Hot Button: Non-invasive Approach for Studying Emotion States

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January 31, 2024

Abstract

Aggression and fear are innate behaviors in most vertebrate animal species, including humans. Various mental health disorders and neurological conditions can be accompanied by pathological aggression or fear. The development of psychiatric drugs that target the brain's emotional circuits requires reliable and non-invasive methods to assess the emotional states. In my research, I demonstrate that an easily accessible thermography camera can provide a measure of the aggressive state of mice.

In my experimental trials I use mice because researchers have identified aggression neurons in a brain region known as the ventromedial hypothalamus, ventrolateral subdivision (VMHvl), as well as fear neurons in the dorsomedial part (VMHdm). These neurons can be optogenetically stimulated, which essentially means that we have the tools to artificially stimulate hardwired primal emotions such as anger and fear in a controlled manner.

My research provides statistical evidence that activation of VMHvl neurons leads to an increase in the mice's head temperature, demonstrating the validity of the non-invasive method I developed. By comparing the temporal responses of pupil dilation and body temperature to VMHvl activation, I found that, unlike pupil dilation, which is caused by arousal, the persistent temperature response is indicative of aggression.

When using a similar approach to study fear in VMHdm neurons, I found that there is a temperature increase in response to VMHdm activation, even greater than that in VMHvl, but no temperature increase in the rat fear natural stimulation. This indicates that there are separate neurons controlling fear and temperature in VMHdm.

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Acknowledgment of Help

This research was conducted at the Tianqiao and Chrissy Chen Institute for Neuroscience of the Califonia Institute of Technology. I am indebted to my mentor, Dr. Amit Vinograd, a Postdoctoral Scholar Fellowship Trainee in Biology and Biological Engineering at Dr. Anderson's Laboratory. He provided the hardware, prepared the mice for the experiment, and guided me throughout my research.

Procedure for Handling Vertebrate Animals

This research involved non-human vertebrates. The care and experimental manipulation of the animals was carried out in accordance with the NIH guidelines and approved by the Caltech Institutional Animal Care and Use Committee. The data collections were conducted under the supervision of experienced researchers and veterinarians and followed state and federal regulatory guidance applicable to the humane and ethical conduct of such research (IACUC protocol number IA21-1552).

The species used for the experiment is laboratory mice (Mus musculus), strain C57BL/6, age 8-20 weeks old. Animals are housed in the Chen Institute and Broad Animal Facilities at Caltech. The Caltech laboratory animal facilities are AAALAC-accredited, and the campus holds an Assurance with the Public Health Service.

The veterinary staff provides animal health care. Animal care staff also provides comprehensive husbandry and breeding management for rodents on campus, including rodent colony management, euthanasia, identification, and genotyping. All experiments involving animals are reviewed by the Caltech Animal Care and Use Committee and are subject to annual review.

Water is provided to rodents via automatic water valves. This system provides fresh water on demand at the animal's request. Drinking water quality is tested at least two times per year in each animal facility.

Laboratory rodents at Caltech are usually fed ad libitum utilizing either stainless steel wire cage lids with integral feeders or modular feeders. All laboratory animals maintained at Caltech

are fed a diet appropriate to their nutritional needs.

All rodent cages are made of polycarbonate or polysulfone which withstands the high temperatures necessary for effective sanitation. The standard static microisolator cage may house 3-4 adult mice depending on the animals' weights. Ventilated cages may house up to 4-5 adult mice depending on cage dimensions. The bedding in each cage is changed at least every 14 days by animal care staff, depending on the number of animals in the cage and the level of soil. Mice are also provided with tissue paper that provides enrichment (mice love to shred it and make a nest that insulates, thereby providing warmth). Unshredded enrichment material is often the first clue that an animal is not feeling well. Many of an animal's biological functions are influenced by photoperiod. Therefore, light intensity and light cycle timing are carefully regulated and monitored in Caltech laboratory animal facilities. Automated light timers ensure a consistent light-dark cycle.

The animals are used for Caltech experimentation before and after my research, and I don't handle the animals at all. The lab process is that when the mice die, mouse carcasses will be double-bagged, and the bag will be marked with a biohazard label and discarded in a biohazard container in a carcass freezer in the animal facilities. Mice will otherwise be euthanized by CO2 asphyxiation followed by immediate cervical dislocation, consistent with the Panel on Euthanasia of the American Veterinary Medical Association.

Introduction

The expression "getting heated," which associates heat with anger, is not unique to English. In Spanish, we say "echar leña al fuego" (add wood to the fire), which refers to aggravating an already tense situation, in Russian, there is Кипеть от злости (boiling from anger), in Hebrew חמום מוח (heated brain), and in Hindi there is an expression गुस्से में आग बबूला होना (being fiery angry). Almost every culture seems to associate anger with heat, but is there a scientific link?

Aggression is an innate behavior in most vertebrate animal species, including humans. Even if we are consciously aware of our current emotion states such as anger, the mechanisms that give rise to these emotions remain largely unresolved and the subject of current research (D. J. Anderson & Adolphs, 2014). Various mental health disorders and neurological conditions can be accompanied by pathological aggression, including some forms of dementia, like Alzheimer's disease, paranoid schizophrenia and psychotic disorders. Progress in understanding these disorders requires an understanding of the brain circuits that control normal aggression (D. J. Anderson, 2012).

Some research on humans has found a correlation between high temperatures potentially causing aggressive behavior (C. A. Anderson et al., 2000). Similarly, in an experiment in which people were asked to self-report where in the body certain emotions were felt, anger was depicted as a red hot map in the upper part of the body (Nummenmaa et al., 2014). However, no study has produced statistical evidence of human body temperature increasing when angry.

One technique that has been instrumental in advancing our understanding of the brain and how different neural circuits contribute to emotions is optogenetic stimulation. Optogenetic stimulation allows researchers to precisely control the activity of specific neurons or cells in complex neural circuits. The method involves genetically modifying target cells to express light-sensitive proteins. When laser light of the appropriate wavelength is shone on the modified cells, it causes the neurons to activate.

This technique has been optimally applied in mice. Groundbreaking research in Professor

Anderson's lab (Lin et al., 2011) demonstrated that optogenetic stimulation of neurons in the ventromedial hypothalamus, ventrolateral subdivision (VMHvl) in mice elicits an aggression response that causes male mice to attack both females and inanimate objects, as well as males. Similarly, pharmacogenetic silencing of VMHvl reversibly inhibits inter-male aggression. It is interesting to note that male mice exhibit a preference for aggression-seeking behavior when VMHvl is stimulated (Falkner et al., 2016)(Martínez et al., 1995). Other states such as fear or anxiety can also be evoked (Kunwar et al., 2015). Similarly, it has been found that optogenetic stimulation in the dorsomedial part of the ventromedial hypothalamus (VMHdm) elicits a fear response that causes mice to freeze in place.

Using this type of research, we can begin modeling the brain through its aggression or fear pathways, paving the path for medical drugs to treat psychological disorders and other brain diseases.

It is important to develop non-invasive methods to correlate emotion states with other vital signs. Previous ways to measure body temperature in mice, for example rectal thermometry (McAllister-Williams et al., 2001) or subcutaneous temperature-sensitive chips (Mei et al., 2018), were highly invasive. This makes the experiment more cumbersome and expensive, and it could introduce anxiety or stress in the animals, thus impacting the data collection. In my research, I developed a novel non-invasive method to measure body temperature, and I demonstrate how it correlates with aggressive states. Then I conducted a similar experiment to measure body temperature with respect to fear states.

My work consists of five main parts:

1. Developing a non-invasive method to measure the mice's temperature.
2. Conducting optogenetic stimulation experiments using this method to collect data.
3. Analyzing the data to assess correlation between temperature and aggression state.
4. Understanding the impact of VMHvl activation on pupil dilation, and how its temporal response deviates from body temperature temporal response.

5. Analyzing the data to assess the correlation between temperature and fear state elicited by VMHdm as compared to the fear state elicited by natural rat stimulation.

I demonstrate that there is statistical evidence that VMHvl neuron activation results in a mouse's head temperature increase. This temperature increase is persistent over several seconds after activation, indicating that it is attributable to an aggression state. This is in contrast with pupil dilation, which goes back to the baseline before the stimulation is over, indicating an arousal state and not anger. I also discover a difference in the activations of VMHdm fear and natural rat fear in mice, implying a specific arrangement of the neurons within VMHdm.

Materials and Methods

For the experiment setup, I used a Mosaic Core Seek Thermal Camera and a Measurement Computing Data Acquisition (DAQ) System. All test animals used in this study were laboratory mice (Mus musculus), strain C57BL/6, aged between 8 to 20 weeks. The care and experimental manipulation of the animals was carried out in accordance with the NIH guidelines and approved by the Caltech Institutional Animal Care and Use Committee.

For the aggression neuron activation trials, the experimental group consisted of three mice. Researchers at Professor Anderson's Lab had expressed channelrhodopsin-2 (ChR2) in VMHvl neurons using stereotactic co-injection of adeno-associated viral vectors fused with green fluorescent protein. Cells in this region can be selectively illuminated with a laser using an implanted fiber-optic cable (Lin et al., 2011). The control group consisted of three mice with the green fluorescent protein but without ChR2 such that the aggression response would not be elicited with laser activation.

Then, for the fear neuron activation trials, the experimental group also consisted of 3 mice in which ChR2 had been expressed in VMHdm neurons using stereotactic co-injection of adeno-associated viral vectors fused with green fluorescent protein. The control group for this experiment consisted of three mice with no modifications. Natural fear was elicited in these mice by bringing a rat in a cage close to the mouse during experiments.

Results

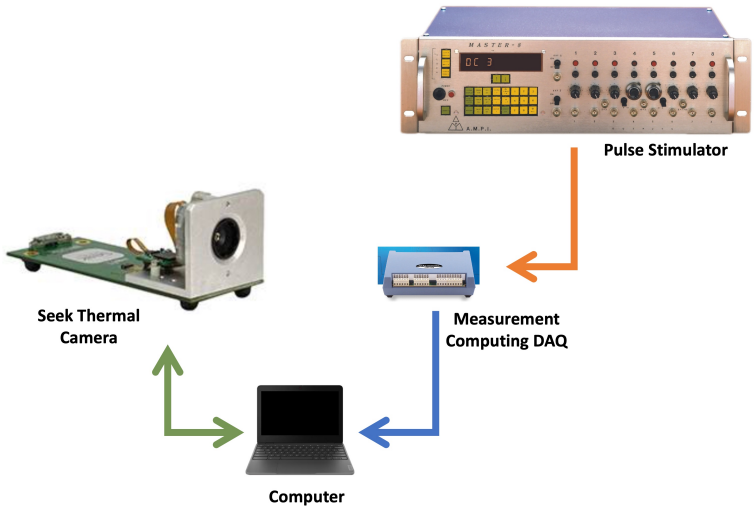
Non-invasive apparatus to measure body temperature

My research primarily aimed to develop a non-invasive apparatus for measuring mouse body temperature, synchronized with the laser stimulation used to optogenetically elicit the anger response.

The thermographic data were collected using an inexpensive Mosaic Core Seek Thermal Camera. In order to synchronize the thermal data capture with the optogenetic neural stimulation, I used a DAQ device from Measurement Computing to receive a trigger from the laser Pulse Stimulator. Figure 1 illustrates how the lab instrumentation is connected and Figure 2 shows a picture of the set up while running a test.

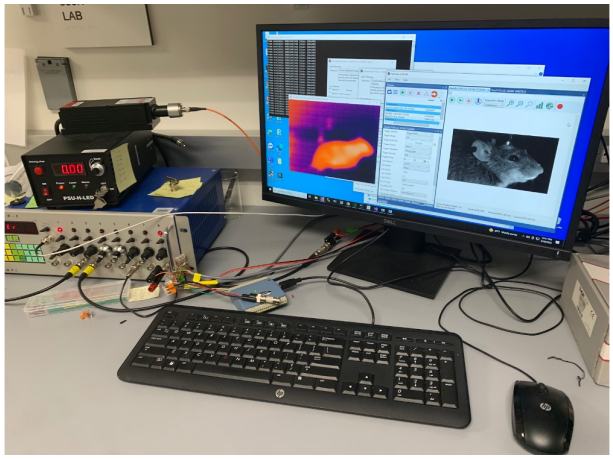
Figure 1

Diagram of experimental set-up. The computer captures a trigger signal from the pulse stimulator (orange arrow) through the DAQ device (blue arrow), and uses it to start a thermal data capture and subsequently read the thermal capture data (green arrow).



Significant amount of work was initially needed to make this camera accurately capture the temperature change in mice over time. Using the Seek Thermal Software Development Kit (SDK) and Measurement Computing DAQ drivers, I created software (in C++) to capture data synchronized with the other lab equipment. I made this code openly available on github (Backer Peral, 2023).

Figure 2
Experimental set-up during data collection.



One problem we encountered early on was that this camera used a shutter to calibrate itself every few seconds, causing the camera to lose about 9 frames with each shutter action. This was problematic because it created missing frames and the recording would lose synchronization with the rest of the lab instrumentation. When we disabled the shutter, the thermal data quality was severely degraded, rendering the data too inaccurate for our needs.

For our experiment, we decided to work around these limitations by activating the shutter by software before each frame to achieve high-quality recordings while maintaining control over the video frame rate. With this approach, the frame rate was limited to 3 Hz, but this was still sufficient for our purposes, since body temperature takes several seconds to change.

Thus, I programmed two capture modes:

1. Continuous mode records continuously at a 27 Hz frame rate upon receiving an external trigger signal or a keyboard press.

2. Trigger mode captures a frame synchronized with each external trigger signal, allowing users to customize the frame rate for the thermal capture.

For both modes, users have several options to select the data type and filtering mode, reducing noise and enhancing data quality.

Aggression (VMHvl) data collection and analysis

We conducted eight trials on the three mice in the experimental group at a 1 Hz sampling rate for thermal recordings. We enabled VMHvl activation for a total duration of 20 seconds with 10-millisecond laser pulses at 20 Hz and 2 mW power. There were 120-second intervals between laser stimulations. I acknowledge with gratitude the tremendous help from my mentor, Dr. Amit Vinograd, in running the trials and collecting the data.

Video 1 shows the live thermal camera data from one mouse as the experiment is conducted (note that viewing the videos requires the free Acrobat Reader and accepting playing media files; other PDF readers, such the Mac reader, will not work). There is also a black box designating the 11x11 pixel area that I used to assess the mouse's temperature. I could not use the overall temperature of the mouse because significant movement during the trials could skew the results, as different parts of the mouse have different temperatures. Instead, I took the average temperature from an 11x11 pixel area centered on the hottest point observed in the video, typically near the eye. Unlike the body, the head position was fairly stable during the trials, providing a more reliable temperature measurement. Furthermore, it is reasonable to assume that the head will better reflect any changes in mouse temperature, since, if anger neuron activations were to increase temperature, that change would manifest first in the head.

As an example, Video 2 shows a single trial measuring a mouse's temperature during laser activation. The area between the red lines indicates the period of laser activation, and the data clearly shows an increase in temperature during and after this period.

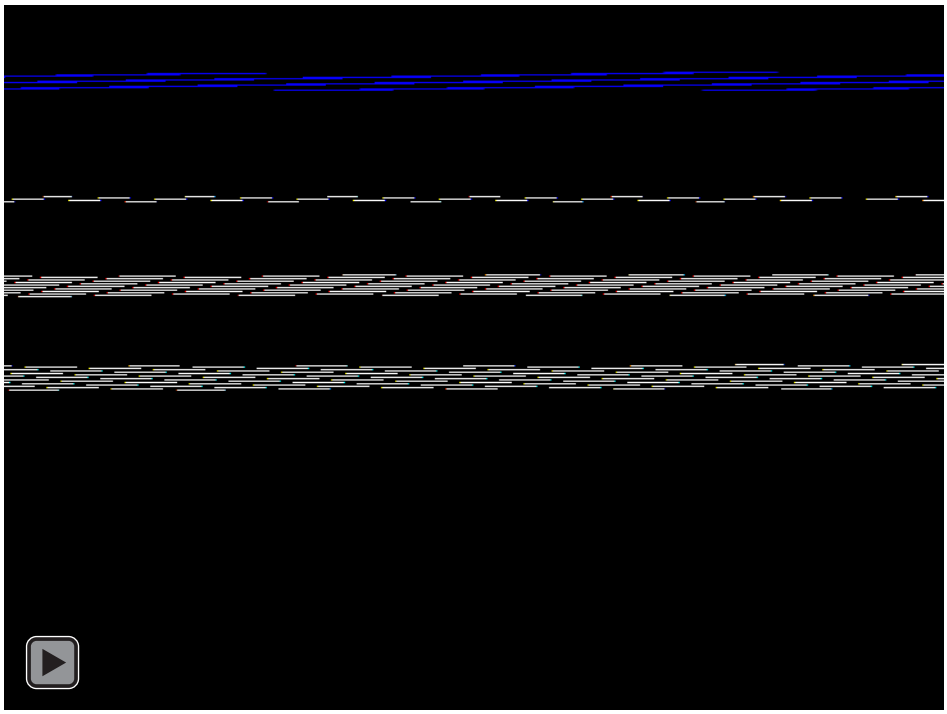
Figure 3 includes snapshots of the three mice used in the experiment, alongside the box selected for assessing temperature. Figure 4 displays the average temperature within the 11x11 pixel box over time (in blue) and the laser activation times (in red boxes).

To assess the statistical validity of my observations, I superimposed the data from all eight trials and three mice, using laser activation as the temporal marker.

Figure 5 displays the average of all eight trials for the three mice. It shows a clear increase in temperature during laser activation, which persists for around 20 seconds after the laser is

Video 1

Thermal camera video of mouse. Small box corresponds to the region used to assess temperature.



activated, before the temperature begins to level off and decrease. The blue curve represents the average temperature in the 11x11 pixel box over time after applying a smoothing filter, with the gray area indicating the standard error of measurement (SEM). The laser activation occurs during the period indicated by the red area.

Control tests for Aggression (VMHvl) data collection

Control test #1: Comparing mouse's temperature to background control temperature

To demonstrate the validity of the result, I compared the mice's temperatures to data collected over a similarly sized background box (see Figure 6). The bottom-left graph in Figure 6 includes the temperatures before and after laser activation for each trial, with the black line displaying the average of all trials. I determined the statistical significance of the results using the Wilcoxon test, which yielded a p-value of 0.002. The same test performed on the background control box resulted in a p-value of 0.7, which is not statistically significant. This validates the

Video 2

Example of increase in temperature during and after optogenetic neural stimulation.

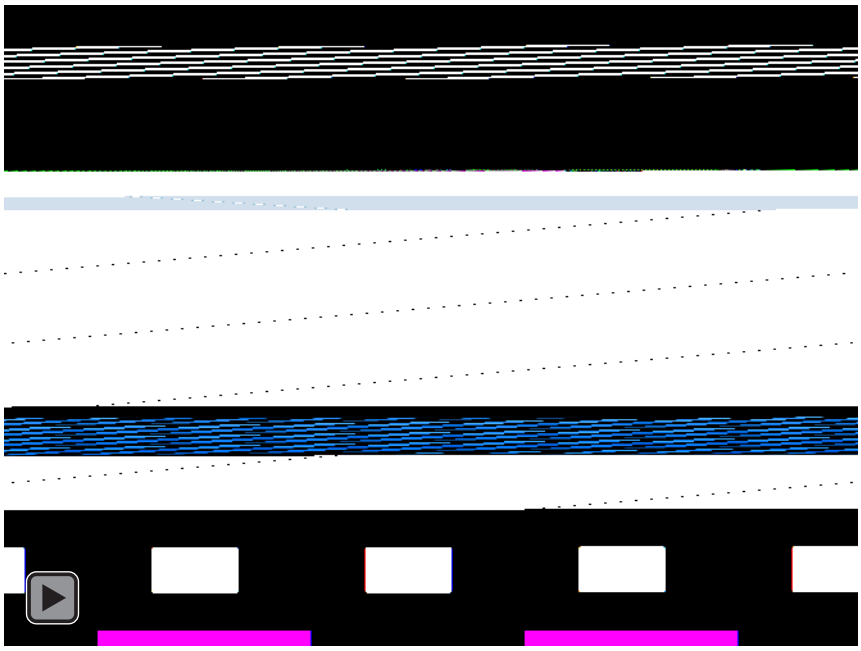
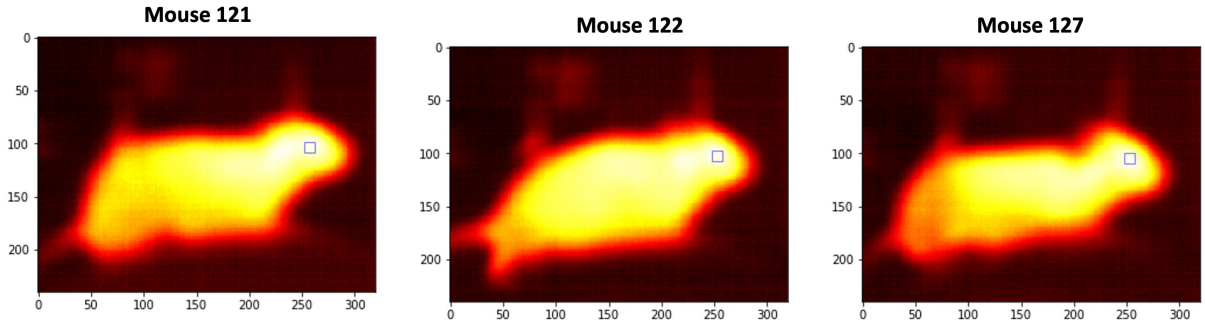


Figure 3

Thermography snapshot for each of the mice with box used to assess temperature.



observation that there is a significant temperature increase in the mice that is not due to an environmental temperature increase.

Control test #2: Increase in temperature due to laser energy

Another potential explanation for this temperature increase could be the heat energy from the laser. To assess this hypothesis, I calculated the expected temperature change in the mouse's

Figure 4

Mouse's temperature in blue as a function of time (average of 11x11 pixel box) for the three mice in the VMHvl experimental group. The red boxes indicate the periods of laser activation.

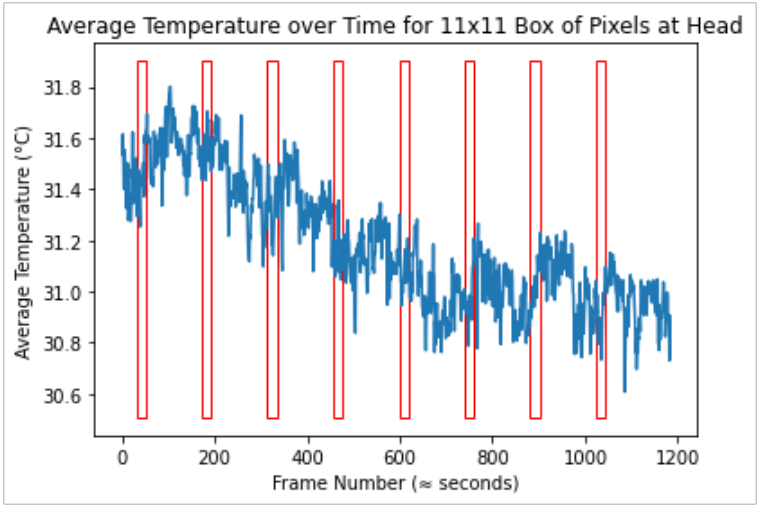
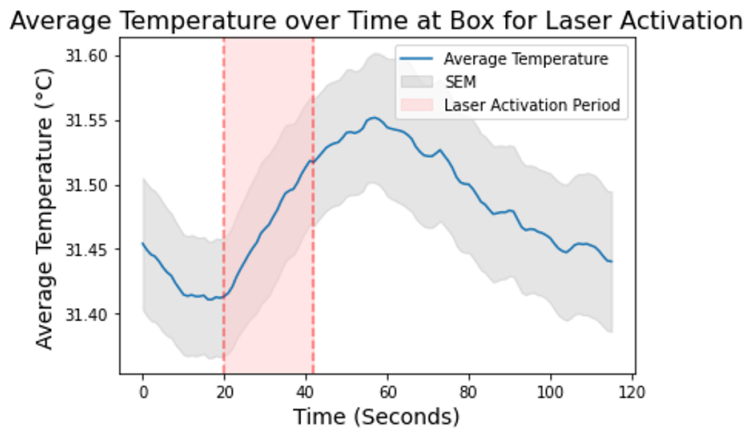


Figure 5

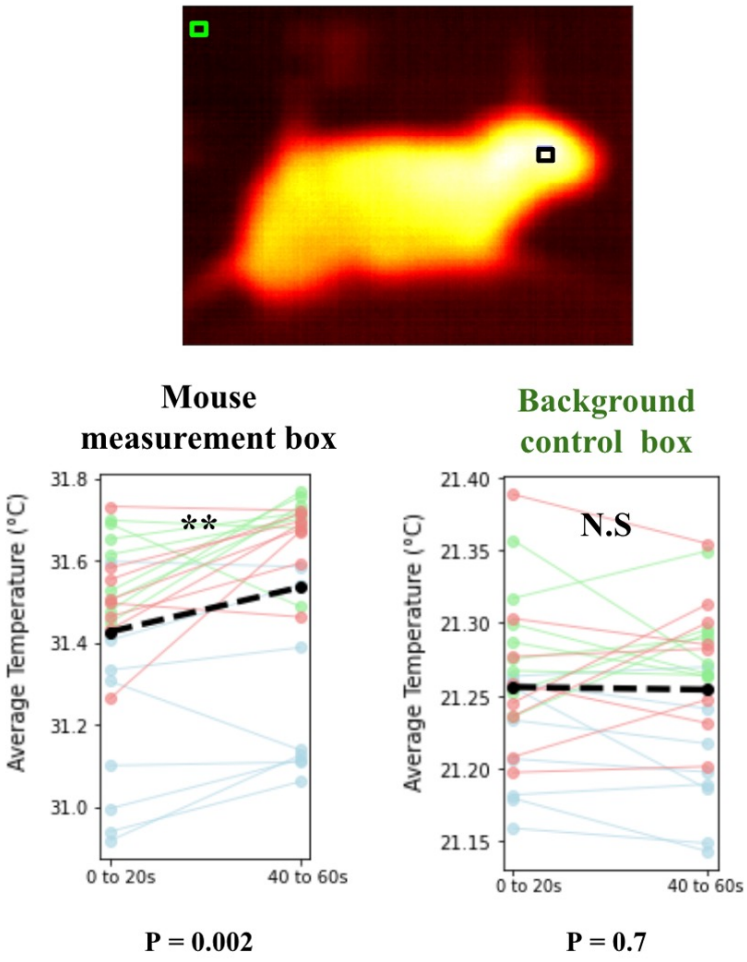
Average temperature in blue and standard error of measurement (SEM) in gray over time for the VMHvl data collection. The red area indicates the laser activation period.



brain by considering the energy in Joules from a 2 mW laser over 20 seconds and the average brain mass of a mouse (0.416 g), using the average specific heat capacity of brain tissue (3.63 J/g°C). This calculation leads to a temperature change of 0.0265 °C over 20 seconds, with a predicted slope of 0.001325 °C/sec, assuming that all the energy contributed to raising the brain's temperature—a conservative assumption indeed. However, the linear regression of the measured data yielded a value nearly four times higher, as seen in Figure 7. This indicates that the observed temperature increase cannot be attributed solely to the injected laser energy.

Figure 6

Control experiment using a box in the background (green in the image) compared the mouse's temperature (black box). The bottom graphs show the temperature data for each trial and all three mice (each mouse a separate color) during and after laser activation. Left is for the VMHvl measurement box and right is for the control box. The dashed black line in each graph is the average. The p-value for the mouse's temperature during VMHvl data collection indicates the significance of the result, whereas the control box temperature shows no significance.



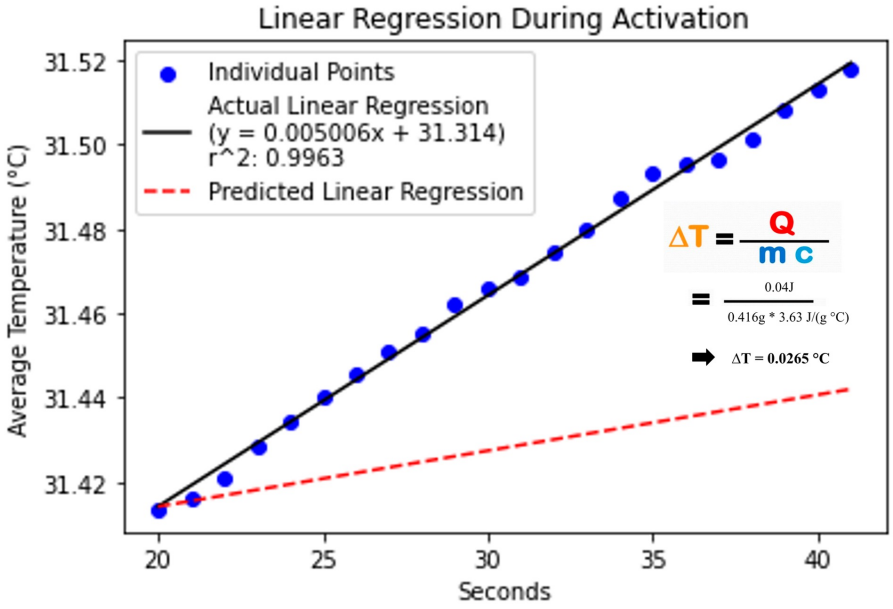
Control test #3: Increase temperature due to mouse's body movement

The increase in body temperature could also be due to the heat produced by the mouse's motion. To disprove this hypothesis, I utilized DeepLabCut (Mathis et al., 2018), an open-source neural network-based software that estimates animal poses.

I selected a few markers to estimate the mouse's velocity (see Figure 8). Tracking the position of these pixels over time and computing the distance between frames allowed me to

Figure 7

The estimated increase in temperature due to the laser activation energy of 0.04 J (2 mW over 20 seconds) is 0.0265 °C. The predicted slope due to the laser energy is 0.001325 °C/sec, whereas the measured slope is 0.005006 °C/sec, demonstrating that this effect cannot be attributed to the laser energy.



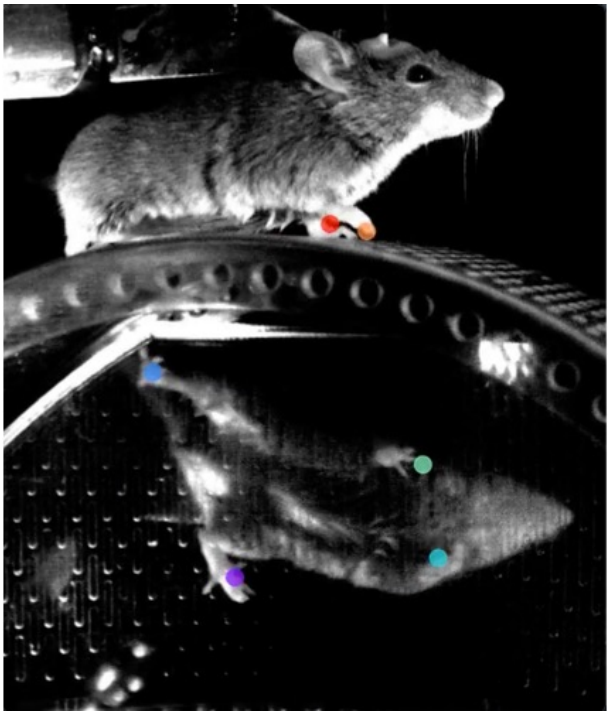
calculate a proxy for the mouse's velocity, by averaging all markers in units of pixels per second.

Video 3 demonstrates the validity of this algorithm: the mouse's motion, as shown, correlates with sharp velocity increases depicted on the right graph. This method yielded data like that shown in Figure 9, which displays the velocity in pixels per second as a function of time.

Figure 9 and Video 3 indicate that mouse motion occurs at velocities greater than approximately 5 pixels per second, peaking at values at least 15 pixels/second. Any velocity less than 5 pixels per second appears to be due to random small motions of the mice and has no statistical significance. To assess the impact of mouse velocity on temperature, I first computed the average speed during and for 20 seconds after laser activation and compared it to the overall speed.

Figure 10 (a) shows that there is actually a decrease in the velocity of the mouse during laser activation compared to the average throughout the 20 minutes of experiments. It also shows a minor increase in the average mouse velocity after laser activation, although the velocity remains relatively low, and certainly well below the 5 pixels per second. The observed velocities

Figure 8
Colored circles are the markers used to estimate the mouse's velocity.



during laser activation and the 20 second period after activation range from 1 to 3.2 pixels per second (including the measurement error). I then calculated the average temperature of the mouse during periods without laser activation at these velocity ranges. Figure 10 (b) indicates that the temperature difference is negligible when comparing mouse speeds of 0 to 1 pixel per second (orange) with speeds of 1 to 3.2 pixels per second (red). Moreover, even at speeds greater than 3.2 pixels per second, the temperature increase is significantly less than what is observed during laser activation.

Control test #4: Impact of laser activation on control group

Ultimately, the best way to demonstrate that the increase in the mice's temperature was due to VMHvl activation was by repeating the experiment with a control mouse population without ChR2 expression, rendering the VMHvl neurons not susceptible to laser illumination. I extend my gratitude to my advisor, Dr. Vinograd, for collecting this data on the control population.

Figure 11 shows the average temperature for each mouse and all trials and Figure 12 the

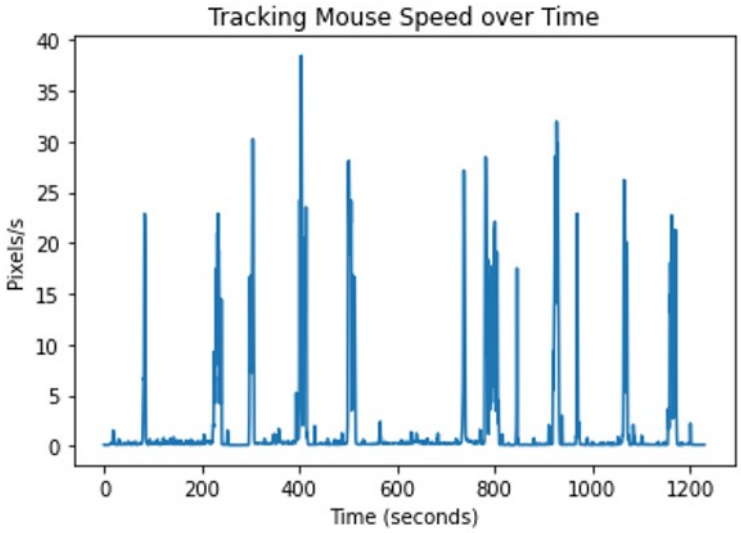
Video 3

Illustration of algorithm to compute mouse's velocity.



Figure 9

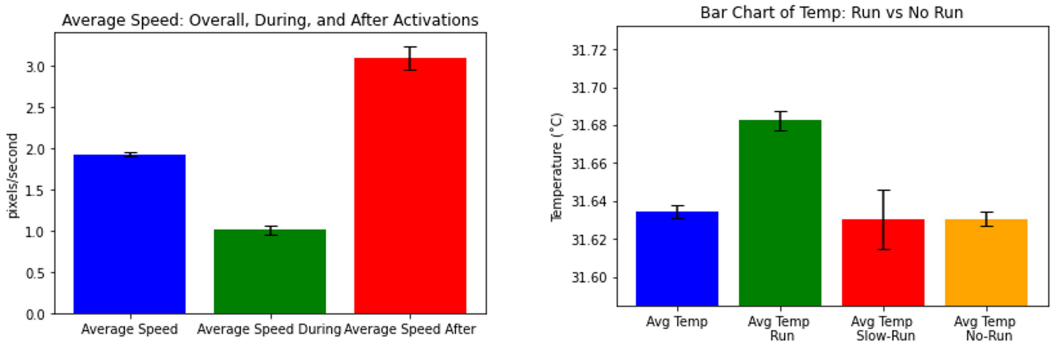
Velocity in pixels per second as a function of time. When the mouse runs, the velocity spikes to over 15 pixels per second.



average for all three mice (bottom) as a function of time. The temperature increase following laser activation (red area) is not statistically significant. A temperature-time plot, similar to the one for

Figure 10

(a) Overall average speed (blue), speed during laser activation (green) and speed for 20 seconds after laser activation (red) with error bars. (b) Overall average temperature (blue), average temperature for velocities larger than 3.2 pixels/second (green), average temperature for velocities between 1 pixel/second and 3.2 pixels/second (red) and temperature for velocities from 0 to 1 pixel/second (orange).

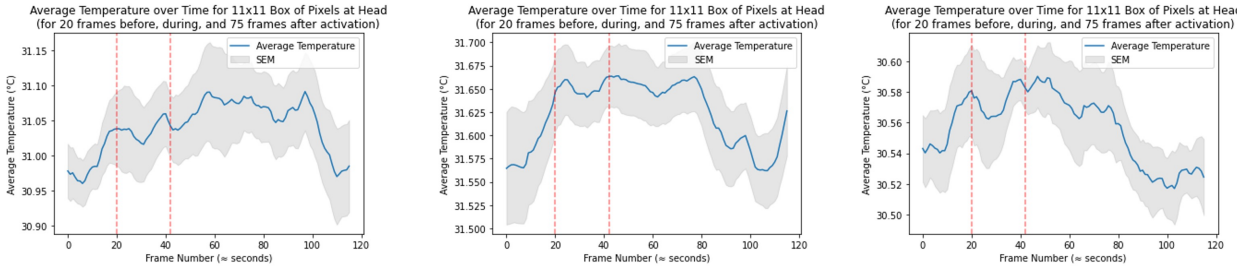


(a) (b)

the experimental group shown in Figure 7, demonstrates temperature variability below any significant increase that could be attributed to the laser energy. This is depicted in Figure 13, with the data in blue and the regression line in black. The fluctuations of the measured temperature data as a function of time, unlike the linear trend in Figure 7, further suggests that these are random temperature variations and not significant increases that could be associated with laser activation.

Figure 11

Temperature as a function of time in the selected 11x11 pixel box for each of the control mice.



Correlation of VMHvl activation and pupil dilation

In order to compare the body temperature response time with that of other vital signs such as pupil dilation, I utilized a neural-network-based open-source package called Facemap (Syeda et al., 2023) to assess pupil size during the experiment.

Figure 12

Average temperature as a function of time in the selected 11x11 pixel box for all the control mice.

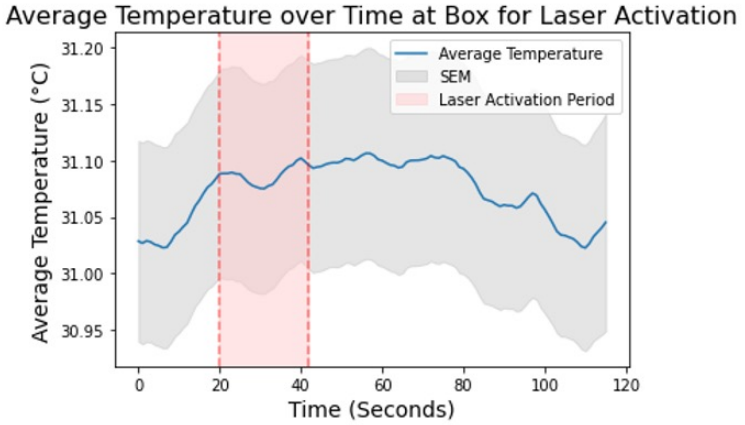


Figure 13

The estimated increase in temperature due to the laser activation energy of 0.04 J (2 mW over 20 seconds) is 0.0265 °C (red). The predicted slope due to the laser energy is 0.001325 °C/sec, whereas the measured slope with the control population (black) is 0.000540 °C/sec. The fluctuations in the data (blue) indicate that these are most likely random variations, not correlated with the laser activation.

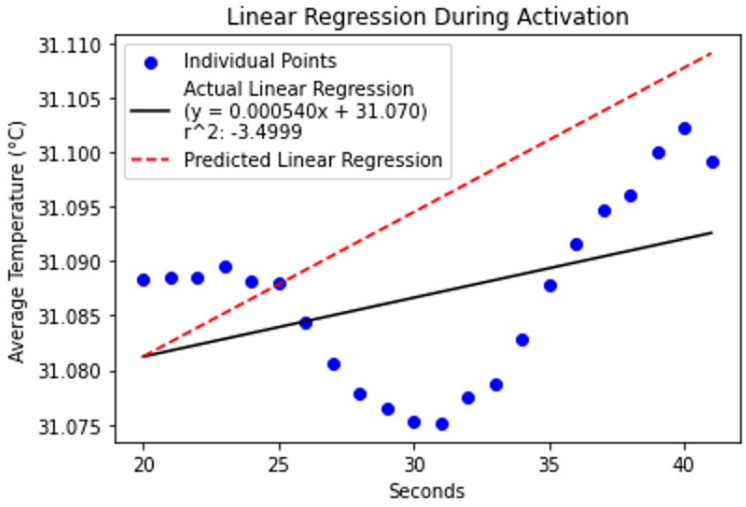


Figure 14 shows snapshots of the mouse's eye, with modified contrast to more clearly observe the pupil. The snapshot on the left shows the eye before activation, while the one on the right shows it 15 seconds after activation, which approximately corresponds to peak pupil size. The middle plot presents the average pupil area (in pixels squared) over time for all trials, with the laser activation time shaded in red. The three panels correspond to the three mice in the

experiment. One mouse (bottom panel) exhibited pupil dilation throughout the experiment. The hypothesis is that this mouse was in an anxiety state and was therefore excluded from the conclusions.

Figure 14

Average pupil area as a function of time for three mice. The first two mice clearly show pupil dilation with laser activation. The third mice (bottom panel) had a dilated pupil over the whole duration of the experiment, likely due to anxiety, so it was discarded.

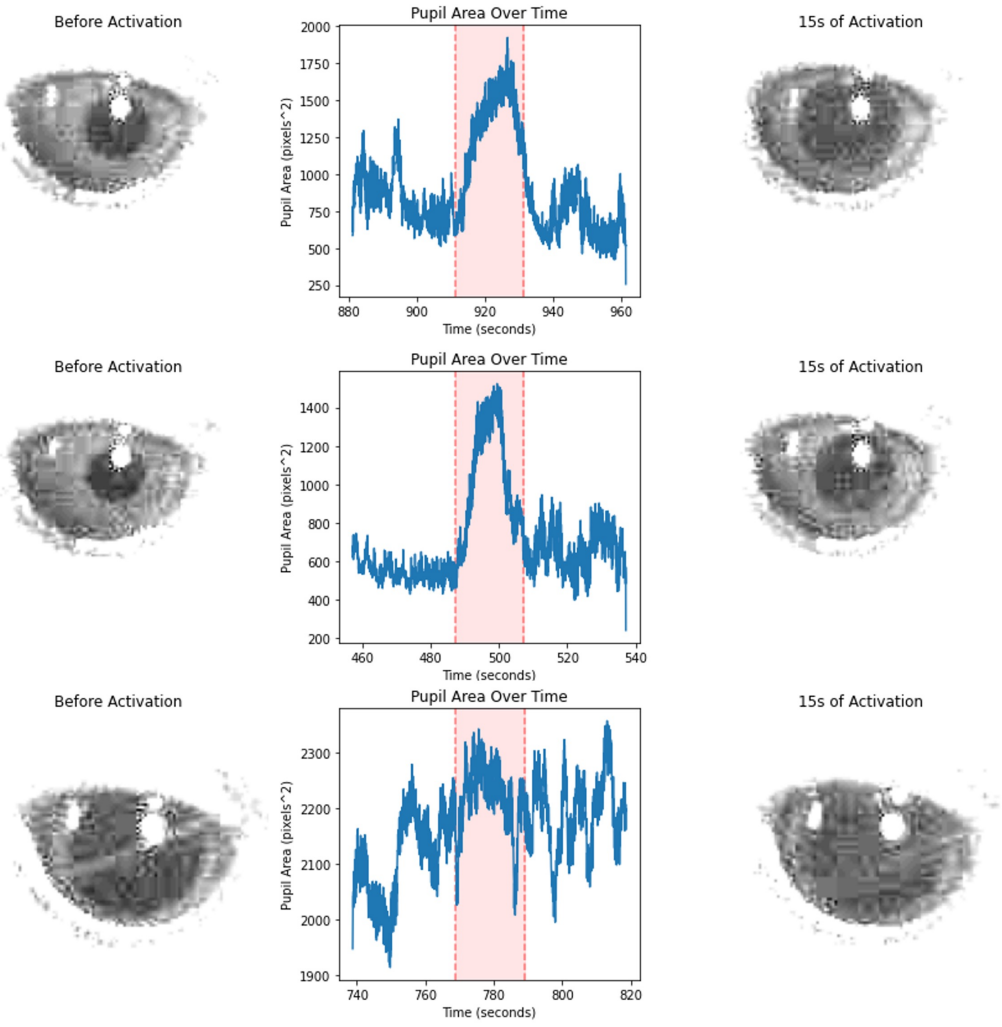
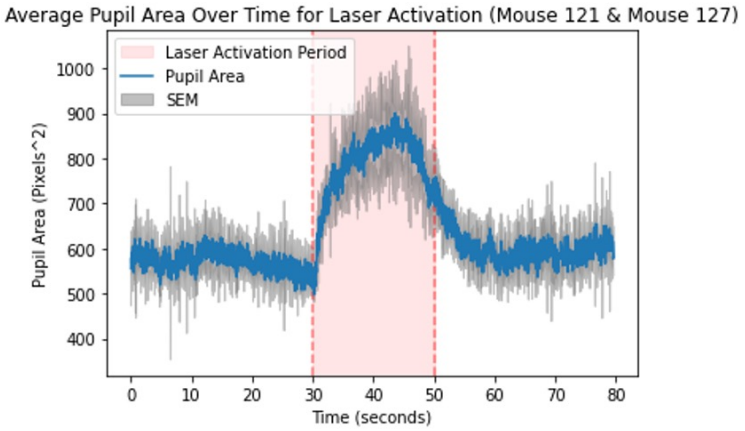


Figure 15 displays the average pupil dilation over time for the two remaining mice. This figure clearly demonstrates a correlation between laser activation and pupil dilation.

Interestingly, the pupil dilation began to decrease before the end of the laser activation period, similar to findings reported in (Zhu et al., 2021), which utilized a different neural circuit.

Figure 15

Average pupil area as a function of time for the two mice that were selected for this experiment. The gray area indicates the standard error measurement (SEM).



This suggests that the response is due to an arousal state rather than anger, which is more persistent.

Fear (VMHdm) data collection and analysis

Similarly to the VMHvl trials, we conducted eight trials on the three mice in the experimental group at a 1 Hz sampling rate for thermal recordings and there were 120-second intervals between laser stimulations.

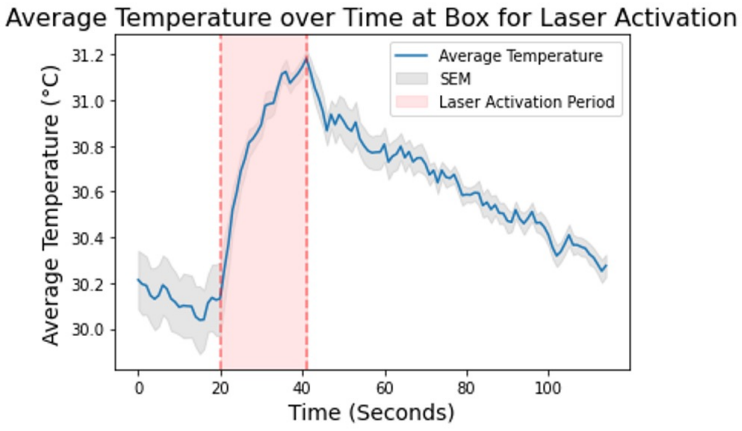
Figure 16 displays the average of all 24 trials for the VMHdm activation. My initial hypothesis was that the temperature would remain similar or drop due to the freeze response causing physiological changes such as decreased metabolic and muscle activity and vasoconstriction to evade predators. However, the results from Figure 16 show a clear increase in temperature during laser activation, over 5 times larger than the temperature increase in VMHvl. The blue curve represents the average temperature in the 11x11 pixel box over time after applying a smoothing filter, with the gray area indicating the standard error of measurement (SEM). The laser activation occurs during the period indicated by the red area.

Control Test for Fear (VHMdm): Natural Rat Fear

Since the experimental set up was identical to the VHMvl data collections, we determined that the laser had little to no impact on temperature. Furthermore, controlling for speed was also

Figure 16

Average temperature of box in blue and standard error of measurement (SEM) in gray over time for the VMHdm data collection.. The red area indicates the laser activation period.



not necessary since in each trial the mice stopped their motion due to the fear response as soon as the VMHdm neural circuit was activated.

As a control, we decided to conduct another experiment with eight trials on the same three mice with natural fear. Since mice are afraid of rats, one can stimulate natural fear by bringing a cage with a rat in front of the mouse as seen in Video 4. I wrote a program to track the frames in which the rat was present and then analyzed the data. Figure 17 shows the average temperature for all trials in the natural rat fear experiment. The result shows no significant change in temperature, contrary to the immediate increase observed with VMHdm activation. This indicates that the VMHdm region of the brain could contain distinct neural circuits—ones that are associated with fear and others that are related to temperature.

As further evidence, once can observe that, contrary to VMHvl where temperature stays high after activation due to aggression being a more permanent state (see Figure 5), Figure 17 shows that in the case of VMHdm the temperature decreases immediately after activation is over, which is indicative of an arousal state. Once the neurons are no longer artificially stimulated by the laser illumination, the temperature begins to decrease. This hypothesis of a different mechanism associated with temperature in VHMdm is also more consistent with the higher temperature increase observed when compared with VMHvl activation.

Video 4

Video of rat fear being elicited in a mouse.

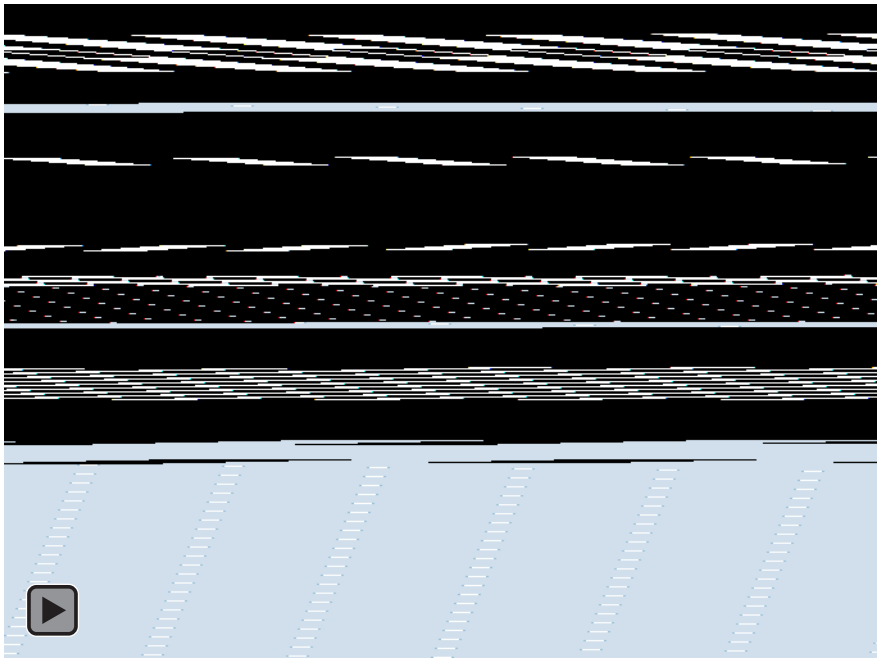
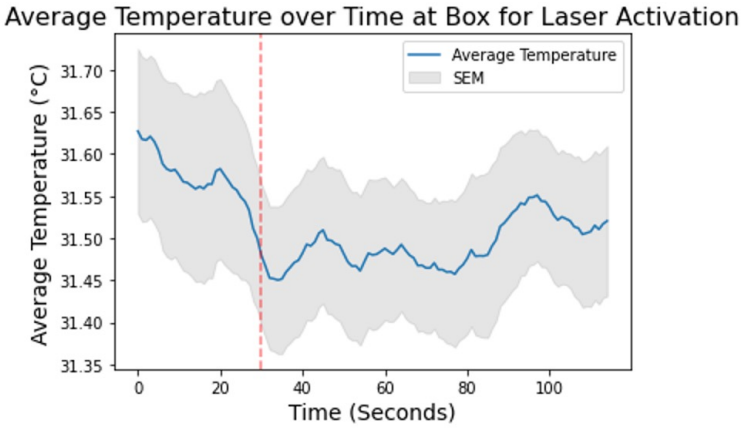


Figure 17

Average temperature in blue and standard error of measurement (SEM) in gray over time for the natural fear experiment. The red dashed line indicates the beginning of the fear activation, which continued for 5-10 seconds after initial activation.



Discussion

During my research, I successfully demonstrated a novel non-invasive method using a thermography camera to capture body temperature changes in mice. Prior methods of performing

these temperature measurements, such as rectal thermometers and implantable microchips, were highly invasive and could affect the mice's behavior due to increased stress or anxiety. They were also more cumbersome and required additional preparation, cost and complexity in the experiment.

Using this non-invasive method, I discovered a statistically significant increase in mice's body temperature following VMHvl activation. By scientifically linking aggressive behavior to this temperature rise, we now have a non-invasive way to both identify and quantify aggression levels in mice. This is particularly important when developing psychiatric drugs that target the brain's aggression circuits. My research indicates that body temperature may serve as a valid indicator of animal behavioral states.

Additionally, this temperature increase during activation prompts significant questions. We believe that this effect results from physiological processes triggered by anger neuron activation. Could the increase be due to neurons that cause blood vessel contraction? Might the temperature rise because anger drives increased blood flow to the head? There could be evolutionary advantages to this mechanism. An angry mouse may need to engage in strenuous activities like fighting, where enhanced blood flow could improve cognitive function and alertness, essential in stressful situations. Further research into the mechanisms behind this temperature rise could be crucial for understanding the brain circuits involved in aggressive behavior.

The evidence also indicates that pupil dilation, typically associated with an arousal state, also correlates with VMHvl activation. Pupil dilation showed a different temporal pattern compared to temperature; it decreased before the activation period ended, while the temperature continued to rise for approximately 20 seconds after laser activation. This further suggests that the aggression circuit, not arousal, drives the temperature increase.

Finally, I also discovered a temperature increase in response to VMHdm activation but no temperature increase in response to natural fear in mice. This indicates that there are separate populations of fear and temperature neurons in VMHdm. An evolutionary advantage of this could be to regulate the temperature of the mice while frozen as they try to avoid a predator. This raises

further questions about the arrangement of different neurons within the different areas of the brain and their function in the regulation of emotions and other processes in animals.

Future Work

We plan to acquire a shutterless thermography camera to enable more precise measurements at a higher frame rate. This will allow us to conduct cross-comparisons with other vital signs such as heart rate or breathing rate and determine whether temperature fluctuations can predict these signs.

We will then compare temperature changes against different laser strengths and durations for VMHvl activation to further explore the anger-temperature relationship and whether temperature continues to rise during extended periods of activation, or at some point it settles and/or decreases. This will give insight into whether temperature can be used as a measure of the extent of the anger of the mouse by tracking the rate of increase in temperature and the overall increase.

Using neural networks for image processing, we also plan to correlate the temperature increase with the mouse's "anger" face. This novel result, previously unpublished, could show if a higher temperature increase correlates with a more pronounced expression of anger, further supporting the idea that body temperature can indicate aggression levels in mice. To better achieve this, I am currently developing a headset with a miniature camera to be able to track the mice facial expressions in a more natural environment since all previous data was collected in an experimental environment.

I am also working on using the neural networks to analyze and track different parts of the bodies of the mice using software such as DeepLabCut to determine if there are any physical cues indicative of specific emotional states.

Through these experiments, we will first gain a better understanding of the internal processes regulated by emotions in mice and how they interact within each other. They will also allow us to create a more complete and reliable method to capture data non-invasively and use it to track the various emotional states in mice. This will be crucial towards any experiments

studying emotion states and their efforts to develop treatments and drugs to conditions involving with these pathways.

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