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Research Article

Establishment of a Non-Human Primate Model for Menopausal Hot Flushes

Istvan Merchenthaler[1,2]*, Christina A Stennett[1], Bethany Haughey[1], Adam Puche[2] and Henryk F Urbanski[3]

[1]Department of Epidemiology and Public Health, University of Maryland, Baltimore, USA

[2]Department of Anatomy and Neurobiology, University of Maryland, Baltimore, USA

[3]Oregon National Primate Research Center, Oregon Health and Science University, USA

***Corresponding Author:** Istvan Merchenthaler, Department of Epidemiology and Public Health, University of Maryland, Baltimore, USA.

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Abstract

Menopause affects the quality of life of millions of women. With modern lifespan the postmenopausal attenuation of circulating estrogen levels can negatively impact a women's life for 30 - 40 years. The major hypoestrogenic consequence is hot flushes but decline in cognitive function, sleep disorders, depression/anxiety, cardiovascular disease, and osteoporosis are also characteristic for the menopause.

Current treatments of hot flushes include estrogen therapy alone or in combination with progestins, soy products, and serotonin and norepinephrine reuptake inhibitors. However, with the exception of estrogens, none of these have satisfactory efficacy. But estrogens come with the unwanted side effects in the periphery, including stimulation of the uterus and breast leading to elevated cancer risk. Therefore, a tremendous effort has been devoted to developing safer therapies and the research has utilized classic rodent models of hot flush with considerable limitations. As hot flushes are primate-specific symptoms, the development of a non-invasive primate hot flush model would have a tremendous impact on drug development. Therefore, our aim was to develop such a non-human primate (NHP) model a hot flush that both recapitulates flushes women experience and is minimally invasive. We investigated if recent developments in thermal imaging have made it possible to accurately monitor skin temperature via camera imaging.

In this study, the skin temperature of an ovariectomized rhesus monkey was measured continuously with an infrared camera in a freely moving animal over long time period. Following mapping skin temperatures of several areas of the neck and face we found that the nose of the monkeys showed that largest changes in skin temperature. In the ovariectomized monkey the temperature of the skin on the nose shows up to 9°C elevations representing hot flushes. In the untreated monkey, hot flushes occurred more frequently in late afternoon/early evening hours than in the morning and last for several minutes. We observed 58 flushes in the 64 evenings of observation. The average number of hot flushes was 0.51 per evening. Oral administration of biotin (niacin) for seven days exaggerated the number of hot flushes to 2.43 per evening. Intramuscular treatment with estradiol benzoate prevented hot flushes and only 2 flushes were detected in the 12 evenings after treatment, averaging 0.17 per evening.

The development of this NHP model of hot flush provides great hope for utilizing it for future drug development and mechanistic studied.

Keywords: *Hot Flush; Hot Flash; Infrared Imaging; Menopause; Non-Human Primate; Estrogen*

Abbreviations

ET: Estrogen Therapy; HT: Hormone Therapy; NHP: Non-Human Primate; NRI: Norepinephrine Reuptake Inhibitor; OVX: Ovariectomy; SSRI: Selective Serotonin Reuptake Inhibitor

Introduction

Menopause affects the quality of life of millions of women all over the world. With increases in the modern lifespan, the postmenopausal attenuation of low circulating estrogen levels can negatively impact a women's life for 30 - 40 years. Major hypoestrogenic consequences include hot flushes (flashes), decline in cognitive function, sleep disorders, depression/anxiety, cardiovascular disease, genitourinary conditions, and osteoporosis. Although menopause primarily impacts women, men are also affected to a lesser extent.

Current treatments of hot flushes include estrogen therapy (ET), hormone therapy (estrogens and progestins; HT), soy products that have estrogenic activity, serotonin and norepinephrine reuptake inhibitors (SSRIs/NRIs), and gabapentin [1]. With the exception of ET and HT, none of the other available treatments have satisfactory efficacy [2]. Estrogens are highly effective at preventing hot flushes, however, they come with the unwanted side effects in the periphery, including stimulation of the uterus and breast leading to elevated cancer risk, making ET approaches sub-optimal. Therefore, a tremendous effort has been devoted to developing safer ET, i.e., estrogen receptor ligands that could have beneficial effects in the brain, without stimulating the uterus and the breast [3,4] or other therapies targeting neurotransmitter and neuropeptide systems [5]. The research into these compounds has utilized classic rodent models of hot flush with considerable limitations, primarily the fact that rodents do not normally exhibit hot flushes and thus do not fully capture the human ontogeny of the condition.

The major impediment to the development of novel and safer alternatives for hot flush therapy, is the lack of animal models that recapitulate human symptoms. As hot flushes are primate-specific symptoms, the development of a non-invasive non-human primate (NHP) hot flush model would have a tremendous impact on drug development. Therefore, our aim was to develop such a non-invasive NHP model of hot flushes. As NHPs undergo a process very similar to what women experience after menopause [6] the use of NHPs for research and drug development to alleviate meno- and andropausal symptoms holds great promise.

More than three decades ago two groups reported that the skin temperature of the head and face of ovariectomized (OVX) NHPs showed cycling changes with elevated temperatures lasting about 4 - 50 minutes and that estrogen treatment prevented them [7,8]. The character and frequency of these changes resembled closely those recorded from women during hot flush stacks. In addition to the undulating changes in skin temperature following OVX and the elimination of them by estrogen this study also reported diurnal changes in skin temperature, occasionally reaching 6 C during nocturnal phases. Both of these studies utilized direct temperature measurements with thermocouples taped on the skin of trained animals. Importantly, both studies reported skin temperature elevations resembling those seen in postmenopausal women suggesting that OVX monkeys do flush. But due to the training and surface instrumentation required and measurement details only from the instrumented location of the skin, the model was impractical.

The field critically needs an NHP hot flush model that both recapitulates flushes characteristic of those experienced by menopausal women and is minimally invasive to reduce disruption to vascular and neural connections at the monitoring site. We investigated if recent developments in thermal imaging technology have made it possible to accurately monitor skin temperature via camera imaging.

In this study, the skin temperature of a female rhesus monkey was measured continuously with an infrared camera starting 5 months post-OVX and continuing over long time period.

Materials and Methods

Skin temperature of the 17-year-old OVX rhesus monkey was monitored non-invasively in the freely moving animal with an 8640 Medical Infrared Camera (Infrared Cameras, Inc., [ICI]) mounted on a block located outside of the cage. Images were captured every second initially and then increased to 10 seconds to provide a more manageable dataset. Recording started five months following OVX. Our pilot experiments ensured us that this monitoring approach was feasible and would generate the expected data.

At the Oregon National Primate Research Center, the animal was housed in its own cage in a room with other monkeys indoors under controlled environmental conditions: 24°C temperature; 12-h light:12-h darkness photoperiods with lights on at 07:00 h. Meals were provided regularly at 08:00h and 15:00h (LabDiet High Protein Monkey Chow; LabDiet, Inc., St Louis, MO, USA) and supplemented with fresh fruit or vegetables; drinking water was available *ad libitum*.

To identify the most responsive area on the neck and skin, the temperatures of several regions, including the forehead, the temporal area, ear, and nose were monitored for weeks. These experiments clearly indicated that the nose of the monkey is the most responsive area; it showed up to 9°C increases representing hot flushes. In the infrared images, the nose appeared black in non-flushing periods and turned white indicating greater temperature during flushes. The next step was to identify the best time of the day when hot flushes occur most frequently. These experiments concluded that the vast majority of hot flushes occurred in the afternoon hours, primarily late afternoon/early evening. When monkeys sleep, they cover their face. Therefore, we could not measure nose temperature while the monkey was at sleep during the night.

To further evaluate our imaging approach, the monkey was treated orally with niacin (vitamin B3; 5.0 mg/kg orally) which caused a 5 - 6°C increase in her nose temperature. Several days later, two doses (42 ug/kg) of estrogen benzoate were given intramuscularly three days apart. This treatment prevented hot flushes (only two were seen in a f12-day period post-treatment). The temperature data were saved on 4-TB portable drives and sent to University of Maryland (UMB) where our IT personnel transferred them to a shared folder accessible for the Oregon and UMB sites. Images were analyzed with IR Flash version 1.0 software by ICI. Since the monkey constantly moved her head, only a small percentage of images in a given night (5-12%) showed clearly her nose without interference from the bars of the cage. However, the temperature changes were clearly detected even in those images where the nose was partially obstructed.

Results and Discussion

The infrared camera used in these studies provided continuous thermal imagery with spatial resolution to approximately 1 mm on the animal and temperature change sensitivity to 0.03ºC. Skin temperature measurements were made through cage meshes with cage bar spacings optimized to the thermal image acquisition procedure. We found that increasing the gap size between rows and columns of the mesh screen improved imaging accuracy, while still retaining sufficient rigidity to avoid compromising the safety of animal handling personnel. However, even with these changes in place, the bars were interfering with many of the images, covering partially or completely the areas of interest. In addition, the monkey was able to move around freely within the cage, which limited the number of continuous images capturing the same body part. When focusing on a specific body part, only a small percentage (5 - 12%) of images without interference of the bars could be analyzed, but the temperature changes were clearly detected even in those images where the bars partially obstructed the body part. Future development of large surface thermally transparent silica sheets is underway to provide unimpeded imaging capability. Initially, images were taken in every second generating several terabits data for analysis. As the image collections progressed, the data volume at 1 second time intervals became problematic (resulting in terabytes of data generated every day), and it was necessary to reduce the temporal sampling interval to every 10 seconds.

To validate our infrared detection approach, during the optimization period, we pharmacologically induced flushes through the administration of a niacin (vitamin B3) dietary supplement (5.0 mg/kg orally), which triggered an up to 5.5ºC increase in the skin temperature

of the nose. Estrogen (17β-estradiol benzoate: 42 ug/kg body weight) was later administered to prevent the increase of nose temperature. Although the pinna of the ear of the monkeys also showed changes in skin temperature, due to the large variability of images due to the frequent moving the animal, we stopped recording from the pinna. The hands/fingers of the animal also showed skin temperature elevations, but we stopped recording them because touching the cage bars or the floor of the cage interfered with our measurements.

Using the nose as a primary measurement point, we continuously collected images every ten seconds for 64 days total duration. Analyzing thermal transients, we found that hot flushes (elevation of skin temperature of the nose by 2 - 9°C) occurred more frequently during the late afternoon/early evening hours, close to a period when the monkey fell asleep. The duration of flushes varied between 1 - 2 to several minutes. Figure 1 shows nose skin temperatures taken within one evening (from 5:00 PM through 8:45 PM) prior to the monkey falling asleep. Of the 1357 images taken during this period, the monkey's nose was visible in 517 images (38%), and 88 (17%) of those nose images were unobstructed by the bar. Figure 2 shows small and large power representative images of the noses of the OVX monkey without (Figure A) and with (Figure B) flushing. We observed 58 flushes in the 64 evenings of observation. The average number of hot flushes before ET was 0.51 per evening. During the 7-day period when niacin was administered daily, we observed a marked increase in flushes, averaging 2.43 per evening. In contrast, only 2 flushes were detected in the 12 evenings after ET, averaging 0.17 per evening.

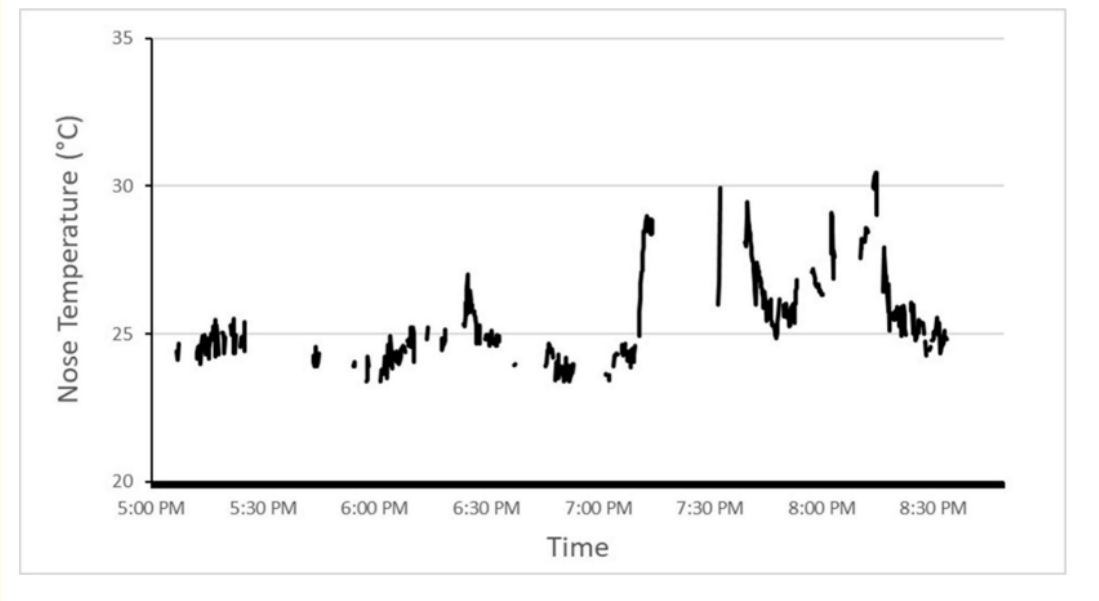

Figure 1: *This figure shows the nose temperature (°C) over time on one evening (5:00 P.M. to 8:45 P.M.) prior to the monkey falling asleep. Red arrows indicate flushes, during which the nose skin temperature increased by 4 - 5°C.*

In this study, the skin temperature of an OVX rhesus monkey was measured continuously with an infrared camera in a freely moving animal over long time period. We have found that the nose of the monkey showed that largest changes in skin temperature that could be attributed to a flushing event. A major advantage of this approach is that the animal is not disturbed, as is the case for surgical or skin surface instrumenting, and that imaging can occur over months or potentially even years within the same subject. The long-term ability to

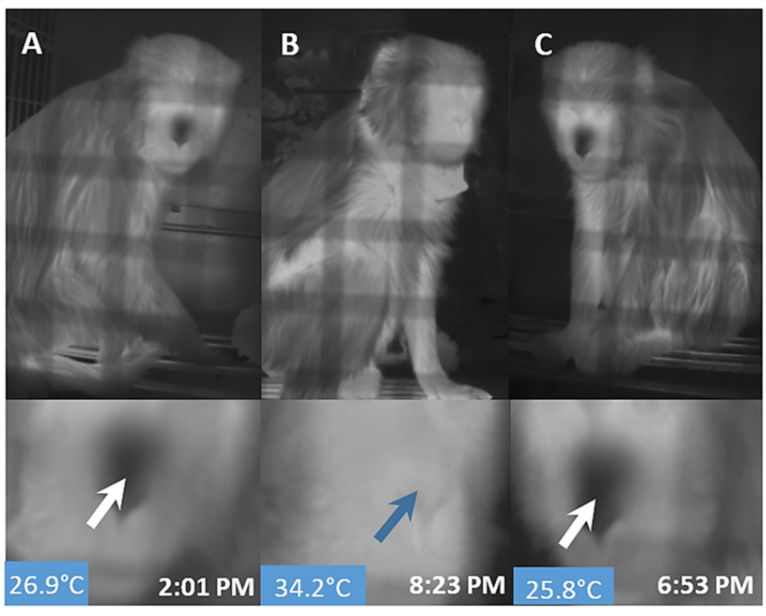

Figure 2: *Thermal images of a freely moving rhesus monkey taken with and ICI 8640 Medical Infrared Camera. The face of the monkey can be seen between the horizontal and vertical bars of the cage. Before the initiation of estrogen treatment, the temperature of the nose of the ovariectomized monkey is 26.9°C during a period when she does not flush (black color). During a flush, the nose temperature increases to 34.2°C (white color). Following estrogen treatment, the monkey does not flush, i.e., her nose temperature goes back to a lower value (25.8°C).*

monitor the same subject makes a deeper investigation of the mechanism of hot flushes possible and also to evaluate how primates may adapt to treatment schedules over weeks, to months, to years.

The vast majority of earlier studies used OVX rats and measured tail skin temperature in pharmacological models (e.g. morphine dependent rats) [9,10]. However, the relevance of these models to the human conditions is questionable. First, rats dissipate heat only by vasodilation and not by sweating as women do, and they do not naturally exhibit hot flushes when they age. Second, morphine and subsequent morphine withdrawal affects many neuronal circuits not involved in heat dissipation, which reduces the relevance of the circuitry investigated due to potential secondary effects from the morphine withdrawal-induced flushing events. This has a great negative impact on studies aimed at exploring the mechanism of hot flushes. Therefore, the availability of a long-term non-invasive primate model will fill these gaps and will advance not only preclinical research but the development of novel therapies.

Although thermal imaging has been shown to be an effective method to measure hot flashes in women [11], as far as we know, this is the first non-invasive macaque model of menopausal hot flushes that provides an invaluable proof of concept that will be a necessary foundation to develop novel therapies for these symptoms. A common method to induce hot flushes in postmenopausal women is to apply heating pads on their abdomen [12,13]. A recent study in marmosets (*Callithrix jacchus)* by Gervais., *et al.* [14] reported similar observations to ours using this method. These authors induced hot flushes by warming up the abdomen of the monkeys and found that the temperature of the nose showed the largest elevation measured with thermo imaging. Other studies showed opposite changes in directions of nose temperature in marmosets following the presentation of negative arousing stimuli (teasing, playback of aggressive vocalizations).

These changes in nasal temperature were correlated with piloerection of the tail as an independent measure of arousal and could not be explained by changes in physical activity [15]. Similarly, in other studies macaques showed a decline in nasal temperature in response to a threatening person [16] or visual and vocal signals of aggressive conspecifics [17]. Kano., *et al*. [18] found that the nose of chimpanzees became colder in response to playback calls and videos of fighting conspecifics.

Thus, it seems that the skin temperature of the nose shows large changes, an elevation during a flush and reduction during emotional reactions.

Conclusion

We have developed a non-invasive NHP hot flush model which makes it possible to study the mechanism of hot flushes and to develop and test novel, better, and safer therapies. Continuous infrared imaging focusing on OVX monkey's nose showed flushing activity, particularly in the evening hours. As current caging partially obstructed images, future development of large surface thermally-transparent silica sheets is underway to provide unimpeded imaging capability. Shortening the post-OVX period and expending the length of estrogen therapy and post-treatment recording period is also planned.

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