide (LPS) treatment in the hypothalamus (206), and this induction is location specific (207). We postulate that an increase in cFOS gene expression is dependent on the proximity to the vasculature. We envision several possible mechanisms that elicit this induction in obesity. An increased level of circulatory cytokines from adipose tissue activates gene expression in neurons and glia specifically in the regions proximal to fenestrated capillaries. Microglia, in response, become activated and increase their own cytokine production that causes synapse stripping and recruitment of peripheral or perivascular macrophages. Another possibility is that macrophages, activated by the increased adiposity, infiltrate the hypothalamus specifically through the circumventricular organs and increase local production of cytokines that activate microglia and alter gene expression. Alternatively, metabolic changes, including increased insulin, leptin, glucose, or free fatty acids, can be sensed by the endothelial cells, astrocytes, or microglia (196, 208), also specifically around the fenestrated capillaries of the circumventricular organs, which in turn activate stress signaling and increased cytokine production in any of these cell types. As we demonstrated, increased cytokines activate cFOS and other genes that affect neuronal responses.

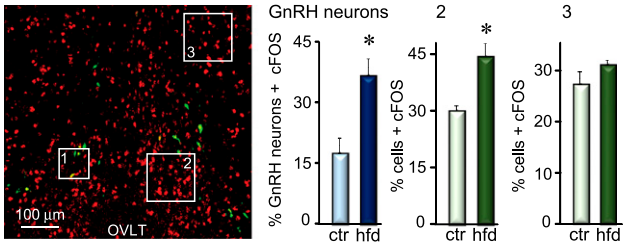

Figure 3. More cells proximal to the OVLT express cFOS following a high-fat diet (hfd). Coronal sections of the preoptic area in the hypothalamus of GnRH-GFP mice following hfd, stained for GFP (green) and cFOS (red). Numbered squares correspond to quantified areas (presented by bar graphs). (1) GnRH neurons (green) that express cFOS (red) were counted following control and an hfd. An increase in the percentage of GnRH neurons with cFOS is observed in obese mice compared with control (ctr); (2 and 3) quantification of the cells, identified by DAPI stain (not shown) that express cFOS, proximal to the OVLT delineated with the no. 2 square, and distal, dorsally from the OVLT delineated with the no. 3 square, following control and an hfd. $*P < 0.05$, reported in Lainez and Coss (203).

Cytokines

We and others reported that obesity causes increased levels of proinflammatory cytokines TNF-α, IL-1β, and IL-6 in the circulation (32, 152) and locally in the hypothalamus (32, 209). These classical, proinflammatory cytokines are involved in both the normal physiology of the nervous system and in inflammatory processes during infection (175). In the CNS, TNF-α and IL-1β regulate synaptic plasticity, neurodegeneration, learning and memory, sleep, food and water intake, and astrocyte-mediated synaptic strength (210–214). During infections, TNF-α and IL-1β mediate the physiological changes, resulting in "sickness behavior" such as fever, reduced food intake, nausea, and fatigue (215–217). As discussed above, in the proximity of the OVLT, these cytokines cause alterations of neuron function and increase thermogenesis, causing fever (184, 218). In several brain diseases, microglia upregulate TNF-α expression, contributing to excitotoxicity by inhibiting glutamate uptake by astrocytes and by increasing localization of ionotropic glutamate receptors to synapses (215). Elevated TNF-α in obesity increases POMC neuron activity, mitochondrial respiration, and ATP production (219). Cytokines may directly affect gene expression in GnRH neurons or neuron function, because GnRH neurons express various cytokine receptors (220). Previous studies have implicated acute inflammation, elicited with an injection of LPS or cytokines themselves, in the impairment of reproductive function and reduced LH (221–228). In particular, repression of GnRH mRNA expression is specifically observed. LPS treatment, resulting in neuroinflammation, represses *Gnrh* mRNA in ewes (229), birds (230) and rats (231). Infusion of the proinflammatory IL-1β cytokine into the rodent hypothalamus also represses *Gnrh* expression (221). More recently, our group determined that low-grade, chronic inflammation

caused by obesity affects GnRH neurons, resulting in reduced levels of LH in circulation and repression of GnRH mRNA in the hypothalamus, specifically in male mice (32). Lower *Gnrh* mRNA expression is consistently observed in obese mice (45, 232). Interestingly, GnRH mRNA is one of the most repressed genes in obesity, detected by the genome-wide analysis of the whole brain (233).

IL-6 plays a role in the regulation of immune reactions and, in the brain, in naive states, IL-6 contributes to normal neuronal function and neurogenesis (234, 235). Of interest, IL-6 is involved in establishment and maintenance of the BBB by increasing tight junction formation (176). In models of brain injury, infection, LPS injection, or diet-induced obesity, IL-6 is upregulated and modulates inflammation, apoptosis, and oxidative stress. Alternatively, IL-6 is also involved in dampening of the immune responses to promote recovery and healing (236, 237), and it is induced by TNF-α and IL-1β as a secondary cytokine (238). Although LPS induces IL-6 in both astrocytes and microglia, TNF-α and IL-1β induce IL-6 production from astrocytes and not microglia (239). Mice that overexpress IL-6 in the CNS exhibit more rapid healing and recovery after traumatic brain injury (240). IL-6 null mice showed increased oxidative stress and impaired repair (241), suggesting a neuroprotective role of IL-6 in brain injury. However, it is still not clear whether IL-6 directly affects neurons involved in reproductive function.

We demonstrated that leukemia inhibitory factor (LIF) represses *Gnrh* gene directly and that LIF levels are increased in the hypothalamus following an HFD (32, 203). LIF is a member of IL-6 family that is induced during the inflammatory response (242). However, its functions are not limited to inflammation: LIF has been demonstrated to play a crucial, nonredundant role in embryo implantation in both mice and humans (243–245). LIF also maintains stem cells and regulates differentiation of germ cells (246, 247). In the brain, LIF regulates neuronal function and neuronal response to injury (248–250). With respect to GnRH neurons, LIF regulates the migration of GN11 cells, a model of immature GnRH neurons, and the release of GnRH in GT1-7 cells, a model of mature GnRH neurons (251–253). LIF binds its specific receptor, which, similarly to the other members of the IL-6 family, recruits and signals through the gp130 signals transducer, activating the JAK-STAT and MAPK pathways (244). Our recent report that showed increased levels of LIF mRNA in the hypothalamus of HFD mice demonstrated this increase only in males that exhibit a reduction in GnRH mRNA and gonadotropin hormones, but not in females that lack changes in GnRH or gonadotropin hormones (32). On the contrary, a family member IL-6 was increased in both sexes. In the following study, we demonstrated that LIF represses GnRH gene via activation of p38 and induction of cFOS in GnRH neurons (203).

Synaptic remodeling in neuroinflammation

Our recent studies postulated that obesity-mediated impairment of reproductive function stems from neuroinflammatory effects on GnRH neurons. We determined that two mechanisms may be at play: (i) reduction in spine density and consequently the connectivity of the GnRH network (Fig. 4) (32), and (ii) from direct cytokine effects on GnRH gene expression, as described above (203). Gene regulation by cytokines may affect expression of synaptic molecules as well as neuropeptides. For example, cytokines directly affect the levels and function of glutamate receptors (236, 254, 255). Alternatively, neuroinflammation may lead to

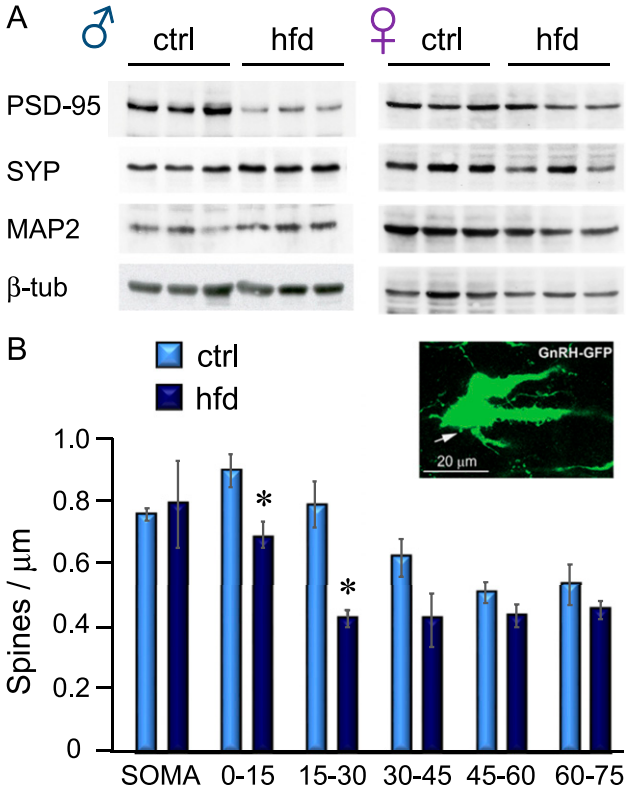

Figure 4. Decreased levels of synaptic proteins in the hypothalami and spines in GnRH neurons in obese male mice. (A) Western blot of hypothalamic lysates indicates lower levels of postsynaptic density protein 95 (PSD-95), but not of presynaptic protein synaptophysin (SYP), or neuronal marker MAP2, in obese males, but not in females. β-Tubulin (β-tub) was used as housekeeping control. (B) Coronal sections of the hypothalamic preoptic area of the GnRH-GFP mice following control and hfd were stained for GFP (green) to allow for spine count. Spines, identified as protrusions by an arrow in the insert, were counted in the soma and along the main axon in 15-µm intervals, which are indicated below the bars, in reference to the distance from the soma. Reported in Lainez *et al.* (32).

synaptic remodeling via synapse engulfment by resident or infiltrating immune cells. This leads to changes in network connectivity. Immune cells strip synapses following deviations in neuronal signals, such as fractalkine, as mentioned above. We demonstrated that an HFD, followed by an increase in cytokines, causes reduced levels of synaptic protein, PSD-95, in the hypothalamus and fewer spines on the GnRH proximal dendron itself in male mice. Fewer spines, sites of excitatory synaptic input, combined with reduced levels of synaptic proteins, indicate potential changes in neuronal activity. The reduction of synaptic connections in this particularly plastic area of the GnRH neuron (256–258) may affect regulation of GnRH secretion by an upstream regulatory network. Previous studies determined that both NPY and POMC neurons also exhibit a decrease in synapses following an HFD (259). Specifically, an HFD caused elimination of inhibitory synapses on POMC neurons and excitatory synapses on NPY neurons. Synaptic stripping, reduced levels of synaptic proteins, and fewer spines in obese male C57BL/6J mice were observed in hippocampal neurons as well (260). A decreased performance of obese mice in cognitive tasks was attributed to the loss of synapses, fewer dendritic spines, and a decrease in synaptic proteins in the prefrontal cortex (261). Given that POMC and NPY neurons are located in the ARC, and that GnRH neurons are located close to the OVLT, the synapses may be eliminated by infiltrating macrophages or activated microglia that occur in these areas (Fig. 5). As stated above, both microglia, brain-resident immune cells and peripheral, monocyte-derived macrophages are involved in synapse stripping.

Conclusion

In this review, we describe neuroendocrine and neuroinflammatory changes underlying sex-specific differences in obesity-induced impairment of the hypothalamic function with potential consequences for reproduction and fertility (Fig. 5). Ovarian estrogen is protective from diet-induced obesity; however, we recently demonstrated that it is not necessary for protection from endocrine and immune changes (32). Females may be protected by a variety of sex differences in the immune system, adipose tissue accumulation, and metabolic rates (61, 65, 66), all of which may be influenced by paracrine, locally produced cytokines, chemokines, adipokines, or hormones, including estrogen. Owing to the complexity in neuronal circuitry that regulates feeding and metabolism, analyses of a single neuropeptide or neuronal population effects on the GnRH neuron or its afferents failed to provide satisfactory explanation for obesity-mediated impairment of reproductive function. Our recent studies focused on neuroinflammatory changes and on the role of glial cells, which may affect neuronal function via synaptic changes (164). Previous studies that demonstrated synaptic changes in feeding circuitry in the ARC of the hypothalamus postulated that synapse elimination and microglia activation stem from the neuronal stress elicited by an altered metabolic state (259). We observed neuroinflammation, microglia activation, and peripheral macrophage infiltration, which may be caused either by metabolic changes in the circulation, or by activated macrophages from the visceral adipose tissue, specifically in the vicinity to the fenestrated capillaries. Given this specificity, we postulate that location is one of the determinants of synaptic changes in neuronal populations located close to circumventricular organs. Given that GnRH neurons are uniquely located in the proximity to fenestrated capillaries in both OVLT and ME, they are primed to respond to the neuroimmune alternations.

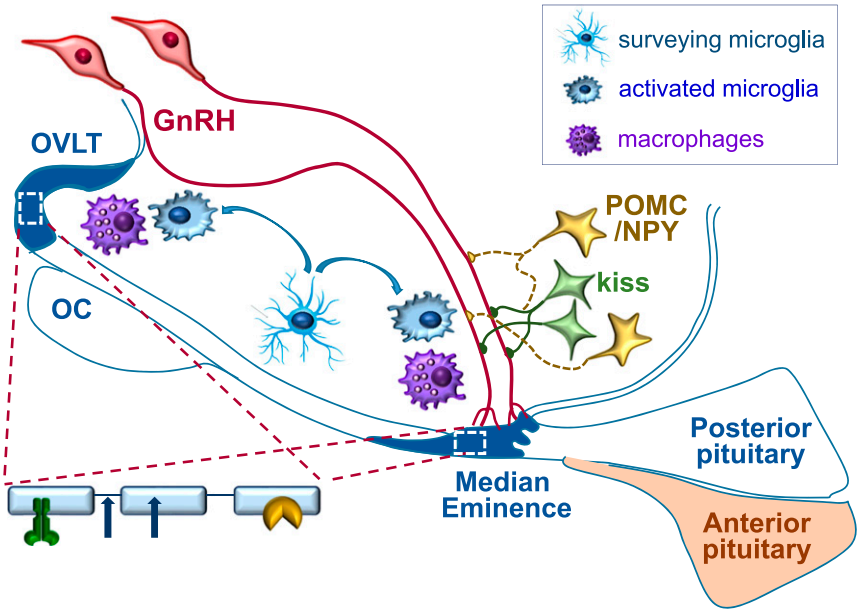

Figure 5. Conclusion. GnRH neurons (red) are uniquely located, proximal to the OVLT at the soma and to the ME at terminals, which are both circumventricular areas with fenestrated capillaries, depicted with dashed lines. Resident immune cells, microglia, are activated following an HFD specifically in the proximity to the OVLT and ME. Change in morphology from resting to activated state is indicated with blue arrows. Peripheral macrophages are recruited to the brain parenchyma, as well. These neuroinflammatory changes, together with changes in the POMC or NPY neurons, which regulate food intake and energy expenditure, or in kisspeptin neurons, which are upstream neurons that regulate GnRH neuron secretion, contribute to alternations in GnRH neuron function and diminished reproductive capacity in obesity.

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Data Availability: All data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

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