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High fat diet increases cognitive decline and neuroinflammation in a model of orexin loss

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Abstract

Midlife obesity is a risk factor for cognitive decline and is associated with the earlier onset of Alzheimer's disease (AD). Diets high in saturated fat potentiate the onset of obesity, microglial activation, and neuroinflammation. Signaling deficiencies in the hypothalamic peptide orexin and/or orexin fiber loss are linked to neurodegeneration, cognitive impairment, and neuroinflammation. Prior studies show that orexin is neuroprotective, suppresses neuroinflammation, and that treatment with orexin improves cognitive processes in orexin/ataxin-3 (O/A3) mice, a transgenic mouse model of orexin neurodegeneration. Our overall hypothesis is that loss of orexin contributes to high fat diet (HFD)-induced hippocampal neuroinflammation and cognitive decline. To examine this, we tested male O/A3 mice (7–8 mo. of age) in a two-way active avoidance (TWAA) hippocampus-dependent memory task. We tested whether 1) orexin loss impaired cognitive function; 2) HFD worsened cognitive impairment; and 3) HFD increased microglial activation and neuroinflammation. O/A3 mice showed significant impairments in TWAA task learning vs. wild type (WT) mice (increased escapes $p < 0.05$, reduced avoidances $p < 0.0001$). Mice were then placed on HFD (45% total fat, 31.4% saturated fat) or remained on normal chow (NC; 4% total fat and 1% saturated fat), and TWAA was retested at 2 and 4 weeks. Learning impairment was evident at both 2 and 4 weeks in O/A3 mice fed HFD for following diet exposure vs. WT mice on normal chow or HFD (increased escapes, reduced avoidances $p < 0.05$). Additionally, O/A3 mice had increased gene expression of the microglial activation marker Iba-1 (measured via qRT-PCR, $p < 0.001$). Further characterization of the microglial immune response genes in hippocampal tissue revealed a significant increase in CX3 chemokine receptor 1 (CX3CR1), tumor necrosis factor- α (TNF- α) and the mitochondria-associated enzyme immune responsive gene-1 (Irg1). Collectively, our results indicate that orexin loss impairs memory, and that HFD accelerates hippocampus-dependent learning deficits and the onset of neuroinflammation.

Keywords

Microglia; Neuroinflammation; Two-Way Active Avoidance Memory Task; Obesity; High Fat Diet

Introduction

Obesity is coupled with peripheral and central chronic low-grade inflammation (Businaro, Ippoliti, Ricci, Canitano, and Fuso, 2012; Uranga, Bruce-Keller, Morrison, Fernandez-Kim, Ebenezer, Zhang, Dasuri, and Keller, 2010). Dietary fats increase the risk of developing obesity and cognitive disorders such as Alzheimer's disease (AD) (Anstey, Cherbuin, Budge, and Young, 2011; Businaro et al., 2012; Xu, Atti, Gatz, Pedersen, Johansson, and Fratiglioni, 2011). Prolonged overnutrition and excess intake of saturated fatty acids (SFA), specifically palmitic acid (PA) increase neuroinflammation and microglial activation (Cai, 2013; Cai and Liu, 2011; Karmi, Iozzo, Viljanen, Hirvonen, Fielding, Virtanen, Oikonen, Kemppainen, Viljanen, Guiducci, Haaparanta-Solin, Nagren, Solin, and Nuutila, 2010; Thaler, Yi, Schur, Guyenet, Hwang, Dietrich, Zhao, Sarruf, Izgur, Maravilla, Nguyen, Fischer, Matsen, Wisse, Morton, Horvath, Baskin, Tschop, and Schwartz, 2012; Valdearcos, Robblee, Benjamin, Nomura, Xu, and Koliwad, 2014). Neuroinflammation, accompanied with progressive neuronal loss, is known to be heightened in cognitive decline and obesity (Stranahan, Cutler, Button, Telljohann, and Mattson, 2011). Moreover, neuroinflammation and microglial activation represent a common link between age-related neurodegenerative diseases and metabolic related disorders.

Microglia (resident immune cells of the brain) play a dynamic role in responding to changes within the surrounding environment leading to constant transitioning between phenotypes (Perry and Teeling, 2013). Microglia have recently been appreciated to have an important role in cognition (Chung, Welsh, Barres, and Stevens, 2015). In the healthy brain, microglia are constantly surveying the surrounding microenvironment to promote structural formation and elimination of neuronal synapses, a process especially important in the hippocampus (Chung et al., 2015). One mechanism in which this could occur is through orexin signaling. Orexin A and B (OXA; hypocretin 1 and OXB; hypocretin 2 respectively) are ligands for the G-protein coupled receptors, orexin receptor 1 and 2 (OX1R and OX2R respectively). Orexin neurons project throughout the central nervous system (CNS), including the hippocampus, and are responsible for mediating cognitive processes, operant tasks, and fear conditioning (Deadwyler, Porrino, Siegel, and Hampson, 2007; Peyron, Tighe, van den Pol, de Lecea, Heller, Sutcliffe, and Kilduff, 1998; Rolls, Colas, Adamantidis, Carter, Lanre-Amos, Heller, and de Lecea, 2011; Sears, Fink, Wigstrand, Farb, de Lecea, and Ledoux, 2013; Sharf, Sarhan, Brayton, Guarnieri, Taylor, and DiLeone, 2010; Thorpe, Cleary, Levine, and Kotz, 2005). Additionally, orexin signaling is altered in AD patients (Kang, Lim, Bateman, Lee, Smyth, Cirrito, Fujiki, Nishino, and Holtzman, 2009; Roh, Jiang, Finn, Stewart, Mahan, Cirrito, Heda, Snider, Li, Yanagisawa, de Lecea, and Holtzman, 2014). Moreover, we and others have demonstrated that orexin loss impairs memory, supporting that orexin mediates cognitive function (Mavanji, Butterick, Duffy, Nixon, Billington, and Kotz, 2017; Yang, Zou, Xiong, Pascual, Xie, Malik, Xie, Sakurai, and Xie, 2013).

Recent studies highlight novel functions of OXA, including neuroprotection and decreased apoptosis and neuroinflammation via activation of orexin receptors (Butterick, Nixon, Billington, and Kotz, 2012; Duffy, Nixon, and Butterick, 2016; Sokolowska, Urbanska, Bieganska, Wagner, Ciszewski, Namiecinska, and Zawilska, 2014; Sokolowska, Urbanska, Namiecinska, Bieganska, and Zawilska, 2012; Xiong, White, Xu, Yang, Sun, Zou, Pascual,

Sakurai, Giffard, and Xie, 2013). Data indicate that orexin-induced neuroprotection could rely upon microglial modulation (Duffy, Yuan, Wisdorf, Billington, Kotz, Nixon, and Butterick, 2015; Xiong et al., 2013). In a cerebral ischemia model, pretreatment with OXA reduces infarct size through a microglial-mediated pathway (Xiong et al., 2013). Additionally, chronic exposure to the potent pro-inflammatory agonist lipopolysaccharide (LPS), reduced orexin signaling indicating orexin has a role in the inflammatory response (Grossberg, Zhu, Leininger, Levasseur, Braun, Myers, and Marks, 2011). Microglia may also become more sensitive to orexin signaling after activation (Duffy et al., 2015). LPS increases TNF- α (tumor necrosis factor- α) in microglia, but also increases OX1R expression, and OXA treatment prior to LPS exposure reduces TNF- α in microglia (Xiong et al., 2013). We have also demonstrated that PA increases OX1R, and that PA treatment causes microglia to shift toward a pro-inflammatory state (Duffy et al., 2015). However, when microglia are pretreated with OXA prior to PA exposure, the PA-induced pro-inflammatory response is attenuated (Duffy et al., 2015). These data indicate increased sensitivity to OXA may allow orexin-mediated attenuation of the toxic microglial phenotype to a protective anti-inflammatory phenotype.

Our overall hypothesis is that orexin effects on memory depend in part upon immunomodulatory control of microglia. To test this, we used orexin/ataxin-3 (O/A3) mice, a rodent model that gradually loses orexin-producing neurons (~84% by 6 mo age) (Hara, Beuckmann, Nambu, Willie, Chemelli, Sinton, Sugiyama, Yagami, Goto, Yanagisawa, and Sakurai, 2001). The O/A3 mice were maintained on a HFD for 4 weeks. We then sought to determine if orexin loss alters neuroinflammation and cognition.

Methods

Animals

7–8-month-old male wild type (WT) and orexin ataxin-3 (O/A3) mice were obtained from a breeding colony at the Minneapolis VA Health Care System (VAHCS). The colony, maintained by Dr. Catherine Kotz, originated from founder animals obtained from Dr. Masashi Yanagisawa at the University of Texas-Southwestern, bred to wild-type C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME USA). Genotype was confirmed using PCR as described in (Hara et al., 2001). Mice were maintained in a 12:12 hour light/dark cycle in a temperature controlled room (21–22°C), and were group-housed until the beginning of behavioral testing. Normal chow (NC; Teklad 8604 Lab Chow; Harlan, Indianapolis, IN USA) or high fat diet (HFD; Research Diets D12452, 45% fat; New Brunswick, NJ USA) and water were provided *ad libitum*. Body weights and food intake were measured thrice weekly. Body composition was recorded using an EchoMRI 700 (Echo Medical Systems, Houston, TX USA) before HFD exposure and 14 and 28 days following HFD exposure, as previously described (Nixon, Zhang, Wang, Kuskowski, Novak, Levine, Billington, and Kotz, 2010). Mice were randomly divided into 4 groups and given either HFD or remained on NC (WT+NC, n = 8; WT+HFD, n = 8; O/A3+NC, n = 9; O/A3+HFD n = 11). The experimental protocol was approved by the Institutional Animal Care and Use Committee at the Minneapolis VAHCS.

Two-Way Active Avoidance (TWAA) Task

Mice were placed in the automated two-way shuttle box (size, 45.7 × 20.3 × 30.5 cm; model 75-FSFX-Fusion; AccuScan Instruments, Columbus, OH USA) and allowed to acclimate for 2 min. Mice were able to move freely throughout the shuttle box during acclimation and training trials. Mice were trained on a massed 20-trial shuttle box two-way active avoidance task. The procedures for the conditioned stimulus and unconditioned stimulus were as follows. A tone (5000 Hz; 45 dB) and a pulsatile light (2.5 Hz) were presented as a conditioned stimulus in the compartment with the animal, paired 5 s later with a 0.3 mA scrambled foot shock (unconditioned stimulus) delivered through the floor grid (steel rods 0.5 cm in diameter, spaced 1.5 cm between centers). The “scrambled” nature of the shock prevents the animals from finding a “no-shock” position on the floor. To avoid receiving a foot shock the mouse had 5 s to move to the opposite compartment. If the animal did not move to the other compartment, the unconditioned stimulus was delivered for a maximum of 7 s, and the conditioned stimulus ended with the unconditioned stimulus. While receiving the unconditioned stimulus, if the animal moved to the other compartment, both conditioned and unconditioned stimuli ended immediately. The inter-trial interval was variable with a mean of 40 s. A computer using remote monitoring system software (Fusion software) was used to control experimental protocols and data collection (Datta, Saha, Prutzman, Mullins, and Mavanji, 2005; Mavanji et al., 2017; Noble, Mavanji, Little, Billington, Kotz, and Wang, 2014; Rodriguez and Wetsel, 2006).

TWAA Data Analysis

To measure performance on the TWAA task, each trial was analyzed for successful shock avoidances and shock latency. Experimental data were analyzed using two-factor (phenotype × diet) ANOVA, followed by Holm-Sidak post-hoc analysis for multiple comparisons. Significance was considered to be achieved where $p < 0.05$. Analyses were performed to determine, a) the differences in acquisition between phenotypes, and b) differences in retention between treatment groups. The improvement of performance between training trials (first session) and test trials (all sessions thereafter) were analyzed for avoidances (crossing after conditioned stimulus, but before shock delivery) and for escape latency (interval between conditioned stimulus and a response). For all experiments, mice underwent 20 trials for training, 24 h later they were retested for TWAA response. Response during the 20 training trials was considered acquisition, while response during the tests 24 h, 14 days, and 28 days later was considered retention. Following 14 d and 28 d of diet exposure mice were retested for TWAA response and body composition. These parameters were also compared between groups during test sessions to see if there was any specific effect of treatment (NC vs. HFD) on memory retention. Statistics were performed using GraphPad Prism 7 (Datta et al., 2005; Noble et al., 2014).

Locomotor activity

Open field chamber tests are commonly used to measure exploration, anxiety, and general locomotor activity. While exploratory activity in a novel environment is expected from rodents, prey animals such as mice can also exhibit anxiety when exposed to an open chamber (Sestakova, Puzserova, Kluknavsky, and Bernatova, 2013). For these tests, mice

were placed in a square chamber (27.3 cm × 27.3 cm × 20.3 cm; Med-Associates). The chamber is divided into a grid by infrared beams. An automated system monitors beam breaks to determine exploratory activity. Mice were allowed to explore for 2 h. All tests were conducted 5 h after lights on.

Reverse transcription polymerase chain reaction (qRT-PCR)

Hippocampal tissue was rapidly dissected from WT and O/A3 mice sacrificed during the light phase (5–8 h after lights on), a time period when orexin expression is expected to be low in a nocturnal animal (Martinez, Smale, and Nuñez, 2002). Total RNA was isolated with the aid of TRIzol reagent (Invitrogen). Real-time thermocycling was performed on ABI 7900HT Fast Real Time PCR System (Applied Biosystems) by two-step RT-PCR using SYBR green. Genes analyzed are indicated in table 1. Data analysis was performed using Sequence Detection System software from Applied Biosystems. The experimental Ct (cycle threshold) was calibrated against the endogenous control product GAPDH. Samples were analyzed for relative gene expression by the 2^{-Ct} method (Pfaffl, 2001).

Results

High fat diet increases obesity in orexin/ataxin-3 mice

We first sought to determine the effects of HFD on food intake, body weight gain and body composition. WT and O/A3 mice have increased cumulative food intake on days 6–10 (Fig 1A). Body weight gain is significantly increased in O/A3-HFD mice compared to WT-NC beginning at day 6 and continue to be significant through day 28 ($p < 0.01$). WT-HFD mice have significantly increased body weight compared to WT-NC at day 8 through day 28 ($p < 0.05$). Both WT and O/A3 mice have increased body weight compared to O/A3-NC and WT-NC at day 10 through day 28 (Fig 1B). Despite no significant difference in body weight, O/A3-NC mice have increased body fat compared to WT-NC mice on days 1, 14, and 28 (Fig 1C). HFD-fed O/A3 and WT mice have increased body fat compared to NC-fed WT and O/A3 mice on days 14 and 28 (Fig 1C).

High fat diet reduces locomotor activity but does not impair anxiety like behavior

To determine if changes in body fat and weight were due in part to orexin signaling, we analyzed locomotor activity in mice following 4 weeks HFD or NC. We demonstrate that locomotor activity is lower in O/A3-NC and reduced even further in O/A3-HFD mice (Fig 2A; $p < 0.001$). To determine if anxiety-like behavior was altered in response to lack of orexin signaling or HFD, the first 5 min of activity was analyzed. We demonstrate that anxiety-like behavior (open field test) is not impaired in WT or O/A3 mice (Fig 2B).

High fat diet impairs cognitive function in mice lacking orexin

Previous studies indicate that orexin is integral in cognitive function (Aitta-Aho, Pappa, Burdakov, and Apergis-Schoute, 2016; Mavanji et al., 2017; Yang et al., 2013). We sought to determine if mice lacking orexin maintained on HFD have impaired cognition. We demonstrated that there was no difference in learning ability between WT and O/A3 mice. When tested 24 h later, cognitive impairment was evident as measured via reduced avoidances and increased latency in O/A3 mice (Fig 2C–D, $p < 0.01$ vs. WT-NC). Mice were

then placed on HFD or maintained on NC. Long term memory was tested following 14 and 28 d of HFD exposure. We demonstrate that HFD significantly impairs long term memory in O/A3 mice as indicated by reduced avoidances and increased latency (Fig 2C–D; * $p < 0.05$ vs. WT-NC, WT-HFD, O/A3-NC; ** $p < 0.01$ vs. WT-NC, WT-HFD, O/A3-NC).

Inflammatory genes are altered in response to HFD

Our previous data indicate that orexin A attenuates PA-induced microglial inflammation (Duffy et al., 2015). We demonstrate that hippocampal orexin 1 receptor is upregulated in mice fed HFD but orexin 2 receptor is unchanged (Fig. 3A–Fig. 3AB, * $p < 0.05$, ** $p < 0.01$ vs. WT-NC). Iba1, a marker of microglial activation, is increased in HFD-fed mice regardless of genotype, and in O/A3-NC mice (Fig 3C, * $p < 0.05$, ** $p < 0.01$ vs. WT-NC). Pro-inflammatory marker TNF- α is increased in O/A3 mice (Fig 3D * $p < 0.05$ vs. WT-NC, # $p < 0.05$, ## $p < 0.01$ vs. WT-HFD). CX3CR1 is upregulated following HFD exposure (Fig 3E, * $p < 0.05$, ** $p < 0.01$ vs. WT-NC). Irg1 expression is upregulated following HFD exposure (Fig 3F, * $p < 0.05$, ** $p < 0.01$ vs. WT-NC).

Discussion

High fat diets and obesity increase neuroinflammation, neurodegeneration, and cognitive decline (Norden and Godbout, 2013; Stranahan et al., 2011; Uranga et al., 2010; Zhang, Li, Purkayastha, Tang, Zhang, Yin, Li, Liu, and Cai, 2013). While hippocampal neuroinflammation normally increases with age, risk of neuroinflammation and cognitive impairment is exacerbated by chronic consumption of diets high in PA (Norden and Godbout, 2013; Uranga et al., 2010). Orexins are important mediators in cognitive function and metabolic function (Aitta-Aho et al., 2016; Mavanji et al., 2017; Yang et al., 2013). Moreover, pro-inflammatory stimuli have been shown to reduce orexin signaling (Gaykema and Goehler, 2009; Grossberg et al., 2011; Ogawa, Irukayama-Tomobe, Murakoshi, Kiyama, Ishikawa, Hosokawa, Tominaga, Uchida, Kimura, Kanuka, Morita, Hamada, Takahashi, Hayashi, and Yanagisawa, 2016; Xiong et al., 2013). Here, we demonstrate that HFD increases body weight gain and fat mass despite genotype (Fig 1). In mice lacking orexin, increased body weight and fat mass is observed compared to WT mice maintained on NC (Fig 1). The loss of orexin also resulted in reduced locomotor activity and this effect is enhanced with exposure to HFD (Fig 2).

It is well accepted that orexins have a distinct role in the regulation of energy metabolism (Sakurai, 2005), and that loss of orexin signaling contributes to increased obesity in part via reduced energy expenditure and movement (Hara et al., 2001). Orexin has pleiotropic effects in the brain as the neurons project to regions involved in cognition, executive function, and learning and memory (Akbari, Naghdi, and Motamedi, 2007; Nixon and Smale, 2007; Yang et al., 2013). Orexin loss is the causal process in the neurodegenerative disease narcolepsy, and loss of orexin has been implicated in comorbidities such as sleep disturbances or metabolic changes noted in other neurodegenerative diseases such as Parkinson's, Alzheimer's, and Huntington's disease (Fronczek, Overeem, Lee, Hegeman, van Pelt, van Duinen, Lammers, and Swaab, 2007; Fronczek, van Geest, Frolich, Overeem, Roelandse, Lammers, and Swaab, 2012; Petersen, Gil, Maat-Schieman, Bjorkqvist, Tanila, Araujo,

Smith, Popovic, Wierup, Norlen, Li, Roos, Sundler, Mulder, and Brundin, 2005; Thannickal, Lai, and Siegel, 2007). While orexin loss has previously been considered largely to be incidental in these latter neurodegenerative diseases, data suggest that orexin loss may actually contribute to disease progression. We and others have demonstrated that OXA exhibits neuroprotective effects (Butterick et al., 2012; Duffy et al., 2016; Duffy et al., 2015; Sokolowska et al., 2014). The loss of orexin could be a mechanism through which the brain becomes more susceptible to neuronal insults and increased degree of neuroinflammation. We demonstrate that mice lacking orexin neurons have increased cognitive deficits, and that exposure to HFD results in impaired long term memory in these mice (Fig 2). We show that both HFD exposure and loss of orexin increase markers of inflammation (Fig 3). Moreover, the upregulation of neuroinflammatory expression indicates that HFD accelerates hippocampal-dependent memory deficits and the onset of neuroinflammation in O/A3 mice. Our results are consistent with prior studies demonstrating upregulation of genes that regulate microglia immune function, including CX3C chemokine receptor 1 (CX3CR1) and the mitochondria-associated enzyme immune responsive gene-1 (Irg1), in response to reduced orexin signaling and pro-inflammatory stimuli (Heydendael, Sengupta, and Bhatnagar, 2012; Lampropoulou, Sergushichev, Bambouskova, Nair, Vincent, Loginicheva, Cervantes-Barragan, Ma, Huang, Griss, Weinheimer, Khader, Randolph, Pearce, Jones, Diwan, Diamond, and Artyomov, 2016). Additionally, the CX3CL1-CX3CR1 system, important in modulating hippocampus-specific memory tasks and synaptic plasticity (Paolicelli, Bisht, and Tremblay, 2014; Sheridan, Wdowicz, Pickering, Watters, Halley, O'Sullivan, Mooney, O'Connell, O'Connor, and Murphy, 2014), is perturbed in HFD exposure, neuroinflammation, aging, and AD (Fuhrmann, Bittner, Jung, Burgold, Page, Mitteregger, Haass, LaFerla, Kretschmar, and Herms, 2010; Lyons, Lynch, Downer, Hanley, O'Sullivan, Smith, and Lynch, 2009; Morari, Anhe, Nascimento, de Moura, Razolli, Solon, Guadagnini, Souza, Mattos, Tobar, Ramos, Pascoal, Saad, Lopes-Cendes, Moraes, and Velloso, 2014; Paolicelli et al., 2014). The CX3CL1-CX3CR1 system could represent a target for microglial-neuronal cross talk and microglial-mediated inflammation. Moreover, the neuroprotective immunomodulatory effects of orexin occur via CX3CL1-CX3CR1 signaling. Here we show mice exposed to HFD also have increased OX1R expression (Fig 3A). This is in agreement with our previous data demonstrating that OX1R expression is upregulated in microglia in response to PA, indicating that OXA effects primarily occur via OX1R signaling (Duffy et al., 2015; Mavanji et al., 2017). Increased OX1R expression could be a mechanism in which microglia enhance orexin responsivity therefore enhancing the capability to appropriately respond to inflammatory insults.

Future studies will focus on the effects of orexin replacement therapy in O/A3 mice, using exogenous orexin to directly test if orexin improves cognition through a neuronal-glial mechanism. Orexin is necessary for consolidation of hippocampal dependent memory (Yang et al., 2013). Orexin treatment has been shown to improve cognitive performance on a variety of tasks (Aitta-Aho et al., 2016; Deadwyler et al., 2007; Flores, Valls-Comamala, Costa, Saravia, Maldonado, and Berrendero, 2014; Palotai, Telegdy, Ekwerike, and Jaszberenyi, 2014; Sharf et al., 2010; Song, Kim, Kim, Song, and Lee, 2015; Thorpe et al., 2005), potentially via changes in long term potentiation (LTP). Other reports have demonstrated that orexin delivery to the CA1 region of the hippocampus improves cognition

via increased LTP (Deadwyler et al., 2007; Yang et al., 2013; Zhao, Zhang, Tang, Ren, Yang, Liu, and Tang, 2014). Microglia have recently been appreciated to have a role in LTP and hippocampal cognition. Additionally, HFDs alter hippocampal LTP and microglial response (Hao, Dey, Yu, and Stranahan, 2016). It is therefore reasonable to predict that orexin effects on cognition depend in part on modulation of microglia. To the best of our knowledge, we are the first to define a pathway linking orexin to phenotypic changes in hippocampal microglia. The data presented here indicate a targetable pathway to reverse diet-induced cognitive decline and treatment for neurodegenerative diseases involving orexin loss.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. High fat diet increases body weight gain and fat mass.

WT and O/A3 mice were given HFD or NC for 28 d. Cumulative food intake was increased after 6, 8, and 10 d HFD exposure regardless of genotype (**A**, HF-fed mice $*p < 0.05$ vs NC-fed mice). Mice fed HFD had significantly increased body weight gain days 10–28 compared to mice fed NC (**B**, HF-fed $*p < 0.001$ vs. WT-NC, $\#p < 0.01$ vs. O/A3-NC). Chow-fed O/A3 mice have a higher percent body fat than WT mice (OA3-NC $*p < 0.01$, $***p < 0.001$ vs WT-NC). Following 14 and 28 d on HFD, both WT and O/A3 mice have significantly more body fat than do WT and O/A3 mice maintained on NC (**C**, HF-fed mice $***p < 0.0001$ vs. NC-fed mice).

Figure 2A-B. High fat diet reduces locomotor activity and impairs cognitive function, but does not impair anxiety like behavior, in mice lacking orexin.

In open-field tests (**A-B**), WT mice move significantly more compared to O/A3 mice fed NC or HFD (**A**, O/A3-HFD * $p < 0.05$ vs. WT; O/A3-NC # $p < 0.05$ vs. WT; OA3-HFD \$ $p < 0.05$ vs. OA3-NC). Anxiety-like behavior (**B**) is not different between strains, as there were no differences in time spent in the chamber center. In Two-Way Active Avoidance task (TWAA, **C-D**), as indicated by training there is no difference in learning ability between WT and O/A3 mice. However, significant differences in short- and long-term retention were observed. When tested for retention 24 h after training, cognitive impairment in OA/3 is evident via reduced avoidances (**C**) and increased latency (**D**, $p < 0.01$ vs. WT-NC). HFD significantly impairs long-term memory in O/A3 mice as indicated by reduced avoidances and increased latency vs. WT mice (**C-D**; * $p < 0.05$ vs. WT-NC, WT-HFD, O/A3-NC; ** $p < 0.01$ vs. WT-NC, WT-HFD, O/A3-NC).

Figure 3. Inflammatory genes are altered in response to HFD.

HFD but not orexin loss increases orexin 1 receptor (A). Orexin 2 receptor levels are unchanged by diet or genotype (B). HFD and loss of orexin induces microglial activation via increased Iba1 (C). CX3CR1 is upregulated following HFD exposure (D). The pro-inflammatory marker TNF- α is increased in O/A3 mice (E). Irg1 expression is upregulated following HFD exposure (F). In all panels, * $p < 0.05$ vs. WT-NC; ** $p < 0.01$ vs. WT-NC; # $p < 0.05$ WT-HFD.

Table 1.

qPCR Primers (5' → 3')

Gene	Forward Primer	Reverse Primer
OX1R (NM_198959.2)	CTCATAGCCTTG GTGGCAA	ACCGACACAGCCTGGAGATA
OX2R (NM_198962.3)	CACGGACTATGACGACGAGG	AGAGCCACAACGAACACGAT
CX3CR1 (NM_009987)	CCGCCAACTCCATGAACAAC	GGATGAGTCTGACGGCTCTG
Iba1 (NM_019467.2)	GGAGATTTCAAAAGCTGATGTGGA	CCTCAGACGCTGGTTGTCTT
TNF- α (NM_001278601)	AGGCACTCCCCAAAAGATG	CCACTTGGTGTTTGTGAGTG
Irg1 (NM_008392.1)	TATGCCAACTACTCCCCCGA	CGGGAAGCTCTTAAAGGCCA