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Regional Astrogliosis in the Mouse Hypothalamus in Response to Obesity

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Abstract

Obesity is associated with chronic low-grade inflammation in peripheral tissues, which contributes to the development of comorbidities such as insulin resistance and cardiovascular disease. While less extensively characterized, obesity also promotes inflammation in the central nervous system (CNS) and the consequences of this inflammation for CNS function are only beginning to be examined. In response to CNS insults such as inflammation, astrocytes undergo a process of hypertrophy and hyperplasia known as reactive astrogliosis. We used immunohistochemistry to examine the differential distribution of the astrocyte marker glial-fibrillary acidic protein (GFAP) in the brains of diet-induced or genetically obese mice compared with their respective lean controls to determine whether different nuclei of the hypothalamus showed comparable astrogliosis in response to obesity. The areas that showed the highest differential GFAP immunoreactivity between lean and obese animals include the medial preoptic, paraventricular, and dorsomedial nuclei. Comparatively, little astrogliosis was seen in the ventromedial nucleus, lateral hypothalamus, or anterior hypothalamic area. In obese animals high levels of GFAP immunoreactivity were often associated with the microvasculature. There were no differences in the differential distribution of GFAP staining between obese animals and their lean controls in the diet-induced compared with the genetic model of obesity. The exact cause(s) of the astrogliosis in obesity is not known. The finding that obesity causes a distinct pattern of elevated GFAP immunoreactivity associated with microvessels suggests that the astrogliosis may be occurring as a response to changes at the blood–brain barrier and/or in the peripheral circulation.

INDEXING TERMS

astrocyte; gliosis; neuroinflammation; high-fat diet; brain; melanocortin 4-receptor

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CONFLICT OF INTEREST

The authors have no competing interests.

Additional Supporting Information may be found in the online version of this article.

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: K.L.J.E. Acquisition of data: L.B.B., M.M.T., H.N.M., and K.L.J.E. Analysis and interpretation of data: L.B.B., M.M.T., and K.L.J.E. Drafting of the article: K.L.J.E. Critical revision of the article for important intellectual content: L.B.B., M.M.T. Statistical analysis: K.L.J.E. Obtained funding: K.L.J.E. Study supervision: K.L.J.E.

Astrocytes play a key role in maintaining the parenchymal environment in the brain and are also a critical component of the neurovascular unit of the blood–brain barrier (BBB) that regulates the influx and efflux of substances from the brain (Abbott et al., 2006). The astrocytic endfeet are in close opposition to endothelial cells of the cerebral vasculature and in vitro astrocytes have been shown to regulate the physical tightness of the BBB as well as the expression of key transporters and enzymes (Sobue et al., 1999; McAllister et al., 2001; Ramsauer et al., 2002; Haseloff et al., 2005). Astrocytes are also known to functionally regulate synaptic plasticity via their interactions with neurons (Theodosis et al., 2006; Prevot et al., 2007; Panatier, 2009; Horvath et al., 2010). The hypertrophy and hyperplasia of astrocytes in response to acute or chronic insults in the brain is known as reactive astrogliosis. Astrogliosis can range from mild changes in astrocyte form and function to severe alterations characterized by the formation of a glial scar, which serves as a barrier between healthy and damaged tissues (Sofroniew, 2009). Astrogliosis is a feature of numerous neuropathologies including stroke, Alzheimer's disease, spinal cord, and traumatic brain injury.

Obesity is the leading public health concern in the U.S. and in many nations of the world. In addition to the more well-accepted comorbidities such as type 2 diabetes and cardiovascular disease, mounting evidence from clinical studies suggests that obesity is also associated with increased vulnerability of the central nervous system (CNS) (Bruce-Keller et al., 2009); for example, obese individuals have a 6% higher incidence of stroke for every unit increase in body mass index greater than 30 (Kurth et al., 2002). Furthermore, obese individuals have an 11% increase in mortality following traumatic brain injury (Brown et al., 2006) and a staggering 74% increased risk of dementia (Whitmer et al., 2005). In peripheral tissues, obesity-associated chronic low-grade inflammation (for review, see Gregor and Hotamisligil, 2011) contributes to the development of insulin resistance and atherosclerosis (Shoelson et al., 2007) but the potential contribution of obesity-associated neuroinflammation to CNS pathologies is only beginning to be recognized (Drake et al., 2011).

In the rodent hypothalamus, diet-induced obesity results in increased expression of proinflammatory cytokines (tumor necrosis factor α [TNF α] and interleukin-1 β [IL-1 β]) (De Souza et al., 2005; Thaler et al., 2012) and activation of the I κ B/NF κ B system (Zhang et al., 2008), which is considered one of the key transcriptional pathways mediating inflammation. Evidence of gliosis (the hypertrophy and hyperplasia of glial cells) in response to high-fat feeding in rodents (Hsueh et al., 2009; Pistell et al., 2010; Drake et al., 2011; Nerurkar et al., 2011; Thaler et al., 2012) is also suggestive of the presence of obesity-associated CNS inflammation. Whether this obesity-associated neuroinflammation arises de novo or in response to the well-characterized peripheral inflammation is not clear. While astrogliosis in response to obesity has been reported in rodents, little information is available about the distribution of astrogliosis in the CNS in response to obesity. The expression of glial-fibrillary acid protein (GFAP), an intermediate filament protein, is a commonly used as a marker of astrocytes. When examining obesity-associated astrogliosis previous studies have either used western blotting for GFAP in homogenates of whole brain (Nerurkar et al., 2011) or hippocampus (Pistell et al., 2010), or have examined GFAP immunoreactivity by immunohistochemistry exclusively in the arcuate nucleus of the hypothalamus (ARC)

(Hsueh et al., 2009; Horvath et al., 2010; Thaler et al., 2012). Due to the distinct roles of different hypothalamic nuclei in the regulation of energy homeostasis and other neuroendocrine processes, we sought to examine the pattern of astrogliosis across the region. In this study we compared the distribution of immunoreactivity for GFAP throughout the hypothalamus in two mouse models of obesity: diet-induced (DIO) and melanocortin-4 receptor (MC4R) deficiency (Huszar et al., 1997).

MATERIALS AND METHODS

Animals

All experiments were conducted in accordance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* and approved by the Animal Care and Use Committee of Vanderbilt University. The animals used in experiments were female C57BL/6J (Stock no. 000664; Jackson Laboratory, Bar Harbor, ME), MC4R-deficient (Huszar et al., 1997), or Tie2-GFP mice (Motoike et al., 2000) (Stock no. 003658, FVB/N background; Jackson Laboratory). MC4R-deficient mice (MC4R^{-/-}; >10 generations on the C57BL/6J background) were bred at Vanderbilt University Medical Center from heterozygous parents, and wildtype littermates were used as controls. Animals were housed at 21 ± 2°C with ad libitum access to standard laboratory chow (13% kcal from fat; Picolab rodent diet 20, PMI Nutrition International, St. Louis, MO) and water, unless mentioned otherwise.

For the DIO studies, at 12–17 weeks of age animals ($n = 3–5$ per diet) were placed on high-fat chow (60% kcal from fat; Cat. no. D12532, Research Diets, New Brunswick, NJ) or maintained on standard laboratory chow and body weights monitored weekly. After 20 weeks of high-fat feeding mice were deeply anesthetized and underwent tissue fixation via transcardial perfusion with 0.9% saline followed by ice-cold fixative (4% paraformaldehyde in 0.01 M phosphate-buffered saline pH 7.4 [PBS]). Brains were postfixed for 2 hours in fixative and were then stored overnight in 30% sucrose in PBS as a cryoprotectant before being frozen at –80°C until use. For the MC4R^{-/-} versus MC4R^{+/+} studies, mice ($n = 5$ /genotype) were maintained on standard laboratory chow and were 24–28 weeks at the time of tissue collection after transcardial perfusion, as described for the DIO animals. The body weights of the animals were measured at the time of euthanasia and are expressed as mean ± standard error. The body weight data were analyzed by an unpaired t -test (Prism 5.04, GraphPad, San Diego, CA) and significance taken as $P < 0.05$.

Immunohistochemistry

Sections were cut at 25 µm from perfused brains on a freezing-sledge microtome (Leica Microsystems, Deer-field, IL) and stored at 4°C, free-floating in PBS containing 0.03% sodium azide as a bactericide. Four sets of sections were generated from each brain; thus, each section in a set was ~100 µm apart. After an initial blocking step, 1 hour at room temperature in 5% normal donkey serum (Pel-Freez, Rogers, AR) in PBS containing 0.3% Triton X-100 (PBST), sections were incubated with primary antibody for 24 hours at 4°C. All primary antibodies (Table 1) were diluted in 5% normal donkey serum in PBST (1:7,500 GFAP and 1:5,000 GFP). After incubation in primary antibody, sections were washed

thoroughly in PBS and incubated for 1 hour at room temperature with the appropriate secondary antibody (Table 2), diluted 1:500 in PBST. After the first primary antibody the procedure was repeated with another primary/secondary combination for double-labeling, where appropriate. For the single-labeling studies GFAP immunoreactivity was detected using ImmPactDAB (Vector Laboratories, Burlingame, CA) according to the manufacturer's instructions. Sections were washed thoroughly with PBS between all incubations. Each secondary antibody was tested in the absence of primary antibody to ensure that there was no cross-reactivity with the tissue. At the end of the incubations the sections were mounted onto gelatin-coated slides, coverslipped using gel-based fluorescence mounting media containing DAPI (Prolong gold; Invitrogen, La Jolla, CA) for fluorescently labeled tissues, or Permount (Fisher Scientific, Pittsburgh, PA) for DAB-stained sections. Sections were viewed using brightfield or fluorescence microscopy as appropriate (AxioImager Z1; Zeiss, Thornwood, NY).

Relative density of GFAP staining was qualitatively assessed and rated independently by two different investigators blinded to the diet/genotype of the animals and scored as follows: +/-, sparse staining; + light staining; ++ moderate staining; +++ extensive staining; ++++ very extensive staining. The scores presented represent the average rating of the two investigators across each anatomic area. A minimum of two sections per animal per brain region were included for the smaller more discrete nuclei. For the larger more extensive nuclei a minimum of five sections per animal were examined for each brain region. To ensure that both investigators were scoring the same anatomic region in each section they both viewed the section consecutively and agreed on the anatomic boundaries before independently scoring the staining. The investigators did not discuss or reveal their scores to each other during this process. The scores of the two investigators were identical 66% of the time; however, when the rating was different it was only by a half-point in either direction. When the average rating resulted in a fraction the score was rounded up to the nearest half-point. Guidance on nomenclature and anatomic boundaries was taken from Paxinos and Franklin (2001).

Brightness and contrast were adjusted in the digital images to improve quality but this manipulation was performed equally across all groups.

Antibody characterization

Western blotting—By western blotting the GFAP antibody detected a single band at ~51 kDa in brain homogenate, corresponding to the predicted size of the GFAP protein (Fig. 1A). A sample of mouse brain was homogenized in RIPA buffer (Sigma-Aldrich, St. Louis, MO) containing protease inhibitors (Complete Protease Inhibitor Cocktail Tablets; Roche Applied Science, Indianapolis, IN) and gently inverted at 4°C for 30 minutes. The homogenate was centrifuged at 13,000g for 10 minutes at 4°C and the protein content of the supernatant assessed by the Bradford method according to the manufacturer's instructions (Bio-Rad, Hercules, CA). Proteins were resolved by loading 10 µg brain homogenate alongside a protein standard (Kaleidoscope ladder; Bio-Rad) on a 4–15% stacking sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (mini-protean TGX gel; Bio-Rad) and electrotransferred using a semidry transfer system (Bio-Rad) onto a

nitrocellulose membrane (Perkin-Elmer, Boston, MA). The membrane was blocked with blocking buffer (Li-Cor Biosciences, Lincoln, NE) and then incubated overnight at 4°C with 1:1,000 anti-GFAP antibody diluted in blocking buffer containing 0.1% Tween 20. The membrane was washed with PBS-Tween and then incubated with 1:20,000 antimouse IRDye 800 (Li-Cor Biosciences) for 1 hour at room temperature. After washing with PBS-Tween the immunoreactivity was detected using the Odyssey scanner (Li-Cor Biosciences).

Other antibody controls—The GFAP immunoreactivity seen in brain slices corresponded to the known morphology of astrocytes (Fig. 1B) (Sofroniew, 2009). Some GFAP immunoreactivity was also detected in ependymal cells around the third ventricle. When the secondary antibody was incubated with the tissue in the absence of the GFAP primary antibody no cellular staining was detected, indicating that the immunoreactivity seen was not due to nonspecific binding of the secondary antibody to the tissue (Fig. 1C). A small amount of noncellular staining was seen at the median eminence, which was likely due the detection of mouse IgG that enters the brain at this circumventricular site.

RESULTS

Distribution of GFAP immunoreactivity in the hypothalamus of lean and diet-induced obese mice

After 20 weeks of high-fat feeding the mean body weight of the DIO C57BL6/J mice was 42.1 ± 1.8 g, which was significantly greater than the standard chow-fed lean group, 20.8 ± 0.8 g ($P < 0.001$, two-tailed unpaired *t*-test). In the lean animals GFAP immunoreactivity was seen at comparatively low levels throughout the hypothalamus, with the highest level seen around the ARC (Figs. 2, 3). In obese animals, increased GFAP immunoreactivity was also seen throughout the rostral-caudal extent of the hypothalamus; however, the astrogliosis was not uniform across all nuclei (Table 3). The most pronounced increases in GFAP immunoreactivity were in nuclei proximal to the third ventricle (3V). In particular, high levels of expression were seen in the medial preoptic area (MPom, vMPO; Fig. 2A,B), paraventricular (Pa; Figs. 2C–F, 4C,D), and dorsomedial nuclei (DMN; Figs. 3A–F, 4E,F) of the hypothalamus. High levels of GFAP immunoreactivity were also seen in the ARC (Figs. 3E,F, 4G,H); however, in the medial portion of the ARC adjacent to the median eminence (ME; Fig. 3E,F) the difference between the lean and DIO animals was less pronounced than in the rostral portion of the nucleus (Fig. 3A,B). The areas with the highest level of GFAP immunoreactivity often fell within the distinct anatomic boundaries of hypothalamic nuclei. For example, while high levels of GFAP immunoreactivity were seen in the DMN and ARC, the obesity-associated increase in expression in the ventromedial hypothalamic nucleus (VMH) was more modest (Fig. 3A–F). The anterior (AH; Fig. 2C–F) and lateral hypothalamic (LH; Figs. 2E,F, 3C,D) areas also showed a less pronounced difference in GFAP immunoreactivity between the lean and DIO groups.

Distribution of GFAP immunoreactivity in the hypothalamus of MC4R^{+/+} and obese MC4R^{-/-} mice

In an independent experiment we examined GFAP immunoreactivity in a genetic model of obesity, the MC4R^{-/-} mouse. The MC4R^{-/-} mice showed a significant increase in body

weight (50.1 ± 1.6 g) compared to their wildtype littermates ($MC4R^{+/+}$; 21.8 ± 0.5 g, $P < 0.001$ by two-tailed unpaired *t*-test). In common with the DIO animals, the $MC4R^{-/-}$ mice showed increased GFAP immunoreactivity throughout the rostral-caudal extent of the hypothalamus, reflecting a similar distribution pattern characterized by high levels of GFAP immunoreactivity in the MPO nuclei, Pa, and DMN (Table 4). In contrast to the DIO study, there was a more pronounced difference in GFAP immunoreactivity in the ARC between the lean $MC4R^{+/+}$ and obese $MC4R^{-/-}$ animals (Fig. 5).

GFAP immunoreactivity associated with microvessels

In areas of high GFAP immunoreactivity the cell bodies of the astrocytes were swollen and their processes in close proximity, in some cases overlapping, indicative of moderate to severe astrogliosis (Sofroniew, 2009). Furthermore, GFAP immunoreactivity was also seen forming distinct staining patterns that resembled the outline of microvessels (Fig. 6). These structures were found predominantly in the obese animals with less pronounced microvascular-associated staining seen in the lean groups. In order to confirm that this distinct GFAP immunoreactivity was in fact associated with microvessels we repeated the DIO experiment in animals expressing green fluorescent protein (GFP) under the control of the promoter for the endothelial marker Tie2 (Tie2-GFP mice) (Motoike et al., 2000). In the DIO Tie2-GFP mice this microvascular-associated GFAP immunoreactivity was adjacent to areas of Tie2-GFP expression, confirming that the GFAP-staining was proximal to microvessels (Fig. 7). In this study the immunoreactivity of the GFP antibody corresponded to the endogenous fluorescence in the Tie2-GFP transgenic mice and was used to intensify the existing signal.

Distribution of GFAP immunoreactivity in extrahypothalamic areas in lean compared with obese mice

The goal of this study was to characterize the distribution of GFAP immunoreactivity in the hypothalamus of lean and obese mice. While a detailed examination of the whole brain is beyond the scope of this study we did see some differences in staining in extrahypothalamic brain regions such as the hippocampus and thalamus, particularly the medial habenula, internal capsule, and reticular thalamic nucleus (Fig. 8). Areas of the forebrain more rostral to the hypothalamus or caudal areas in the midbrain, pons, and medulla were not examined in this study.

DISCUSSION

Inflammation in the hypothalamus is being increasingly recognized as a pathologic feature of obesity in animals (De Souza et al., 2005; Zhang et al., 2008; Hsueh et al., 2009; Horvath et al., 2010; Thaler et al., 2012) and possibly humans (Thaler et al., 2012). Reactive astrogliosis occurs in response to inflammation and injury to the CNS. While changes in GFAP immunoreactivity have been documented using immunohistochemistry in the ARC in response to obesity (Hsueh et al., 2009; Horvath et al., 2010; Thaler et al., 2012), this study is the first to examine the relative distribution of reactive astrogliosis throughout the hypothalamus. The finding that some hypothalamic nuclei, such as the MPO, Pa, and DMN show a profound up regulation in GFAP immunoreactivity in response to obesity compared

with other areas, such as the VMH, AH, and LH, which showed comparatively less astrogliosis, suggests that these nuclei have distinctly different inflammatory responses to obesity. One potential reason for this difference may be the proximity of the different nuclei to the third ventricle. In general, the hypothalamic nuclei that showed the highest increases in GFAP immunoreactivity in this study were situated proximal to the third ventricle. The relative expression (lean vs. obese) and distribution of GFAP immunoreactivity was very similar between the diet and genetic (MC4R^{-/-}) obesity models, suggesting that it is the obesity, not the high-fat diet per se, that leads to the increased GFAP immunoreactivity; however, the immunohistochemistry was performed on different occasions so the intensity of the staining cannot be directly compared.

In this study we used GFAP immunoreactivity as a marker of astrocytes/astrogliosis. While GFAP immunoreactivity is commonly used for this purpose, we acknowledge that GFAP is not a completely comprehensive marker of astrocytes and that some astrocytes do not express GFAP (Cahoy et al., 2008); however, for the purposes of this study we believe that GFAP immunoreactivity provides an indication of the behavior of this subset of astrocytes in response to obesity.

In this study we utilized female mice. Glial plasticity has been shown to vary with the stage in the estrous cycle (Prevot et al., 2007; Garcia-Segura et al., 2008). The stage of the estrous cycle of the animals used in this study was not controlled; thus, it may be partially responsible for the relatively higher GFAP immunoreactivity seen in the ARC of the lean female mice compared to other hypothalamic nuclei. Other studies have utilized male mice, and while they did not document nuclei outside of the ARC, they did see increases in GFAP immunoreactivity in the ARC of high-fat fed mice in their studies (Horvath et al., 2010; Thaler et al., 2012). The goal of the present study was not to examine the sexual dimorphism in GFAP immunoreactivity in response to obesity, so only one sex was used. This study will need to be repeated in male mice in order to confirm that the same pattern of GFAP immunoreactivity is seen in response to obesity.

Whether hypothalamic inflammation in obesity arises de novo or in response to the well-documented peripheral inflammation has not been completely elucidated. A recent study by Thaler et al. (2012) performed a time-course of the development of inflammation and reactive astrogliosis in the CNS. Their study indicated that the hypothalamic inflammation in response to high-fat feeding in rats precedes the onset of white adipose tissue inflammation; however, as adipose tissue inflammation was only examined at a single timepoint, further investigation is needed. The distinct pattern of GFAP immunoreactivity seen in this study associated with microvessels (Figs. 6, 7) suggests that the reactive astrogliosis can occur, at least in part, in response to changes at the level of the BBB/periphery. It is likely that in chronic obesity inflammation in the brain occurs both de novo and in response to chronic adipose tissue-derived inflammation. As our study only examined a single timepoint, reflecting chronic obesity, further studies are needed to characterize the development of reactive astrogliosis in the different hypothalamic nuclei over time.

Increased CNS inflammation associated with obesity is likely to have profound repercussions for neuropathology and may contribute to the increased vulnerability of the

CNS of obese individuals to damage associated with diseases such as dementia and cerebral ischemia (Bruce-Keller et al., 2009). Furthermore, the regional differences in astrogliosis in response to obesity may have important consequences for the regulation of energy homeostasis. Astrocytes are known to regulate synaptic plasticity in other neuroendocrine systems such as the reproductive axis (Prevot et al., 2007; Garcia-Segura et al., 2008) and hypothalamo-neurohypophysial system (Theodosis et al., 2006; Panatier, 2009); thus, astrogliosis seen in response to obesity may have a significant impact on modulating neuronal communication. Indeed, this has already been demonstrated for the melanocortin system of the ARC (Horvath et al., 2010), which is critical for the regulation of energy homeostasis (Cone, 2006). The consequences of obesity-associated astrogliosis outside of the ARC have yet to be determined but are likely to have wide-ranging implications for the control of metabolism (Yi et al., 2011) and other neuroendocrine axes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Characterization of the glial-fibrillary acidic protein (GFAP) antibody. **A:** Western blotting for GFAP in whole brain homogenate resulted in a band at 51 kDa corresponding to the predicted size of GFAP. **B:** GFAP immunoreactivity seen in brain slices corresponded to the known morphology of astrocytes. **C:** Incubation of the tissue with secondary antibody in the absence of primary did not result in any cellular staining, indicating that the immunoreactivity seen was not due to nonspecific binding of the secondary antibody to the tissue. Scale bars = 25 µm in B; 200 µm in C.

Figure 2.

Regional increases in glial-fibrillary acidic protein (GFAP) immunoreactivity in the rostral hypothalamus in diet-induced obese compared with lean mice. Diet-induced obesity was associated with the most pronounced increases in GFAP immunoreactivity in the medial preoptic nucleus (**A**, lean; **B**, obese), periventricular nucleus (**A,C,E** lean; **B,D,F**, obese) and paraventricular nucleus (**C,E**, lean; **D,F**, obese) compared with the anterior and lateral hypothalamic areas (**C,E**, lean; **D,F**, obese), which showed a lower relative increase. Third ventricle = 3V, medial preoptic nucleus = MPO (medial portion = MPOM; ventromedial portion = vMPO), OX = optic chiasm, Pe = peri-ventricular nucleus, Pa = paraventricular nucleus, Sch = suprachiasmatic nucleus, AHC = anterior hypothalamic area central portion, LH = lateral hypothalamic area, opt = optic tract. Distance from bregma is according to Paxinos and Franklin (2001). A,B = bregma -0.1 mm; C-F = bregma 30.82. Scale bars = 100 μ m.

Figure 3.

Regional increases in glial-fibrillary acidic protein (GFAP) immunoreactivity in the medial hypothalamus in diet-induced obese compared with lean mice. Diet-induced obesity was associated with the most pronounced increases in GFAP immunoreactivity in the dorsomedial hypothalamus (**A,C,E**, lean; **B,D,F**, obese) and periventricular nucleus (**A,C,E**, lean; **B,D,F**, obese) compared with the ventromedial and lateral hypothalamic areas (**A,C,E**, lean; **B,D,F**, obese), which showed a lower relative increase. Third ventricle = 3V, ARC = arcuate nucleus, DMN = Dorsomedial hypothalamus, LH = lateral hypothalamic area, Pe = Periventricular nucleus, opt = optic tract, VMH = ventromedial hypothalamus. Distance from bregma is according to Paxinos and Franklin (2001). A–D = bregma –1.46 mm; E,F = bregma –1.94 mm. Scale bars = 200 μ m.

Figure 4.

Higher-magnification images of regions with the most pronounced difference in glial-fibrillary acidic protein (GFAP) immunoreactivity between lean and diet-induced obese mice. Medial preoptic area (**A**, lean; **B**, obese); paraventricular nucleus (**C**, lean; **D**, obese); dorsomedial hypothalamus (**E**, lean; **F**, obese), and arcuate nucleus (**G**, lean; **H**, obese). Distance from bregma according to Paxinos and Franklin (2001). A,B = -0.1 mm; C,D = -0.82 mm; E,F = -1.46 mm; G,H = -1.94 . Scale bars = $100\ \mu\text{m}$.

Figure 5.

Regional increases in glial-fibrillary acidic protein (GFAP) immunoreactivity in the medial hypothalamus of melanocortin-4 receptor deficient (MC4R^{-/-}) mice compared with wildtype (MC4R^{+/+}) littermates. Genetic obesity caused by loss of MC4R signaling was associated with the most pronounced increases in GFAP immunoreactivity in the dorsomedial hypothalamus (A–D) and arcuate nucleus (A,B) compared with the ventromedial and lateral hypothalamic areas, which showed a lower relative increase. MC4R^{+/+} = Panels A,C; MC4R^{-/-} = panels B,D. Bregma = -1.46 mm, according to Paxinos and Franklin (2001). Scale bars = 200 μm in A,B; 50 μm in C,D.

Figure 6. Obesity is associated with increased microvascular associated glial-fibrillary acidic protein (GFAP) immunoreactivity. A representative image taken from a diet-induced obese mouse illustrating the distinct pattern of microvascular associated GFAP staining. Scale bar = 100 μ m.

Figure 7.

Increased glial-fibrillary acidic protein (GFAP) immunoreactivity is seen surrounding endothelial cells in diet-induced obese Tie2-GFP animals. A distinct pattern of GFAP immunoreactivity (**A**) is seen surrounding endothelial cells (**B**; Tie2-GFP expression) in diet-induced obese mice. **C**: Nuclei stained with DAPI. **D**: All panels overlaid. See Supporting Fig. 1 for a magenta-green version of this image. Scale bar = 50 μ m.

Figure 8.

Increased expression of glial-fibrillary acidic protein (GFAP) is also seen in extrahypothalamic regions in obese compared with lean animals. A side-by-side comparison of GFAP immunoreactivity across an entire brain slice reveals an increased expression in the multiple brain regions in the diet-induced obese animals including the hippocampus, medial habenula, internal capsule, and reticular thalamic nuclei. Scale bar = 500 μ m.

TABLE 1

Primary Antibodies Used in These Studies

Primary antibodies			
Name	Immunogen	Manufacturer	Host species
Glial-fibrillary acidic protein (GFAP)	Purified GFAP from bovine spinal cord	Cat. # MAB360, Millipore, Bedford, MA	Mouse monoclonal, Clone GA5
Green-fluorescent protein (GFP)	Purified GFP from <i>Aequorea victoria</i>	Cat. # A-11122, Invitrogen Life Technologies, La Jolla, CA	Rabbit polyclonal, IgG fraction

TABLE 2

Secondary Antibodies Used in These Studies

Name	Manufacturer	Host species
Antimouse IgG (H+L) HRP	Cat. # W4021, Promega, Madison, WI	Donkey
Antirabbit IgG (H+L) AlexaFluor 488	Cat. # A-21206, Invitrogen Life Technologies, La Jolla, CA	Donkey
Antimouse IgG (H+L) AlexaFluor 594	Cat. # A-21203, Invitrogen Life Technologies	Donkey
Antimouse IgG (H+L) IRDye 800CW	Cat #. 926-32212, Li-Cor Biosciences, Lincoln, NE	Donkey

TABLE 3

Relative Expression of Glial-Fibrillary Acidic Protein (GFAP) Immunoreactivity in Lean Compared With Diet-Induced Obese (DIO) Mice

Region	Bregma	Mean lean	Mean DIO
Anteroventral periventricular nucleus (AVPe)	0.38 - 0.02	+/-	+
Anterodorsal preoptic nucleus (ADP)	0.14 - 0.02	+/-	+,+/-
Ventromedial and medial preoptic nuclei (vMPO, MPOM & MPOL)	0.38 - -0.46	+	+,+/-
Medial preoptic area (MPA)	0.26 - -0.58	+	+
Periventricular hypothalamic nucleus (Pe)			
rostral	0.26 - -0.46	+	+,+/-
medial	-0.58 - - 1.34	+, +/-	+,+/-
caudal	-1.46 - -1.94	+/-	+, +/-
Suprachiasmatic nucleus (Sch)	-0.22- -0.82	+,+/-	++,+/-
Paraventricular hypothalamic nucleus (Pa)			
rostral	-0.58 - -0.82	+,+/-	++,+/-
caudal	-0.94 - -1.22	+	++
Anterior hypothalamic area (AH)			
Anterior	-0.34 - -0.58	+	+
Central	-0.70 - -0.82	+	+
Posterior	-0.94 - -1.34	+	+
Lateroanterior hypothalamic nucleus (LA)	-0.34 - -0.70	+	+
Lateral hypothalamic area (LH)			
rostral	-0.34 - -0.94	+/-	+
medial	-1.06 - - 1.70	+/-	+,+/-
caudal	-1.82 - - 2.46	+/-	+/-
Arcuate hypothalamic nucleus (ARC)			
rostral	-1.22 - -1.82	+++	+++
caudal	-1.94 - -2.46	++,+/-	++,+/-
Dorsomedial hypothalamic nucleus (DMN)			
rostral	-1.34 - -1.70	+	++,+/-
caudal	-1.82 - -2.18	+,+/-	++, +/-
Posterior hypothalamic area (PH)	-1.82 - -2.46	+	+
Premammillary nucleus, entral part (PMV)	-2.30 - -2.46	+/-	+
Ventromedial hypothalamic area (VMH)			
rostral	-1.06 - -1.58	+	+, +/-
caudal	-1.70 - -2.06	+	+, +/-

TABLE 4

Relative Expression of Glial-Fibrillary Acidic Protein (GFAP) Immunoreactivity in Obese Melanocortin-4 Receptor Deficient Mice (MC4R^{-/-}) Compared With Lean Wildtype (MC4R^{+/+}) Littermates

Region	Bregma	MC4R ^{+/+}	MC4R ^{-/-}
Anteroventral periventricular nucleus (AVPe)	0.38 - 0.02	+/-	++,+/-
Anterodorsal preoptic nucleus (ADP)	0.14 - 0.02	+/-	+,+/-
Ventromedial and medial preoptic nuclei (vMPO, MPOM & MPOL)	0.38 - -0.46	+	+,+/-
Medial preoptic area (MPA)	0.26 - -0.58	+	+,+/-
Periventricular hypothalamic nucleus (Pe)			
rostral	0.26 - -0.46	+	+
medial	-0.58 - -1.34	+,+/-	++
caudal	-1.46 - -1.94	+	+,+/-
Suprachiasmatic nucleus (Sch)	-0.22 - -0.82	++	++,+/-
Paraventricular hypothalamic nucleus (Pa)			
rostral	-0.58 - -0.82	+,+/-	+++
caudal	-0.94 - -1.22	++	++
Anterior hypothalamic area (AH)			
Anterior	-0.34 - -0.58	+	+
Central	-0.70 - -0.82	+	+
Posterior	-0.94 - -1.34	+	+,+/-
Lateroanterior hypothalamic nucleus (LA)	-0.34 - -0.70	+	+
Lateral hypothalamic area (LH)			
rostral	-0.34 - -0.94	+	+
medial	-1.06 - -1.70	+	+
caudal	-1.82 - -2.46	+/-	+
Arcuate hypothalamic nucleus (ARC)			
rostral	-1.22 - -1.82	++	+++ , +/-
caudal	-2.06 - -2.46	+,+/-	+++
Dorsomedial hypothalamic nucleus (DMN)			
rostral	-1.34 - -1.70	+,+/-	++, +/-
caudal	-1.82 - -2.18	+,+/-	++, +/-
Posterior hypothalamic area (PH)	-1.82 - -2.46	+	+
Premammillary nucleus, ventral part (PMV)	-2.30 - -2.46	+	+,+/-
Ventromedial hypothalamic area (VMH)			
rostral	-1.06 - -1.58	+	++
caudal	-1.70 - -2.06	+	++