Title

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Journal

Cell Reports, 9(6)

ISSN

2639-1856

Authors

Valdearcos, Martin
Robblee, Megan M
Benjamin, Daniel I
et al.

Publication Date

2014-12-01

DOI

10.1016/j.celrep.2014.11.018

Peer reviewed

HHS Public Access

Author manuscript

Cell Rep. Author manuscript; available in PMC 2015 December 24.

Published in final edited form as:

Cell Rep. 2014 December 24; 9(6): 2124–2138. doi:10.1016/j.celrep.2014.11.018.

Microglia Dictate the Impact of Saturated Fat Consumption on Hypothalamic Inflammation and Neuronal Function

Martin Valdearcos[1], **Megan M. Robblee**[1,2], **Daniel I. Benjamin**[3], **Daniel K. Nomura**[3], **Allison W. Xu**[1,4], and **Suneil K. Koliwad**[1,2,5,*]

[1]Diabetes Center, University of California San Francisco, San Francisco, CA 94143, USA

[2]Biomedical Sciences Graduate Program, University of California San Francisco, San Francisco, CA 94143, USA

[3]Department of Nutritional Sciences and Toxicology, University of California Berkeley, Berkeley, CA 94720, USA

[4]Department of Anatomy, University of California San Francisco, San Francisco, CA, 94143, USA

[5]Department of Medicine, University of California San Francisco, San Francisco, CA, 94143, USA

Abstract

Diets rich in saturated fat produce inflammation, gliosis, and neuronal stress in the mediobasal hypothalamus (MBH). Here we show that microglia mediate this process and its functional impact. Although microglia and astrocytes accumulate in the MBH of mice fed a diet rich in saturated fatty acids (SFAs), only the microglia undergo inflammatory activation, along with a build-up of hypothalamic SFAs. Enteric gavage specifically with SFAs reproduces microglial activation and neuronal stress in the MBH, and SFA treatment activates murine microglia, but not astrocytes, in culture. Moreover, depleting microglia abrogates SFA-induced inflammation in hypothalamic slices. Remarkably, depleting microglia from the MBH of mice abolishes inflammation and neuronal stress induced by excess SFA consumption, and in this context, microglial depletion enhances leptin signaling and reduces food intake. We thus show that microglia sense SFAs and orchestrate an inflammatory process in the MBH that alters neuronal function when SFA consumption is high.

Introduction

Fatty acids (FAs) serve as energy substrates and as signals controlling metabolic processes. For example, dietary FAs signal through intestinal GPCRs to stimulate pancreatic insulin

*Correspondence: skoliwad@diabetes.ucsf.edu, phone: (415) 476-9623, fax: (415) 450-1447.

Author Contributions

M.V. performed the experiments, analyzed data, generated the figures, and wrote the paper. M.M. R. assisted M.V. with experiments and creative input. D.I.B. and D.K.N. performed and interpreted lipidomic analyses. A.W.X. helped obtain and interpret physiological and histological data. S.K.K. conceived the project, oversaw the experiments, analyzed data, edited figures, and wrote the final paper.

secretion (Talukdar et al., 2011). FAs also act in the brain. In particular, the mediobasal hypothalamus (MBH), including the arcuate nucleus (ARC) and median eminence (ME), can sense FAs and transduce this to control food intake, thermogenesis, and intermediary metabolism (Lam et al., 2005). The fenestrated nature of the blood brain barrier (BBB) surrounding the MBH may facilitate this capacity.

However chronic consumption of FAs, particularly when saturated, leads to inflammation in the white adipose tissue, liver, and skeletal muscle that is implicated in the pathogenesis of metabolic diseases including type 2 diabetes (Bergman and Ader, 2000), non-alcoholic steatohepatitis (de Almeida et al., 2002), and atherosclerosis (Singh et al., 2002). Chronic "metabolic inflammation", marked by macrophage accumulation, has been targeted to mitigate diseases linked to overnutrition (Dali-Youcef et al., 2013).

Diet-induced obesity also produces metabolic inflammation in the MBH (Thaler et al., 2012), and work targeting TLR4, TNFα, NF-κB, and NLRP3 suggests that controlling inflammation in the MBH can limit metabolic complications of diet-induced obesity (Milanski et al., 2012; Vandanmagsar et al., 2011). Reaching this objective, however, requires identifying what factors trigger inflammation in the MBH and the responsive cell types that mediate this process.

Treating cultured macrophages with long-chain saturated FAs (SFAs), but not unsaturated species, stimulates an inflammatory response reminiscent of what occurs in tissues responding to dietary excess, and feeding mice diets rich in SFAs induces insulin resistance (Kennedy et al., 2009). Interestingly, dietary SFAs also exert pro-inflammatory effects in the MBH that are linked to impaired control over peripheral metabolism (Milanski et al., 2012; Ross et al., 2010), but it is unknown which cell type(s) mediate this process.

Metabolic inflammation in the MBH is marked by accumulation of astrocytes and microglia, the CNS analogs of macrophages. Based on the role of macrophages in metabolic inflammation, it is intriguing to consider targeting microglia to control CNS metabolic inflammation. However microglial responses to FAs are not understood, and tools to manipulate hypothalamic microglia have been lacking.

Here we use new approaches to both enrich and deplete microglial content in the MBH of mice in order to demonstrate their unique inflammatory responsiveness to enteric SFAs. By doing so, we identify microglia as sensors that dictate the intensity of metabolic inflammation in the MBH and mediate key changes in hypothalamic function that occur in response to consuming excess saturated fat.

Results

Excessive dietary SFA consumption specifically activates microglia in the MBH, which is mirrored by hypothalamic SFA accumulation

Rats express classical (M1) inflammatory markers and develop hypothalamic gliosis when consuming excess dietary fat for as little as 7 days (Thaler et al., 2012). To examine the role of long-chain dietary SFAs in triggering metabolic inflammation in the MBH, we fed 10

week-old mice a high-fat diet (HFD) in which 42% of calories were from milk fat, which is highly enriched in SFAs (mostly C16:0 palmitic (PA) and C18:0 stearic acids) for 1, 4, and 16 weeks.

Mice consuming this HFD accumulated microglia (and astrocytes; not shown) in the MBH, reaching a plateau by 4 weeks (Figure 1A, B). These microglia displayed morphological features of M1 activation (Figures 1A, B). We also measured hypothalamic inflammation in this setting by staining for TNFα, an M1 cytokine which when neutralized in the brain produced beneficial metabolic effects in high-fat fed mice (Milanski et al., 2012). High-fat feeding increased TNFα in the MBH, and this co-localized strongly with microglia ($Iba1^+$ cells) but not at all with cells expressing the astrocyte marker GFAP (Figures 1C, D). Moreover, the HFD specifically increased the mRNA levels of M1 genes in the hypothalamus, and this increase was retained when only hypothalamic microglia were analyzed (Figure 1E). Together these findings indicate that hypothalamic microglia specifically undergo M1 activation in mice consuming a diet rich in SFAs, marking a form of diet-induced metabolic inflammation that is localized to the MBH.

To determine whether dietary SFAs activate hypothalamic microglia directly, we first tested the impact of consuming excess dietary SFAs on lipid levels in the brain. A targeted analysis of lipids extracted from the hypothalami and cerebral cortices of mice consuming standard chow or the SFA-rich diet for 28 days revealed that a minority of analytes were altered by high-SFA feeding (Figure S1). Of these, most were either altered similarly in both the cortex and hypothalamus, or only in the cortex with hypothalamic levels trending in the same direction (Figure S1). On the other hand, high dietary SFA intake specifically increased hypothalamic SFA levels. PA, the most abundant lipid in the high-SFA diet, was the only species the level of which was increased solely in the hypothalamus, and this increase (~40% over chow), was the largest of any FA in either the hypothalamus or cortex (Figure 1E). These findings indicate that dietary SFAs build up in the hypothalamus when consumed in excess, and prompted us to wonder whether this build-up could trigger microglial activation in the MBH.

Enteric SFAs trigger microglial activation in the MBH independent of caloric intake

To determine whether consuming excess SFAs stimulates M1 activation of MBH microglia independently of its effect on body weight, we provided mice with a variety of FAs by enteric gavage while holding total fat and caloric intake constant. We first provided mice with clarified milk fat (primarily PA) that included a radioactive (^{14}C-PA) label. In this context, we observed a rapid flux of PA into the hypothalamus and cortex, with kinetics similar to those for PA flux into peripheral tissues (Figure S2A). These findings indicate that the brain, including the hypothalamus, is a site for postprandial flux of enteric SFAs. Moreover, increasing daily milk fat consumption enhanced PA flux into the brain as it did in other tissues, mirroring what was seen with a HFD (Figure S2A).

The rapidity with which enteric PA fluxed into the hypothalamus suggested that it might be transported there by post-prandial lipoproteins. We therefore tested whether hypothalamic microglia take up lipoprotein-associated lipids by injecting mice with very low-density lipoproteins (VLDL) containing lipids labeled with a fluorescent lipophilic dye (DiI).

Hypothalamic microglia took up the DiI within 1 hour of VLDL injection, and this increased over 6 hours, indicating that lipoprotein-associated lipids rapidly access the MBH and that microglia take up these lipids (Figure S2B).

In concert with its flux into the brain, milk fat gavage for 3 days also remarkably increased the hypothalamic levels of M1 markers (Figure 2A) and induced accumulation of MBH microglia with features of activation resembling those in mice fed a high-SFA diet (Figure 2B). Moreover, this short-term SFA gavage did not increase TNFα, MCP-1, or IL-6 levels in the blood, indicating that the effects in the MBH were not due to systemic inflammation (data not shown). By contrast, isocaloric gavage with olive oil (primarily oleic acid; OA) did not induce either the transcription of M1 genes (Figure 2A) or the accumulation of microglia in the MBH (Figure 2B). Taken together, these findings indicate that excess dietary SFA intake directly triggers hypothalamic metabolic inflammation, and is not a byproduct of increased total fat or caloric intake, or inflammation elsewhere in the body.

As with a chronic high-SFA diet, enteric milk fat gavage markedly increased TNFα in the MBH, and this again localized to microglia (Figure 2C), but not astrocytes (Figure 2D), confirming that microglia are uniquely responsive to SFAs in initiating metabolic inflammation. Gavage with coconut oil, (mainly short-chain SFA), on the other hand, stimulated a response in between that of olive oil and milk fat (Figure 2A), indicating that some of the pro-inflammatory effects of dietary SFAs may be a function of chain length.

Excess enteric SFA consumption induces neuronal stress in the MBH

Consumption of a high-fat diet induced expression of heat-shock protein 72 (Hsp72), a chaperone involved in the response to neuronal stress (Sharp et al., 1999), in the MBH of rats (Thaler et al., 2013). We found that feeding mice a high-SFA diet induced Hsp72 in neurons specifically in the MBH (Figure 2E), a response rapidly reproduced by administering milk fat to chow-fed mice by enteric gavage (Figure 2E). By contrast, gavage with OA-rich olive oil did not induce neuronal Hsp72, even though both olive oil and milk fat increased daily fat intake by ~38% (Figure 2E). Thus increased SFA intake, and not fat intake *per se*, accounts for the stress response of neurons in the MBH to excess saturated fat consumption.

Moreover, the neuronal stress response to gavage with SFAs involved neurons in the ARC that phosphorylate STAT3 upon systemic leptin treatment (Figure 2F), suggesting that excessive SFA consumption may impact the leptin-responsive neurocircuitry of the MBH.

Long-chain SFAs exert direct and specific inflammatory effects on microglia

Given that dietary SFAs accumulate in the hypothalami of mice and induce M1 microglial activation in the MBH even when their intake is dissociated from caloric excess, we sought to determine if SFAs directly activate microglia. We measured cytokine release by primary murine microglia treated with FAs of varying chain lengths and saturation status for 24 hours. We found that only SFA treatment induced M1 cytokine secretion by microglia, and that long-chain SFAs were generally more potent than short-chain SFAs (Figure 3A). Similarly, only SFA treatment activated NF-κB, a transcription factor for M1 genes, as

measured by monitoring the phosphorylation both IKKβ (p-IKKβ) and the p65 subunit of NF-κB (p-RelA) (Figure 3B). Indeed PA treatment dose-dependently increased p-RelA between 100 and 300 μM (Figure 3C). These findings indicate that long-chain SFAs directly stimulate microglial M1 activation at concentrations that might be reached in the hypothalami of mice consuming excess dietary fat.

We next examined whether SFAs can activate astrocytes, another glial cell type accumulating in the MBH of mice consuming an SFA-rich diet. We first eliminated microglia from freshly harvested, astrocyte-enriched, mixed glial cultures by treatment with liposomal clodronate (Lip-CLO; 100 μg/ml), or used diphtheria toxin (DT; 20ng/ml) to treat similar cultures harvested from mice expressing the human diphtheria toxin receptor (DTR; a.k.a hbEGF) under control of the CD11b promoter (CD11b-DTR). Immunostaining clearly revealed that both strategies eliminated microglia, leaving behind a pure population of $GFAP^+$ cells without discernable impairment in viability (Figures 3D and S3A). Primary astrocytes and microglia from the same mice were treated with vehicle (BSA), OA, PA, or LPS (control), respectively. Whereas PA treatment induced M1 cytokine secretion from microglia, this response was nearly absent in astrocytes (Figures 3E and S3B), illustrating that microglia directly and specifically respond to long-chain SFAs (e.g. PA) and supporting their role as CNS sensors capable of initiating inflammation in response to rising SFA levels in the MBH.

Microglia are responsible for the inflammatory response of hypothalamic slice cultures to PA treatment

To test the direct role of microglia in FA-driven hypothalamic inflammation, we first depleted microglia from 300 μM-thick murine organotypic hypothalamic slice cultures using Lip-CLO. Microglial depletion was confirmed by documenting a greater than 90% loss of $Iba1^+$ cells vs. control (Figure 4A) and a marked reduction in the mRNA levels of microglial markers, including *Aif1*/Iba1, *CD68*, and *Itgam*/CD11b (Figure 4B). Moreover, the effects of Lip-CLO treatment were specific, as no changes were seen in the numbers of $NeuN^+$ mature neurons (Figure 4A) or in the mRNA levels of *Gfap* (astrocyte marker) or either *Agrp* or *Npy* (specific neuropeptides) (Figure 4B). Treating hypothalamic slices cultured from CD11b-DTR mice with DT produced similar results (Figure 4C).

PA treatment, vs. treatment with vehicle or OA, stimulated M1 cytokine secretion from hypothalamic slice cultures, and microglial depletion nearly abolished this response (Figures 4D and E), indicating that microglia are essential for SFA-driven hypothalamic metabolic inflammation. On the other hand, microglial depletion only modestly reduced the inflammatory response to LPS treatment, indicating that non-microglial cell types may contribute to the response to other inflammatory stimuli.

Specifically depleting hypothalamic microglia in mice

In attempting to manipulate microglial content in the MBH of mice, we first lethally irradiated adult CD11b-DTR and WT (control) mice while shielding their heads and necks with lead in order to protect the BBB from irradiation-induced disruption (Figure S5A). We then reconstituted the bone marrow of these mice with hematopoietic cells from WT mice in

which RFP was expressed under control of the C-C chemokine receptor 2 (CCR2) promoter (CCR2-RFP). The resulting mice (DTR^{BMT}) expressed DTR in microglia, but not in donor-derived cells, the $CCR2^+$ monocytes of which could be tracked by monitoring RFP. After recovering for 6 weeks, we used FACS to confirm that >90% of $CD11b^+$ cells in the blood of both DTR^{BMT} mice and WT^{BMT} controls were also RFP^+, indicating high reconstitution efficiency (Figure S5B). In this way, we were able to confine DTR expression to microglia and exclude its expression from peripheral immune cells. Moreover, whereas RFP-labeled monocytes infiltrated the brains of transplanted mice that were not head-shielded during irradiation, head shielding eliminated such post-transplant monocyte infiltration over 8 weeks (Figure S5C).

DTR^{BMT} and WT^{BMT} mice were either treated with I.P. vehicle (control) or DT (10ng/g body weight) daily for 3 days, and the microglia from hypothalamic sections were analyzed by immunostaining 24 hours after the last injection. As expected, the number and morphology of hypothalamic $Iba1^+$ microglia was unaffected in WT^{BMT} and DTR^{BMT} mice treated with vehicle or in WT^{BMT} mice receiving DT (Figures 5A and B). By contrast, treating DTR^{BMT} mice with DT reduced the number of $Iba1^+$ cells in the MBH by ~60% and the mRNA levels of microglial markers in the MBH by ~50%. (Figures 5A-C). On the other hand, DT-treated DTR^{BMT} mice had no alteration in hypothalamic levels of the astrocyte marker *Gfap*, indicating specific depletion of microglia (Figure 5C). DT-induced depletion of hypothalamic microglia in DTR^{BMT} mice was also associated with gross alterations in microglial morphology (Figure 5A) and with microglial apoptosis (co-localization of $TUNEL^+$ and $Iba1^+$ cells) not seen in controls (Figure S5D). Microglial depletion in DT-treated DTR^{BMT} mice was also specific to the MBH, and not seen in the cortex or hippocampus (Figure S6A), likely owing to more effective transit of DT into the MBH vs. other brain regions under the conditions of this study.

Manipulating the number of microglia in the MBH of DTR ^{BMT} mice

BrdU labeling of DT-treated DTR^{BMT} mice revealed that microglial depletion was followed within 72 hours of the final dose of DT by proliferation (Figures 5D-E), leading to a re-emergence of MBH microglia within 5 days. These $BrdU^+/Iba1^+$ cells did not express RFP, indicating they did not arise from donor-derived monocytes and supporting the concept that adult microglia can self-renew and maintain their own numbers within the CNS (Ajami et al., 2007).

Interestingly, post-depletive microglial proliferation in the MBH of DT-treated DTR^{BMT} mice resulted over 10 days in increased microglial numbers, both over baseline and vehicle- or DT-treated controls (Figures S5E and S5F). As for the initial depletion, the post-depletive increase in microglial content was specific to the MBH (Figures S5G and S6A). Moreover, the microglia that accumulated in the MBH were not M1-activated, but rather had features similar to those of microglia in the MBH of chow-fed controls (Figure S6B). Thus simply increasing the basal number of microglia in the MBH of chow-fed mice is not sufficient to induce inflammation. On the other hand, post-depletive microglial proliferation was associated with increases in hypothalamic mRNA levels of the chemokine gene *Ccl2* (MCP-1; Figure S6B), which was shown to induce microglial proliferation but not M1-type

activation *in vitro* (Hinojosa et al., 2011). Overall, this process allowed us to generate mice with an increased basal number of quiescent of microglia, specifically in the MBH.

Supporting the tissue-autonomous self-renewing capacity of microglia (Eliason et al., 2002), post-depletive microglial proliferation also occurred in hypothalamic slices, restoring microglial content within 8 days of Lip-CLO-induced depletion (Figures S4A and B). Moreover, post-depletive re-emergence of hypothalamic microglia, which occurred without altering mRNA levels of astrocyte- or neuronal markers, restored the responsiveness of hypothalamic slices to PA and LPS treatment (Figure S4C), underscoring the key role of microglia in triggering hypothalamic inflammation.

To stabilize microglial depletion in the MBH, we administered DTR^{BMT} mice a specific BBB-permeable colony-stimulating factor 1 receptor (CSF1R) antagonist (Ki20227, 30mg/kg) by gavage following DT-induced MBH microglial depletion. CSF1R blockade dramatically reduced post-depletive MBH microglial proliferation in DTR^{BMT} mice, assessed by the number of both $Iba1^+$ and $Iba1^+/BrdU^+$ cells in hypothalamic sections vs. control (Figures 5D and E). Concordantly, hypothalamic microglial mRNA levels in DT-treated DTR^{BMT} mice given Ki20227 were 80% lower than those in matched mice receiving vehicle (Figure 5F). These findings indicate that antagonizing CSF1R can sustain DT-induced microglia depletion in the MBH of DTR^{BMT} mice.

Microglial content in the MBH dictates the intensity of the inflammatory response to excess dietary SFAs

We next tested the impact of manipulating microglial content upward or downward specifically in the MBH (Figure 6A) on the intensity of hypothalamic inflammation induced by excess consumption of dietary SFAs. 3 days of SFA gavage increased the mRNA levels of hypothalamic M1 markers similarly in both DT-treated WT^{BMT} mice and vehicle-treated DTR^{BMT} mice (Figure 6B), and to a degree similar that seen in un-transplanted WT mice (Figure 2A). By contrast, DT-treated DTR^{BMT} mice in which microglial content in the MBH was greatly increased by post-depletive proliferation had an augmented hypothalamic inflammatory response to SFA gavage (Figure 6B). On the other hand, when MBH microglial depletion was sustained by Ki20227 treatment, the inflammatory response to SFA gavage within the hypothalamus was remarkably abolished, and not different from control mice gavaged with saline (Figure 6B). These data indicate that the microglial content of the MBH dictates the intensity of the hypothalamic inflammatory response to SFA excess.

Microglia mediate neuronal injury induced by excess dietary SFAs in the MBH

We also tested the role of microglia in the response of neurons in the MBH to excess SFA intake. Remarkably, depleting microglia from the MBH of DTR^{BMT} mice nearly abolished the marked induction of Hsp72 otherwise seen in ARC neurons following enteric SFA gavage (Figures 6C-E). Moreover, when basal microglial content in the MBH was increased, the neuronal stress response to enteric SFA challenge was accentuated (Figure 6E), and extended beyond the ARC to affect other hypothalamic regions, such as the VMH (not shown). Taken together, these results indicate that microglia mediate the mechanism by which excess SFA consumption triggers neuronal stress in the MBH.

Microglia mediate the impact of excess SFA consumption on hypothalamic function

Because enteric gavage with SFAs produced neuronal stress in leptin-responsive ARC neurons, we wanted to test the effect of microglial depletion on leptin signaling in the context of high SFA consumption. Mice were fed a normal chow diet containing PLX5622, a CSF1R antagonist with oral bioavailability superior to that of Ki20227 and that inhibits the tyrosine kinase activity of CSF1R at nanomolar concentrations (Cavnar et al., 2013; Coniglio et al., 2012; Hamilton and Achuthan, 2013). PLX5622 acts similarly to other CSF1R blockers (Elmore et al., 2014; Zhang et al., 2013a), one of which (PLX3397) was recently shown to deplete microglia in mice on its own (Elmore et al., 2014). Consistent with this, feeding mice a diet containing PLX5622 depleted over 98% of microglia within 7 days without altering circulating monocyte numbers (Figure S7A and B).

We then supplemented the diet of PLX5622-treated and control mice with milk fat by twice-daily gavage for 10 days, after which the mice were injected with I.P. leptin. Interestingly, microglial depletion in this context enhanced leptin-induced STAT3 activation by ARC neurons, indicating that microglia act to restrain leptin responsiveness in the ARC when SFA consumption is high (Figures 7A and 7B).

Based on our analysis of leptin signaling in the ARC, we wondered whether microglia have a concomitant impact on food intake, which is regulated by the hypothalamic actions of leptin, when SFA intake is high. Whereas PLX5622-induced microglial depletion did not impact daily food intake in mice fed a standard low-fat diet chow diet (Figure 7C), microglial depletion progressively reduced daily food intake in mice consuming excess SFAs (Figure 7D), a finding that was confirmed by placing the mice in metabolic cages (Figures 7E and F). Together, these data indicate that microglia control hypothalamic function to maintain consistent food intake specifically in the face of excessive SFA consumption.

Discussion

Diet-induced inflammation in the MBH is marked by an accumulation of astrocytes and activated microglia, but it was unclear what orchestrates this inflammation. Here we identify microglia as sensors activated by rising levels of dietary SFAs in the MBH that control the intensity of a highly localized form of inflammation. In this setting, microglia mediate the stress-inducing effects of dietary SFAs on neurons residing in the ARC and reduce their responsiveness to leptin, thus impacting food intake. Metabolic inflammation in the MBH occurs more acutely in response to the steady consumption of excess saturated fat than in peripheral tissues, where it occurs in conjunction with obesity. There is interest in mitigating MBH inflammation due to this temporal primacy and because it is linked to metabolic dysregulation. Our findings provide strong mechanistic evidence to support targeting microglia to reach this objective.

Our focus on SFAs was prompted by a lipidomic analysis of the brains of mice fed a high-fat diet. Prior work showed that mice consuming a lard-based diet (~60% of calories from fat) for 3 months accumulate hypothalamic lipids, many of which are linked to peripheral tissue inflammation and/or insulin resistance (Borg et al., 2012). This lard-based diet also

produced hypothalamic inflammation in rats (Thaler, et al., 2012). The mice in our study were fed a high-SFA milk fat-based diet for only 28 days, and gained much less weight than would have been seen with a more chronic diet. Despite the short duration of this dietary challenge, the mice in our study had altered levels of several lipid species, including specific phospholipids, sphingolipids, ether lipids, and fatty acids in both the cerebral cortex and the hypothalamus. Most of diet-induced lipid alterations were directionally similar in the hypothalamus and cortex, likely reflecting common patterns of lipid metabolism throughout the brain.

On the other hand, C16:0 PA was the only lipid species that was increased by diet specifically in the hypothalamus. This finding is notable, as although the diet in this study (42% of calories from fat) is less calorically dense than standard lard-based HFDs, it is specifically enriched in SFAs (long-chain SFA: monounsaturated ratio of ~2.3:1, as opposed to ~1.4:1 in the prior study) the most abundant of which is PA. The enrichment of dietary PA specifically in the hypothalamus may reflect the emerging concept that the MBH is more amenable to the entry of nutritional factors than other brain areas, allowing it to sample dietary composition in order to coordinate physiological processes.

Our study also demonstrates that the transit of dietary SFAs into the brain is fast enough to stimulate the rapid inflammatory activation and proliferation of microglia previously reported (Thaler, et al., 2012). We used radiolabeling to show that saturated fats flux into the brain within hours of enteral consumption, and used fluorescent labeling to show that fats are readily transported into the hypothalamus by circulating lipoproteins (VLDL in our model), where they are taken up by microglia. Other work shows that hypothalamic microglia also accumulate IgG in response to a HFD, supporting the concept that these cells can sense peripheral signals in response to nutritional and/or environmental changes (Yi et al., 2012).

Excess SFA consumption induced microglial activation in the MBH despite controlling for total fat and caloric intake, and without increasing body weight. These data support our *in vitro* data, indicating that dietary SFAs directly stimulate M1 activation of MBH microglia, whereas peripheral tissue inflammation may depend, at least in part, on the presence of obesity. Additionally, enteric SFAs induced MBH inflammation despite not increasing circulating levels of inflammatory cytokines, indicating that SFA-induced MBH inflammation is not a byproduct of systemic inflammation.

Importantly, metabolic inflammation in the MBH could be induced by enteric SFAs without the need for I.V. or I.C.V. administration, underscoring the physiological relevance of our findings to people, many of whom consume high levels of saturated fat on a daily basis. Our findings are supported by recent work showing that hypothalamic microglial activity in obese mice is influenced by dietary composition and by fat- and gut-derived hormonal cues, but not body weight *per se* (Gao et al., 2014).

Tracking $CCR2^{+}$ monocytes showed that accumulation of MBH microglia in mice receiving SFA gavage was not due to infiltrating monocytes differentiating into microglia-like cells. Instead, our data suggest that this accumulation is due to local proliferation in the MBH, a

capacity that was inducible by depleting hypothalamic microglia using Lip-CLO or DT. Indeed, a nestin^{+} precursor cell type was recently found to be responsible for microglial self-renewal and proliferation in mice (Elmore et al., 2014), directly supporting this concept. Diet-induced microglial accumulation in the MBH is in some ways similar to macrophage accumulation in the white adipose tissue, a process also recently shown to involve local proliferation (Amano et al., 2014).

We also observed that post-depletive microglial proliferation is associated with the secretion of cytokines and chemokines independent of inflammatory activation. For example, basal *Il6* mRNA levels were slightly elevated in CD11c-DTR hypothalamic slices following microglial depletion with DT. This was not seen when microglia were depleted with clodronate, perhaps indicating that post-depletive microglial proliferation is milder in this model. Similarly, *Ccl2* mRNA levels were elevated in the hypothalami of DTR^{BMT} treated with DT. It will be interesting to test the role of cytokines and chemokines in modulating microglial proliferation.

Do other cell types sense FAs in the MBH? Unlike SFAs, which stimulated microglial M1 activation in culture, in hypothalamic slices, and in the MBH of mice, monounsaturated FAs failed to do so in any context. By contrast, monounsaturates given I.V. or I.C.V. reduce food intake and hepatic glucose production in rodents, whereas SFAs do not (Obici et al., 2002). Therefore, whereas microglia sense SFA levels and transduce this into an inflammatory response, other cell types in the MBH may respond to monounsaturates. For example, neurons in the MBH are implicated in sensing FAs (Lam et al., 2005), and neuronal lipoprotein lipase may be needed for this sensing to occur (Wang et al., 2011).

A recent study suggested that SFA treatment could induce cytokine secretion by cultured astrocytes (Gupta et al., 2012). However, eliminating microglia from astrocyte cultures is difficult because astrocytes produce CSF-1, a potent microglial growth factor (Hao et al., 1990), and traces of microglia in astrocyte cultures can confound experimental results (Saura, 2007). We treated CD11b-DTR astrocyte cultures with DT and WT astrocyte cultures with Lip-CLO to eliminate all microglia. In doing so, we found that hypothalamic microglia, and not astrocytes, secrete inflammatory cytokines when directly stimulated by SFAs. This finding suggests that astrogliosis in the MBH under conditions of dietary excess may be a response to signals from activated microglia and/or neurons responding to metabolic stress. Identifying such signals is a key area for exploration.

On the other hand, both astrocytes and microglia responded to LPS, indicating that astrocytes can sense certain inflammatory stimuli. Indeed astrocyte activation, for example during infection, may stimulate responses including cachexia, that are distinct from those induced by microglial activation. Moreover, emerging evidence suggests that crosstalk between M1-like microglia and astrocytes can amplify inflammatory responses and synergistically increase the production of neurotoxic factors (Pascual et al., 2012; Saijo et al., 2009). The significance of microglia-astrocyte crosstalk in SFA-induced MBH inflammation remains to be defined.

Excess SFA consumption also specifically induced MBH neurons to express the cellular stress chaperone Hsp72, a finding with two possible interpretations. One is that neurons directly respond to SFAs by expressing Hsp72 and producing factors that attract local microglia and trigger their activation. Our findings instead support the idea that neuronal stress in the MBH is dictated by microglia responding directly to SFAs. Future studies should focus on whether activated microglia mediate SFA-induced neuronal stress through physical interaction, cytokine secretion (supported by data shown here), or both of these.

Diet-induced obesity is associated with hyperphagia and dysregulated hepatic glucose production, processes controlled by neuronal circuits in the MBH that respond to afferent inputs from leptin and insulin (Samuelsson et al., 2008). The current study indicates that excessive SFA consumption can stimulate microglia to alter the function of neurons within these circuits. Specifically, we found that microglia are required for mice consuming excess saturated fat to maintain a consistent daily food intake. Without microglia, mice with high SFA intake had a progressive reduction in total daily food intake over several days. Remarkably, microglial depletion did not impact food intake in control mice. Together, these findings indicate that while not a dominant determinant of food intake, microglia regulate the function of hypothalamic neurons controlling food intake when SFA consumption is high.

ARC neurons that expressed Hsp72 in response to high SFA consumption included those activated by leptin, a hormonal regulator of food intake. Moreover, leptin signaling in the ARC of mice consuming excess SFAs was enhanced when microglia were depleted, highlighting the importance of microglia-neuronal crosstalk in the response to SFAs. Future research should focus on the role of this crosstalk in long-term energy balance, metabolic function, and the complications of dietary excess.

Beyond promoting pathology, it is intriguing to consider the potential physiological role of microglial activation and proliferation in the MBH. Microglia respond rapidly to a variety of physiological stimuli, and are implicated in the acute remodeling of neuronal circuits. It is therefore worth noting that systemic SFA levels are not only elevated in the setting of obesity, but also following individual fatty meals. We observed the flux of dietary fats into the brain, microglial activation, and neuronal stress in the MBH following acute bouts of SFA intake. These factors may act in concert to remodel hypothalamic circuitry and rapidly tailor the response to dietary intake. Such remodeling may be required, for example to reduce satiety in predatory mammals consuming a large meal after a prolonged fast, or to allow newborns to thrive solely on maternal milk. If microglia help remodel neuronal circuits under such circumstances, then the MBH inflammation in obesity may reflect the hijacking of a physiological process, as is suggested for metabolic inflammation in peripheral tissue.

Whereas hypothalamic inflammation induced by HFD is associated with obesity, inflammation due to injury, infection, cancer, or autoimmunity is paradoxically associated with cachexia and weight loss. Our findings suggest this contrast may reflect differences in the amplitude of metabolic inflammation vs. other forms. For example high levels of IL-6, as seen in profound inflammation, are implicated in reducing body weight(Franckhauser et

al., 2008). Consistently, global *Il6*$^{-/-}$ mice are prone to obesity, and forced overexpression of *Il6* in the brain protects against it(Hidalgo et al., 2010). On the other hand, we show that hypothalamic *Il6* levels are also increased by HFD, however to a potentially much smaller degree than that due to infection or transgenic overexpression. Whereas IL-6 in the brain may produce cachexia when dramatically elevated or accompanied by systemic inflammation, small rises in IL-6 specifically in the MBH may produce different effects. This concept is analogous to the low-grade nature of diet-induced inflammation in peripheral metabolic tissues(Gregor and Hotamisligil, 2011).

The current study also points to the promise of targeting microglia to control MBH inflammation. Prior approaches to alter gene expression or deplete microglia have depended on administering reagents into the brain (Zhang et al., 2013b) that disrupt the BBB and produce nonspecific inflammation. BBB disruption also permits monocyte infiltration into the brain (Varvel et al., 2012). We depleted and increased the basal number of quiescent microglia in the MBH without altering peripheral myeloid cell numbers or injecting reagents into the brain. Indeed, I.P. DT injection specifically targeted microglia in the MBH of CD11b-DTR^{BMT} mice, and microglial depletion in this model was sustained by CSF1R antagonism.

Recent work using reporter mice shows that microglia, but not neurons, express CSF1R in the adult brain, including the hypothalamus (Erblich et al., 2011; Sierra et al., 2007). Highly potent CSF1R antagonists are now being examined for their ability to control microglial self-renewal, and one such agent was recently shown to rapidly deplete microglia from the brains of mice by blocking the proliferative capacity of local microglia-specific progenitors (Elmore et al., 2014). As shown for the first time here, such tools represent a new way to limit microglial proliferation and hypothalamic inflammation in the context of dietary excess.

The role of microglia in the MBH prompts comparisons to macrophages in tissues, such as the adipose, liver, and skeletal muscle. Targeting macrophage activation ameliorates diet-induced inflammation in these tissues and insulin resistance in obese models. Diet-induced inflammation in the MBH precedes that in peripheral tissues. Our finding that microglia mediate the inflammatory impact of dietary SFAs and their effect on neuronal function in the MBH suggests that targeting hypothalamic microglia may be a promising new way to mitigate diet-induced metabolic dysfunction.

Experimental procedures

Mice

All studies used male C57BL/6 mice. Slice studies and bone marrow (BM) transplants used WT and CD11b-DTR mice, which have been used to deplete CD11b-expressing cells (Cailhier et al., 2005; Duffield et al., 2005). CX3CR1-GFP mice were used to confirm the effect of CSF1R antagonism on microglial depletion *in vivo*. Primary microglia and astrocytes were from WT mice. Mice were group housed (12h/12h light/dark) and age-matched. Mice were fed either a standard chow diet (13.2 % of calories from fat; LabDiet 5053) or a high-fat diet containing extra calories from milk fat (HFD; 42% of calories from

fat, TD.88137; Harlan-Teklad). Anesthesia was by isofluorane or by 100mg/kg ketamine and 10mg/kg xylazine or Avertin (terminal procedures). The University of California San Francisco Institutional Animal Care and Use Committee approved all procedures.

Measurements of Food Intake and Energy Metabolism

Metabolic parameters in mice were assessed using a Comprehensive Lab Animal Monitoring System (Columbus Instruments). The mice were singly housed and received water and food *ad libitum*, along with enteric supplementation with saline or milk fat twice a day (below). Cages were maintained at 20-22°C under a 12:12-h light-dark cycle (light period 07:00-19:00), and mice acclimatized for 48 hours before being studied. The cages continuously weighed food for each mouse and daily intake was measured as change in food weight over successive 24-hour periods. All data were normalized to body weight per manufacturer guideline.

Lipidomic analysis

For lipidomic analyses (Benjamin et al., 2013), mice underwent cervical dislocation, and their brains were removed and flash frozen in liquid nitrogen. Hypothalamic and cortical tissues were dissected, and nonpolar lipids extracted in chloroform:methanol:PBS (2:1:1 v:v:v;) containing 10 nmoles each of C12:0 dodecylglycerol and pentadecanoic acid (internal standards). 10μl of this extract was injected into a triple quadrupole LC-MS/MS for targeted lipidomic analysis.

Gavage Feeding

Mice on a normal *ad libitum* chow diet received intragastric gavage with 200 μl of clarified milk fat [>60% long-chain SFAs, 35% of which is C16:0 PA], or isocaloric volumes of coconut oil [>70% short-chain SFAs, mostly lauric acid (C12:0)] or olive oil [>80% unsaturated FAs, mostly oleic acid (OA, C18:1)]. Oils (vs. PBS control) were delivered by gavage needle (Instech Laboratories, Inc) in two equal doses (9:00 AM and 6:00PM) per day for 3-10 days. Each oil increased daily calories by 38%.

Bone Marrow (BM) Chimeras

Eight week-old CD11b-DTR mice were anesthetized and individually placed in lead tubes (RPI Corp.) to shield their heads and necks from irradiation (Mildner et al., 2007). The mice were lethally irradiated in two 5-Gy doses 3 hours apart and underwent BM transplant the next day by tail-vein injection with 3×10^6 BM cells flushed from the femurs and tibiae of mice that did not express DTR, thus restricting DTR expression to recipient microglia (DTR^{BMT}). Donor BM was from CCR2-RFP mice in order to track donor-derived leukocytes in recipient tissues (Saederup et al., 2010). After transplant, DTR^{BMT} and WT^{BMT} mice received antibiotics (Polymyxin B and Neomycin) for 1 month, and reconstitution efficiency confirmed by FACS.

Microglial Depletion

To specifically deplete hypothalamic microglia, DTR^{BMT} mice were injected I.P. with 10 ng/gram body weight of DT (Sigma-Aldrich) in PBS every 24 hours for 3 doses, and the

mice were dissected either 3 or 10 days after the final dose. To prevent proliferation of hypothalamic microglia following DT-induced depletion in mice receiving oil gavage, a CSF1R tyrosine kinase inhibitor, Ki20227 (30mg/kg/day in 0.5% methyl cellulose), was administrated once orally during each day of the 3-day protocol. Microglia were also depleted from mice by treatment with another CSF1R antagonist, PLX5622 (Plexxikon), formulated in AIN-76A rodent chow (Research Diets) at a dose of 1.2 g/kg. Microglia were depleted by exposure to PLX5622 in this way for 7 days.

Culture and treatment of primary glia

Glial cultures were prepared as described (Deierborg, 2013). Cerebral cortices were harvested from p1-4 pups, the meninges and blood vessels were removed, and the parenchyma minced and triturated in DMEM + GlutaMAX (Invitrogen) with 4.5 g/l glucose, 10% fetal bovine serum, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. Suspended cells were filtered (70 μm) and plated on poly-L-lysine-coated flasks with media replenished twice weekly, resulting in mixed glial monolayers. 6-10 days later, the flasks were shaken (200 rpm) for two hours (37°C) to specifically release microglia, which were re-plated. Pure astrocyte cultures were obtained either using liposomal clodronate to remove residual adherent microglia from WT glial monolayers (Kumamaru et al., 2012), or using DT (20ng/ml) for 3 days to remove microglia in glial monolayers from CD11b-DTR mice. Both microglia and astrocyte cultures were 70-80% confluent prior to treatment with FAs, which were added to serum-free medium as a 4:1 BSA complex (Listenberger et al., 2001).

Isolation of hypothalamic microglia from adult mice

Murine hypothalamic microglia were also isolated from PBS-perfused adult brains as described (Cardona et al., 2006). At least 5 hypothalami per group were pooled, digested for 30 min (37°C) with 1mg/ml collagenase and 0.1 mg/ml DNAse I, homogenized in Hank's Balanced Salt Solution, and passed through a 70 μm strainer. Homogenates were then centrifuged at 500 × g for 5 min, after which supernatants were removed and cells re-suspended in 37% isotonic Percoll. Cells were then passed through a discontinuous Percoll gradient (70%, 37%, 30% and 0% layers) by centrifugation (500 × g for 30 min), and microglia were collected from the 70-37% interphase. Extractions yielded ~5×10^5 viable cells, of which 90% were $CD11b^+/CD45^{low}$ microglia.

Hypothalamic organotypic slice culture

Organotypic hypothalamic slices were isolated and cultured essentially as described (Fukuda et al., 2008). WT and CD11b-DTR pups (p8-11) underwent rapid brain dissection and removal. Hypothalami were blocked and cut into 300 μm sections on a vibratome (VT1000 S, Leica) while floating in chilled Gey's Balanced Salt Solution with glucose (0.5%) and KCl (30 mM). Coronal slices containing the ARC and ME were then placed on 0.4 μm Millicell-CM filters (Millipore), and cultured at an air-media interface in DMEM/F12 (1:1) supplemented with heat-inactivated horse serum (25%), Glucose (32 mM) and GlutaMAX (2 mM). Cultures were maintained for 10 days, and medium was replaced 3 times a week. Slices were incubated overnight in low-serum (2.5%) medium with 2 mM GlutaMAX prior to experiments.

Depleting microglia from hypothalamic slice cultures

Microglia were depleted from hypothalamic slice cultures without altering other cell types by modifying prior approaches (Okamura et al., 2012; Vinet et al., 2012). In one method, slices were incubated with 0.5 mg/ml of liposomal clodronate (Lip-CLO) or control liposomes (Lip-C) (Encapsula NanoSciences) for 48 hours. Slices were then washed in PBS to remove residual liposomes, placed in fresh medium, and studied 48 hours later. In another method, CD11b-DTR slices were treated with 10ng/ml DT for 48 hours, after which washing and subsequent culture were as for the first method. Vehicle-treated CD11b-DTR slices and DT-treated WT slices were controls.

Immunohistochemistry

Anesthetized mice were perfused with 4% paraformaldehyde in 100 mM phosphate buffer, and their brains were dissected, post-fixed in the same fixative overnight (4°C), and immersed in 30% sucrose. Hypothalami were then separated from other regions, embedded in OCT, immediately frozen on dry ice, and stored at −80°C. 10 μm-thick hypothalamic coronal sections were cut on a cryostat, blocked for 1 hour with 5% BSA in PBS containing 0.1% Triton X-100, and incubated with primary antibodies (Supplementary Procedures) overnight at 4°C. TUNEL assays were done using the *In situ* Cell Death Detection Kit Fluorescein (Roche) per manufacturer's instructions, and sections were washed 3 times with 0.05% Tween 20 in PBS and incubated with secondary antibodies for 1 hour. Images were acquired with a fluorescence- (Olympus BX51, DP71) or confocal laser-scanning microscope (Leica TCS SP5).

Immunoblot

Proteins were extracted by homogenizing cells in RIPA buffer with 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 1mM Na_3VO_4, 1 mM PMSF, and a protease inhibitor cocktail (Sigma)] at 4°C. Homogenates were then centrifuged at 12,000 × g for 10 min, and protein concentrations from supernatants were quantified (BCA Kit; Thermo Scientific; Supplemental Procedures). Leptin-induced STAT3 phosphorylation (pSTAT3) was measured in mice fasted for 16 hours, injected with I.P. leptin (3 mg/kg) or saline, and perfused 45 min later. Hypothalamic homogenates were then made as above.

Cytokine and Chemokine Measurements

Levels were measured by ELISA (eBioscience), using manufacturer's instructions.

Real-time quantitative (qPCR)

Hypothalamic RNA was extracted in Trizol (manufacturer's instructions), quantified by spectrophotometry, and reverse-transcribed (SuperScript III; random hexamers) to make cDNA. qPCR was performed using an ABI Prism 7900 HT machine and SYBR Green detection of amplicons (Applied Biosystems). Relative mRNA abundance was normalized to cyclophilin by the ΔΔCT method per manufacturer, and analyzed using the Sequence Detection System software (SDS version 2.2; Applied Biosystems). Primer sequences are in Table S1.

Statistics

Data are presented as the mean ± SEM. Two groups were compared using 2-tailed Student's t-tests. For more than two groups, one- or two-way ANOVA was used, as appropriate, followed by *post hoc* adjustment. All analyses were performed with GraphPad software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Koliwad and Xu labs for suggestions and helpful discussions, Dr. Lauren Martens for the initial protocol for microglial culture, Drs. Kimberly Cordes and Israel Charo for CCR2-RFP mice, Dr. Qizhi Tang for CX3CR1-GFP mice, the UCSF Diabetes Center Flow Cytometry and Imaging Cores for technical assistance, and Plexxikon (Parmveer Singh and Dr. Brian West) for freely and openly providing PLX6522-containing and control diets. This work was supported by a National Institutes of Health Grant (5K08DK080174) to S.K.K. and a UCSF Diabetes Family Fund Award (to S.K.K, and A.W.X).

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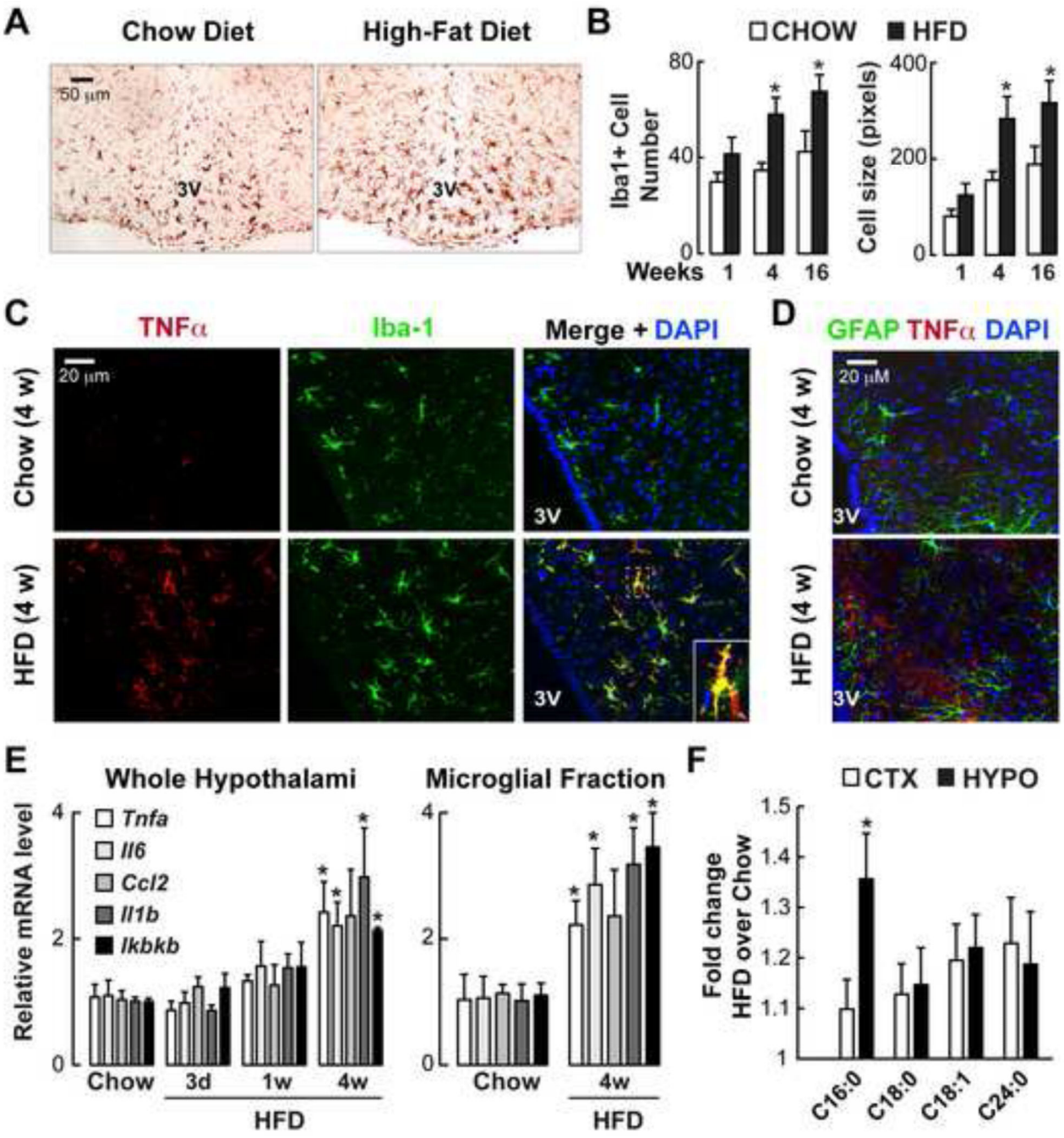

Figure 1. High-fat diet (HFD) induces the inflammatory activation of microglia specifically in the hypothalamic ARC

(A) Increased ARC staining of Iba1 in hypothalamic sections from mice fed a HFD for 4 weeks (3V: third ventricle). (B) Quantification of ARC microglial number and size in A (n=5/group *p< 0.05 vs. chow). (C) Representative immunostained ARC sections, illustrating diet-induced induction of TNF-α and its strong co-localization with Iba1 (see also inset), but not GFAP (D). (E) qPCR analysis, showing increased mRNA levels of M1 mediators, cytokines, and chemokines in whole hypothalami and microglial fractions of mice fed a 4-week HFD (n=5/group *p< 0.05 vs. chow). (F) Fold change in individual FA

levels (LC-MS) in the cerebral cortices (CTX) and hypothalami (HYPO) of mice following 4 weeks of HFD (n=5/group *p< 0.05 vs. CTX). See also Figure S1.

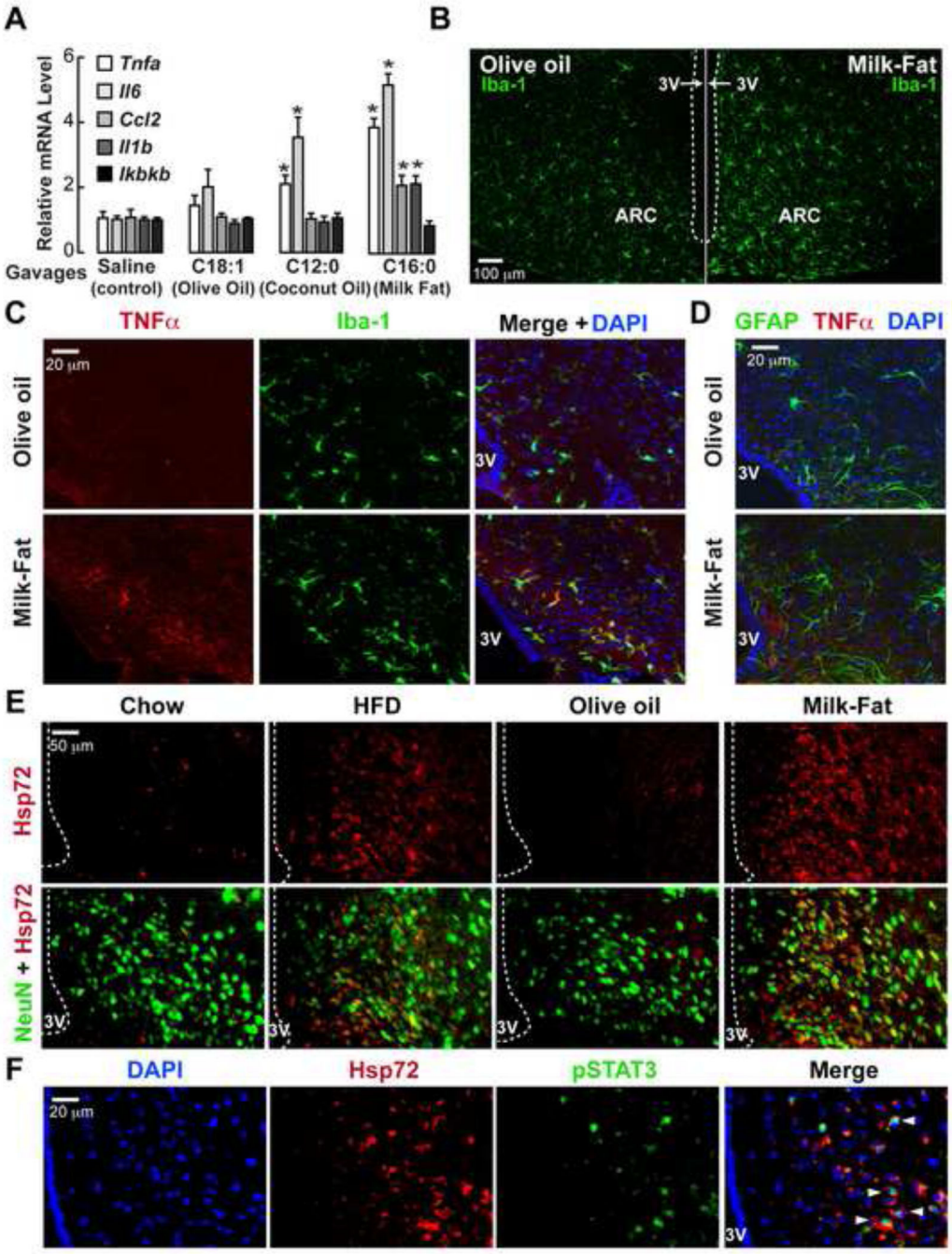

Figure 2. SFA gavage reproduces diet-induced hypothalamic inflammation and neuronal stress in mice

(A) Increased mRNA levels of M1 markers in the MBH of mice receiving isocaloric OA (C18:1; olive oil), lauric acid (C12:0; coconut oil), or PA (C16:0; milk fat) vs. saline by enteric gavage twice daily for 3 days (n=4/group *p< 0.05 vs. control). (B) Increased Iba1 staining in the ARC of mice receiving milk fat (vs. olive oil) by gavage as above. (C) Immunostaining, showing specific TNF-α induction in the ARC by milk fat gavage, and co-localization with Iba1, but not GFAP (D). (E) Induction of Hsp72 in $NeuN^+$ cells

specifically in the ARC by feeding mice a 4-week high-SFA diet or by milk fat by gavage for 3 days (vs. chow diet and olive oil gavage, respectively; n=4/group).

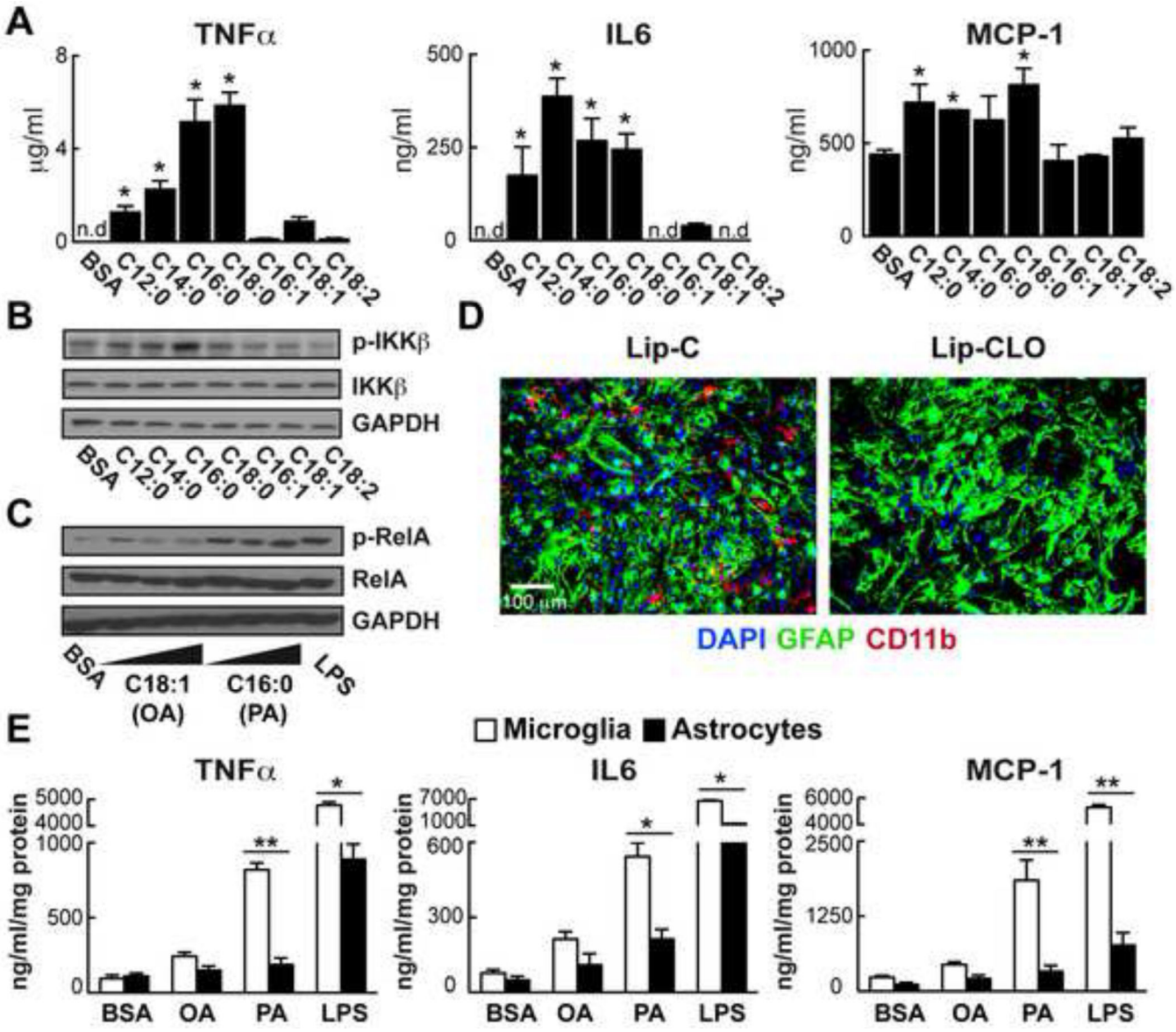

Figure 3. Long-chain dietary SFAs specifically stimulate the inflammatory activation of primary microglia

(A) M1 cytokine secretion (ELISA) by primary murine microglia treated for 24 hours with 100 μM of the listed FAs (n=3/group; *P<0.05 vs. BSA). Representative immunoblots, showing phosphorylation of (B) IKK-β and (C) p65 (Rel-A) induced specifically by treatment with SFAs between 100-300 μM (total protein and GAPDH as loading controls). (D) Immunostaining for GFAP (green), CD11b (red) and DAPI (blue) in primary astrocyte cultures treated for 72 hours with a control liposome (Lip-C) or Lip-CLO (200ng/ml), showing microglial elimination. (E) Differential M1 cytokine secretion by microglia and astrocytes treated for 24 hours with PA (100μM). LPS and OA were positive and negative controls, respectively (n=3/group, *P<0.05 or **P<0.01 vs. astrocytes). See also Figure S3.

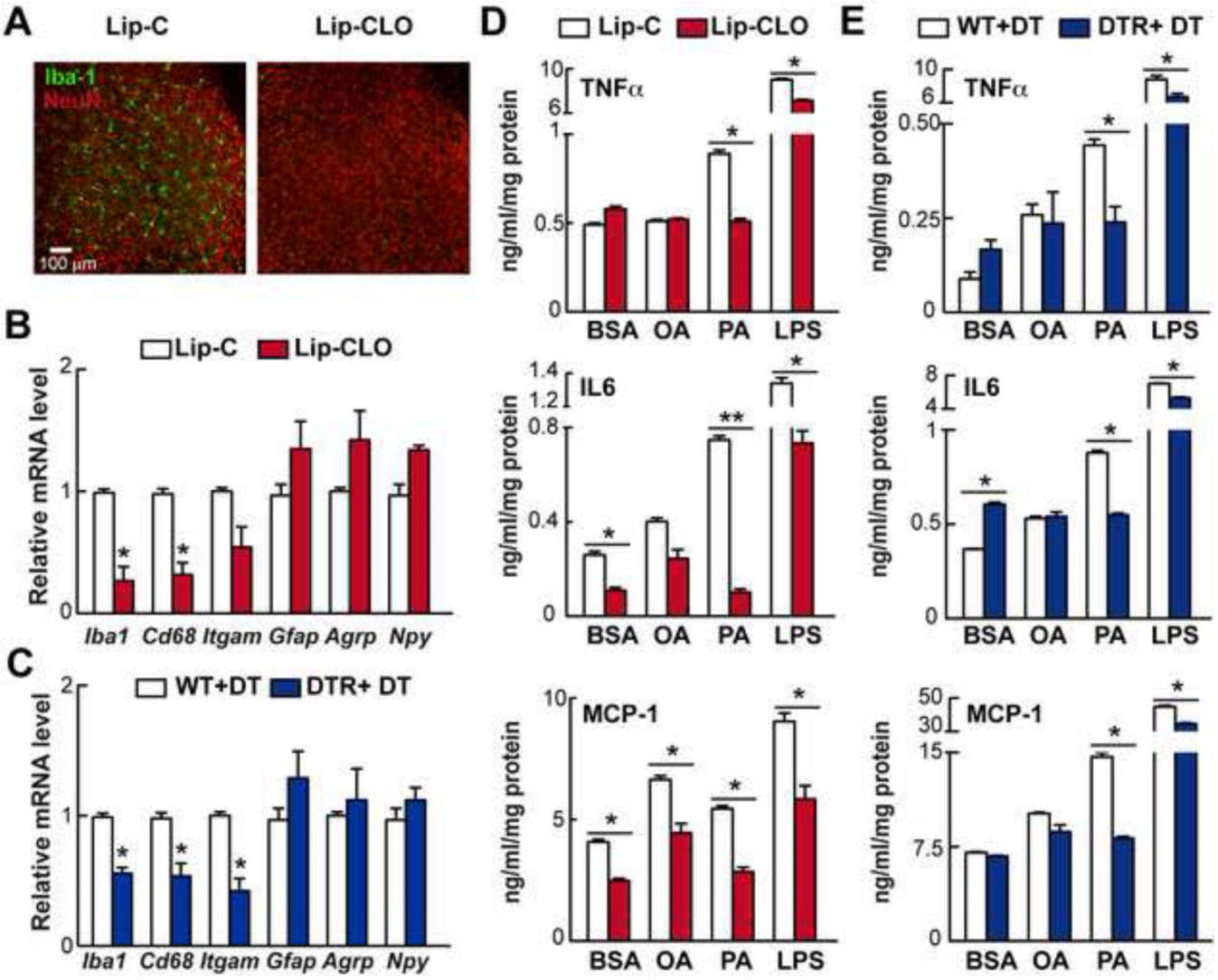

Figure 4. Microglial depletion abolishes the inflammatory response of hypothalamic slice cultures to SFA treatment

(A) Immunostaining for Iba1 (green) and NeuN (red) in 5 day-old hypothalamic slices cultures treated with either Lip-C or Lip-CLO (3 days), showing profound microglial depletion. qPCR analysis of (B) Lip-C and Lip-CLO-treated WT slice cultures and (C) DT-treated CD11b-DTR and WT slice cultures, showing reduced mRNA levels of microglial markers (*Iba1, CD68, Itgam*), but not astrocyte (*Gfap*) or neuronal (*Agrp, Npy*) markers. Microglial depletion by (D) Lip-CLO treatment (WT) or (E) DT treatment (CD11b-DTR) specifically abolishes M1 cytokine secretion by hypothalamic slices treated with 100μM PA or OA. BSA (negative control); LPS (positive control) (n=3/group, *P<0.05 or **P<0.01 vs. control or as indicated). See also Figure S4.

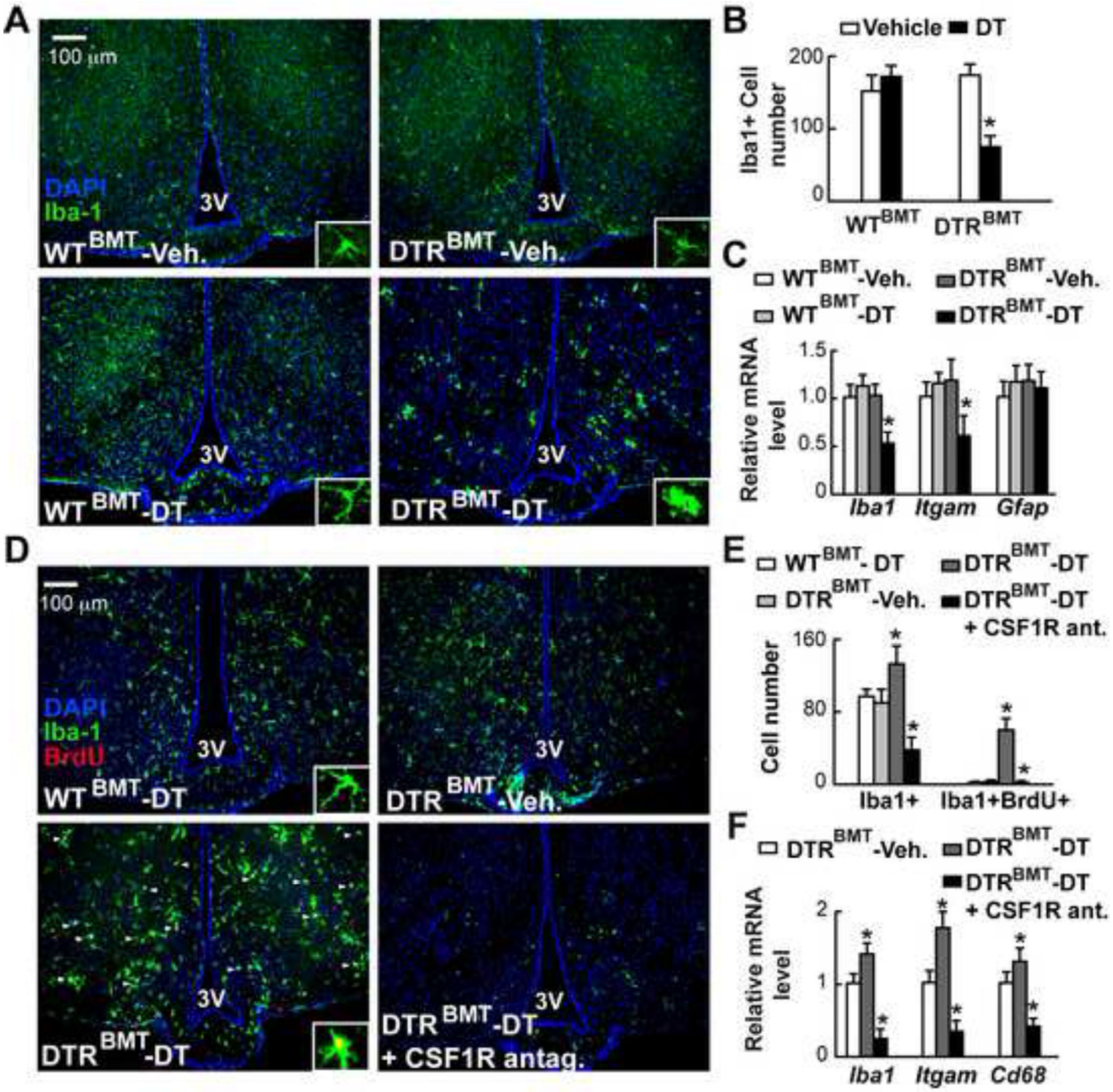

Figure 5. Selectively and stably depleting microglia in the MBH of DTR^{BMT} mice

(A) Hypothalamic sections 24 hours after BM-transplanted WT and DTR^{BMT} mice were given DT (10ng/g I.P.) daily for 3 days, showing depletion of Iba1^{+} cells (green) in DT-treated DTR^{BMT} mice along with abnormal microglial morphology (inset). Reduction in (B) hypothalamic microglial number and (C) microglial mRNA levels (*Iba1*, *Itgam*), but not the astrocyte marker *Gfap* in DT-treated DTR^{BMT} mice (n=3/group, *P<0.05 vs. control). (D) Stabilization of microglial depletion in the MBH of DTR^{BMT} mice by preventing post-depletive microglial proliferation [Iba1+/BrdU+ cells] with Ki-20227 treatment (30mg/kg/day for 3 days post-DT). (E) Quantification of D (n = 5/group). (F) Reduced

microglial mRNA levels in hypothalami of DT-treated DTR^{BMT} mice also treated with Ki20227 (*P<0.05 vs. control). See also Figure S5.

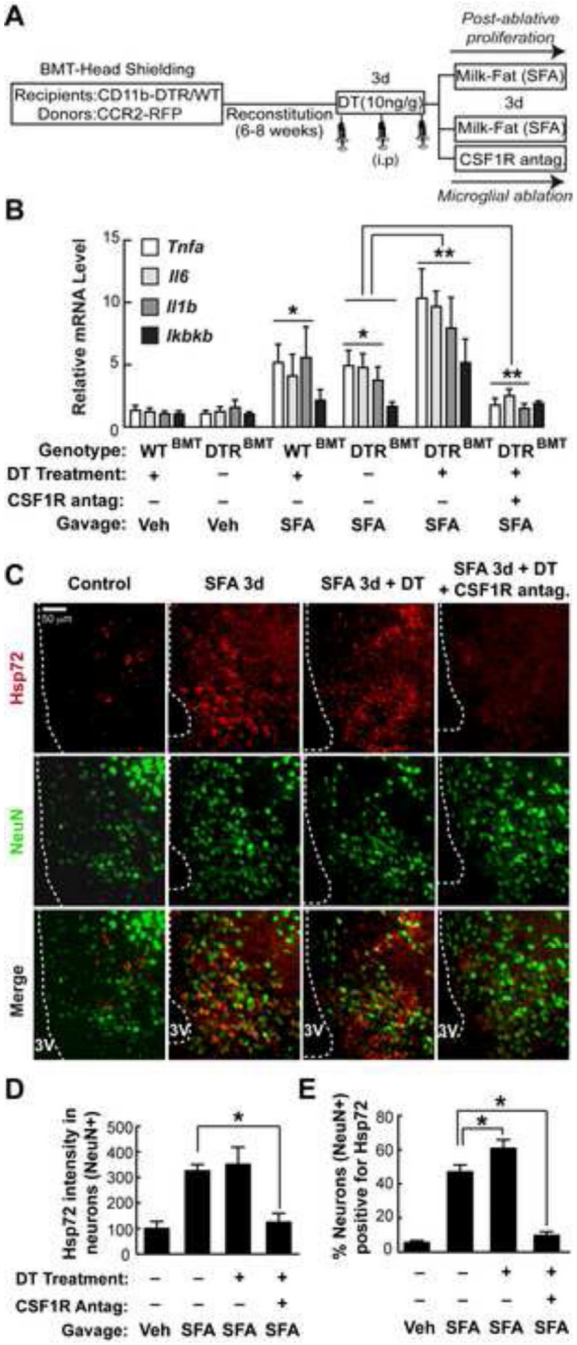

Figure 6. Hypothalamic microglial content modulates inflammatory response and neuronal injury to dietary SFAs

(A) Protocol for administering DT (10ng/g I.P.) followed by enteric SFA gavage under conditions where post-depletive microglial proliferation in the MBH was allowed to occur, or in which Ki20227-treatment sustained microglial depletion. (B) Analysis of M1 markers in DT-treated DTR^{BMT} mice, showing that the inflammatory response to SFA gavage is enhanced by increasing microglial content in the MBH and abolished by depleting microglia from the MBH (DT-treated WT and vehicle-treated CD11b-DTR mice as reference). (C) Immunostaining of ARC sections, illustrating induction of Hsp72 in $NeuN^{+}$ cells by SFA

gavage, the accentuation of SFA-induced neuronal stress by increasing basal microglial content in the MBH, and the complete prevention of SFA-induced neuronal stress by local depletion of microglia. Quantification of C, showing reduced (D) Hsp72 intensity and (E) percentage of $Hsp72^{+}/NeuN^{+}$ cells induced by microglial depletion in the MBH (n=6/group, *P<0.05 vs. matched control or as indicated and **P<0.05 vs. DTR control). See also Figure S6.

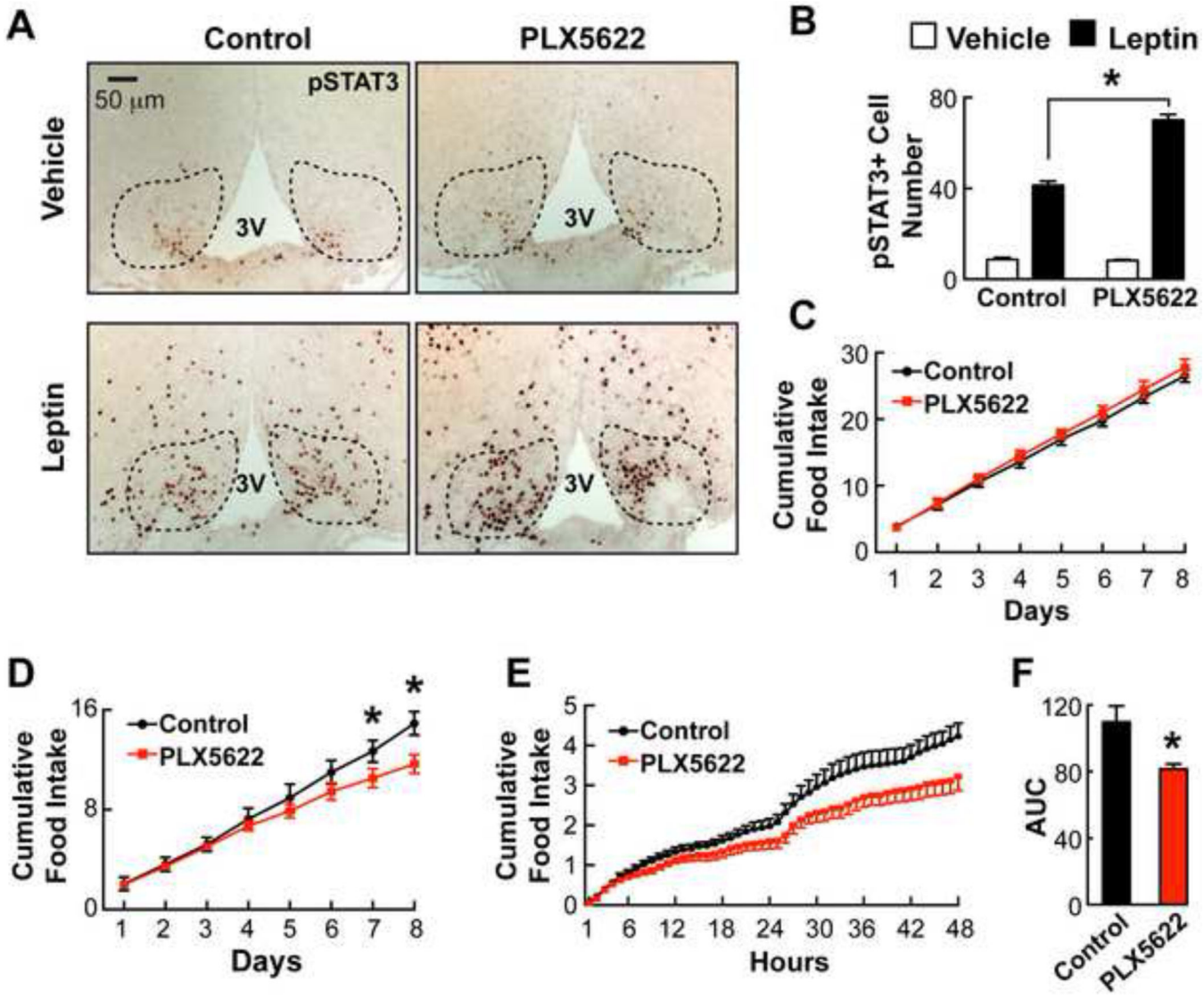

Figure 7. Microglial depletion enhances leptin signaling and decreases food intake in response to excess dietary SFAs

A) Hypothalamic sections from mice fed a chow diet or a matched diet compounded with PLX5622 for 7 days followed by continuation of the respective regimens with milk fat (twice-daily gavage) supplementation for 10 more days, showing pSTAT3 staining 45 min after injection with I.P. leptin (3mg/kg) or vehicle B) pSTAT3 quantification, showing enhanced leptin responsiveness in the ARC of mice consuming excess milk fat in the setting of microglial depletion. C) Equivalent food intake in PLX5622-treated and control mice consuming chow alone. D) Reduced food intake in PLX5622-treated mice receiving SFA gavage for 8 days. E) Reduced food intake measured by metabolic cages in PLX5622-treated mice with excess SFA intake. F) Area under the curve (AUC) analysis for E. G) RER (CO_2 exhaled/O_2 inhaled) and H) AUC analysis of RER during light and dark cycles for G. I) Energy expenditure (heat) and J) AUC analysis of energy expenditure during light and dark cycles for I. (n=6 mice/group, *P< 0.05). See also Figure S7.