Published in final edited form as:

Pain. 2016 January ; 157(1): 235–246. doi:10.1097/j.pain.0000000000000355.

Abnormal trigeminal sensory processing in obese mice

Heather L Rossi, PhD^{a,b,c}, Kimberly A Broadhurst, BS^c, Anthony SK Luu, BS^c, Orlando Lara, BS^c, Sunny D Kothari, BS^c, Durga P Mohapatra, PhD^d, and Ana Recober, MD^{a,b,c,*}

^aDepartment of Neurology, The Children's Hospitals of Philadelphia and the University of Pennsylvania, Philadelphia, PA 19104

^bDepartment of Pediatrics at The Children's Hospitals of Philadelphia and the University of Pennsylvania, Philadelphia, PA 19104

^cDepartment of Neurology, Carver College of Medicine, University of Iowa, Iowa City, IA 52240

^dDepartment of Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, IA 52240

1. Introduction

Obesity is associated with chronic pain^{18,26,44,47}, yet this relationship remains understudied despite its significant burden to individuals and society. Importantly, the impact of obesity on pain is not restricted to weight bearing structures. There is strong epidemiological evidence of an association between migraine and obesity.^{3,4,35} Obese migraineurs have more frequent and severe headaches, and report more photophobia than migraine sufferers with normal weight.^{2,51} Photophobia, or sensitivity to light, is not exclusive of migraine and is commonly associated with other primary headache disorders such as cluster headache, tension-type headaches, and also with secondary headaches, such as post-traumatic headache or subarachnoid hemorrhage. Photophobia has also been reported in trigeminal neuralgia, in some cases even when the pain distribution spared the first division of the trigeminal nerve.¹⁷ Human studies have demonstrated a strong relationship between photophobia pathways and the trigeminal system in migraine patients and healthy subjects.^{5,12,14–16,20,29,43} In migraineurs, photophobia is enhanced by trigeminal pain^{14,15} and pain thresholds in all trigeminal dermatomes are decreased by exposure to light.²⁰ In healthy subjects, sodium chloride injection into the frontalis muscle enhanced involuntary winking on exposure to bright light.¹⁶

From neuroanatomical and electrophysiological studies in rats, we know that light and trigeminal afferents potentially interact within the eye itself¹³, that bright light evokes activity in second order neurons of the trigeminal nucleus caudalis^{31,32}, and that a subset of third order neurons in the thalamus respond to light and dural stimulation.³⁰ However,

* **Corresponding Author:** Ana Recober, MD, Colket Translational Research Building – Rm. 10200, 3501 Civic Center Boulevard, Philadelphia, PA 19104, Phone: 1-215-590-4384, recobera@email.chop.edu.

Conflict of Interest Statement

Dr. Recober has received licensing fees from Alder Biopharmaceutical, LLC (for anti-CGRP antibodies in the treatment of photophobia) unrelated to current manuscript.

behavioral studies haven't confirmed whether nociceptive activation of the trigeminal system changes light sensitivity in rodents as it does in humans.

Furthermore, it is not known what direct effect obesity has on any of the structures mentioned above.^{13,30–32} Our previous work demonstrated that obesity increases nociceptive activation of the trigeminal system.⁴¹ We showed that stimulation of the trigeminal afferents in the whisker pad with 0.01% capsaicin, which had no effect on control mice, caused over three-fold increase in Fos expression in the caudal portion of the trigeminal nucleus (TNc) in obese mice.⁴¹ Second order neurons in this region of the TNc (trigeminal subnucleus caudalis/upper cervical cord junction) also showed increased Fos upon activation by bright light in rats³³ and are required for light-induced blink reflexes.³⁶ The pattern of light-evoked Fos³³ overlaps with the vibrissal dermatome in the rat TNc⁴⁸ and the area of Fos expression in our previous study.⁴¹

In this study, we sought to investigate the effects of obesity on trigeminal sensory processing. We used two well-established mouse models of obesity, high-fat diet induced obesity and a genetic model of leptin deficiency, to dissect the effects of obesity from the effects of high-fat diet (Fig. 1). First, we determined the effects of obesity on capsaicin-induced nocifensive behavior³⁴ and photophobic behavior in mice.^{19,27,37,38} Then, we used calcium imaging to determine the effects of obesity on the activity of trigeminal primary afferents *in vitro*. Our findings suggest that obesity increases the sensitivity of trigeminal afferent neurons and results in photophobic behavior. These findings have implications for the relationship between obesity and headache disorders associated with photophobia.

2. Methods

2.1 Animals

Male C57BL/6J mice were purchased from Jackson Laboratories or bred in our colony. Mice were maintained on either regular chow (RD; Teklad) or a 60% fat diet (HFD; Research Diets Inc. #D12491) from weaning (4 weeks of age). In order to distinguish the relative contribution of HFD versus obesity to photophobia (Fig. 1) some mice were tested at 8–11 weeks of age before they become obese (lean HFD) and some were tested at 20–25 weeks of age, after they become obese (obese HFD). We also used a cohort of genetically obese mice (*ob/ob*) that develop obesity despite being fed regular chow. Male *ob/ob* mice (B6.VLep^{ob}/OlaHsd) and age-matched C57BL/6 from Harlan were purchased at 5 weeks old, maintained on regular chow, and tested at 6–8 weeks of age, when the *ob/ob*'s body weight was almost double than lean controls. All weights are listed in Table 1 for comparison between different experimental groups (Table 1).

All mice were maintained on a standard 12-hour light-dark cycle (lights on 06:00–18:00), with food and water available *ad libitum* when not undergoing behavioral testing. All protocols were approved by the University of Iowa IACUC and follow the guidelines established by the NIH⁹.

2.2 Nocifensive Behavior

To determine if diet affects the acute nocifensive response to capsaicin, obese HFD and lean RD control mice were injected with 10 μ L of either capsaicin (0.01 or 0.1%) or vehicle (5% each ethanol and Tween80 in phosphate buffered saline, PBS) in one whisker pad. Each mouse was placed in a 600 mL glass beaker filled with a small amount of their home cage bedding and their behavior was recorded for ten minutes. An investigator, blinded to the treatment, reviewed the videos and scored the following behaviors: number of ipsilateral wipes, total number facial wipes (including unilateral and bilateral), and number of times the animal reared on hindpaws. We assessed the total number of facial wipes to control for possible diet-related differences in motor or grooming behavior.

2.3 Light Avoidance Assay

Photophobia is a key clinical feature of headache disorders and light avoidance in mice can be a surrogate of photophobia^{19,27,37,38}. Here we assessed photophobia in mice as previously described^{19,37}, using a modified commercially available apparatus (Med Associates) where mice are given a choice between light and darkness. To avoid the potential anxiety-inducing effects of the clear walls and open top on the lit side, we covered the walls of the chambers with black opaque foam panels and the top with clear Plexiglas to allow illumination of that side while being enclosed like the dark side. Mice were always placed first in the lit compartment and allowed to move freely between the lit and dark compartments for the duration of the test (20 minutes). Mice acclimated in the testing room for at least an hour prior to testing, with food available ad libitum in their cages. We tested mice between 09:00 and 14:00 each day, during the light cycle, to avoid interruption of the dark cycle. All mice were tested two times to establish a baseline prior to any treatment. The second baseline was used to compare with post-treatment behavior tests. All tests were performed with a light intensity of 775 lux.

We measured the percentage of time that mice spend in the lit compartment (% time in light) as a surrogate of photophobic behavior. Additionally, we quantified other parameters of locomotor activity in each compartment to confirm that there were no motor impairments that could interfere with the test. These parameters included percentage of time spent resting (“% time resting”), percentage of time spent ambulating (“% time moving”), and distance traveled (cm) relative to the time spent in each zone (s).

2.4 Induction of Photophobic Behavior with Capsaicin

After baseline testing in the light-dark assay, several cohorts of mice received an intradermal injection of capsaicin (0.01% or 0.1%) or vehicle in one whisker pad and tested 18–20 hours later. Five to seven days later, they were treated with the other drug on the opposite side and tested 18–20 hours later. The rationale to assess photophobia 18–20 hours after capsaicin injection was: 1. To allow the animals enough time to recover from the stress of the facial injections; 2. To avoid the possible confounding factor of acute facial pain; and 3. To keep the testing time consistent with baseline, during the first part of the light cycle.

2.5 Measurement of Serum Corticosterone

To determine if anxiety contributes to photophobic behavior in this paradigm we assessed activation of the hypothalamic-pituitary-adrenal (HPA) axis by measuring serum corticosterone levels under different conditions. First, to determine if there were basal differences in corticosterone levels between the two diet groups, we collected blood from naïve mice after being transported from the housing facility to the testing room and acclimating there as usual. A different cohort of naïve mice was used to measure corticosterone levels immediately after being tested in the light avoidance assay. Finally, to determine the possible effects of anxiety on capsaicin-induced photophobia, blood was collected from mice 18–20 hours after treatment with either vehicle or 0.01% capsaicin. Half of the mice were tested in the light avoidance assay before blood collection and the other half were not tested. We used different cohorts and collected blood in the morning to avoid variability related to multiple blood collections and the circadian rhythm. Blood was collected from the submandibular vein of conscious, gently restrained male obese HFD and lean RD control mice. The blood was kept on ice in serum separating tubes (BD) until collection from all mice was completed. To isolate the serum, the blood was allowed to coagulate at room temperature for 20–30 minutes and then centrifuged for 10 minutes at 2700xg at 4°C. We determined corticosterone concentration of diluted samples (1:100) using a commercially available ELISA (Arbor Assays), following the manufacturer's instructions.

2.6 Primary Culture of Adult Trigeminal Ganglia Neurons

Primary cultures of TG cells from obese HFD and lean RD control mice were prepared according to a published protocol⁵², with some modifications. Mice were euthanized with CO₂ and their TG were harvested into 10mL of ice-cold Dubecco's Modified Eagle Medium (DMEM, high glucose, Gibco) with 25mM HEPES (pH 7.2–7.4). The ganglia were treated with dispase (Gibco, 10mg/mL) for 30 minutes at 37°C, with gentle rocking to break up the connective tissue. The dispase solution was replaced with fresh plating media and the cells were further dissociated by triturating through a 5 mL pipette until the media was cloudy, approximately 100–120 times. Cells were centrifuged at 250xg for 5 minutes, and the pellet was resuspended in Leibovitz's-15 medium (L-15, Gibco) containing 10% FBS, 50mM glucose, 250µM ascorbic acid, 8 µM glutathione, 10ng/mL nerve growth factor, 100U/mL penicillin, 100µL/mL streptomycin. For calcium imaging, cell suspensions from three pairs of ganglia/diet were distributed across three laminin-coated 25mm glass coverslips, in a volume of 400µL/coverslip. Cultures were maintained at 37°C and ambient CO₂ for at least 24 hours prior to calcium imaging.

2.7 Calcium Imaging of Primary Cultures

Calcium imaging of primary cell cultures from obese HFD and lean RD control mice was performed as previously described²³, with some modifications. Coverslips containing cultured cells were incubated in HEPES-buffered HBSS (HH buffer; for full recipe see Schnizler *et al.*⁴⁵, containing 2µM of the calcium sensitive dye Fura-2- AM (Invitrogen) for at least 20 minutes. The coverslips were secured in the treatment delivery chamber mounted on the stage of an inverted microscope (Olympus IX-71) for signal recording. Fluorescence was alternately excited at 340 and 380 nm using the Polychrome IV monochromator (TILL

Photonics), through a 10× objective. Emitted fluorescence was collected at 510nm using an IMAGO CCD camera (TILL Photonics). Pairs of 340/380 images were sampled at 0.2 Hz. Treatment of the cells was as follows: 1 min of baseline recording (HH buffer only), followed by 15 sec of 500nM capsaicin, 2 min wash (HH buffer), 15 sec of 60mM potassium chloride (KCl), and 2 min wash. KCl was used to ensure that all cells selected in the field of view were functional. A positive response to capsaicin was considered to be an increase of more than 25% above baseline, indicating specific activation of the transient receptor potential family vanilloid 1 (TRPV1). Capsaicin-insensitive cells that did not respond to 60mM KCl with an increase from baseline of at least 50% were considered inviable and excluded from the total cell count. TILLvisION (v. 4.0.1.2, TILL Photonics) was used to process the data. ImageJ was used to determine the diameter of cells recorded for each coverslip from the transmission images. Calcium imaging data represents experiments performed in duplicate.

2.8 Statistical Analysis

We used two-way ANOVAs to evaluate significant effects of diet and treatment on nocifensive grooming and corticosterone levels in mice with Sidak post-hoc tests. We used two-way ANOVAs with repeated measures to evaluate significant effects of diet or genotype and treatment on light aversive behavior. For calcium imaging, we used un-paired *t*-tests with Welch's correction to determine if neurons from the two diets exhibited significant differences in evoked calcium responses. For all tests, $p < 0.05$ was considered significant and Prism (v.6, Graph Pad) was used.

3. Results

3.1 Diet-induced obesity does not increase acute nocifensive behavior

We have previously shown that obese HFD mice had over 3-fold increase in Fos expression in the TNC in response to 0.01% capsaicin, which had no effect on control RD mice.⁴¹ The first step to investigate the behavioral correlates of this finding was to assess acute nocifensive response to capsaicin injected in the skin innervated by the trigeminal nerve.³⁴ We found that capsaicin injection increased the number of ipsilateral facial wipes (treatment: $F_{(2,72)} = 6.656$, $p = 0.0022$; diet: $F_{(1,72)} = 0.2294$, $p = 0.6334$; interaction: $F_{(2,72)} = 0.007584$, $p = 0.9924$) (Fig. 2A) and decreased the number of rearings (treatment: $F_{(2,72)} = 24.26$, $p < 0.0001$, diet: $F_{(1,72)} = 0.06774$, $p = 0.7954$; interaction: $F_{(2,72)} = 0.1539$, $p = 0.8576$) (Fig. 2B) similarly in both diet groups and in a dose-dependent manner. We also measured the total number of facial wipes to control for possible differences in motor or grooming behavior. Total number of facial wipes were similar in both diet groups and decreased with capsaicin (treatment: $F_{(2,72)} = 6.656$, $p = 0.0022$; diet: $F_{(1,72)} = 0.2294$, $p = 0.6334$; interaction: $F_{(2,72)} = 0.007584$, $p = 0.9924$). This indicates that diet-induced obesity does not change acute nocifensive responses to capsaicin injection. Alternatively, it could suggest that acute nocifensive behavior is not sensitive enough to detect subtle differences between the two diet groups.

3.2 Diet-induced obesity increases basal photophobia

Before testing the effects of capsaicin on photophobic behavior, we sought to determine if diet-induced obesity has any effect on basal photophobia in naïve mice. We tested several cohorts of untreated obese HFD and lean RD control mice ($n = 9\text{--}18/\text{diet/cohort}$), and observed a small and not always statistically significant difference. When we considered all these groups together ($n = 74$ RD and 80 HFD), we found that obese HFD mice spent 7% less time in the light than lean RD mice ($t_{152} = 3.592$, $p = 0.0004$). We also found that 74% of obese HFD versus 47% of lean RD mice showed a preference for the dark side at baseline (i.e. spend $>50\%$ time in dark side) (Fig. 3A). Considering that the chamber is divided in two compartments of equal size, mice are expected to spend half of the time on each side unless there is a preference for the light or the dark. This suggests that diet-induced obesity increases photophobia in naïve mice.

To control for possible differences in locomotor activity, we measured percentage of time resting, percentage of time ambulating, and distance traveled in each compartment relative to the time spent in each side during the light avoidance test (Fig. 3B–D). We observed, as expected, that obese HFD mice move slightly less and rest slightly more than control mice, but these differences in the percentage of time resting and moving were not statistically significant in any zone. Furthermore, both diets exhibit similar patterns of locomotor activity in each zone. Mice on either diet spent less time resting and more time moving in the light than the dark (For % time resting - diet: $F_{(1,152)} = 5.18$, $p = 0.0242$; zone: $F_{(1,152)} = 16.02$, $p < 0.0001$; interaction: $F_{(1,152)} = 0.0001861$, $p = 0.9891$. For % time moving - diet: $F_{(1,152)} = 5.328$, $p = 0.0223$; zone: $F_{(1,152)} = 89.48$, $p < 0.0001$; interaction: $F_{(1,152)} = 0.02756$, $p = 0.8684$) (Fig. 3B, C). Obese HFD mice moved significantly slower than controls in both zones during the baseline test (diet: $F_{(1,152)} = 11.73$, $p = 0.0008$; zone: $F_{(1,152)} = 121$, $p < 0.0001$; interaction: $F_{(1,152)} = 0.1114$, $p = 0.7390$) (Fig. 2D).

3.3 Capsaicin induces photophobic behavior in obese mice

We hypothesized that obese mice have enhanced photophobic behavior in response to trigeminal stimulation. We tested photophobia in obese HFD and lean RD mice 18–20 hours after capsaicin (0.01%) or vehicle injection in the whisker pad. We found that capsaicin significantly reduces time in light in obese HFD mice, but not in lean RD mice (treatment: $F_{(2,160)} = 4.351$, $p = 0.0145$; diet: $F_{(1,80)} = 5.623$, $p = 0.0201$; interaction: $F_{(2,160)} = 5.640$, $p = 0.0043$) (Fig. 4A).

Careful assessment of locomotor activity during the test revealed that differences in locomotor activity cannot explain capsaicin-induced photophobia. At baseline, there were no differences between the two diet groups in the dark compartment in any of the parameters measured (Fig. 4B–D). The obese HFD group displayed significantly higher time resting and total distance traveled was lower in the light compartment at baseline (Fig. 4B–D), which is consistent with known effects of obesity on motor activity in mice⁶. However, the percentage of time that mice spent moving was similar at baseline in both diet groups in each compartment (Fig. 4C), suggesting that obese HFD move slower than lean RD mice, but there is no motor impairment that could interfere with the assay. Notably, we found that after capsaicin injection only obese HFD mice spent more time resting, less time moving,

and traveled less distance in the dark compartment. Moreover, the fact that their motor activity did not change in the light compartment after capsaicin injection indicates that their ability move between compartments was not affected. Table 2 includes all the statistical outcomes for the locomotor activity parameters (Table 2).

To determine if there was a relationship between the degree of obesity and photophobia in response to trigeminal stimulation, we performed a linear regression analysis comparing total body weight to percent of time in light after capsaicin injection (Fig. 4E, F). A significant correlation between total body weight and time in light was only observed in the obese HFD group ($R^2 = 0.25$, $p = 0.0001$) (Fig. 4F). Among obese HFD mice, higher total body weight correlated with lower time in light. No correlation was observed in the lean RD group ($R^2 = 0.01$, $p = 0.46$) (Fig. 4E). This suggests that abnormally high total body weight is necessary for capsaicin-induced photophobia. However, it is possible that high-fat diet also contributes to this behavior.

In summary, the data indicate that obese HFD mice develop photophobia in response to trigeminal stimulation with low dose capsaicin (0.01%). This behavior seems to correlate to some extent with the degree of obesity. Obese HFD mice also avoid locomotor activity when they are in the dark compartment but demonstrate normal locomotor activity while in the light. In contrast, lean RD mice do not develop photophobia following 0.01% capsaicin injection, nor does this treatment affect their locomotor activity. This suggests that diet-induced obesity contributes to enhanced light sensitivity following trigeminal stimulation independent of changes in locomotor activity.

3.4 A higher dose of capsaicin is required to induce photophobia in lean RD mice

Next, we wanted to determine whether or not photophobia could be induced in the absence of obesity. We hypothesized that a higher dose of capsaicin would induce light avoidant behavior in lean RD mice and this would be more pronounced in obese HFD mice. To assess this, we tested a new cohort of obese HFD and lean RD mice after receiving 0.1% capsaicin injection in the whisker pad. Both obese HFD and lean RD mice spent 7% less time in light following 0.1% capsaicin compared to vehicle injection, and there was no significant difference between the two diet groups in any of the testing conditions (baseline, vehicle, 0.1% capsaicin). There was a significant overall effect of diet ($F_{(1,55)} = 8.361$, $p = 0.0055$) and a significant effect of treatment ($F_{(1,55)} = 15.36$, $p = 0.0002$), but no significant interaction ($F_{(1,55)} = 0.005572$, $p = 0.9408$). This suggests that diet induced obesity decreases the threshold for capsaicin-induced photophobia, but not the magnitude of the response.

3.5 Contribution of obesity versus high fat diet to photophobic behavior

In order to dissociate the contribution of the diet from obesity, we used two different approaches. First, to assess the possible contribution of high-fat diet, we tested C57BL/6J mice fed HFD for 8 weeks (lean HFD), before they typically develop significant obesity, compared with RD controls. Alternatively, to assess the possible contribution of obesity independent of the diet, we tested leptin-deficient mice (*ob/ob*), a genetic model that develops significant obesity while fed regular chow.

Lean HFD mice spent about 8% less time in light than lean RD control mice at baseline (Fig. 5A). However, both diet groups were similar and did not exhibit photophobia in response to capsaicin 0.01% or 0.1% compared with vehicle (diet: $F_{(1,31)} = 1.946$, $p = 0.1729$; treatment: $F_{(3,93)} = 4.362$, $p = 0.0064$; interaction ($F_{(3,93)} = 1.497$, $p = 0.2205$). There were no post-hoc differences between capsaicin and vehicle in either diet group (Fig. 5A). Locomotor activity was not significantly affected by diet (Fig. 5B–D). While there were some statistically significant overall treatment effects and interactions, post-hoc testing revealed no capsaicin-specific effect (i.e. no difference between vehicle and capsaicin) in either diet group on locomotor parameters (Table 2) (Fig. 5B–D). These findings suggest that HFD alone, in the absence of significant weight gain, is not sufficient to increase the trigeminal sensitivity to capsaicin and photophobia.

Leptin-deficient mice (*ob/ob*) spent 23% less time in light at baseline than lean controls, which is in part driven by these young control mice spending more than 50% of time in the light (Fig. 6). Additionally, 11 out of 16 *ob/ob* mice spent > 50% of the time in the dark versus 1 out of 13 control mice. *ob/ob* mice significantly decreased their time in light and spent more than 90% of the time in the dark in response to 0.1% capsaicin (genotype: $F_{(1,27)} = 77.19$, $p < 0.0001$; treatment $F_{(4,108)} = 14.14$, $p < 0.0001$; interaction: $F_{(4,108)} = 6.069$, $p = 0.0002$) (Fig. 6A). As previously described by others^{8,10,11,28}, *ob/ob* mice displayed lower locomotor activity than lean controls (Fig. 6B–D) (Table 2). Notably, after 0.1% capsaicin injection locomotor activity in the light increased in the *ob/ob* group to the level of the control group. Therefore, their photophobic behavior is not likely to be a result of their overall decreased ambulatory activity.

Taken together, these findings further support the hypothesis that excessive total body weight contributes to capsaicin-induced photophobia. While a contribution of high fat diet is possible, our results suggest that obesity is necessary for the development of enhanced trigeminal sensitivity and capsaicin-induced photophobia in this paradigm. Additionally, the lack of response of the younger control groups to the higher dose of capsaicin reveals potential age-related effects on capsaicin-induced photophobic behavior.

3.6 Capsaicin-induced photophobia in obese mice cannot be explained by anxiety

An important consideration when using the light avoidance test is the potential role of anxiety-like behavior in this assay. To address this, we measured serum corticosterone levels in each diet group under different conditions (Fig. 7). We found that the serum levels of corticosterone in naïve mice were similar in both diet groups before and after behavioral testing, although they were almost twice as high after behavioral testing ($F_{(1,67)} = 42.03$, $p < 0.0001$) (Fig. 7A). Interestingly, the day after 0.01% capsaicin injection only obese HFD mice had a two-fold increase in corticosterone levels ($p < 0.0005$) while lean RD mice and all vehicle treated mice had levels similar to baseline (Fig. 7B). This may suggest that obese HFD mice have higher stress levels after capsaicin injection and before undergoing behavioral test. However, the levels of corticosterone are similar after light avoidance test in the vehicle-treated and capsaicin-treated mice within each diet group ($F_{(1,22)} = 2.984$, $p = 0.098$) (Fig. 7C). This is in contrast with the effects of capsaicin on photophobic behavior in

the obese HFD mice. These data suggest that stress alone cannot explain the effects of capsaicin injection on photophobic behavior, although we cannot rule out some contribution.

3.7 Trigeminal ganglia neurons from obese mice exhibit an enhanced response to stimulation in vitro

Our previous finding of enhanced capsaicin-induced Fos expression in TNc of obese HFD mice could be due to enhanced sensitivity of second order neurons in the TNc or increased afferent drive from primary sensory neurons in the trigeminal ganglia.⁴¹ Here, we sought to determine if diet-induced obesity has an effect on capsaicin-evoked calcium influx in cultured trigeminal ganglia neurons. We investigated the proportion of trigeminal afferents that respond to capsaicin, and the magnitude of their response. Overall, the percentage of capsaicin-responsive (TRPV1+) neurons was similar in both diet groups (32% in obese HFD vs. 27% in lean RD) (Fig. 8A, B). However, when we examined the size distribution of TRPV1+ neurons in each diet group, we found that TRPV1+ neurons from obese HFD animals exhibit a rightward shift in cell diameter compared with lean RD mice (Fig. 8A–D). The proportion of TRPV1+ neurons with diameter over 29 μm was five times higher in the obese HFD group (25% in obese HFD vs 5% in lean RD). This was at the expense of a decrease in the proportion of small diameter neurons (32% in obese HFD vs 50% in lean RD) (Fig. 8A–D).

We also found that capsaicin-evoked calcium influx was significantly higher in neurons from obese HFD mice compared to lean RD controls (Fig. 8E, G, H). The peak of calcium influx was 25% higher in trigeminal neurons from obese HFD mice in response to capsaicin ($t_{104.4} = 2.268$, $p = 0.0254$) (Fig. 8E). Unexpectedly, when we used KCl to assess neuronal viability/excitability, we also found 25% higher response in the obese HFD group (TRPV1+ cells $t_{114.5} = 3.906$, $p = 0.0002$; TRPV1– cells $t_{261.5} = 5.144$, $p < 0.0001$) (Fig. 8D). This may suggest that obesity affects the overall excitability of sensory neurons.

4. Discussion

This is the first study to assess photophobic behavior in obese mice and begin to explore the underlying mechanisms. We found that intradermal injection of capsaicin in the face induces photophobic behavior at a lower dose in obese HFD mice than in lean RD mice. Unexpectedly, *ob/ob* mice also required a higher dose of capsaicin than HFD mice, but their photophobic behavior was then more pronounced. At a cellular level, we found that calcium influx is increased and functional TRPV1 expression is altered in trigeminal ganglion neurons from obese HFD mice.

We used two different approaches to determine if the cellular changes we found in the TNc of obese mice⁴¹ correlate with changes in behavior. First, we assessed acute nociception by quantifying nocifensive facial wipes and found no difference between obese HFD and lean RD mice after capsaicin injection. This suggests that acute nocifensive responses are normal in obese HFD mice. These results are consistent with our previous finding of intact thermal nociception in obese HFD mice.⁴² These observations may seem at first to conflict with our current findings in trigeminal neuronal cultures and our previous immunohistochemical findings in the TNc.⁴¹ However, it should be noted that nocifensive wipes may not be

sensitive enough to detect subtle differences between the two diet groups. Additionally, a greater nociceptor activation may not produce enhanced acute pain, but could have downstream effects in the pain modulatory processes.

Then, we investigated the effects of capsaicin injection in trigeminal-innervated skin using a validated behavioral assay of photophobia.^{19,27,37,38} We found that obese HFD mice are slightly, but significantly more photophobic than lean controls at baseline. We also found that only obese HFD mice displayed photophobia in response to low dose capsaicin (0.01%), while lean RD controls required a higher dose (0.1%). Similarly, *ob/ob* mice displayed marked photophobic behavior in response to the higher dose of capsaicin. In contrast, photophobia did not occur in lean HFD mice with either dose of capsaicin, suggesting that obesity, rather than high fat diet, is driving this phenomenon.

Remarkably, this capsaicin-induced photophobic behavior was present 18–20 hours after unilateral facial injection of capsaicin. The time of onset of photophobia after capsaicin injection and the duration of the behavior in obese mice remains unknown. The onset of photophobia in humans after painful trigeminal stimulation has been demonstrated acutely, within minutes, but it has not been assessed over time.^{14,15} Unfortunately, human studies investigating photophobia have not controlled for body mass index. The 2 studies that have explored photophobia in migraineurs in relation to body mass index have found an association. Bigal *et al.*, in a large population-based study, found that obese migraineurs report photophobia and phonophobia, but not nausea, more frequently during the attacks than patients with normal weight.² Winter *et al.* found the same in the Women's Health Study.⁵¹

By assessing locomotor behavior and corticosterone levels, we confirmed that the difference in time in light cannot be explained solely by locomotor deficits or enhanced HPA axis activity. These assessments did indicate some differences between obese and lean mice that may have important implications. It is noteworthy that only obese mice (HFD or *ob/ob*) display decreased locomotor activity in the dark compartment after capsaicin injection, while they are able to move normally in the light compartment. We have observed similar behavior previously in a model of photophobia evoked by calcitonin gene-related peptide (CGRP).^{19,37,38} Corticosterone did not differ between the obese HFD and lean RD mice at baseline, and it was not different between vehicle and capsaicin treated obese HFD mice after behavioral testing. Thus we conclude that HPA axis activation alone cannot explain the effect of obesity on photophobia. However, it is noteworthy that corticosterone levels in the untested obese HFD mice were significantly elevated 18–20 hours after capsaicin treatment. Others have observed normalization of capsaicin-evoked corticosterone by 3 hours in C57BL/6J mice¹, suggesting that obesity prolongs HPA axis activation evoked by capsaicin. Prolonged HPA axis activation in response to pain could have implications for pain modulation, especially in disorders of recurrent or chronic pain. The contribution of this phenomenon to obesity's effect on photophobia or pain in other regions of the body remains to be determined.

While capsaicin-induced photophobia occurred in both models of obesity, we observed some differences between the dietary and genetic models that warrant further investigation of

leptin. Obese HFD mice responded to a low dose of capsaicin (0.01%), while *ob/ob* mice required a higher dose of capsaicin (0.1%) and displayed more pronounced photophobic behavior. Leptin has a variety of functions beyond energy homeostasis and has been recently implicated in pain modulation peripherally and centrally.^{21,22,24,49} *ob/ob* mice fail to develop neuropathic pain following nerve injury, which has been attributed to impaired macrophage mobilization to the injured nerve and lack of leptin-mediated upregulation of NMDA and IL-beta in the spinal cord.^{22,24} The differences between the two models of obesity may be related to several inherent characteristics, such as the congenital lack of leptin and potential effects on development, the more rapid and severe weight gain in the *ob/ob* mice, and the presence of other metabolic and immune-related abnormalities in the *ob/ob* mice. Both models of obesity are associated with chronic systemic inflammation which could contribute to pathological interaction between light-sensing and trigeminal pain pathways.

We reasoned that increased input from primary afferents in obese mice may lead to greater downstream activation that could result in enhanced light sensitivity. To investigate this, we used calcium imaging to determine if the properties of trigeminal nociceptors were affected by obesity. We found enhanced capsaicin-evoked calcium influx in the trigeminal afferents of obese HFD mice which could indicate enhanced primary afferent activity. Obesity-related inflammation could explain altered activation properties of TRPV1 channels and increased capsaicin-evoked calcium influx. Inflammatory mediators lead to phosphorylation of TRPV1, causing sensitization or increase trafficking to the plasma membrane.⁷ The enhanced calcium influx following *in vitro* stimulation with potassium chloride was an unexpected finding and suggests that obesity may have additional effects on the properties of voltage-gated ion channels. This is an important finding because it suggests that trigeminal primary afferents in obese individuals may display an abnormally high response to depolarizing stimulation. An important limitation of these *in vitro* experiments is that the neurons are not in their normal environment within the ganglia. Nevertheless, the differential response of neurons from obese HFD and lean RD mice warrants further investigation.

We also observed a shift in functional TRPV1 expression from small to medium/large diameter neurons in TG cultures from obese HFD mice. A similar shift has been observed in rats following neuropathic injury.⁵⁰ This may have implications for nociceptive transmission since medium/large diameter neurons have faster conduction velocity. Future studies with retrograde neuronal labelling and electrophysiological assessment of neuronal excitation and firing will investigate the functional changes in specific subpopulations of trigeminal neurons. Moreover, electrophysiological recordings from second order neurons will help confirm whether central sensitization is also present.

Certain aspects of the photophobic behavior may suggest that central sensory processing is important in our findings. First, the presence of photophobia 18–20 hours after capsaicin injection cannot be explained by secondary hyperalgesia alone. Intradermal injection of capsaicin causes secondary hyperalgesia that typically resolved within 2–3 hours.⁴⁶ Secondary hyperalgesia is attributed to loss of descending inhibition on the TNc or dorsal horn of the spinal cord.^{25,39} However, important questions remain to explain how secondary hyperalgesia resolves. This “reset” mechanism may prevent trigeminal activity from

engaging light sensing pathways under healthy conditions and it may be impaired in obese mice.

Second, the fact that injection of capsaicin in the skin innervated by the second branch of the trigeminal nerve (V2) induced photophobia, may also suggest altered central processing in obesity. Photophobia is presumed to involve structures innervated by the ophthalmic branch. However, others have shown that neuronal populations in the TNc³³ that respond to light lie in areas that also receive V2 or vibrissal input.⁴⁸ Moreover, the TNc has reciprocal connections with the rostroventral medulla (RVM), implicated in pain modulation, and also connects to the posterior thalamus⁴⁰, where neurons responsive to light and dura stimulation have been identified.³⁰ Additional work is needed to investigate whether light-responsive TNc neurons send projections to those areas, what consequence this has for regulating light sensitivity, and how this may be modulated by obesity.

In summary, our interpretation of these results is that obesity may lead to changes in trigeminal sensory processing. The specific molecular mediators and pathophysiologic mechanisms remain to be determined. This study has laid the foundation for future in-depth work that will provide a better understanding of the effects of obesity on the trigeminal system, and how it might contribute to headache disorders. Additionally, we are exploring the effects sex and age in trigeminal sensory processing. Given the current epidemic of obesity and the high prevalence of headache disorders, further research in this area is crucial.

Acknowledgments

This work was supported by NINDS/NIH grants NS066087 to AR and NS069898 to DPM.

We thank Drs. Andrew Russo and Randy Kardon for the use of equipment and reagents. We also thank the University of Iowa Pain Research Group for their feedback.

References

1. Benedetti M, Merino R, Kusuda R, Ravanelli MI, Cadetti F, dos Santos P, Zanon S, Lucas G. Plasma corticosterone levels in mouse models of pain. *Eur J Pain*. 2012; 16:803–815. [PubMed: 22337291]
2. Bigal ME, Liberman JN, Lipton RB. Obesity and migraine: a population study. *Neurology*. 2006; 66:545–550. [PubMed: 16354886]
3. Bigal ME, Lipton RB. Obesity is a risk factor for transformed migraine but not chronic tension-type headache. *Neurology*. 2006; 67:252–257. [PubMed: 16864817]
4. Bigal ME, Tsang A, Loder E, Serrano D, Reed ML, Lipton RB, Prevalence ftAM, Prevention Advisory Group. Body Mass Index and Episodic Headaches: A Population-Based Study. *Arch Intern Med*. 2007; 167:1964–1970. [PubMed: 17923596]
5. Bouloche N, Denuelle M, Payoux P, Fabre N, Trotter Y, Géraud G. Photophobia in migraine: an interictal PET study of cortical hyperexcitability and its modulation by pain. *J Neurol Neurosurg Psych*. 2010; 81:978–984.
6. Brownlow BS, Petro A, Feinglos MN, Surwit RS. The role of motor activity in diet-induced obesity in C57BL/6J mice. *Physiology & behavior*. 1996; 60:37–41. [PubMed: 8804640]
7. Cheng J-K, Ji R-R. Intracellular signaling in primary sensory neurons and persistent pain. *Neurochem Res*. 2008; 33:1970–1978. [PubMed: 18427980]
8. Clark LD, Gay PE. Activity and body-weight relationships in genetically obese animals. *Biol Psychiatry*. 1972; 4:247–250. [PubMed: 5041920]
9. Committee, NRC. *Guide for the Care and Use of Laboratory Animals*. Washington, (DC): National Academies Press; 2011.

10. Dauncey MJ. Activity-induced thermogenesis in lean and genetically obese (ob/ob) mice. *Experientia*. 1986; 42:547–549. [PubMed: 3709761]
11. Dauncey MJ, Brown D. Role of activity-induced thermogenesis in twenty-four hour energy expenditure of lean and genetically obese (ob/ob) mice. *Q J Exp Physiol*. 1987; 72:549–559. [PubMed: 3423201]
12. Denuelle MM, Bouloche NM, Payoux PMDP, Fabre NM, Trotter YP, Geraud GM. A PET study of photophobia during spontaneous migraine attacks. *Neurology*. 2011; 76:213–218. [PubMed: 21148120]
13. Dolgonos S, Ayyala H, Evinger C. Light-induced trigeminal sensitization without central visual pathways: another mechanism for photophobia. *Investigative Ophthalmology & Visual Science*. 2011; 52:7852–7858. [PubMed: 21896840]
14. Drummond PD. Photophobia and autonomic responses to facial pain in migraine. *Brain*. 1997; 120:1857–1864. [PubMed: 9365375]
15. Drummond PD, Woodhouse A. Painful stimulation of the forehead increases photophobia in migraine sufferers. *Cephalalgia*. 1993; 13:321–324. [PubMed: 8242724]
16. Eckardt LB, McLean JM, Goodell H. Experimental studies on headache: the genesis of pain from the eye. *Res Publ-Assoc Res Nerv Ment Dis*. 1943; 23:209–227.
17. Gutrecht JA, Lessell IM. Photophobia in Trigeminal Neuralgia. *J Neuro-Ophthalmol*. 1994; 14:122–123.
18. Hitt HC, McMillen RC, Thornton-Neaves T, Koch K, Cosby AG. Comorbidity of Obesity and Pain in a General Population: Results from the Southern Pain Prevalence Study. *J Pain*. 2007; 8:430–436. [PubMed: 17337251]
19. Kaiser EA, Kuburas A, Recober A, Russo AF. Modulation of CGRP-induced light aversion in wild-type mice by a 5-HT1B/D agonist. *The Journal of Neuroscience*. 2012; 32:15439–15449. [PubMed: 23115181]
20. Kowacs P, Piovesan E, Werneck L, Tatsui C, Lange M, Ribas L, da Silva H. Influence of Intense Light Stimulation on Trigeminal and Cervical Pain Perception Thresholds. *Cephalalgia*. 2001; 21:184–188. [PubMed: 11442552]
21. Lim G, Kim H, McCabe MF, Chou C-W, Wang S, Chen LL, Marota JJA, Blood A, Breiter HC, Mao J. A Leptin-Mediated Central Mechanism in Analgesia-Enhanced Opioid Reward in Rats. *J Neurosci*. 2014; 34:9779–9788. [PubMed: 25031415]
22. Lim G, Wang S, Zhang Y, Tian Y, Mao J. Spinal leptin contributes to the pathogenesis of neuropathic pain in rodents. *J Clin Invest*. 2009; 119:295–304. [PubMed: 19139561]
23. Loo L, Shepherd AJ, Mickle AD, Lorca RA, Shutov LP, Usachev YM, Mohapatra DP. The C-Type Natriuretic Peptide Induces Thermal Hyperalgesia through a Noncanonical G β -dependent Modulation of TRPV1 Channel. *J Neurosci*. 2012; 32:11942–11955. [PubMed: 22933780]
24. Maeda T, Kiguchi N, Kobayashi Y, Ikuta T, Ozaki M, Kishioka S. Leptin derived from adipocytes in injured peripheral nerves facilitates development of neuropathic pain via macrophage stimulation. *PNAS*. 2009; 106:13076–13081. [PubMed: 19620723]
25. Mainero C, Zhang W-T, Kumar A, Rosen BR, Sorensen AG. Mapping the spinal and supraspinal pathways of dynamic mechanical allodynia in the human trigeminal system using cardiac-gated fMRI. *NeuroImage*. 2007; 35:1201–1210. [PubMed: 17336547]
26. Marcus DA. Obesity and the impact of chronic pain. *Clin J Pain*. 2004; 20:186–191. [PubMed: 15100595]
27. Markovics A, Kormos V, Gaszner B, Lashgarara A, Szoke E, Sandor K, Szabadfi K, Tuka B, Tajti J, Szolcsanyi J, Pinter E, Hashimoto H, Kun J, Reglodi D, Helyes Z. Pituitary adenylate cyclase-activating polypeptide plays a key role in nitroglycerol-induced trigeminovascular activation in mice. *Neurobiol Dis*. 2011
28. Morton GJ, Kaiyala KJ, Fisher JD, Ogimoto K, Schwartz MW, Wisse BE. Identification of a physiological role for leptin in the regulation of ambulatory activity and wheel running in mice. *American Journal of Physiology - Endocrinology and Metabolism*. 2011; 300:E392–E401. [PubMed: 21062956]
29. Moulton EA, Becerra L, Borsook D. An fMRI case report of photophobia: Activation of the trigeminal nociceptive pathway. *Pain*. 2009; 145:358–363. [PubMed: 19674842]

30. Nosedá R, Kainz V, Jakubowski M, Gooley JJ, Saper CB, Digre K, Burstein R. A neural mechanism for exacerbation of headache by light. *Nat Neurosci.* 2010; 13:239–245. [PubMed: 20062053]
31. Okamoto K, Tashiro A, Chang Z, Bereiter DA. Bright light activates a trigeminal nociceptive pathway. *PAIN.* 2010; 149:235–242. [PubMed: 20206444]
32. Okamoto K, Tashiro A, Thompson R, Nishida Y, Bereiter DA. Trigeminal interpolaris/caudalis transition neurons mediate reflex lacrimation evoked by bright light in the rat. *Eur J Neurosci.* 2012; 36:3492–3499. [PubMed: 22937868]
33. Okamoto K, Thompson R, Tashiro A, Chang Z, Bereiter DA. Bright light produces Fos-positive neurons in caudal trigeminal brainstem. *Neuroscience.* 2009; 160:858–864. [PubMed: 19285114]
34. Pelissier T, Pajot J, Dallel R. The orofacial capsaicin test in rats: effects of different capsaicin concentrations and morphine. *Pain.* 2002; 96:81–87. [PubMed: 11932064]
35. Peterlin BL, Rosso AL, Williams MA, Rosenberg JR, Haythornthwaite JA, Merikangas KR, Gottesman RF, Bond DS, He J-P, Zonderman AB. Episodic migraine and obesity and the influence of age, race, and sex. *Neurology.* 2013; 81:1314–1321. [PubMed: 24027060]
36. Rahman M, Okamoto K, Thompson R, Bereiter DA. Trigeminal pathways for hypertonic saline- and light-evoked corneal reflexes. *Neuroscience.* 2014; 277:716–723. [PubMed: 25086311]
37. Recober A, Kaiser EA, Kuburas A, Russo AF. Induction of multiple photophobic behaviors in a transgenic mouse sensitized to CGRP. *Neuropharmacology.* 2010; 58:156–165. [PubMed: 19607849]
38. Recober A, Kuburas A, Zhang Z, Wemmie JA, Anderson MG, Russo AF. Role of calcitonin gene-related peptide in light-aversive behavior: implications for migraine. *The Journal of Neuroscience.* 2009; 29:8798–8804. [PubMed: 19587287]
39. Rempe T, Wolff S, Riedel C, Baron R, Stroman PW, Jansen O, Gierthmühlen J. Spinal fMRI Reveals Decreased Descending Inhibition during Secondary Mechanical Hyperalgesia. *PLoS ONE.* 2014; 9:1–9.
40. Ren, K.; Dubner, R. The Role Of Trigeminal Interpolaris-Caudalis Transition Zone In Persistent Orofacial Pain. In: Kobayashi, NKKI Masayuki; John, LW., editors. *International Review of Neurobiology.* Vol. 97. Academic Press; 2011. p. 207-225.
41. Rossi HL, Luu AK, DeVilbiss JL, Recober A. Obesity increases nociceptive activation of the trigeminal system. *European Journal of Pain.* 2013; 17:649–653. [PubMed: 23070979]
42. Rossi HL, Luu AKS, Kothari SD, Kuburas A, Neubert JK, Caudle RM, Recober A. Effects of diet-induced obesity on motivation and pain behavior in an operant assay. *Neuroscience.* 2013; 235:87–95. [PubMed: 23333672]
43. Sava SL, de Pasqua V, Magis D, Schoenen J. Effects of Visual Cortex Activation on the Nociceptive Blink Reflex in Healthy Subjects. *PLoS ONE.* 2014; 9:e100198. [PubMed: 24936654]
44. Scher AI, Stewart WF, Ricci JA, Lipton RB. Factors associated with the onset and remission of chronic daily headache in a population-based study. *Pain.* 2003; 106:81–89. [PubMed: 14581114]
45. Schnizler K, Shutov LP, Van Kanegan MJ, Merrill MA, Nichols B, McKnight GS, Strack S, Hell JW, Usachev YM. Protein Kinase A Anchoring via AKAP150 Is Essential for TRPV1 Modulation by Forskolin and Prostaglandin E2 in Mouse Sensory Neurons. *J Neurosci.* 2008; 28:4904–4917. [PubMed: 18463244]
46. Simone DA, Baumann TK, LaMotte RH. Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. *Pain.* 1989; 38:99–107. [PubMed: 2780068]
47. Stone AA, Broderick JE. Obesity and Pain Are Associated in the United States. *Obesity.* 2012; 20:1491–1495. [PubMed: 22262163]
48. Strassman AM, Vos BP. Somatotopic and laminar organization of fos-like immunoreactivity in the medullary and upper cervical dorsal horn induced by noxious facial stimulation in the rat. *J Comp Neurol.* 1993; 331:495–516. [PubMed: 8509507]
49. Tian, Yab; Wang, Sa; Ma, Ya; Lim, Ga; Kim, Ha; Mao, Ja. Leptin enhances NMDA-induced spinal excitation in rats: A functional link between adipocytokine and neuropathic pain. *Pain.* 2011; 152:1263–1271. [PubMed: 21376468]

50. Urano H, Ara T, Fujinami Y, Hiraoka BY. Aberrant TRPV1 Expression in Heat Hyperalgesia Associated with Trigeminal Neuropathic Pain. *Int J Med Sci.* 2012; 9:690–697. [PubMed: 23091405]
51. Winter A, Berger K, Buring J, Kurth T. Body Mass Index, Migraine, Migraine Frequency and Migraine Features in Women. *Cephalalgia.* 2009; 29:269–278. [PubMed: 19143772]
52. Zhang Z, Winborn CS, Marquez de Prado B, Russo AF. Sensitization of Calcitonin Gene-Related Peptide Receptors by Receptor Activity-Modifying Protein-1 in the Trigeminal Ganglion. *J Neurosci.* 2007; 27:2693–2703. [PubMed: 17344407]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Fig. 1. Experimental groups to determine the relative contribution of obesity and high fat diet to photophobia.

Fig. 2.

Effects of diet-induced obesity on nocifensive behaviors in C57BL/6J mice (10 min test).

Intradermal capsaicin in the whisker pad (A) Increases ipsilateral facial wipes and (B)

Decreases rearing events in both diet groups equally. * $p < 0.05$ ** $p < 0.0001$ versus vehicle.

(vehicle $n = 15-17$ /group, capsaicin $n = 10-12$ /group)

Fig. 3.

Effects of diet-induced obesity on basal photophobic behavior and locomotor parameters in C57BL/6J mice. (A) More obese HFD mice ($n = 80$) than RD mice ($n = 74$) are photophobic at baseline (i.e. they spent less than 50% time in light). (B–D) Locomotor parameters (B, percentage of time resting, C, percentage of time moving, and D, cm/s) exhibited by mice for both zones at baseline. There were no post-hoc differences between the diets in percentage of time resting or percentage of time moving in either zone. Obese HFD mice traveled less distance relative to the time they spent in each zone than RD mice. $*p < 0.05$ versus control mice.

Fig. 4.

Effects of trigeminal stimulation with capsaicin on photophobic behavior and locomotor activity in obese HFD mice. (A) Percentage of time in light decreases after facial injection of 0.01% capsaicin in obese HFD ($n = 44$) but not RD ($n = 38$) mice. (B–D) Locomotor parameters: B, percentage of time resting increases after 0.01% capsaicin in obese HFD mice in the dark compartment only; C, percentage of time moving decreases after 0.01% capsaicin in obese HFD mice in the dark compartment only; and D, distance travelled relative to the time spent in each side (cm/s) decreases after 0.01% capsaicin in obese HFD

mice in the dark compartment only. (E, F) Correlation of % time in light after capsaicin with body weight in RD and obese HFD mice respectively.

(E) For RD mice, there is no significant correlation between body weight and % time in light ($R^2 = 0.01$, $p = 0.46$). (F) For obese HFD mice, there is a significant correlation of body weight with % time in light ($R^2 = 0.25$, $P = 0.0001$). $*p < 0.05$ versus vehicle for the indicated diet. $^+p < 0.05$ versus control mice.

Fig. 5.

Effects of trigeminal stimulation with capsaicin on photophobic behavior and locomotor activity in lean HFD ($n = 17$) and RD ($n = 16$) mice. (A) Percentage of time in light is similar in both diet groups for all treatments. (B–D) Locomotor parameters (B, percentage of time resting, C, percentage of time moving, and D, cm/s) are similar in both diet groups for all treatments. * $p < 0.05$ versus vehicle for the indicated diet.

Fig. 6.

Effects of trigeminal stimulation with capsaicin on photophobic behavior and locomotor activity in *ob/ob* ($n = 16$) and age-matched control mice ($n = 13$). (A) *ob/ob* spend less time in light than controls and display a significant decrease following 0.1% capsaicin injection compared with vehicle. (B–D) Locomotor parameters (B, percentage of time resting, C, percentage of time moving, and D, cm/s) of *ob/ob* and control mice indicate that *ob/ob* mice move less in general and that their movement is altered by capsaicin injection. * $p < 0.05$ versus vehicle for *ob/ob* mice. $^+p < 0.05$ versus control mice.

Fig. 7.

Serum corticosterone levels in obese HFD and RD mice under various testing and treatment conditions. (A) Baseline corticosterone levels are similar in both diet groups and they increase after light avoidance test ($n = 12-22$). (B) Corticosterone levels are higher in obese HFD mice 18 hours after capsaicin injection but before behavioral testing ($n = 5-7$). (C) Corticosterone levels after the light avoidance test are higher in obese HFD mice regardless of treatment ($n = 5-7$). * $p < 0.05$

Fig. 8. Calcium imaging of primary trigeminal ganglia cultures from obese HFD ($n = 6$) and RD ($n = 6$) mice. (A, B) Percentage of TRPV1+ cells from six obese HFD (A) and six RD (B) coverslips and their distribution by cell diameter. (C, D) Histograms of cell diameter size distribution. (C) TRPV1+ neurons from obese HFD mice exhibit rightward shift in cell diameter compared to RD. (D) TRPV1- neurons from both diets exhibit normal distribution of cell diameter. (E) Change in relative calcium level in response to capsaicin is higher in neurons from obese HFD mice. (F) Change in relative calcium levels in response to 60mM

KCl is higher in TRPV1+ and – neurons from obese HFD mice. These values are normalized to the initial baseline signal before capsaicin treatment. (G, H) Representative coverslips from obese HFD and RD mice. * $p < 0.05$ versus control diet.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

Average weights of each experimental group (mean $\pm$ SEM). Weights at weaning were only available for mice that were bred in our colony (some were purchased and weaning weights are not available).

Experiment	Experimental Group	Weight at Weaning	Weight at Test
Nocifensive behavior	Obese HFD ($n = 44$) Lean RD ($n = 39$)	Not available	45.02 $\pm$ 1.1 * 30.8 $\pm$ 0.4
Photophobia	Obese HFD ($n = 28-82$) Lean RD ($n = 29-76$)	9.8 $\pm$ 0.2 9.1 $\pm$ 0.4	42.7 $\pm$ 0.6 * 30.9 $\pm$ 0.5
	Lean HFD ($n = 17$) Lean RD ($n = 16$)	9.6 $\pm$ 0.5 10.1 $\pm$ 0.8	29.7 $\pm$ 0.8 * 26.0 $\pm$ 0.4
	Obese <i>ob/ob</i> ($n = 16$) Lean control ($n = 13$)	Not available	44.2 $\pm$ 0.3 * 23.5 $\pm$ 0.4

* $p < 0.05$ (obese compared with lean control).

Statistical outcomes of two-way ANOVAs with repeated measures (*F* values) for locomotor parameters of all groups tested in the light avoidance test.

Table 2

Group	Locomotor Parameter	Light Side			Dark Side		
		Diet/Genotype	Treatment	Interaction	Diet/Genotype	Treatment	Interaction
Obese HFD	% resting	5.319 *	7.615 *	4.609 *	2.316	29.33 **	5.985 *
	% moving	0.7896	13.73 **	1.302	3.109	12.17 **	3.100 *
	cm/s	5.182 *	3.573 *	3.091 *	4.150 *	16.60 **	3.817 *
Lean HFD	% resting	0.06684 *	1.277	0.6714	0.4852	5.999 *	3.328 *
	% moving	1.616	3.088 *	0.2738	0.3717	5.381 *	3.306 *
	cm/s	1.666	2.815 *	0.3383	0.2681	6.348 *	1.812
<i>Ob/ob</i>	% resting	50.40 **	8.185 **	6.002 *	316.8 **	11.57 **	9.515 **
	% moving [‡]	5.339 *	10.45 **	9.380 **	233	4.795 **	3.411 *
	cm/s	49.56 **	9.535 **	8.264 **	266.7 **	5.837 *	3.164 **

* *p* < 0.05,

** *p* < 0.0001.

[‡] one statistical outlier (Grubb's Test) was removed from % time moving in light.