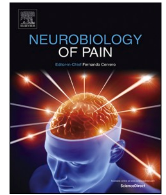
Neurobiology of Pain 13 (2023) 100111

Contents lists available at ScienceDirect

Neurobiology of Pain

journal homepage: www.sciencedirect.com/journal/neurobiology-of-pain



Brainstem pain-modulating neurons are sensitized to visual light in persistent inflammation

Gwen Hryciw [a,b,c], Jennifer Wong [c], Mary M. Heinricher [c,d,*]

[a] School of Dentistry, Oregon Health & Science University, Portland, OR, USA
[b] Dept. Biomedical Engineering, Oregon Health & Science University, Portland, OR, USA
[c] Dept. Neurological Surgery, Oregon Health & Science University, Portland, OR, USA
[d] Dept. Behavioral Neuroscience, Oregon Health & Science University, Portland, OR, USA

ARTICLE INFO

Keywords:
Rostral ventromedial medulla
Descending control
Photosensitivity
Photophobia
Sex differences
Rat

ABSTRACT

Many individuals with chronic pain report abnormal sensitivity to visual light, referred to as “photosensitivity” or “photophobia,” yet how processing of light and nociceptive information come together remains a puzzle. Pain-modulating neurons in the rostral ventromedial medulla (RVM) have been shown to respond to bright visual light in male rats: activity of pain-enhancing ON-cells is increased, while that of pain-inhibiting OFF-cells is decreased. Since the RVM is the output node of a well-known pain modulation pathway, light-related input to these neurons could contribute to photosensitivity. The purpose of the present study was to fully characterize RVM ON– and OFF-cell responses to visual light by defining stimulus–response curves in male and female rats across a range of intensities (30 to 16,000 lx). We also determined if light-evoked responses are altered in animals subjected to persistent inflammation. We found that ON– and OFF-cells responded to relatively dim light (<1000 lx in naïve animals), with no difference between the sexes in threshold for light-evoked changes in firing or the percentage of responsive cells. Second, light-evoked suppression of OFF-cell firing was enhanced in persistent inflammation, with no change in light-evoked activation of ON-cells. These data indicate that pain-modulating neurons can be engaged by dim light, even under normal conditions. Further, they suggest that decreased descending inhibition during light exposure could contribute to reduced nociceptive thresholds in chronic pain states, resulting in light-induced somatic discomfort and aversion to light. Lastly, our findings argue for differences in how light and somatic stimuli engage RVM, and suggest that light-related input acts as a “top-down” regulatory input to RVM.

Introduction

Many individuals with chronic pain report heightened sensitivity to multiple sensory modalities, including light. Increased sensitivity to light is best documented in migraine, but photosensitivity is prominent in patients with fibromyalgia, and strongly associated with the perceived impact of chronic pain in some populations (Gutrecht et al., 1990; Woodhouse and Drummond, 1993; Main et al., 1997; Wilbarger and Cook, 2011; Martenson et al., 2016; Staud et al., 2021; Balba et al., 2022). Dysfunction of central pain-*transmission* circuitry is argued to account for some of the observed photophobia, particularly in migraine (Woodhouse and Drummond, 1993; Digre and Brennan, 2012; Matynia et al., 2012; Noseda et al., 2018), but there is also evidence for changes in pain-*modulating* circuitry (Martenson et al., 2016). The output node of an important pain-modulating circuit is the rostral ventromedial medulla (Heinricher and Fields, 2013), which has been shown to contain a subset of neurons that respond to both noxious stimuli and bright visual light (Martenson et al., 2016).

The RVM amplifies or suppresses nociceptive transmission through two classes of neurons, termed “ON-cells” and “OFF-cells”, respectively. Activation of pain-facilitating ON-cells and suppression of pain-inhibiting OFF-cells together act as a positive feedback loop (Hernandez and Vanegas, 2001; Jinks et al., 2007), facilitating responses to subsequent inputs, and contributing to pathological pain (Ramirez and Vanegas, 1989; Heinricher et al., 2009; Cleary and Heinricher, 2013; Heinricher and Fields, 2013). Bright light exposure has been shown to modulate RVM cell activity in male rats, and also produces measurable thermal hyperalgesia (Martenson et al., 2016). Given that ON-cells exert a net pro-nociceptive effect, and that OFF-cells exert a net anti-nociceptive effect, activation of ON-cells and suppression of OFF-cell

* Corresponding author at: Department of Neurological Surgery, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA.
E-mail address: heinricm@ohsu.edu (M.M. Heinricher).

https://doi.org/10.1016/j.ynpai.2022.100111
Received 6 November 2022; Received in revised form 23 December 2022; Accepted 24 December 2022
Available online 26 December 2022
2452-073X/© 2022 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

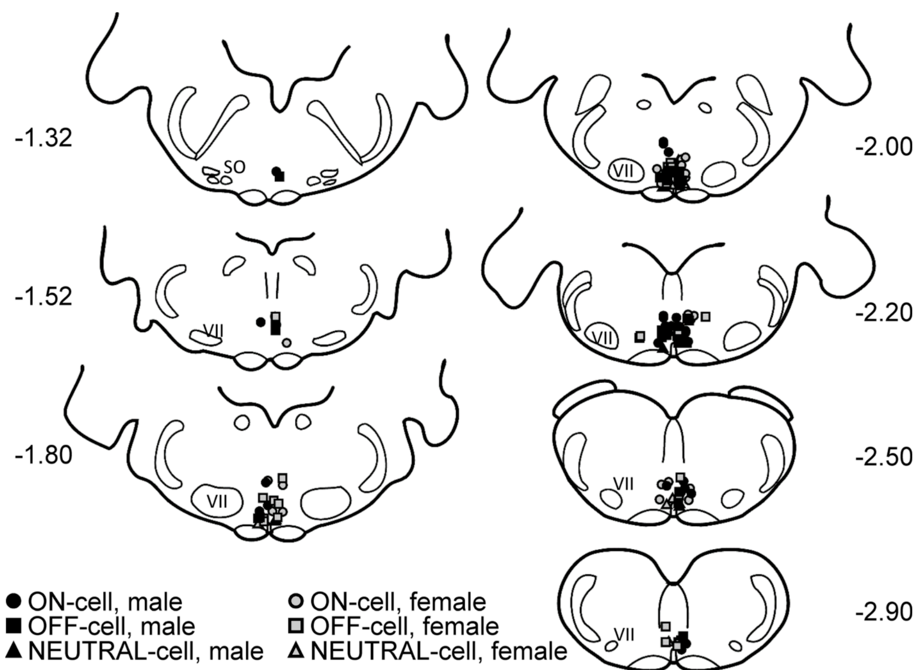


Fig. 1. Locations of ON–, OFF-, and NEUTRAL-cells recorded in males and females. Cells were recorded at sites ranging from −1.32 mm to −2.90 mm relative to the interaural line, with the majority of the cells recorded between −1.80 mm and −2.5 mm. Cells in males and females were evenly distributed across different rostro-caudal levels. There were 29 ON-cells, 25 OFF-cells, and 10 NEUTRAL-cells recorded from 49 male animals and 28 ON-cells, 26 OFF-cells, and 10 NEUTRAL-cells recorded from 53 female animals. SO: superior olive; VII: nucleus of the facial nerve.

firing by light would provide a means by which ambient light influences pain transmission.

There are substantial sex differences in the prevalence and presentation of many functional pain disorders associated with photosensitivity (Fillingim et al., 2009; Buse et al., 2013). While we know that there are molecular and structural differences in pain-modulation circuitry between the sexes (Loyd and Murphy, 2006; Loyd and Murphy, 2014), potential differences in the *functional* output of this system have been understudied. We recently described RVM cell responses to *somatic* stimuli in male and female rats, finding them to be similar (Hryciw et al., 2021). However, light likely engages RVM through *non-somatic* pathways (Martenson et al., 2016). This makes it important to compare RVM responses to light in the two sexes to understand whether the fundamental neural "machinery" is comparable, or distinct. In the present studies, we generated light-evoked stimulus response curves, and directly compared responses in males and females.

In addition, although photosensitivity is commonly associated with chronic pain disorders, the initial report of RVM responses to light did not employ a chronic pain model. Therefore, the second goal of the present study was to test the effect of persistent inflammation on RVM cell responses to light. ON– and OFF-cells are "sensitized" to somatic inputs in animals subjected to nerve injury or persistent inflammation, demonstrating lowered thresholds for responses to cutaneous stimulation (Carlson et al., 2007; Cleary and Heinricher, 2013). Thus, it is reasonable to ask whether RVM cell responses to light are also enhanced during persistent inflammation. If photoresponsiveness of RVM pain-modulating neurons is heightened during persistent inflammation, light could further lower nociceptive thresholds, contributing to the widespread hypersensitivity observed in many chronic pain conditions.

Materials and methods

All experiments followed the guidelines of the National Institutes of Health and the Committee for Research and Ethical Issues of the International Association for the Study of Pain, and were approved by the Institutional Animal Care and Use Committee at the Oregon Health & Science University. Male and female rats from Charles River were used in all experiments. Rats were housed in 12 h light/12 h dark cycles, with food and water available *ad libitum*. Experiments were performed during the light phase.

Inflammation

A subset of male and female rats weighing < 315 and 200 g, respectively, were briefly anesthetized with isoflurane (4 %, 4–5 min), and saline (0.1 ml) or CFA (heat-killed Mycobacterium tuberculosis in mineral oil, 1 mg/ml, 0.1 ml, Sigma-Aldrich) was injected subcutaneously into the plantar surface of the left hindpaw. Rats were returned to their home cage for 5 to 6 days to model persistent inflammation, since hypersensitivity peaks at this time (Ren, 1999; Ren and Dubner, 1999). Animals were randomly assigned to saline or CFA treatment.

Lightly anesthetized preparation

Following previously described methods (Cleary and Heinricher, 2013; Martenson et al., 2016), animals were anesthetized (4 % isoflurane, Piramal) and a catheter placed in the external jugular vein for subsequent infusion of methohexital (Par Pharmaceutical). Animals were then transferred to a stereotaxic apparatus and kept deeply anesthetized while a small craniotomy posterior to the lambda suture was drilled to gain access to RVM. After surgery, anesthesia was adjusted so that noxious heat elicited a paw withdrawal, although there was no spontaneous movement. Animals were maintained at this stable anesthetic plane for the duration of the experiment by infusion of methohexital at a constant rate. Heart rate and body temperature were also monitored. All testing was performed in low ambient light conditions (<5 lx) and the pupils were dilated (1 % atropine sulfate ophthalmic solution, 20 μl/eye, Akorn Pharmaceuticals) to eliminate differences in amount of light reaching the retina due to pupillary light reflexes.

Electrophysiological recording

A gold- and platinum-plated stainless-steel microelectrode was placed in the RVM to record cell activity. Signals were amplified and band-pass filtered (Neurolog, Digitimer ltd., Welwyn Garden City, UK) then transmitted to a computer for real-time spike detection and monitoring using Spike2 software (CED, Cambridge, UK). EMG activity, heart rate, and paw heat-stimulus temperature were also recorded using

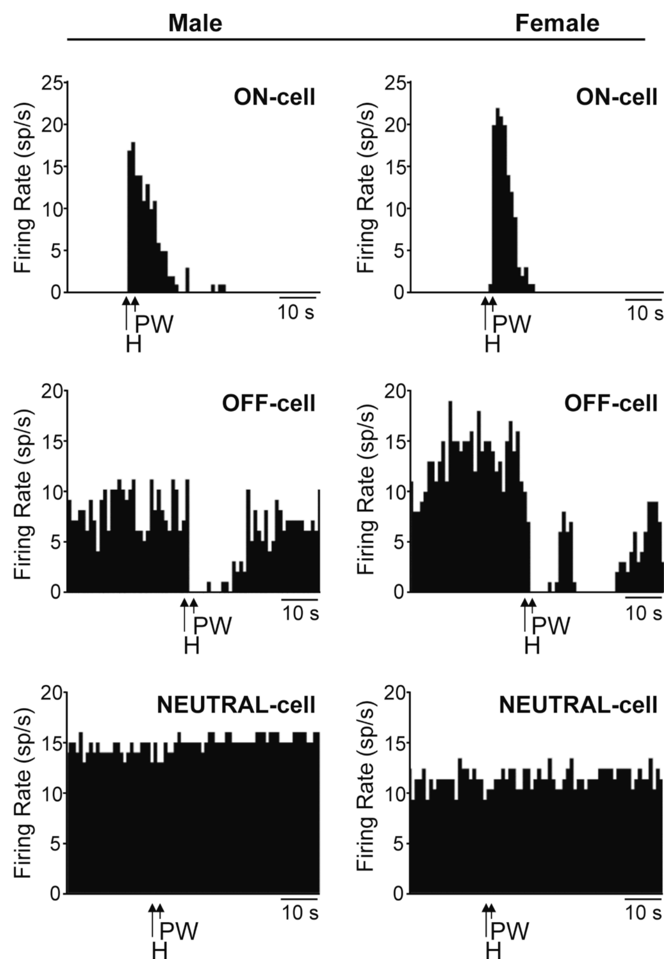


Fig. 2. Representative heat-evoked responses of ON-cells (top), OFF-cells (middle), and NEUTRAL-cells (bottom). Ratemeter records (1-s bins) with heat onset (H) and paw withdrawal (PW) show heat-related cell responses recorded from RVM in male (left) and female (right) rats. ON-cells exhibited a burst of activity while the OFF-cells showed a pause or suppression of ongoing activity just after heat onset and prior to paw withdrawal. NEUTRAL-cell firing rate was not changed in association with noxious stimulation. Sp/s: spikes per second.

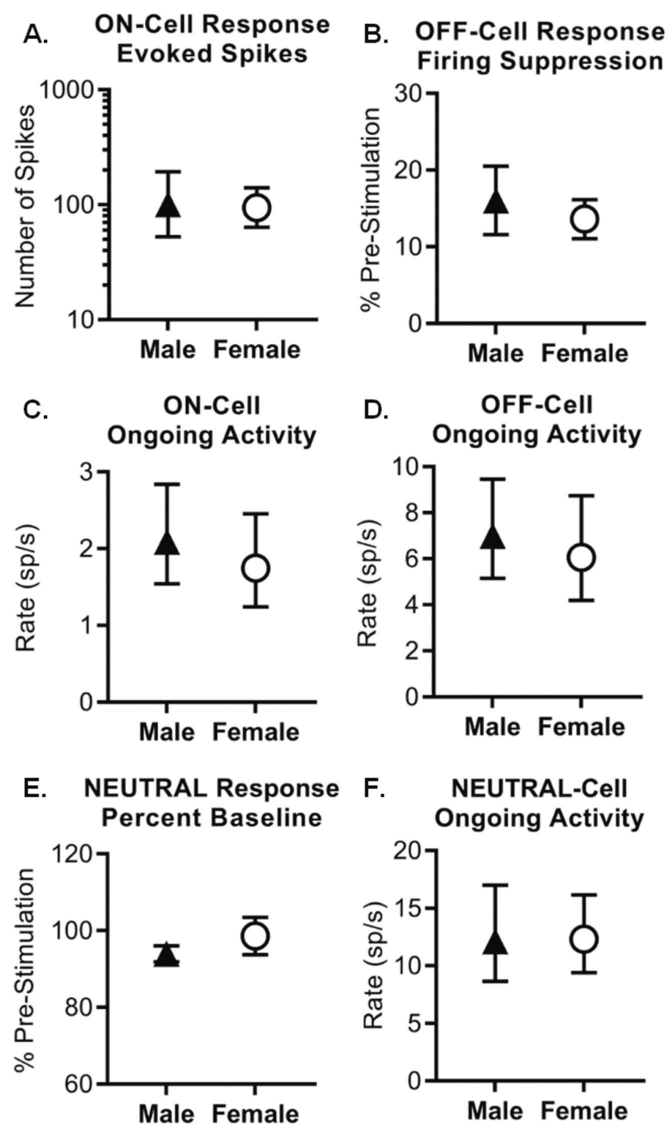


Fig. 3. Noxious somatic stimulus-related responses and ongoing activity of ON– and OFF-cells in male and female animals. Heat-evoked ON-cell burst (A) and OFF-cell suppression (B) were not significantly different between males and females. NEUTRAL-cell firing was not changed during noxious stimulation in either sex (E). Average ongoing firing rates of ON-cells (C), OFF-cells (D) and NEUTRAL-cells (F) during baseline (unstimulated) periods were also not significantly different between males and females. Sp/s: spikes per second. Analysis. **A.** Mann-Whitney *U* test, U = 395, p = 0.86. **B.** U = 292, p = 0.54. **C.** U = 334.5, p = 0.24. **D.** U = 311, p = 0.80. **E.** U = 32.5, p = 0.20. **F.** U = 45, p = 0.74.

Spike2. Identified neurons were classified as ON–, OFF-, or NEUTRAL-cells, as originally defined based on changes in firing rate associated with withdrawal from noxious heat stimulation (Fields et al., 1983; Cleary and Heinricher, 2013; Martenson et al., 2016). ON-cells are defined by a burst in activity beginning just prior to withdrawal from a noxious stimulus. OFF-cells stop firing just prior to withdrawal. NEUTRAL-cell firing does not change in response to noxious stimuli.

In the first set of experiments, naïve males and females were used. After isolating and identifying a cell, two heat trials were performed (2.5 min apart) to determine magnitude of response to noxious heat. The heat stimulus involved lightly resting a Peltier device (Yale Instruments, New Haven, CT) on the plantar surface of the paw, with surface temperature raised at a constant rate of 1.5 °C/s from 35 °C to a maximum of 53 °C. To avoid damage to the paw, the Peltier device was removed when the paw moved (determined using EMG). Next, the neuronal response to light was tested by placing a fiber-optic light source (Dolan-Jenner Fiber-Lite; Dolan-Jenner Industries, Buxborough, MA) 5 cm from the left eye to deliver diffused light at a range of intensities in ascending order (330, 575, 900, 6,000, 10,500, and 16,000 lx). Each intensity was applied for 30 s at approximately 2.5-min intervals, with each intensity repeated two times. Some trials were delayed (approx. 1 min) in order to capture an ON-cell in a quiet state or an OFF-cell in an active state. A final heat trial was performed at the end of the experiment to confirm anesthetic stability. Males required a higher anesthetic rate than females ($t(100) = 18.74$, $p < 0.0001$, n = 102; M: 88.77 ± 7.85 mg/kg/h, F: 61.0 ± 7.07 mg/kg/h, mean ± SD to achieve a similar anesthetic depth based on nociceptive withdrawal (Merkel and Eger, 1963). A total of 64 cells were recorded from 49 males, and 64 cells from 53 females (1–3 cells per animal).

In the second set of experiments testing the effect of persistent inflammation, male and female animals treated with CFA or saline (control) were used. After isolating and identifying a cell, spontaneous activity was recorded for 10 min during which time animals were not stimulated. Stimuli were subsequently delivered at approximately 2.5-min intervals, with some trials delayed in order to capture an ON-cell in a quiet state or an OFF-cell in an active state. Two thermal trials

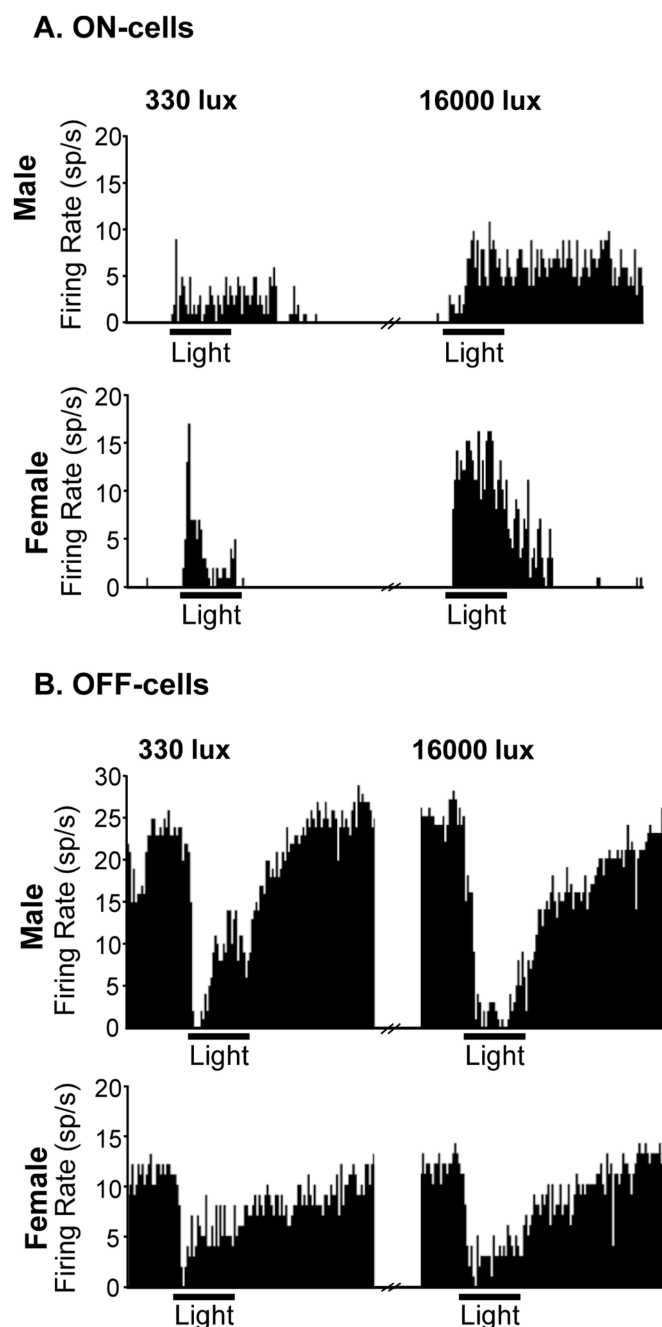


Fig. 4. Representative ON-cell (A) and OFF-cell (B) response to 330 lx (left) and 16,000 lx (right). Ratemeter records (1-s bins), with the time of the light stimulus application below each trace, show examples of light-evoked responses recorded from RVM in male and in female animals. There was a clear burst in ON-cell activity and suppression of OFF-cell activity during light exposure, even with the 330 lx stimulus. Sp/s: spikes per second.

were performed to determine the magnitude of the cell response to noxious heat and the latency to paw withdrawal. Next, cell responses to light were tested by placing a fiber-optic light source (Dolan-Jenner Fiber-Lite; Dolan-Jenner Industries, Buxborough, MA) 5 cm from the left eye to deliver diffused light at a range of intensities in ascending order (30, 140, 330, 900, and 6,000 lx). Each light trial lasted 30 s, with each intensity repeated three times. Intensities used in this set of experiments were lower than those employed in naïve animals to avoid the possibility of a floor effect. Inflammation was confirmed visually and paws were measured at the widest point across the dorsal-plantar surface. CFA injection did not significantly affect weight gain compared to saline injection ($F(1,98) = 3.38$, $p = 0.069$, n = 52 M, 50F; pre-CFA weight not obtained for some animals) or anesthetic requirement ($F(1,111) = 0.21$, $p = 0.65$, n = 115). However, as in the first set of experiments, males required a higher rate of anesthetic delivery ($F(1,111) = 217.6$, $p < 0.0001$, n = 115, males: 76.91 ± 8.04 mg/kg/h, females: 58.51 ± 4.93 mg/kg/h) to achieve a similar anesthetic depth. A total of 147 ON– and OFF-cells were recorded from 115 animals (1–3 cells per animal). Twenty-three, 17, 20, and 18 ON-cells were recorded from CFA-treated males, saline-treated males, CFA-treated females, and saline-treated females, respectively. Seventeen, 19, 14, and 20 OFF-cells were recorded from CFA-treated males, saline-treated males, CFA-treated females, and saline-treated females, respectively.

Histology

At the end of each experiment, the recording site was marked with an electrolytic lesion. Animals were euthanized by methohexital overdose and perfused transcardially with saline and 10 % formalin. Brains were removed, and brainstems were sectioned on a Leica CM3050 S cryostat (60 μm sections). RVM lesion was photographed with an Optronics Microfire camera attached to an Olympus BX51 microscope. The RVM was defined as the nucleus raphe magnus and adjacent reticular formation medial to the lateral boundary of the pyramids at the level of the facial nucleus (Paxinos and Watson, 2009).

Data processing and analysis

Action potential waveforms were individually examined to verify correct waveform sorting. Paw withdrawal latency was defined as the average time from heat onset until paw withdrawal based on EMG activity, and averaged across all trials for each animal.

In the first set of experiments, spontaneous activity was found by taking the average firing rate over the 30-s periods prior to the heat trials. In the second set of experiments, spontaneous activity was found by taking the average firing rate during three 30-s periods 2.5 min apart during the 10-min baseline period. Heat-evoked, reflex-related neuronal activity for ON-cells was defined as the total number of spikes in the burst and the duration of the burst, where a “burst” was defined as beginning with the first action potential after heat onset until the last action potential that preceded a 2-s quiet period. If a cell was active prior to heat onset, then the number of action potentials in the 3-s period beginning 0.5 s prior to the paw withdrawal was used to define the number of spikes in the burst, and burst duration was not defined for this trial. Heat evoked reflex-related change in neuronal activity for OFF-cells was defined using the percent of baseline (using firing rate in the 3-s period beginning 0.5 s prior to the paw withdrawal relative to the firing rate 10-s prior to heat onset) as well as the duration of the pause. A “pause” was considered to begin at the last spike after heat onset and to end when there were two action potentials within 2 s. Percent pre-stimulus firing was determined for NEUTRAL-cells based on the firing rate in the 3-s period around the paw withdrawal compared to the 10-s period preceding heat onset.

Overall a cell was considered to be “light-responsive” if it had a positive response at the highest intensity tested, defined as a change of at least 50 % during light exposure relative to the 30-s period preceding light onset. In addition, we required a minimum of 10 action potentials during the light trial for ON-cells, and 10 action potentials preceding the light trial for OFF-cells for a response to be considered positive. The percentage of cells that responded at each light intensity was also determined using these criteria.

For light-responsive cells, stimulus–response curves for light-related firing were generated based on the average of the light trials at each intensity. For ON-cells, the total number of action potentials in the burst was defined the same way as in heat trials. If an ON-cell was active prior to a light trial, then the total number of spikes during the light stimulus was used to define the number of spikes in the burst. For OFF-cells, we

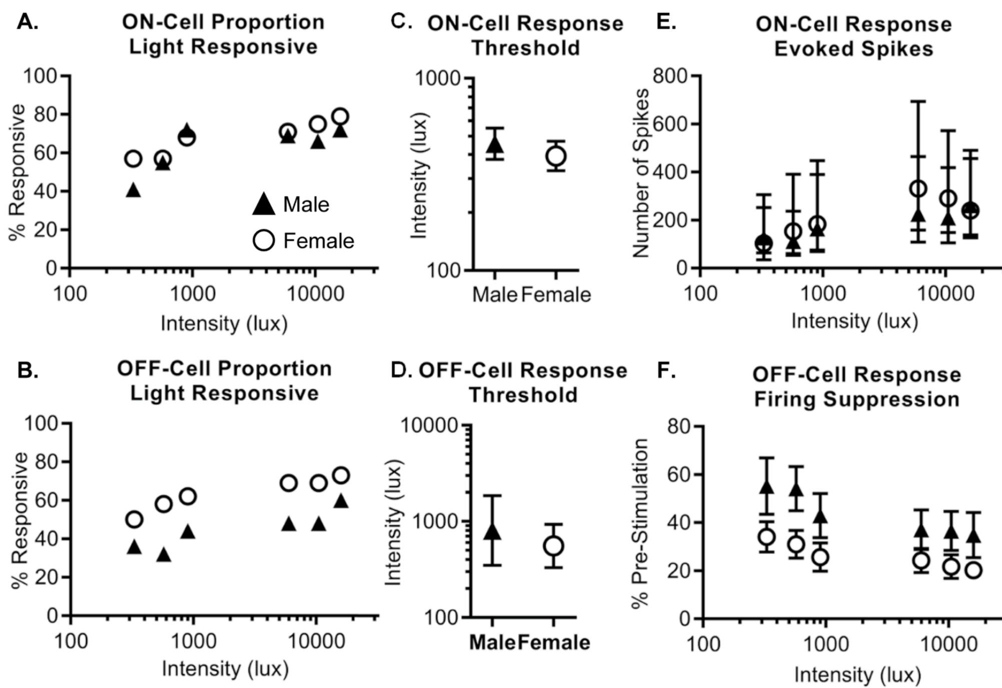


Fig. 5. Comparison of light-evoked responses of ON– and OFF-cells in male and female animals. The overall proportion of light-responsive cells was not different in males and females, although there was a significant stimulus–response relationship within both cell classes (A,B, one-sample *t*-test, slope significantly > 0). There was no significant difference between males and females in response threshold (C,D), or in changes in firing during light (E,F). Analysis. **A**. Proportion of ON-cells responsive to light: Fisher's exact test, $p = 0.76$; stimulus–response effect: $X^2(1) = 10.83$, $p = 0.0010$. **B**. Proportion of OFF-cells responsive to light: Fisher's exact test, $p = 0.38$; stimulus–response effect: $X^2(1) = 8.76$, $p = 0.0031$. Proportion data is displayed as the percent cells with a positive response at each intensity. **C**. Threshold, ON-cells: Mann-Whitney *U* test, $U = 167.5$, $p = 0.19$. **D**. Threshold, OFF-cells: $U = 127.5$, $p = 0.56$. **E**. ON-cell burst (sex: $t(41) = 0.29$, $p = 0.77$; stimulus response: $t(42) = 4.40$, $p < 0.0001$). **F**. OFF-cell suppression (sex: $t(32) = 1.99$, $p = 0.055$; stimulus–response: $t(33) = 3.40$, $p = 0.0018$).

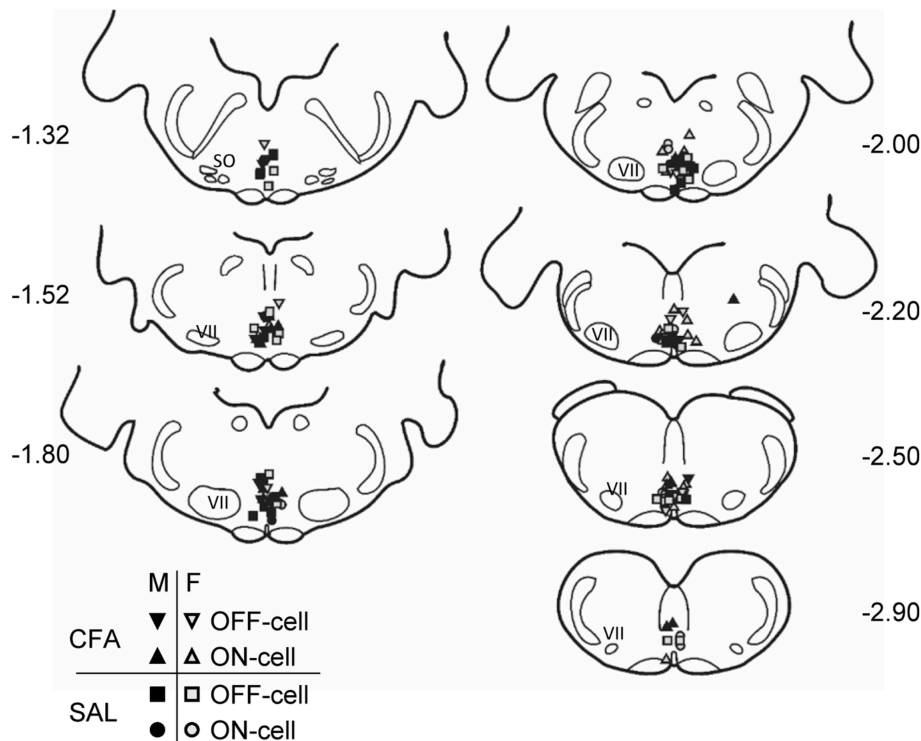


Fig. 6. Locations of recorded neurons in males and females with persistent inflammation. ON–, OFF-, and NEUTRAL-cells were distributed between −1.32 mm and −2.90 mm relative to the interaural line, with the majority of the cells recorded between −1.80 mm and –2.5 mm. Cells in males and females were evenly distributed across rostro-caudal levels. SO: superior olive; VII: nucleus of the facial nerve.

calculated percent of baseline (firing rate during light exposure relative to the firing rate in the 30-s period preceding the light trial).

Cell parameters with skewed distributions were analyzed using non-parametric statistics or log-transformed for analysis with a parametric test. In the first set of experiments, characterization of light-evoked responses in males and females under basal conditions, spontaneous activity and magnitude of heat responses were compared using a Mann-Whitney *U* test. The proportion of light-responsive cells was compared between the sexes with Fisher's exact test, and the stimulus–response relationship for the proportion of light-responsive cells was determined using a Chi-squared test for trend. The threshold for light-evoked responses was determined as the mean lowest intensity required to evoke a response from light-responsive cells, and compared between the sexes using a Mann-Whitney *U* test. Area under the curve was determined for

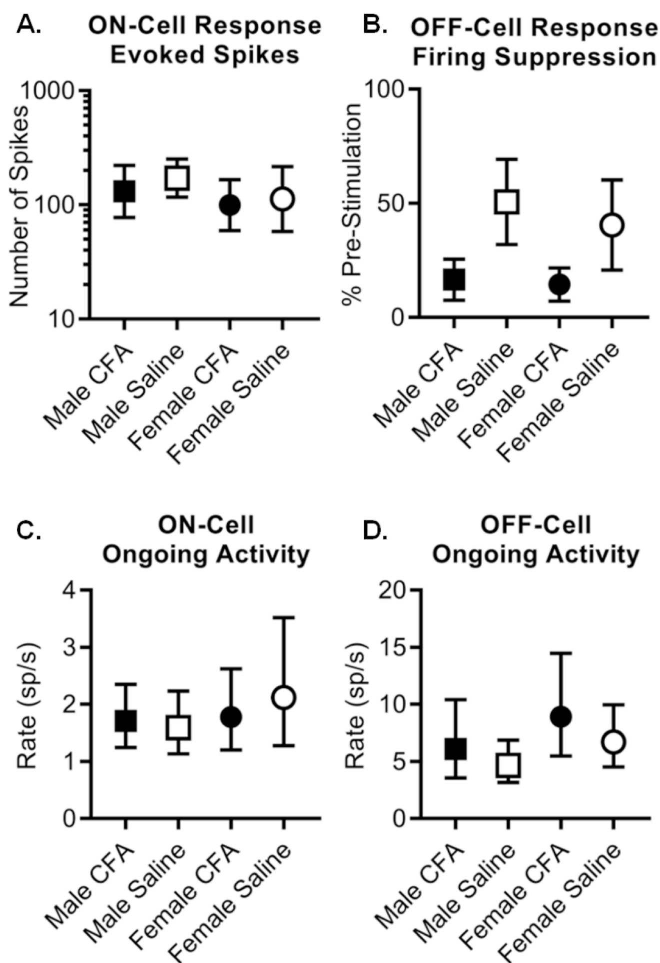


Fig. 7. Responses during heat-evoked withdrawal and ongoing activity of RVM OFF-and ON-cells recorded in males and females with and without persistent inflammation. Heat-evoked ON-cell burst (A) and OFF-cell suppression (B) were not significantly affected by sex or persistent inflammation. Average ongoing firing rates of ON-cells (C) and OFF-cells (D) were also not significantly affected by sex or persistent inflammation. Sp/*s*: spikes per second. Analysis. **A.** ON-cell burst. No significant effect of treatment (F(1,74)=0.59, p = 0.45), sex (F(1,74)=1.88, p = 0.17), or sex X treatment interaction (F(1,74)=0.085, p = 0.77). **B.** OFF-cell suppression. No significant effect of treatment (F(1,66)=3.42, p = 0.069), sex (F(1,66)=0.14, p = 0.71), or sex X treatment interaction (F(1,66)=0.061, p = 0.80). **C** ON-cell ongoing activity. No significant effect of treatment (F(1, 74)=0.079, p = 0.78), sex (F(1,74)=0.77, p = 0.38), or sex X treatment interaction (F(1,74)=0.44, p = 0.51). **D.** OFF-cell ongoing activity. No significant effect of treatment (F(1,66)=2.04, p = 0.16), sex (F(1,66)=2.98, p = 0.089), or sex X treatment interaction (F(1,66)=0.000094, p = 0.99).

the magnitude of light-related cell activity responses. This, and duration of evoked responses were compared between the sexes with a *t*-test for independent means. A one-sample *t*-test was used to determine if the average slope of the stimulus–response curves were significantly different from zero.

For the second set of experiments examining the effect of persistent inflammation on light-evoked cell responses, spontaneous firing and heat-evoked changes in cell activity, and paw withdrawal latency in CFA-treated and control animals were compared using a 2-factor ANOVA with sex and treatment as factors. The proportion of light-responsive cells was compared between the sexes with Chi-squared test, and the stimulus–response relationship for proportion of light-responsive cells was determined using a Chi-squared test for trend. Area-under-the-curve was determined for light-evoked response magnitudes and compared using a 2-factor ANOVA with sex and treatment as factors. If the overall ANOVA revealed significant differences, Sidak's post-hoc test was used to compare between groups at each intensity. A stimulus–response relationship was considered significant if a one-sample *t*-test determined average slope was significantly different from zero.

Data are presented as geometric mean ± 95 % confidence intervals unless otherwise indicated. For all tests, $p < 0.05$ was considered statistically significant.

Results

Experiment 1. Light-evoked responses of RVM neurons in male and female rats

Neurons were recorded in the RVM of male and female rats. ON–, OFF-, and NEUTRAL-cells were sampled at random, classified based on responses to noxious heat, and then tested for responses to a range of light intensities. Distribution of recording sites is shown in Fig. 1.

No differences in RVM cell spontaneous firing or noxious stimulus-related responses in males and females

Examples of ON–, OFF-, and NEUTRAL-cell responses associated with heat-evoked withdrawal in males and females are shown in Fig. 2. There were no differences between males and females in the ON-cell burst (Fig. 3A) or suppression of OFF-cell firing (Fig. 3B). The duration of the ON-cell burst and OFF-cell pause were not significantly different (ON-cells: $t(46) = 1.82$, $p = 0.075$; OFF-cells: $t(49) = 0.77$, $p = 0.45$). Spontaneous activity of both classes was also not significantly different in the two sexes (Fig. 3C,D). NEUTRAL-cells, were observed in both males and females, with no difference in firing rates (Fig. 3E,F). These data are consistent with our recent report that spontaneous firing rates and noxious-evoked changes in firing rates of RVM neurons are not substantially different in male and female rats (Hryciw et al., 2021).

RVM ON– And OFF-cell responses to light are graded with stimulus-intensity, and comparable in males and females

Representative examples of ON– and OFF-cell responses to low (330 lx) and high (16,000 lx) light intensities are shown in Fig. 4. Light-responsive ON-cells display a burst of activity, while firing of light-responsive OFF-cells is suppressed during light exposure. The percentage of light-responsive cells was intensity-dependent in both sexes (Fig. 5A,B), with the proportions of responsive cells not significantly different in males and females. The mean threshold for activation for both cell classes was < 1000 lx, and this was true in both males and females (Fig. 5C,D). The magnitude of light-evoked changes in firing was also determined for light-responsive cells, and there was no difference between males and females in the number of evoked spikes (ON-cells, Fig. 5E) or the suppression of spontaneous activity (OFF-cells, Fig. 5F). There were no NEUTRAL-cells in either sex that responded to light.

In sum, this first set of experiments demonstrates that many RVM pain-modulating neurons respond to light at intensities below 1000 lx, and that they respond in females as they do in males.

Experiment 2. Effect of persistent inflammation on light-evoked responses of RVM neurons

Persistent inflammation following CFA injection does not produce thermal hypersensitivity or change the thermal stimulus-related responses of ON– or OFF-cells

The second set of experiments was designed to determine whether light-evoked stimulus–response curves for RVM neurons are altered in the CFA model of persistent inflammation. Animals were treated with an injection of CFA or saline (control) in one hindpaw 5–6 days prior to recording. The injected paw of CFA-treated animals was significantly larger than that of saline-treated animals ($F(1,111) = 506.9$, $p < 0.0001$, n = 115; M SAL: 6.71 ± 0.45; M CFA: 8.90 ± 0.48 mm; F SAL: 6.06 ± 0.68; F CFA: 8.78 ± 0.69 mm) on the day of recording. Recording sites

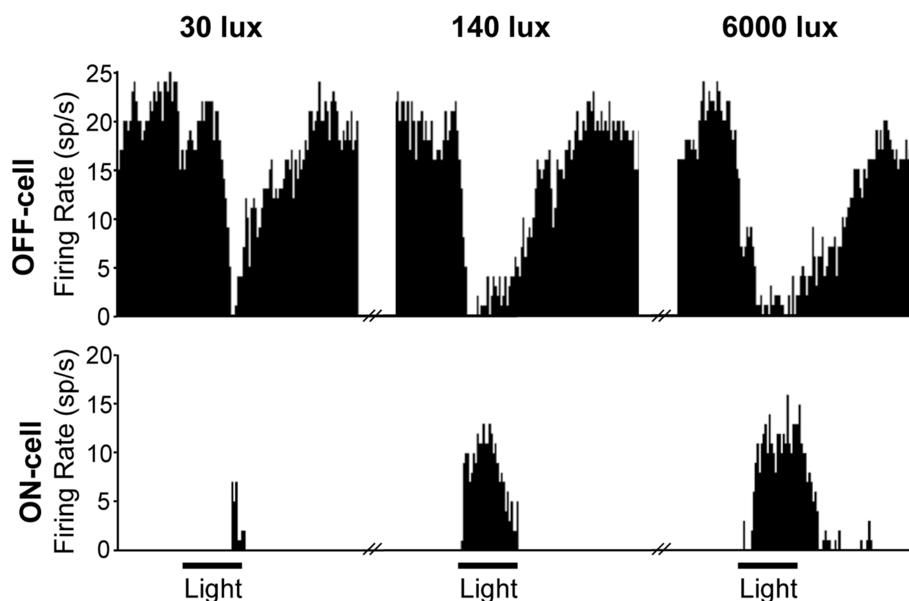


Fig. 8. Representative RVM OFF-cell (top) and ON-cell (bottom) responses to visual light. Rate meter records (1-s bins) from an OFF- and ON-cell recorded simultaneously in RVM in a male CFA-treated animal, with 30-s light stimulus denoted below the traces. Examples show clear light-evoked suppression of OFF-cell activity and activation of ON-cell activity during exposure to light at 30, 140, and 6000 lx. Sp/s: spikes per second.

are shown in Fig. 6.

While it was shown previously that males with persistent inflammation exhibit hyperalgesia to mechanical but not thermal stimulation of the inflamed paw, and that RVM neurons are sensitized to mechanical, but not thermal stimuli (Pinto-Ribeiro et al., 2008; Almarestani et al., 2011; Cleary and Heinricher, 2013), we used noxious heat as a rapid and reliable way to characterize RVM cells. Consistent with these prior reports, there was no effect of CFA-treatment on cell response during withdrawal from noxious thermal stimulation (Fig. 7A,B) or spontaneous firing rate (Fig. 7C,D). There was also no effect of CFA-treatment ($F(1,111) = 0.016$, $p = 0.90$) or sex ($F(1,111) = 0.60$, $p = 0.44$) on withdrawal latency.

Light-evoked stimulus–response curves of RVM pain-inhibiting OFF-cells, but not ON-cells, are shifted in animals with persistent inflammation

We next quantified light-evoked changes in firing in animals with and without persistent inflammation. Fig. 8 shows representative responses recorded simultaneously from a light-responsive ON-cell and OFF-cell in a male CFA-treated rat during exposure to 30, 140, and 6,000 lx. The ON-cell shows a characteristic “burst”, while the OFF-cell exhibits a “pause” in activity to visual light stimulation, with the responses graded with light intensity. When considering group data, we first compared the overall proportions of cells that were photoresponsive, and defined stimulus–response curves for each class, in both males and females with and without CFA treatment.

The proportion of OFF-cells responding to light was altered in animals with inflammation (Fig. 9A), with a significant increase in the proportion of cells responding to a low intensity (140 lx) stimulus, indicating that OFF-cell activity is inhibited by lower light intensities during inflammation, although mean threshold for the population was not altered (Fig. 9B). Among light-responsive OFF-cells, stimulus–response curves for light-evoked suppression of firing were also left-shifted (Fig. 9C), with a significant greater suppression at 140 lx in CFA-treated animals of both sexes.

By contrast with the increased sensitivity to relatively dim light exhibited by OFF-cells in animals with persistent inflammation, light-evoked changes in ON-cell firing were not altered. There was no significant effect of inflammation on the proportion of ON-cells that responded to light of any intensity (Fig. 10A), ON-cell response threshold (Fig. 10B), or on the magnitude of the light-evoked activity in light-responsive ON-cells (Fig. 10C).

In sum, the second experiment demonstrates a shift in the light-evoked stimulus–response curve for OFF-cells, but not ON-cells, after CFA.

Discussion

A recent report that exposure to bright visual light evokes a response in a substantial proportion of RVM pain-modulating neurons (Martenson et al., 2016) raised the possibility that recruitment of the RVM contributes to photosensitivity in some common pain states. However, that initial study employed a single, relatively bright stimulus in animals under basal conditions. Moreover, only male animals were used. In the present study, we established stimulus–response curves for light-evoked changes in activity of RVM pain-modulating neurons in males and females, under basal conditions and in a persistent inflammatory state. We determined that RVM neurons show graded responses to light stimuli across a broad range, and that stimulus–response curves for light-evoked changes in firing of these neurons are not different in males and females. Additionally, the proportion of OFF-cells responding to dim light is increased and these neurons exhibit an enhanced response to dim light following CFA treatment, whereas the ON-cell response to light is not altered.

Characterization of light-evoked responses in RVM pain-modulating neurons

RVM pain-modulating neurons responded even to dim levels of light as reflected by approximately 50 % of cells responding to 330 lx, the lowest light intensity tested in naïve animals. These low intensities are well below the threshold shown to activate trigeminal nociceptive pathways in rats (Okamoto et al., 2010) and are generally well tolerated by healthy human subjects (Woodhouse and Drummond, 1993; Kowacs et al., 2001; Martenson et al., 2016). Additionally, the RVM response to light, even bright light, is independent of the trigeminal ganglion and posterior thalamus, and is instead dependent on the olivary pretectal nucleus (Martenson et al., 2016). Thus, although the simplest mechanism to explain the RVM cell response to light would be that light is acting as a noxious stimulus and evoking a response in RVM cells via the trigeminal nociceptive system, this seems unlikely. Rather, these

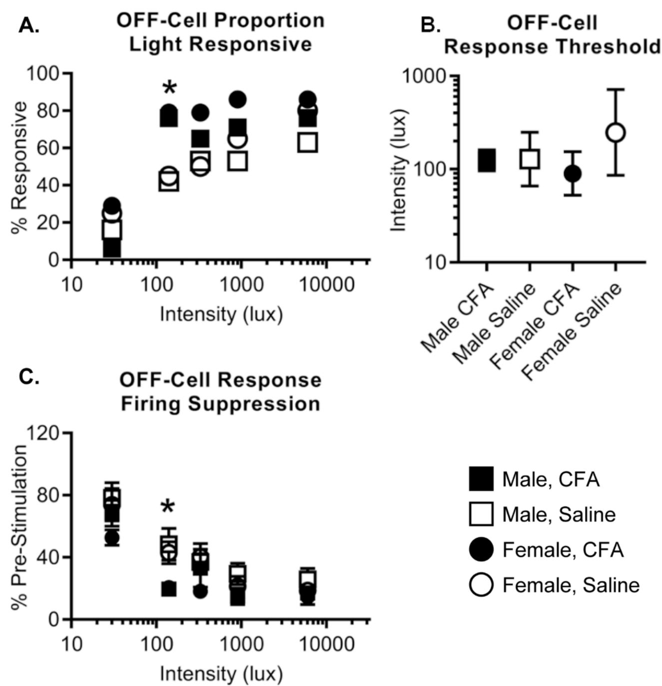


Fig. 9. RVM OFF-cell response to light is enhanced during persistent inflammation. There was a significant effect of inflammation on the proportion of OFF-cells that respond to dim light (A), but no effect on response threshold (B). There was also a significant effect of inflammation on the magnitude of OFF-cell response to dim light (C). There was no effect of sex on any parameter. Analysis. **A.** Percent of OFF-cells responsive to light of different intensities. Significant effect of inflammation on the percent of OFF-cells that responded to 140 lx (Fisher's exact test, $p = 0.0043$ compared to saline in both sexes). Additionally, there was a significant stimulus–response relationship (SAL: $X^2(1) = 21.71$, $p < 0.0001$; CFA: $X^2(1) = 20.19$, $p < 0.0001$). **B.** OFF-cell response threshold. No significant effect of treatment ($F(1,49) = 2.15$, $p = 0.15$), sex ($F(1,49) = 0.22$, $p = 0.64$), or sex X treatment interaction ($F(1,49) = 1.90$, $p = 0.17$). **C.** Light-evoked OFF-cell suppression. Significant effect of treatment ($F(1,49) = 6.64$, $p = 0.013$), but no significant effect of sex ($F(1,49) = 0.99$, $p = 0.32$), or group X intensity interaction ($F(1,49) = 0.044$, $p = 0.83$). Post hoc testing revealed a significant effect of treatment at 140 lx (Sidak's multiple comparisons, $p = 0.0027$). Additionally, there was a significant stimulus–response relationship (CFA: $t(24) = 7.96$, $p < 0.0001$; SAL: $t(27) = 8.70$, $p < 0.0001$), displayed as mean +/- SEM.

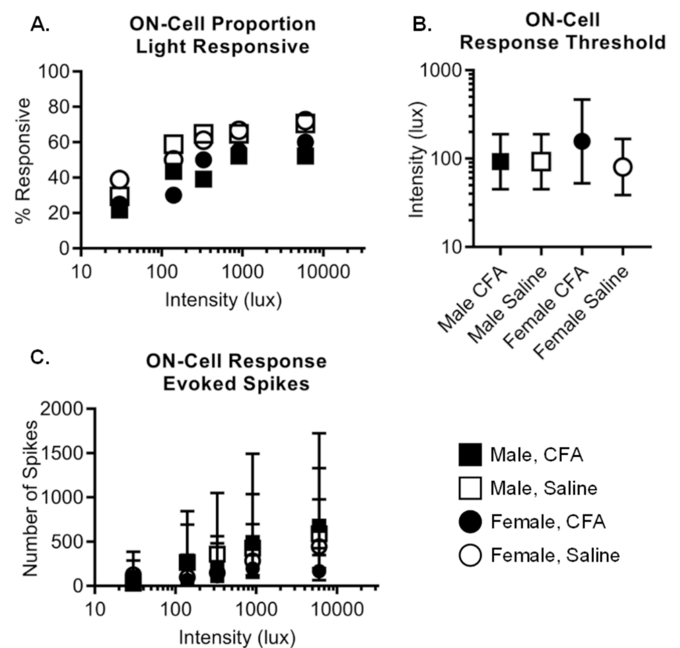


Fig. 10. RVM ON-cell response to light is not altered in persistent inflammation. There was no effect of inflammation on the proportion on ON-cells responding to light (A), response threshold (B), or magnitude of ON-cell response (C). There was also no effect of sex. Analysis. **A.** Percent of ON-cells responsive to light of different intensities. There was a significant stimulus–response relationship $X^2(1)=21.67$, $p < 0.0001$, but no differences between groups. **B.** No significant effect of treatment ($F(1,45) = 0.79$, $p = 0.38$), sex ($F(1,45) = 0.27$, $p = 0.61$), or sex X treatment interaction ($F(1,45) = 0.79$, $p = 0.38$) on ON-cell response threshold. **C.** Light-evoked ON-cell burst. No significant effect of treatment ($F(1,45)=0.47$, $p = 0.50$), sex ($F(1, 45) = 2.89$, $p = 0.096$), or sex X treatment interaction ($F(1,45) = 1.28$, $p = 0.26$). There was a significant stimulus–response relationship ($t(48) = 9.18$, $p < 0.0001$).

findings indicated that light and noxious somatic stimulation access RVM via different pathways.

Additional support for differing pathways is that the responses to somatic and visual inputs are qualitatively different. We found that the RVM ON– and OFF-cell response to light is graded with stimulus intensity. The graded response to light, and the response to dim levels of light, are in contrast with the response to somatic stimulation, in which RVM cells respond in an all-or-nothing manner and only to stimuli that evoke a nocifensive withdrawal (Fields et al., 1983; Cleary and Heinricher, 2013). Noxious somatic input forms a recurrent loop with RVM in which noxious somatic stimuli act as a bottom-up input to RVM, causing a change in RVM cell activity that in turn modulates nociceptive transmission, and ultimately influences the behavioral response threshold (Hernandez et al., 1994; Chen and Heinricher, 2022). The present data suggest that light instead acts as a top-down input that can influence the excitability of the pain-modulation system through graded changes in RVM cell activity. Engagement of RVM pain-modulating neurons has the potential to produce widespread changes in nociceptive sensitivity, because individual RVM neurons project diffusely to multiple spinal levels (Huisman et al., 1981; Skagerberg and Björklund, 1985). Indeed, nociceptive withdrawal threshold is lowered during light exposure even in lightly anesthetized animals, and prolonged exposure to red light has been reported to produce RVM-mediated hyperalgesia (Martenson et al., 2016; Khanna et al., 2019).

The pathways through which light engages RVM remain to be fully elucidated. As noted above, light-evoked responses in RVM require the olivary pretectal nucleus, a relay in the pupillary light reflex that is part of the irradiance detection system, and does not contribute to pattern vision (Clarke and Ikeda, 1985a; Clarke and Ikeda, 1985b; Martenson et al., 2016). A direct connection from the olivary pretectal nucleus to RVM has not been documented, but intervening circuits such as through centrally-projecting Edinger-Westphal nucleus or the parabrachial complex area are reasonable possibilities (Kozicz et al., 2011; Dos Santos Júnior et al., 2015; Roeder et al., 2016; Chen et al., 2017; Chiang et al., 2019; Cano et al.v 2021). It is possible that this irradiance input to RVM contributes to the modest but measurable circadian variations in pain that have been reported (Bumgarner et al. 2021).

RVM OFF-cells are "sensitized" to light in persistent somatic inflammation

RVM pain-modulating neurons in both males and females develop enhanced responses to *somatic* mechanical stimuli in models of persistent pain (Carlson et al. 2007; Cleary and Heinricher 2013; Hryciw et al. 2021). The second experiment demonstrated that OFF-cells are also "sensitized" to low intensity *light* in a persistent inflammatory state. Although responses to relatively bright light stimuli were unchanged, the proportion of OFF-cells responding to dim light was increased in animals subjected to inflammation, as was the degree to which OFF-cell firing was suppressed. This parallels the effect of persistent inflammation on responses to somatic tactile stimulation (Montagne-Clavel and Oliveras 1994; Cleary and Heinricher 2013; Hryciw et al. 2021), in that there are no differences observed at higher intensities or in the

maximum evoked-response, but the response to lower intensities is enhanced. Interestingly, within the light-responsive population, there was no effect of inflammation on the ON-cell stimulus–response curve. This latter finding is in contrast to the effects of persistent inflammation on mechanically-evoked responses, since ON-cells, as well as OFF-cells, are sensitized to innocuous cutaneous probing. These findings suggest that any RVM contribution to photosensitivity in chronic pain reflects a decrease in descending inhibition rather than increased light-evoked facilitation.

A further distinction between the sensitized RVM response to somatic versus light stimuli in persistent inflammation comes from the fact that with somatic stimuli, the RVM neurons demonstrate increased responses only to stimulation of the inflamed paw (Cleary and Heinricher 2013), and do not show enhanced responses to stimulation of normal tissues. This further supports the notion that light engages RVM through a "top-down" pathway separate from the somatosensory system.

One implication of the present findings is that even dim light could have behavioral effects, given that a not insignificant portion of RVM pain-modulating neurons respond to light intensities <300 lx. Particularly after injury, very low levels of light could diminish descending inhibition and have net effects on behavior. Moreover, given that RVM neurons are sensitized to innocuous somatic stimuli in persistent and chronic pain models, behavioral experiments in such models would be useful to determine if light can interact with somatic stimuli in an additive or even multiplicative fashion. Consistent with this idea, pressure-pain thresholds are reported to be further lowered during exposure to light in some chronic pain disorders (Kowacs et al., 2001).

RVM pain-modulating neurons respond to light in female as well as male rats

Finally, this study included female animals and explicitly compared the response properties of RVM neurons in female animals with those recorded in males. Although there are likely few sex differences in basal pain threshold in humans, chronic pain disorders disproportionately impact women (Fillingim et al., 2009; Mogil, 2012; Racine et al., 2012). Indeed, women reporting chronic pain are more likely to report photophobia (Buse et al., 2013). The present data indicate that the physiological properties and responsiveness of RVM pain-modulating neurons are unlikely to explain these sex differences, since stimulus–response functions for light-evoked changes in activity of pain-modulating neurons were comparable in males and females, and since the effects of persistent inflammation were also similar in the two sexes. Thus, as with responses to somatic inputs (Hryciw et al., 2021), the responses to light are not fundamentally different between males and females. Although this system may very well be differentially recruited in males and females in chronic pain, the present data support the idea that the same fundamental "machinery" for descending control of pain is in place in females as well as males.

Conclusions

The present experiments demonstrate that pain-modulating neurons in the RVM respond to visual light with a graded, stimulus-related response that is distinct from the response to somatic noxious stimuli, but comparable in males and females. At the same time, OFF-cells exhibit a sensitized response to light in animals subjected to persistent inflammation. These data also provide a framework through which photosensitivity, or aversion to light, could be produced by a convergence of somatic and visual inputs in the RVM.

CRediT authorship contribution statement

Gwen Hryciw: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jennifer Wong:** Data curation, Formal analysis, Visualization. **Mary M. Heinricher:** Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing.

Funding

This work was supported by the National Institutes of Health (grant number NS098660). GH was supported by fellowships from the NIDCR (F30 DE027584) and American Association for Dental Research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Almarestani, L., Fitzcharles, M.A., Bennett, G.J., Ribeiro-da-Silva, A., 2011. Imaging studies in Freund's complete adjuvant model of regional polyarthritis, a model suitable for the study of pain mechanisms, in the rat. Arthritis Rheum. 63, 1573–1581.

Balba, N.M., McBride, A.A., Callahan, M.L., Mist, S.D., Jones, K.D., Butler, M.P., Lim, M. M., Heinricher, M.M., 2022. Photosensitivity is associated with chronic pain following traumatic brain injury. J. Neurotrauma 39, 1183–1194.

Bumgarner, J.R., Walker 2nd, W.H., Nelson, R.J., 2021. Circadian rhythms and pain. Neurosci. Biobehav. Rev. 129, 296–306.

Buse, D.C., Loder, E.W., Gorman, J.A., Stewart, W.F., Reed, M.L., Fanning, K.M., Serrano, D., Lipton, R.B., 2013. Sex differences in the prevalence, symptoms, and associated features of migraine, probable migraine and other severe headache: Results of the American Migraine Prevalence and Prevention (AMPP) study. Headache 53, 1278–1299.

Cano, G., Hernan, S.L., Sved, A.F., 2021. Centrally projecting Edinger-Westphal nucleus in the control of sympathetic outflow and energy homeostasis. Brain Sci. 11.

Carlson, J.D., Maire, J.J., Martenson, M.E., Heinricher, M.M., 2007. Sensitization of pain-modulating neurons in the rostral ventromedial medulla after peripheral nerve injury. J. Neurosci. 27, 13222–13231.

Chen, Q., Roeder, Z., Li, M.H., Zhang, Y., Ingram, S.L. and Heinricher, M.M. (2017). Optogenetic evidence for a direct circuit linking nociceptive transmission through the parabrachial complex with pain-modulating neurons of the rostral ventromedial medulla (RVM). *eNeuro* **4**: ENEURO.0202-0217.2017.

Chen, Q., Heinricher, M.M., 2022. Shifting the balance: How top-down and bottom-up inputs modulate pain via the rostral ventromedial medulla. Front. Pain Res. 3, 932476.

Chiang, M.C., Bowen, A., Schier, L.A., Tupone, D., Uddin, O., Heinricher, M.M., 2019. Parabrachial complex: a hub for pain and aversion. J. Neurosci. 39, 8225–8230.

Clarke, R.J., Ikeda, H., 1985a. Luminance and darkness detectors in the olivary and posterior pretectal nuclei and their relationship to the pupillary light reflex in the rat. I. Studies with steady luminance levels. Exp. Brain Res. 57, 224–232.

Clarke, R.J., Ikeda, H., 1985b. Luminance detectors in the olivary pretectal nucleus and their relationship to the pupillary light reflex in the rat. II. Studies using sinusoidal light. Exp. Brain Res. 59, 83–90.

Cleary, D.R., Heinricher, M.M., 2013. Adaptations in responsiveness of brainstem pain-modulating neurons in acute compared with chronic inflammation. Pain 154, 845–855.

Digre, K.B., Brennan, K.C., 2012. Shedding light on photophobia. J. Neuroophthalmol. 32, 68–81.

Dos Santos Júnior, E.D., Da Silva, A.V., Da Silva, K.R., Haemmerle, C.A., Batagello, D.S., Da Silva, J.M., Lima, L.B., Da Silva, R.J., Diniz, G.B., Sita, L.V., Elias, C.F., Bittencourt, J.C., 2015. The centrally projecting Edinger-Westphal nucleus–I: efferents in the rat brain. J. Chem. Neuroanat. 68, 22–38.

Fields, H.L., Bry, J., Hentall, I., Zorman, G., 1983. The activity of neurons in the rostral medulla of the rat during withdrawal from noxious heat. J. Neurosci. 3, 2545–2552.

Fillingim, R.B., King, C.D., Ribeiro-Dasilva, M.C., Rahim-Williams, B., Riley 3rd, J.L., 2009. Sex, gender, and pain: a review of recent clinical and experimental findings. J. Pain 10, 447–485.

Gutrecht, J.A., Lessell, I.M., Zamani, A.A., 1990. Central dazzle in trigeminal sensory neuropathy. Neurology 40, 722–723.

Heinricher, M.M., Fields, H.L., 2013. Central nervous system mechanisms of pain modulation. In: McMahon, S., Koltzenburg, M., Tracey, I., Turk, D.C. (Eds.), Wall and Melzack's Textbook of Pain, 6th ed. Elsevier, London, pp. 129–142.

Heinricher, M.M., Tavares, I., Leith, J.L., Lumb, B.M., 2009. Descending control of nociception: specificity, recruitment and plasticity. Brain Res. Rev. 60, 214–225.

Hernandez, N., Dmitrieva, N., Vanegas, H., 1994. Medullary on-cell activity during tail-flick inhibition produced by heterotopic noxious stimulation. Pain 58, 393–401.

Hernandez, N., Vanegas, H., 2001. Encoding of noxious stimulus intensity by putative pain modulating neurons in the rostral ventromedial medulla and by simultaneously recorded nociceptive neurons in the spinal dorsal horn of rats. Pain 91, 307–315.

Hryciw, G., De Preter, C.C., Wong, J., Heinricher, M.M., 2021. Physiological properties of pain-modulating neurons in rostral ventromedial medulla in female rats, and responses to opioid administration. Neurobiol. Pain 10, 100075.
Huisman, A.M., Kuypers, H.G., Verburgh, C.A., 1981. Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with the multiple fluorescent retrograde tracer technique. Brain Res. 209, 271–286.
Jinks, S.L., Carstens, E.E., Antognini, J.F., 2007. Glutamate receptor blockade in the rostral ventromedial medulla reduces the force of multisegmental motor responses to supramaximal noxious stimuli. Neurosci. Lett. 426, 175–180.
Khanna, R., Patwardhan, A., Yang, X., Li, W., Cai, S., Ji, Y., Chew, L.A., Dorame, A., Bellampalli, S.S., Schmoll, R.W., Gordon, J., Moutal, A., Vanderah, T.W., Porreca, F., Ibrahim, M.M., 2019. Development and characterization of an injury-free model of functional pain in rats by exposure to red light. J. Pain 20, 1293–1306.
Kowacs, P.A., Piovesan, E.J., Werneck, L.C., Tatsui, C.E., Lange, M.C., Ribas, L.C., da Silva, H.P., 2001. Influence of intense light stimulation on trigeminal and cervical pain perception thresholds. Cephalalgia 21, 184–188.
Kozicz, T., Bittencourt, J.C., May, P.J., Reiner, A., Gamlin, P.D., Palkovits, M., Horn, A. K., Toledo, C.A., Ryabinin, A.E., 2011. The Edinger-Westphal nucleus: a historical, structural, and functional perspective on a dichotomous terminology. J. Comp. Neurol. 519, 1413–1434.
Loyd, D.R., Murphy, A.Z., 2006. Sex differences in the anatomical and functional organization of the periaqueductal gray-rostral ventromedial medullary pathway in the rat: a potential circuit mediating the sexually dimorphic actions of morphine. J. Comp. Neurol. 496, 723–738.
Loyd, D.R., Murphy, A.Z., 2014. The neuroanatomy of sexual dimorphism in opioid analgesia. Exp. Neurol. 259, 57–63.
Main, A., Dowson, A., Gross, M., 1997. Photophobia and phonophobia in migraineurs between attacks. Headache 37, 492–495.
Martenson, M.E., Halawa, O.I., Tonsfeldt, K.J., Maxwell, C.A., Hammack, N., Mist, S.D., Pennesi, M.E., Bennett, R.M., Mauer, K.M., Jones, K.D., Heinricher, M.M., 2016. A possible neural mechanism for photosensitivity in chronic pain. Pain 157, 868–878.
Matynia, A., Parikh, S., Chen, B., Kim, P., McNeill, D.S., Nusinowitz, S., Evans, C., Gorin, M.B., 2012. Intrinsically photosensitive retinal ganglion cells are the primary but not exclusive circuit for light aversion. Exp. Eye Res. 105, 60–69.
Merkel, G., Eger 2nd, E.I., 1963. A comparative study of halothane and halopropane anesthesia including method for determining equipotency. Anesthesiology 24, 346–357.
Mogil, J.S., 2012. Sex differences in pain and pain inhibition: Multiple explanations of a controversial phenomenon. Nat. Rev. Neurosci. 13, 859–866.
Montagne-Clavel, J., Oliveras, J.L., 1994. Are ventromedial medulla neuronal properties modified by chronic peripheral inflammation? A single-unit study in the awake, freely moving polyarthritic rat. Brain Res. 657, 92–104.
Noseda, R., Copenhagen, D., Burstein, R., 2018. Current understanding of photophobia, visual networks and headaches. Cephalalgia 39, 1623–1634.
Okamoto, K., Tashiro, A., Chang, Z., Bereiter, D.A., 2010. Bright light activates a trigeminal nociceptive pathway. Pain 149, 235–242.
Paxinos, G., Watson, C., 2009. The Rat Brain in Stereotaxic Coordinates, Compact, 6th ed. Academic Press, Amsterdam.
Pinto-Ribeiro, F., Ansah, O.B., Almeida, A., Pertovaara, A., 2008. Influence of arthritis on descending modulation of nociception from the paraventricular nucleus of the hypothalamus. Brain Res. 1197, 63–75.
Racine, M., Tousignant-Laflamme, Y., Kloda, L.A., Dion, D., Dupuis, G., Choiniere, M., 2012. A systematic literature review of 10 years of research on sex/gender and experimental pain perception - part 1: are there really differences between women and men? Pain 153, 602–618.
Ramirez, F., Vanegas, H., 1989. Tooth pulp stimulation advances both medullary off-cell pause and tail flick. Neurosci. Lett. 100, 153–156.
Ren, K., 1999. An improved method for assessing mechanical allodynia in the rat. Physiol. Behav. 67, 711–716.
Ren, K., Dubner, R., 1999. Inflammatory models of pain and hyperalgesia. ILAR J. 40, 111–118.
Roeder, Z., Chen, Q., Davis, S., Carlson, J.D., Tupone, D., Heinricher, M.M., 2016. The parabrachial complex links pain transmission to descending pain modulation. Pain 157, 2697–2708.
Skagerberg, G., Björklund, A., 1985. Topographic principles in the spinal projections of serotonergic and non-serotonergic brainstem neurons in the rat. Neuroscience 15, 445–480.
Staud, R., Godfrey, M.M., Robinson, M.E., 2021. Fibromyalgia patients are not only hypersensitive to painful stimuli but also to acoustic stimuli. J. Pain 22, 914–925.
Wilbarger, J.L., Cook, D.B., 2011. Multisensory hypersensitivity in women with fibromyalgia: implications for well being and intervention. Arch. Phys. Med. Rehabil. 92, 653–656.
Woodhouse, A., Drummond, P.D., 1993. Mechanisms of increased sensitivity to noise and light in migraine headache. Cephalalgia 13, 417–421.