# CHARACTERIZATION OF THE LIGHT RESPONSE OF ROSTRAL VENTROMEDIAL MEDULLA PAIN-MODULATING NEURONS

by

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# ABSTRACT

Despite substantial progress in understanding pain mechanisms, inadequate pain management persists as a problem affecting millions of people globally.[1] One reason that pain is difficult to treat is that the experience of pain is subjective and unique to every individual, representing the interaction between two systems: pain transmission and pain modulation. A key part of the brain circuit responsible for modulation of pain is the rostral ventromedial medulla (RVM), which modulates pain transmission at the spinal cord through the actions of two classes of cells: pain-facilitating "ON-cells" which activate just before the occurrence of nociceptive reflexes, and pain-inhibiting "OFF-cells" which cease activity just before the occurrence of nociceptive reflexes. It has been widely accepted that these RVM pain-modulating neurons respond only to noxious somatic stimulation. However, the Heinricher lab has recently shown that a subset of these neurons respond to a white light visual stimulus: light can activate ON-cells and suppress the firing of OFF-cells.[48]

The experiments presented here were designed to pursue the preliminary finding that light can cause changes in the firing of pain-modulating RVM neurons. The goal was to further characterize this response to light, under the hypothesis that light-responsive cells would show differences in response to light of different wavelengths and intensities.

From our preliminary findings we know that light is accessing this modulatory center, but we do not know what effect, if any, this response is having on the pain-modulatory function of the RVM. If light can contribute to pain sensation through the RVM, it would represent a novel factor in the explanation of how pain sensation is unique to every individual, and light exposure could become a new component of pain management.

*Chapter 1*

# INTRODUCTION

## Descending Pain Modulation

The sensation of pain is dictated by the brain, and the level of pain felt in response to any painful event is the result of an ever-changing compromise between the intrinsic pain transmission and pain modulation systems. It is the burden of the intrinsic pain modulation system to take stock of and evaluate the body's situation and context, such as sickness or stress, and based on this information either amplify or suppress incoming pain signals in order to protect the body from harm. A key part of the brain circuit responsible for pain modulation is a collection of nuclei in the brainstem that make up the "rostral ventromedial medulla" (RVM). Inputs from many brain areas, especially the periaqueductal grey region, terminate in the RVM.[2,3,4] The RVM is the output of the pain modulation system, projecting to the dorsal horn of the spinal cord to modulate nociceptive transmission by exerting bidirectional control,[2,5,7,12] and is known to be involved in hypersensitivity following nerve injury, inflammation,[13] sickness,[14] or stress.[15] The RVM consists of three cell types: ON-cells, OFF-cells, and NEUTRAL-cells (Fig. 1). Noxious inputs have been shown to alter RVM neuronal activity: ON-cells fire rapidly ('burst') in response to a noxious input, preceding a nocifensive behavior.[5] Activation of these neurons produces hyperalgesia,[16] indicating that they have a net facilitatory influence on nociception. OFF-cells exhibit a pause in firing immediately before a nocifensive response,[5] and disinhibition of these cells can prevent a nocifensive response,[17,18,19] thus they have an inhibitory influence on nociception. NEUTRAL-cells do not respond to nociceptive stimuli and their role in descending pain modulation, if any, remains unclear.[20]

The overall motivation behind the pain modulation system is always survival of the organism. For example, if you twist your ankle while being chased by a bear, the neurons in the RVM will change their firing to suppress the pain signal coming from your ankle: OFF-cells will increase firing and cease to pause, ON-cells will not burst, which will tip the scales of pain transmission and allow you to escape from the bear. However, once you escape, the RVM neurons will change again: ON-cells will fire more and burst in response to normally non-painful stimuli, and OFF-cells will fire less, this time to amplify the pain caused by your ankle injury, so that you are inclined to rest this part of your body and

give it a chance to heal. This is how ON- and OFF-cells play a crucial role in both hyperalgesic and analgesic states,[6,7] contributing to the way in which the experience of pain manages to be unique to every individual.

**Light is Aversive, but is it Painful?**

Light-induced pain states, such that the presence of light can cause or intensify pain is referred to as "photosensitivity"[8,21,22] and is not a new concept. Photosensitivity has been most extensively studied in the context of migraine headache, though it is also reported anecdotally in several other functional pain states.[8,21,22,49,50] Research into the anatomical substrates of photosensitivity in migraine has considered trigeminal afferents,[23] the posterior thalamus,[24] and the non-image-forming visual pathway.[24,25] The possibility that pain-modulating circuits respond to light or contribute to photosensitivity has not been investigated.

It had been widely accepted that RVM pain-modulating neurons respond only to somatic stimulation. However, our lab has recently published data showing that a subset of these neurons respond to a white light visual stimulus.[48] In preliminary experiments, we observed that approximately half of ON- and OFF-cells, but no NEUTRAL-cells respond to a white light shone into the eye. This response was defined as a change in firing rate of at least 50% relative to ongoing firing rate, with activation of ON-cells and suppression of OFF-cell firing. These findings represent a novel observation, that visual light input can regulate activity of pain-modulating cells in the RVM, and raised the possibility that light modifies pain by recruiting brainstem pain-modulating systems. Since ON-cells have a net pro-nociceptive influence and OFF-cells exert a net anti-nociceptive influence, this observation opens up the possibility that light exposure could engage RVM modulating circuits to intensify pain.

To investigate the RVM pathway as a potential photosensitivity gateway, we must first consider how light signals reach the RVM. Light is detected by photoreceptors and relayed to the brain via the optic nerve. The majority of the light signals leaving the retina are for pattern vision, and synapse in the lateral geniculate nucleus (LGN) to be processed in the primary visual cortex. Other light signals are transmitted through a non-image-forming pathway, which originates in a specific class of retinal ganglion cell called intrinsically photosensitive retinal ganglion cells (ipRGCs) that contain the photopigment melanopsin, which responds maximally to blue light (460 - 480 nm).[51] ipRGCs can sense irradiance[26,27] and project to LGN-independent brain areas[27,28] for functions such as regulation of

circadian rhythms and the pupillary light reflex.[51] The non-image-forming pathway has emerged as the primary candidate for photosensitivity in some clinical pain states such as migraine, due to the observation that migraineurs who are blind but with intact retina and non-image-forming pathways can demonstrate powerful photophobia.[24] Because the non-image-forming pathway is thought to be the photosensitivity pathway in migraine, we hypothesized that this might be the path through which visual information reaches and affects the RVM.

**Lightly Anesthetized Recording: Gathering Cellular and Behavioral Data in an Intact System**

The RVM is not a sensory structure, meaning that the firing of the pain-modulating cells in this brain area is not signaling the presence or absence of incoming noxious stimulation. These pain-modulating cells of the RVM are changing their ongoing firing patterns based on the state of the body. By recording these firing patterns we can gain insight into the motive of the pain modulatory system, in the context of how that will affect pain transmission, which is represented by a response to a noxious stimulation. Therefore, we need the complete pain-sensory and pain-modulatory systems as well as a withdrawal reflex to study the RVM, which can only be accomplished in an intact system in an animal. In addition, the neurons under study must be physiologically characterized according to their spinal reflex-related activity and responses to cutaneous stimulation. Thus, an intact nervous system is required for these studies.

One potential confound is the effect of anesthesia on in vivo recording. The lightly anesthetized model used here, in which a spinal reflex to noxious stimuli is used as the behavioral outcome, has been well-established.[5,6,11,40] ON-, OFF-, and NEUTRAL-cell activity has been recorded under a variety of anesthetics[41,42,43] as well as awake, freely moving rats[44] to show that all cell types exist under these conditions and function similarly. Anesthesia affects the basal firing pattern of cells, but not the facilitatory or inhibitory firing of ON-and OFF-cells,[41] thus the qualitative relationship between RVM activity and nociceptive sensitivity is preserved in the lightly anesthetized model.

**Are ON- and OFF-cells Sensitive to Light Intensity or Wavelength?**

The data presented here pursued our preliminary observation that light can evoke changes in the firing of ON- and OFF-cells of the RVM, which are known to modulate pain. This series of experiments explored the ON- and OFF-cell response to specific wavelengths of light and differing levels of intensity, to understand whether melanopsin cells of the retina were involved in RVM

response to light, and whether the light response of ON- and OFF-cells responded to intensity of the light stimulus (Fig. 4).

*Chapter 2*

# METHODS

All methods described here were based on established protocols from the Heinricher lab.[6,11,15] Experiments were performed in custom neurophysiological recording rooms, each electrically shielded and on a dedicated transformer. Each room included appropriate amplifiers, oscilloscopes, a hydraulic microdrive, feedback-controlled radiant heat stimulator system, a stereotactic frame on an air table, and a Windows-based computer for on-line data acquisition and control as well as off-line analysis (CED Spike 2, v.7). My room included a ThermoOriel light source, adapted for use with my recording setup, and adjustable brightness with multiple filter wheels to alter the light stimulus.

## Animals

All experiments were performed on adult male Sprague–Dawley rats (Charles River) weighing between 250 and 350g, ages 8 weeks upon arrival, housed 2 per cage. Rats were given a minimum of five days to acclimate to the housing after arrival, during which time they had ad libitum access to food and water. Animals were housed in an AALAC-approved colony facility two floors below the laboratory, which included full-time animal caretakers and veterinary staff.

## Extracellular Recording

The lab space included a separate room for surgical preparation of animals for recording. Rats were anesthetized throughout the experiments, which typically last three to seven hours. Rats were first deeply anesthetized with inhaled isoflurane (5% in oxygen, 5 minutes). An incision was made to expose the external jugular vein, and a catheter was inserted for subsequent infusion of anesthetic. This procedure lasts 15-25 minutes, and the rats were maintained with isoflurane (2% in oxygen) for the duration. Upon removal to the stereotaxic frame, anesthetic was switched to methohexital, a short-acting barbiturate.

The animals were maintained in a lightly anesthetized state using a continuous infusion of methohexital at a rate gradually titrated over an hour (15-30 mg/kg per h) until brief noxious stimuli applied to the skin (pinch with toothed forceps or radiant heat of up to 50 °C) would elicit a brisk withdrawal without other signs of discomfort (for example, the animals do not move spontaneously,

vocalize, or produce vigorous or prolonged withdrawal reflexes following noxious pinch). During recording, body temperature was maintained by a circulating water pad, heart rate (derived from EKG), and core temperature were monitored,[6] and breathing rate was monitored throughout the surgery to produce an appropriate level of anesthesia. Using these procedures, anesthetized animals showed no spontaneous movement, and noxious stimulation such as pinch elicits only a brief withdrawal reflex. The experiment was begun after an anesthetic stabilization period of at least 30 minutes, and the methohexital infusion rate was not altered during the protocol.[11]

A small craniotomy and opening in the dura was made to allow stereotaxic placement of an electrode in the brainstem. A gold- and platinum-plated stainless steel recording microelectrode (Microprobe) was lowered into the RVM for extracellular single unit recording, cells were found by moving the electrode through the RVM in 2.5 μm steps with the micropositioner. RVM neurons were classified as previously described.[5] Spike waveforms were monitored and stored for off-line analysis (Spike2, CED, Cambridge, UK). The recording protocol was only initiated once a well-isolated and identified ON- or OFF-cell with a robust reflex-related change in activity was identified.[11]

**Heat and Light Stimuli**

Nociceptive responsiveness was evaluated by running a heat ramp (37 → 50 °C over 13 seconds) using a radiant heat box device on the rat hindpaw and measuring latency to withdraw. The rat's paw was placed over a light bulb kept at a holding temperature of 37 °C. During a heat trial, the bulb turns on in a temperature-sensitive feedback-controlled way to heat the area above from 37 °C to a maximum of 50 °C at a rate of 1 °C/second. The rat was free to withdraw his paw, which is measured by the device and allows us to determine the precise temperature at which the rat withdrew. In lightly anesthetized animals this normally occurs between 40 and 45 °C.[6] After the rat withdraws, the bulb turns off and the apparatus returns to the holding temperature. Baseline firing rate is calculated from the 10 seconds before initiation of the heat ramp, while response to noxious heat is calculated by the number of spikes in a 3 second period around the paw withdrawal.

Atropine was applied to both eyes to dilate the pupil (atropine sulfate 1%, 14 mM, Bausch & Lomb Inc, 20 μL per eye) and prevent any confound due to the pupillary light reflex. The light source (Thermo Oriel) included an Isotemp Water Circulator, two sets of filter wheels, and a shutter controller box. It emits light of 425-750 nm, which was the 'light on' stimulus (Fig. 4). During

experiments, the output of the light source, a liquid light guide, was placed 5 cm away from the surface of the left eye of the rat. The light source was checked for consistency with a powermeter (Thor Labs) and adjusted if needed. The 'light off' condition was the low ambient light in the recording room (0.016 mW). Following a baseline period in which the white light stimulus was applied in alternation with a noxious heat trial (at least 3 minute intervals between stimuli, three applications of each), other light stimuli were tested, interspersed with additional heat trials.

There were 4 white light stimuli of varying intensity used in this experiment, the brightest named 'Level 4' and the least bright called 'Level 1'. All white light stimuli had the same wavelength range (425-750 nm), and their varying brightness was achieved through the use of neutral density filters, which change light intensity of all wavelengths equally. There are 2 light stimuli that were not white, named 'blue blocked' (425-475 + 490-750 nm) and 'blue' (475-510 nm). More information about the intensity, measured in mW or Lux, wavelength, or names of all light stimuli can be found in Fig. 4.

**Anatomical Verification**

Recording sites in the RVM were marked with an electrolytic lesion. Rats do not regain consciousness after completion of the recording session, they were euthanized with an anesthetic overdose of barbiturate, perfused transcardially with saline and 10% formalin, and their brains removed. This was consistent with the recommendation of the Panel on Euthanasia of the American Veterinary Medical Association. The brain was allowed to post-fix for at least 24 hours. Brains were blocked and sectioned at 60 μm with a cryostat (Leica CM3050 S). Lesion sites were imaged using an Olympus BX51 fluorescence microscope and recording sites were verified by referencing the rat brain atlas of Paxinos and Watson.

**Data Analysis**

Heat and light stimuli were repeated many times over the course of the experiment. Repetitions were averaged for each stimulus type, so a particular rat's average response could be the result of anywhere between 1 to 13 presentations of the same stimulus. Averages and the standard error of the mean (SEM) were calculated for RVM cell responses to heat and light, presented as total spikes during the stimulus and percent of baseline, which was calculated by dividing the cell response during stimulus by an equal amount of time immediately preceding initiation of the stimulus. Response to light for 30 second trials was characterized by comparing the firing rate in spikes per second of the 30 seconds before the light to the 30 seconds during the light. Response to light for the 10 second trials

was calculated the same way, by comparing firing rate in spikes per second for the 10 seconds before the light and 10 seconds during light.

These data have been presented in two ways, as total spikes during the light and as percent of ongoing activity. I prefer the percent of baseline measurement because it incorporates the cell's ongoing activity into the measurement of the response. Cells can go through different active or quiet phases spontaneously in the course of a recording, which can affect the response to a stimulus. Using the change in activity relative to the activity in the moment just before insures that the ongoing activity of the cell in the moment does not skew the representation of the response. Also, as shown by the response to heat and bright light (Fig. 2 and 3), cells vary in their ongoing firing rate. Representing the response as a percent of ongoing activity helps to compare numbers between cells that fire at disparate rates.

At the same time, when I did these recordings I would adjust the initiation of the heat or light stimulus if the cell was not 'ready'. I waited a minimum of 5 minutes between 30 second light trials and 3 minutes in the 10 second experiments, but the 5 and 3 minute interstimulus intervals were a minimum amount of time between noxious stimuli, for the rat and the system to recover. If, for example, the cell had not returned to its baseline activity, which I spend 30 minutes characterizing at the beginning of the experiment, I would wait to initiate the next stimulus. This means that to a certain extent I am picking my ongoing activity, which is open to experimenter bias. For this reason, I have also presented the data as total spikes during the light stimulation, which does not involve any information from the moments leading up to the initiation of the light. By presenting these two measures I hope to give a complete picture of my data.

*Chapter 3*

# RESULTS

## ON- and OFF-cells Respond to Light

For all ON- and OFF-cells, the response to heat was established by 3 heat trials in the first 30 minutes of the recording, to characterize the cellular response to a noxious stimulus and prove ON- or OFF-cell identity. Heat trials were repeated throughout the rest of the experiment, with an average of 6 heat trials per recording, with a minimum of 4 and a maximum of 13. During heat trials, quality of flick and temperature of paw withdrawal were assessed to evaluate the rat's condition, especially in terms of anesthetic stability. If a rat displays any signs of being anesthetically unstable (spontaneous movements and vocalizations, inability to withdraw paw in response to strong pinch or 50 °C heat), the experiment was terminated and no data recorded during the 'unstable' phase were analyzed. Average withdrawal temperature was 41.8 °C, with a minimum of 37.2 °C and a maximum of 47.2 °C.

Firing rate (spikes per second) in response to noxious heat is shown in Fig. 2, which includes the average of all heat trials over the course of the recording for each cell, as well as group averages for ON- and OFF-cells. ON-cells have an average baseline firing rate of 0.49 spikes per second. They increase firing in response to noxious heat, to an average firing rate of 9.67 spikes per second. OFF-cells have an average baseline firing rate of 6.51 spikes per second, which decreases to 0.632 in response to noxious heat. As is shown in Fig. 2, in response to noxious heat, all ON-cells increase firing and all OFF-cells decrease firing, but baseline firing rate and changes in firing rate in response to heat varies between cells.

The response to light was evaluated by shining 'Level 4' white light (425-750 nm, 10.93 mW, Fig. 4) into the left eye of the rat for either 10 seconds or 30 seconds. There are 2 experimental groups, one where all light stimuli were on for 30 seconds, and the other where all light stimuli were on for 10 seconds, as shown in Fig. 3. This light stimulus is the brightest light used in this experiment, was presented 3 times in the first 30 minutes of the experiment to characterize the cell's baseline response to light. This stimulus was repeated throughout the experiment, so each cell's response to light as shown in Fig. 3 is an average of multiple presentations of this light stimulus. Each cell has an average

of 6 presentations of this light stimulus. All OFF-cells decreased firing during the light stimulus, and most ON-cells increased firing during light (Fig. 3).

Of 15 ON-cells sampled, 12 increased firing rate in response to light and 3 decreased firing rate in response to light. The average baseline firing rate for all 15 ON-cells was 0.95 spikes per second, and the average firing rate during light was 6.04 spikes per second. Of the 3 ON-cells that did not respond to light, the average baseline firing rate was 1.23 spikes per second, while the firing rate during light was 0.87 spikes per second. These 3 cells were considered non-light-responsive and were excluded from analysis of intensity and blue light responses. For the 12 light-responsive ON-cells, the average baseline firing rate was 0.88 spikes per second, with 7.34 spikes per second during light.

**ON-cell Stimulus Response Curve**

Of the ON-cells that respond to 'Level 4' light (n=12), will they respond to lower intensity light, and if so, is the response graded? Responses to brightness of light varied slightly between the 30 second and 10 second groups (Fig. 5 and 6). In the 30 second group, ON-cells responded to all light levels, and seemed to give a graded response to light intensity (Fig. 6B, blue points), a trend which is echoed by the 10 second group (Fig. 6C, blue points), with the exception of an increase to 'Level 3' light. The response to 'Level 3' light in the 10 second group represents the average of just 2 cells, so more recordings need to be done to justify this trend. The data can be pooled if presented as percent of baseline (Fig. 6A), which shows that ON-cell response to white light peaks at 'Level 3', but is still high at 'Level 1' (average 155% change during light as compared to baseline). This suggests that ON-cells are sensitive to white light at all levels, and may respond differently according to light intensity.

**OFF-cell Stimulus Response Curve**

OFF-cell response to intensity of white light was similar in both 30 second and 10 second groups (Fig. 7 and 8). For both groups light caused a decrease in firing, and the more bright the light, the larger the decrease (Fig 8, blue points). The strongest response was to 'Level 4' light, which caused an 85.7% decrease in firing, while the response to the least amount of light, 'Level 1', caused a 47.7% decrease in firing compared to ongoing activity. This suggests that OFF-cells are also sensitive to white light at all levels, and may respond to light intensity.

**ON-cell Response to Blue Light**

The blue light stimulus used is created by placing filters over 'Level 4' light, meaning that the blue light stimulus represents all the blue light in 'Level 4' (brightest) light, so though its measured intensity is much lower than 'Level 4' (3.82 mW compared to 10.93 mW). In theory it represents 100% of the light from 475-510 nm that was coming through in the 'Level 4' stimuli (Fig. 4). When measured by total spikes during the light stimulus, the 30 second group shows an enhanced response to blue light (Fig. 6B, magenta points). When the light stimuli are arranged by intensity it is apparent that just as many spikes are evoked by 30 second of blue light as of 'Level 4' light, despite the large decrease in intensity (Fig. 5B). The 10 second trials do not show this trend, the total spikes during blue light are less than blue blocked or 'Level 4' light (Fig. 5C, 6C magenta points). When all cells are pooled and represented as percent of baseline (Fig. 5A, 6A), it seems as though there is no enhanced response to blue light, as the percent change is very close to that of 'Level 2' white light, and in fact the greatest percent change was in response to blue blocked light, even more than white light of similar intensity (Fig. 5A).

**OFF-cell Response to Blue Light**

OFF-cells appear to show no enhanced response to blue light. During 30 second and 10 second light groups, when represented as total spikes during light, OFF-cells do respond to blue light, but less than white light (Fig. 5B, 5C, 6B and 6C magenta points). When all cells are pooled and represented as percent baseline, it is clear that the response to blue light is less than 'Level 4' light, and again blue blocked light caused the greatest change in firing (13.7% of baseline compared to 14.3% of baseline for 'Level 4' white light, Fig. 5A and 6A).

*Chapter 4*

# DISCUSSION

## Almost All Cells Sampled Respond to Light

All OFF-cells and 80% of ON-cells sampled showed a response to light, which contradicts our recently published data,[64] where we found 61% of OFF-cells and 50% of ON-cells respond to light. In this data set, as with our recently published work, more OFF-cells than ON-cells were light responsive, but there is still a discrepancy in these proportions. One potential explanation is that these data were collected from a recording setup with a different light source. However, even with 'Level 1' light, the lowest intensity used in this experiment, there was more than a 50% change in response for all light responsive ON- and OFF-cells. In our recently published work, the definition for a light responsive cell was a 50% change in firing rate, meaning that all OFF-cells and 80% of ON-cells reported here qualify as light responsive, even at 'Level 1' light. In addition, the three ON-cells from this dataset that were classified as not light responsive were excluded because they decreased firing during light, rather than failed to meet the 50% increase (for ON-cells) in firing rate cutoff.

## ON- and OFF-cell Light Response is Modulated by Intensity

ON- and OFF-cells were sensitive to light at all intensity levels tested by this protocol. The light responsive ON- and OFF-cells of this dataset responded in a graded manner to light intensity, which suggests that the input from the retina that drives this response is not a binary signal, it includes information about the intensity of the incoming light, and pain-modulating cells of the RVM respond accordingly. This finding is particularly interesting, because up to this point the understanding of the response of the pain-modulating cells of the RVM is that they do not respond in a graded manner to stimuli. The current belief is that ON- and OFF-cells respond in an all-or-nothing fashion around a noxious threshold: when the stimulus is non-noxious they do not change their firing, and when the stimulus becomes noxious they respond by increasing (ON-cells) or decreasing (OFF-cells) firing rate. Conditions such as inflammation or anesthesia can move the threshold of the cellular response, but the response is still binary, not graded. Then again, this is the first time we have found a non-noxious stimulus that can cause ON-cells to burst and OFF-cells to pause, so analysis and understanding of these data may require a new set of rules.

These data do not support the theory that pain-modulating cells of the RVM specifically respond to blue light. If melanopsin cells, which are excited by blue light (460-480 nm),[51] were specifically driving the ON- and OFF-cell response, the response to the blue light stimulus should be the same as the response to 'Level 4' light, since in theory the blue light stimulus represents 100% of the light between 475 and 510 nm in 'Level 4' light. If melanopsin cells were driving the light response of RVM cells, then 'blue blocked' light would precipitate a reduced response compared to the 'Level 4' response, as no melanopsin cells should be directly activated by this light stimulus. Instead, these data show that ON- and OFF-cell responses are not especially responsive to blue light. The pain-modualting cells of the RVM still respond to blue and blue blocked light, but in a manner that is graded by intensity (Fig 5A and 7A), which is the same way that they respond to white light.

**30 second and 10 second Light Stimulation Groups**

For OFF-cells, the 30 second and 10 second groups seem quite similar. For ON-cells, some of the observed trends in the 30 second and 10 second groups went in opposite directions. For example, in the 30 second group there is a pronounced increase in the spikes during blue light when compared to 'Level 4' or blue blocked light, while in the 10 second group there is an obvious decrease in spikes during blue light compared to 'Level 4' and blue blocked stimuli. This could be because these two groups are capturing different responses: the 10 second group could represent the initial response, while the 30 second group could represent a prolonged response.

*Chapter 5*

# CONCLUSIONS

The data presented here are part of a picture that is far from complete, but the insights gained from them are an important first step on the path to understanding light activation of ON-cells and light suppression of OFF-cells. These experiments were designed to pursue questions from our preliminary discovery and recent publication.[48] We found evidence to suggest that the RVM cell response to light is sensitive to intensity, and that ON- and OFF-cells respond to intensity of light in a graded manner. We did not find any evidence to support the theory that melanopsin cells are driving this response. We are certain that light is accessing this pain-modulatory center of the brain, but whether light is contributing to pain sensation through the RVM is unknown. If light is able to influence our intrinsic pain modulation system, it could be one of the reasons that pain sensation is unique to every individual, and light exposure could become a new component of pain management.

**Future Directions**

Continuing work on the stimulus response curve is paramount. These initial results are exciting, but more cells and a consistent methodology are required to make these data significant. The 10 second light stimulus protocol is best, and should be used exclusively going forward. It would be interesting to add more neutral density filters to test more levels of light as well.

Though we have observed ON- and OFF-cell responses to light many times, no knowledge of a connection between the visual systems and the RVM exists currently. Two likely relays are the olivary pretectal nucleus (OPt) and the parabrachial complex (PB). One major target of the non-image-forming pathway is the OPt,[29] which is known to control the pupillary light reflex[30] and has been implicated in light-evoked activation of the trigeminal subnucleus caudalis, which is hypothesized to have a nociceptive effect.[31] The OPt may be involved in the relay of light to the RVM. A downstream target of OPt is the PB, which is an important nociceptive relay in the pons. The PB projects to the RVM,[32,33] and is known to be responsive to noxious stimulation.[34,35] Therefore, PB represents a potential site of integration for light and pain signals, and it could be relaying these signals to the RVM to modulate pain. Once we know how light information is reaching the RVM we can better

understand how light affects the pain modulatory system, and potentially develop new therapeutic targets.

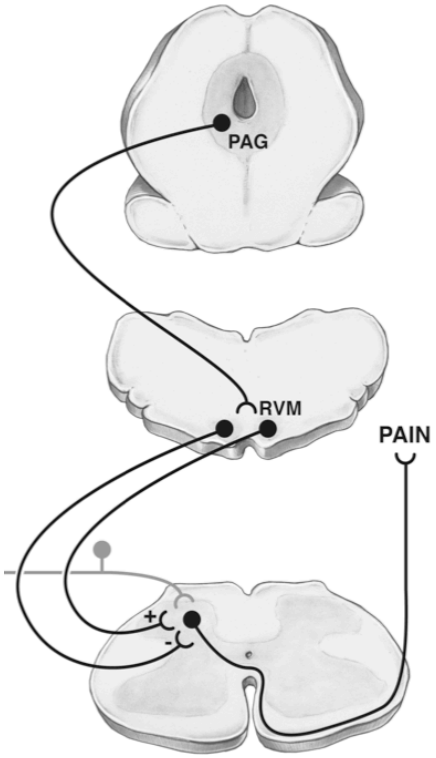
The observation that light can activate ON-cells and suppress OFF-cell firing suggests that light exposure would potentiate pain-related behaviors. Many groups have shown that a particular drug or genetic intervention can cause light aversion,[36,37,38,39] and other groups have shown that light can activate a specific somatosensory brain area,[21,23,24,31] but none have demonstrated a functional connection between these responses and nociceptive behavior. If we could connect the light-evoked neuronal changes to pain-related behavior, we could tie light-related pain behavior to this pain modulating system, providing a mechanistic explanation for photosensitivity-related pain.

RVM neurons become sensitized in many enhanced pain states, such as inflammation or nerve injury, by responding to normally subthreshold stimuli such as innocuous warming or light touch.[7,9,10,45] Sensitizing the RVM recruits ON- and OFF-cells to thermal stimulation, so perhaps it could also unmask a light response in cells that were non-responsive to light in normal conditions.

Understanding how light inputs reach the RVM and affect the RVM circuit in normal and hyperalgesic conditions could help us understand and create new therapeutic targets for pain states which are known to involve a light-sensitive component[8] as well as many chronic pain conditions that have not been associated with light sensitivity before.

FIGURES

Figure 1: **Functional organization of the brainstem pain-modulating system.** Inputs from many brain areas converge on the periaqueductal grey region (PAG) in the midbrain and are sent to the rostral ventromedial medulla (RVM) in the brainstem. ON- and OFF-cells of the RVM project to the dorsal horn of the spinal cord where they exert their influence: ON-cells increase (+) and OFF-cells decrease (-) incoming pain transmission at this synapse in the spinal cord, which changes the ascending pain signal being sent to the brain.



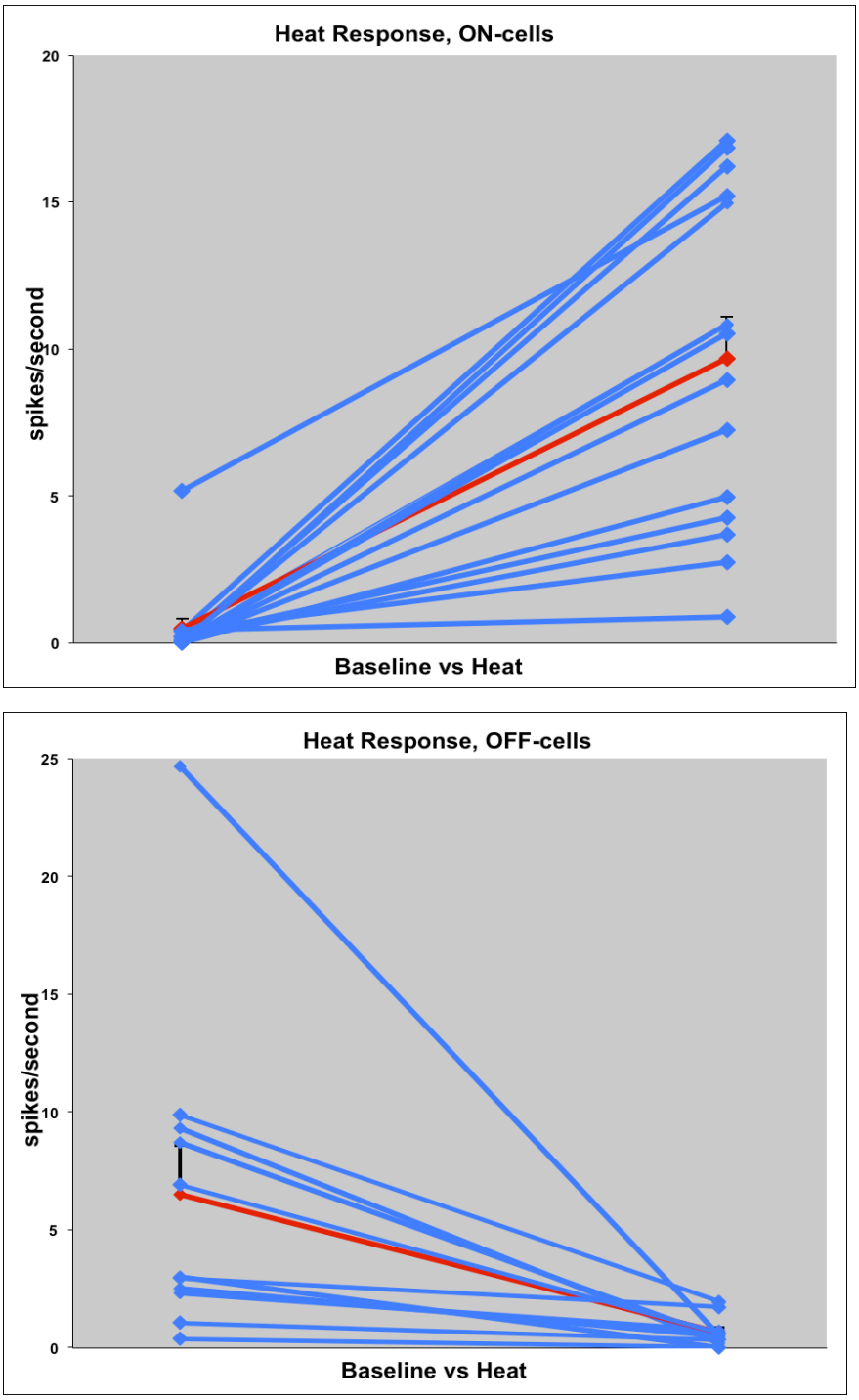



Figure 2: **ON- and OFF-cell response to noxious heat.** All ON-cells increase firing to noxious heat stimuli, all OFF-cells decrease firing to noxious heat stimuli. Blue lines represent individual cell averages of all heat trials during recording. Red lines are averages of all blue lines. Error bars are SEM. ON-cell n = 15, OFF-cell n = 11.

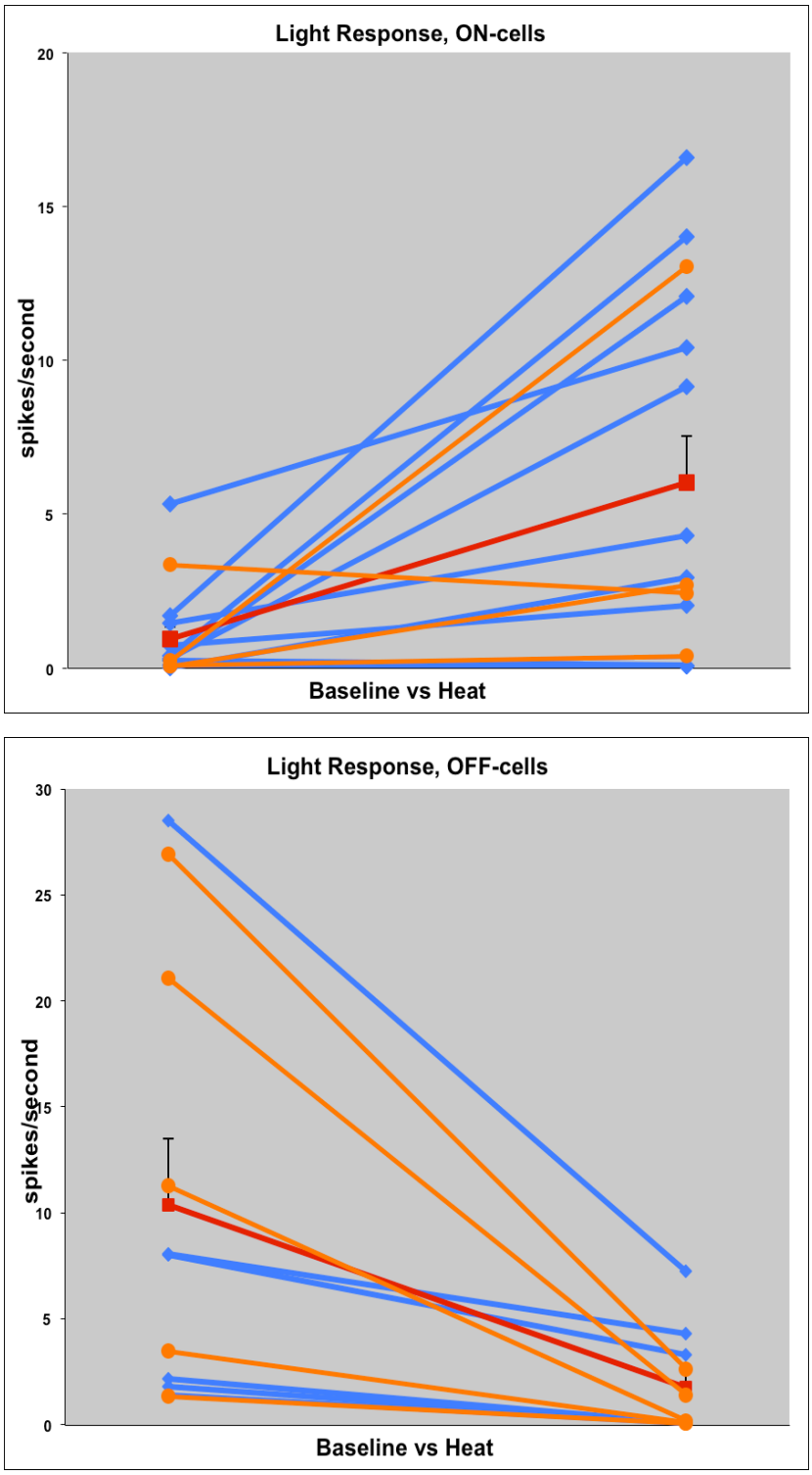
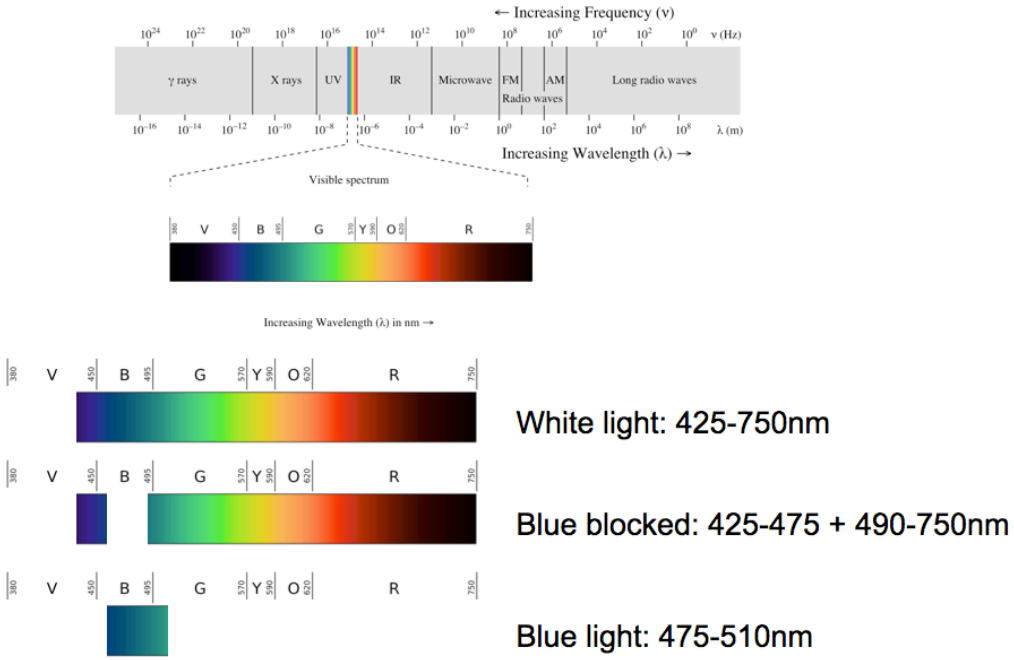



Figure 3: **ON- and OFF-cell response to light.** Most ON-cells increase firing to light (n = 15). All OFF-cells decrease firing rate in response to light (n = 11). Blue lines represent 30 second light stimulus, orange lines represent 10 second light stimulus, red line is average for all, error bars are SEM.



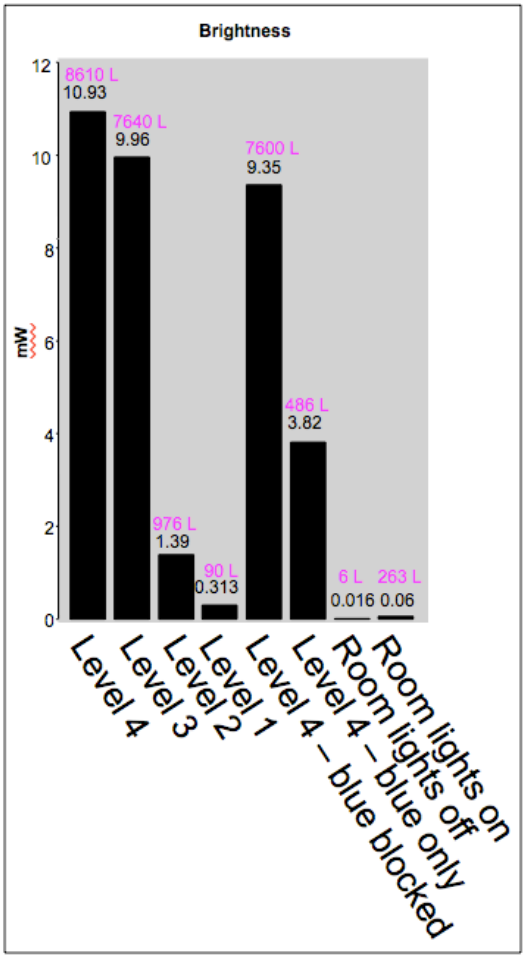




Figure 4: **Power and wavelength of light stimuli.**

Above: basic diagram of the electromagnetic spectrum, emphasis on visible light. Not-to-scale representation of the wavelength of my light stimuli. Adapted with permission.[46,47]

Left: Power measured in Watts (black text) and Lux (magenta text). The 'Levels' refer to 4 different neutral density filters, which change the intensity evenly across all wavelengths. Levels 4-1 represent white light stimuli of varying brightness used to create the stimulus response curves (425-750 nm), 'blue blocked' (425-475 + 490-750 nm) and 'blue only' (475-510 nm) are the stimuli used to assess the cellular response to blue light. 'Room lights off' represents the light level in between stimuli.

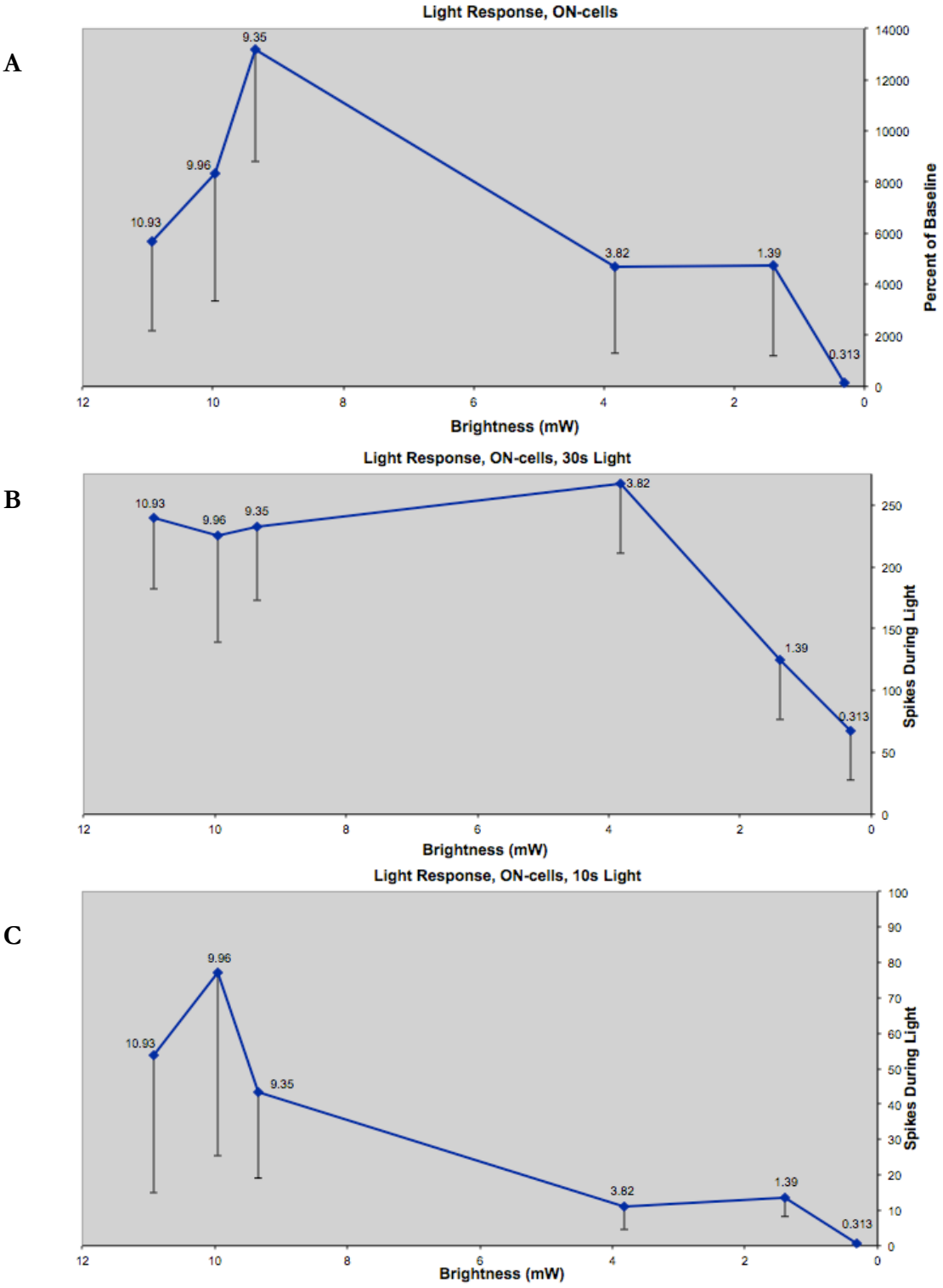



Figure 5: **ON-cell response to all light stimuli, arranged by intensity.** The 6 light stimuli are arranged by power (in mW) and include 'blue blocked' (9.35 mW) and 'blue' light (3.82 mW), all others are white light. (A) All ON-cells (n=9-12) response to intensity as percent of baseline. (B) and (C) are separated into the 30 second (n=7-9) or 10 second (n=2-3) light trials, and response is reported as number of spikes during light. All error bars are SEM.

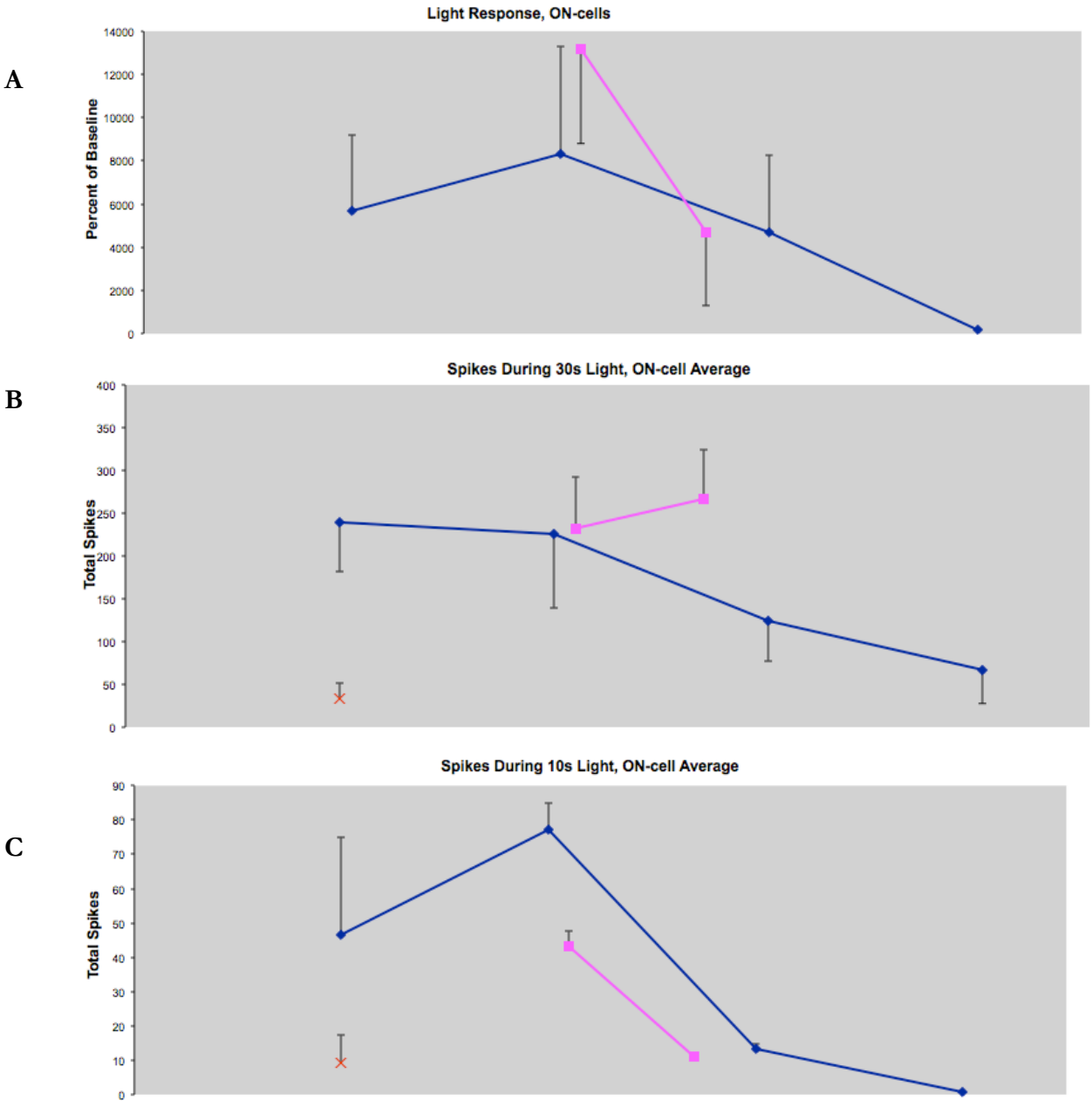
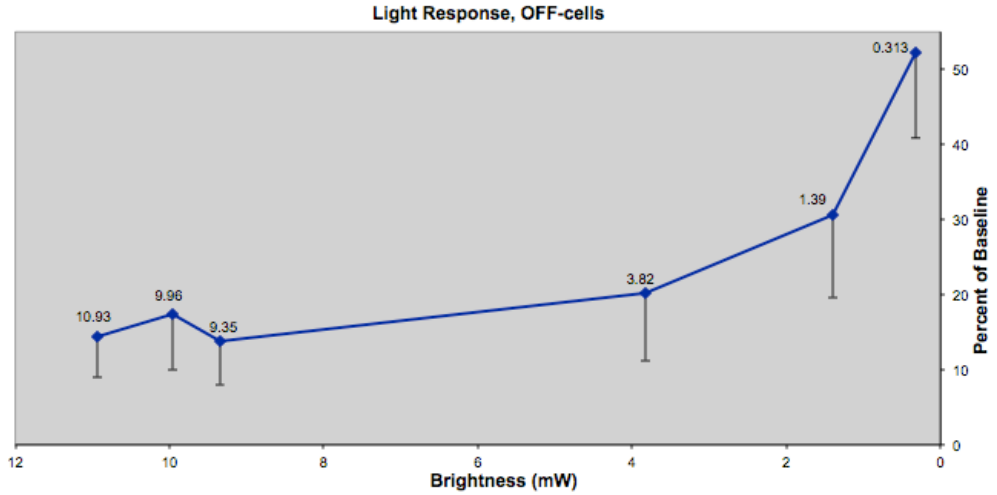
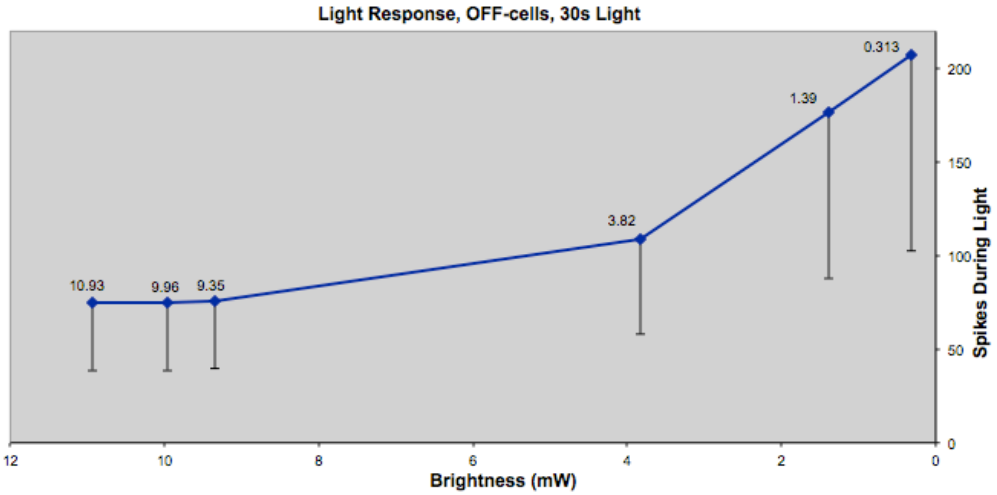



Figure 6: **ON-cell response to light stimuli, arranged by wavelength.** Blue points represent the 4 stimuli of white light presented in order from most (10.93 mW) to least bright (0.313 mW). Magenta points are 'blue blocked' followed by 'blue' light. Red x represents average total spikes for 30 second or 10 second before light, used as 'baseline' reference. (A) All ON-cells (n=9-12) response to intensity as percent of baseline. (B) and (C) are separated into the 30 second (n=7-9) or 10 second (n=2-3) light trials, and response is reported as number of spikes during light. All error bars are SEM.

A




B




C

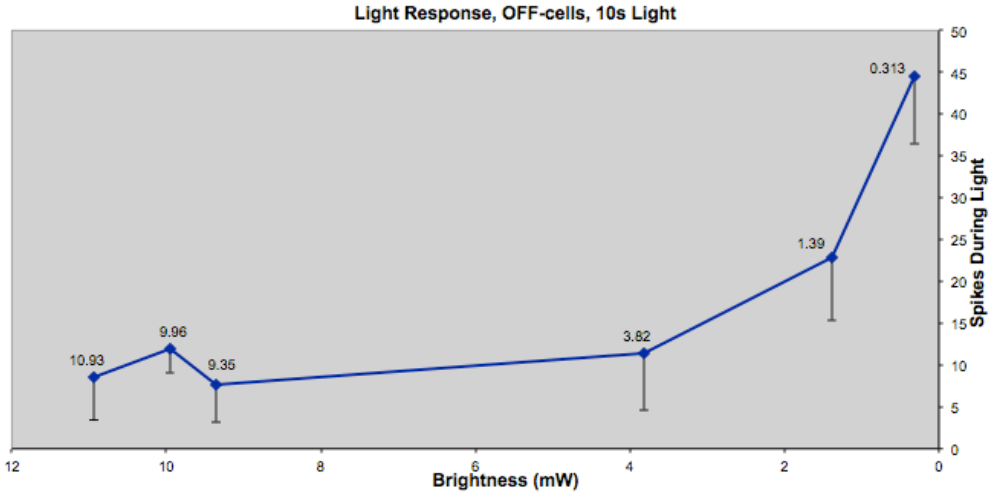
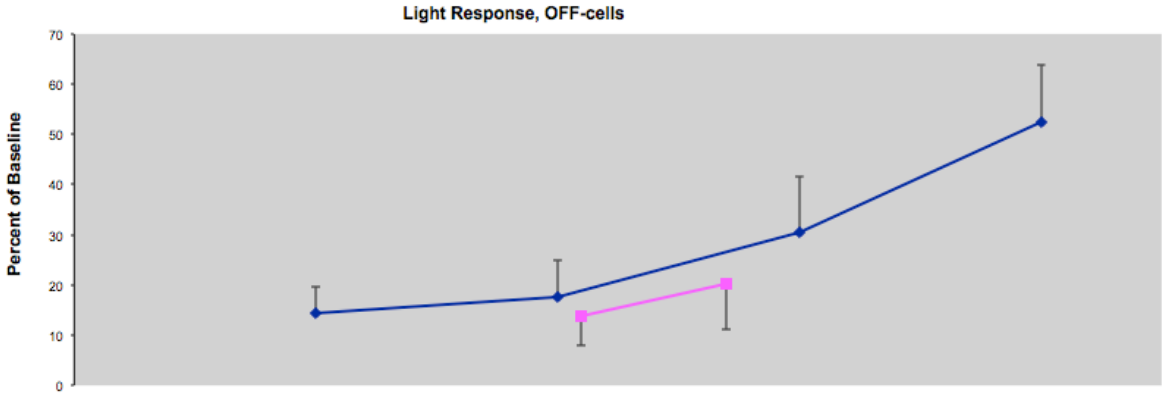
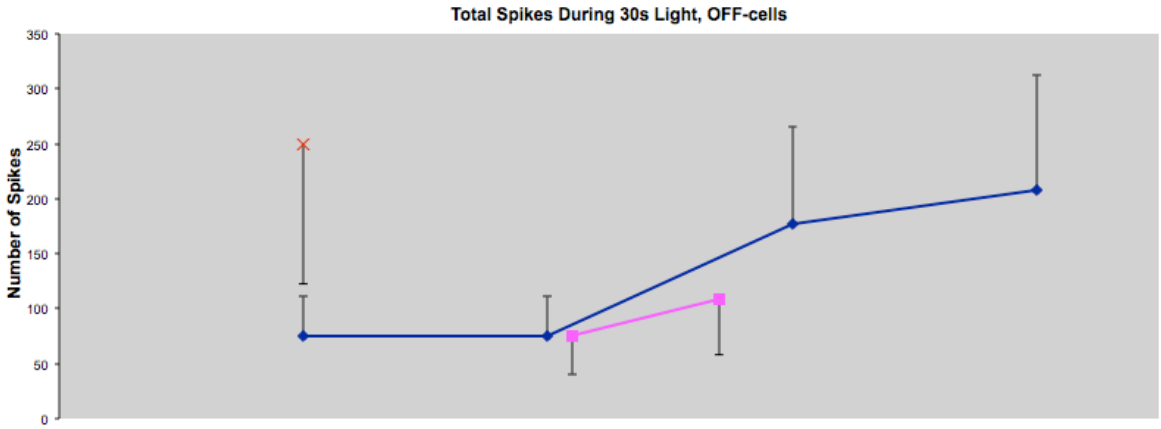



Figure 7: **OFF-cell response to all light stimuli, arranged by intensity.** The 6 light stimuli are arranged by power (in mW) and include 'blue blocked' (9.35 mW) and 'blue' light (3.82 mW), all others are white light. (A) All OFF-cells (n=9-11) response to intensity as percent of baseline. (B) and (C) are separated into the 30 second (n=6) or 10 second (n=3-5) light trials, and response is reported as number of spikes during light. All error bars are SEM.

A




B

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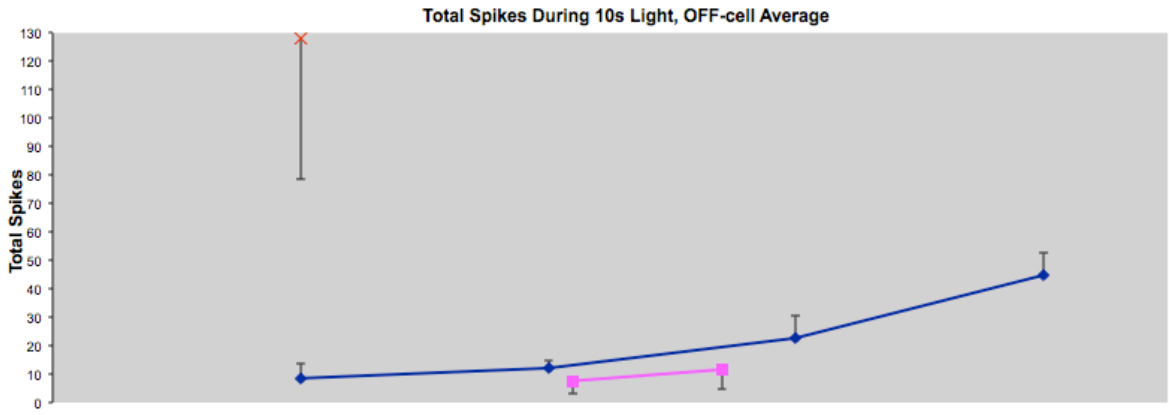


Figure 8: **OFF-cell response to light stimuli, arranged by wavelength.** Blue points represent the 4 stimuli of white light presented in order from most (10.93 mW) to least bright (0.313 mW). Magenta points are 'blue blocked' followed by 'blue' light. Red x represents average total spikes for 30 second or 10 second before light, used as 'baseline' reference. (A) All OFF-cells (n=9-11) response to intensity as percent of baseline. (B) and (C) are separated into the 30 second (n=6) or 10 second (n=3-5) light trials, and response is reported as number of spikes during light. All error bars are SEM.

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