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Estrogen Sensitive Neurons in the Medial Preoptic Area of the Hypothalamus Regulate Temperature in Mice

A thesis submitted in partial satisfaction

of the requirements for the degree Master of Science

in Physiological Science

by

Johnathon Roy DiVittorio

2020

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2020

ABSTRACT OF THE THESIS

Estrogen Sensitive Neurons

in the Medial Preoptic Area of the Hypothalamus

Regulate Temperature in Mice

by

Johnathon Roy DiVittorio

Master of Science in Physiological Science

University of California, Los Angeles, 2020

Professor Stephanie Correa Van Veen, Chair

Reduced estrogen levels in menopausal and perimenopausal women are associated with hot flashes. The medial preoptic area (MPA) of the hypothalamus is region of the brain with many physiological functions including temperature regulation. While this region of the brain is rich in estrogen responsive neurons, it is unknown whether or not estrogen plays a role in the MPA's temperature regulation. Thus, we hypothesized that temperature sensitive neurons in the MPA are responsive to estrogen and could mediate thermoregulation via thermogenesis and heat dissipation. To define the role of estrogen sensitive neurons in the MPA we selectively activated the neurons using chemogenetics or ablated by delivering a virus

expressing caspase3 into Esr1-Cre mice. We recorded core body temperature using intraperitoneal telemetry probes. Ablation of the neurons led to an increase in core body temperature. In contrast, chemogenetic activation of the neurons led to a drop in core body temperature. Thermogenesis of brown adipose tissue (BAT) and head dissipation of skin temperature on the tail were observed using an infrared camera and analysis software. Upon activation, there was a decrease in temperature of BAT suggesting a reduction of thermogenesis. We also observed an initial increase in tail temperature suggesting vasodilation and a release of heat, lowering core body temperature. To further analyze the MPA's role in temperature regulation we took advantage of the physiological state of torpor which mice enter upon fasting. Torpor is associated with a decrease in temperature, metabolism, activity, cardiac function, and mental activity. We saw that ablation of estrogen sensitive neurons in the MPA impedes the temperature drop seen in torpor. These complementary loss of function and gain of function studies suggest a critical role for estrogen signaling in the MPA in temperature regulation. Building an understanding of the estrogen-sensitive temperature populations of the brain will help model and study hot flashes.

The thesis of Johnathon Roy DiVittorio is approved.

David Walker

Barnett Schlinger

Stephanie Correa Van Veen, Committee Chair

University of California, Los Angeles

2020

DEDICATION

I would like to dedicate this thesis to my family, specifically my parents, Adrienne and Roy DiVittorio. Your continuously support and encouragement over the years has allowed me to try new experiences and grow academically and personally.

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ACKNOWLEDGMENTS

I would like to thank my principal investigator, Dr. Stephanie Correa Van Veen for your guidance and support over the past two years. Your consistent belief in me has guided me in this program through the highs and lows to get me where I am today. You have inspired me to work hard at whatever I do, pursue my passion, and enjoy the good times. I would also like to thank my other committee members, Dr. David Walker and Dr. Barney Schlinger for their help and influence.

I would also like to thank all of the members of the Correa Laboratory. Specifically, I want to thank my mentor, Dr. Zhi Zhang. You embody all the characteristics of a perfect mentor: patience, kindness, vast knowledge, and an unwavering confidence in myself. All of your advice and support has aided me in the completion of this thesis and also prepared me for my next steps in life.

This research was funded by the UCLA Division of Life Sciences, the Iris Cantor-UCLA Women's Health Center/UCLA National Center of Excellence in Women's Health, and NIH National Center for Advancing Translational Science (NCATS) UCLA CTSI Grant Number UL1TR001881.

BACKGROUND

Reduced estrogen levels in menopausal and perimenopausal women are associated with a variety of metabolic, vasculature, and neuroendocrine changes. The symptom most associated with menopause is a hot flash, which include sudden feeling of heat, vasodilation, sweating, and shivering [1] and are the primary source of menopausal distress [2]. It is believed that the decreased levels of estrogens are what lead to the sudden dissipation of heat via peripheral vasodilation [3]. The increased peripheral heat leads to a drop in core body temperature [4]. Hot flashes are reported by 75-80% of menopausal women in the US and occur over an average span of 7.4 years [5]. The mechanism behind how the onset of hot flashes is associated with fluctuating estrogen levels is not fully understood.

The strongest treatment for hot flashes is hormone therapy, but the process comes with risks, especially for patients with cardiovascular diseases, liver disease, or previous cancer patients [1]. Another non-hormonal treatment uses anti-depressants; these selective serotonin reuptake inhibitors have been shown to decrease the side effects of hot flashes, but are less effective than hormone therapy [6, 7]. An alternative, non-hormonal treatment is behavioral treatment involving changes in lifestyle habits. This includes wearing layers, carrying fans, and minimizing stressful environments. One study looked at the practice of breathing exercises, finding a slight reduction in episodes of hot flashes [8]. The lack of a safe and effective treatment suggests that more research into the mechanism of hot flashes be conducted.

We plan to fill the knowledge gap by studying a particular group of estrogen sensitive neurons, which express estrogen receptor alpha (ERα) from the gene *Esr1*, in the medial

preoptic area (MPA) of the hypothalamus in female mice. The MPA is believed to have thermostat like qualities [9]. Neurons in the MPA sense temperature and drive thermoregulation through thermogenesis, heat conservation, or heat dissipation [10]. Neurons of the MPA also richly express estrogen receptors [11]. Although it has been shown that changes in estrogen levels can affect heat generation, heat dissipation, and body temperature in mice [12], the connection of estrogens directly affecting temperature regulation via the MPA is still unclear.

Mice can be used as a model for temperature regulation by measuring the temperature of tail skin (T-skin), core body temperature (T-core), and ambient temperature (T-ambient) [13]. T-skin is the primary method of heat dissipation in mice via vasodilation and losing heat to the environment. By genetically manipulating key thermoregulatory regions of the mouse brain, we hope to gain insight into the complexity of temperature regulation and the contribution of estrogens.

CHAPTER 1: ERα NEURONS IN THE MPA ARE REQUIRED FOR THERMOREGULATION

Introduction

The medial preoptic area (MPA) of the hypothalamus is a key regulatory center for thermoregulation and is also rich in neurons expressing the *Esr1* gene encoding estrogen receptor alpha (ERα). Declined levels of estrogen have been shown to have profound effects on temperature in the form of a hot flash in peri- and postmenopausal women. We believe that there is a connection between the two. Estrogens may be involved in keeping temperature at a homeostatic range via the neurons in the MPA. In order to test this hypothesis, we ablated the neurons in the MPA expressing ERα, thus making the MPA insensitive to estrogens, then monitored temperature in female mice.

Materials and Methods

Animals

Wildtype (WT) C57BL/6J mice were purchased from Jackson Laboratories. Mice expressing the *Esr1 Cre* knock in allele (*Tm1.1(Cre)And*) was maintained on a C57BL/6 genetic background and bred in our colony at University of California, Los Angeles (UCLA). All mice were maintained under a 12:12 hour L/D schedule, and allowed to receive food and water ad libitum unless otherwise indicated. Mice were at 8-10 weeks old at the start of all the experiments.

Mouse Procedures

All studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. UCLA is AALAS

accredited and the UCLA Institutional Animal Care and Use Committee (IACUC) approved all animal procedures. Mice were anaesthetized with isoflurane and received combinatorial analgesics (0.01mg/mL buprenorphine, 0.58mg/mL carprofen) pre- and post- any surgeries.

Stereotactic surgery

The pAAV-flex-taCasp3-TEVp were bought from UNC Vector core (Addgene plasmid # 45580). AAVs (150nL) were injected bilaterally under stereotaxis to the MPA (coordinates: AP 0.2 mm, ML ±0.35 mm, DV -5.3 mm from the surface of skull) of adult *Esr1*-Cre or WT mice.

Temperature recording

A G2 eMitter (Starr Life Sciences) was implanted to the abdominal cavity and attached to the inside body wall of a mouse. Mice were single-housed in cages placed on top of ER4000 Energizer/Receivers. Nesting material was held constant to normalize behavioral temperature regulation. Gross movement and core body temperature were measured every 5 min using VitalView software (Starr Life Sciences).

ERα Immunohistochemistry

Mice were perfused transcardially with ice-cold DEPC treated PBS (PH=7.4) followed by 4% paraformaldehyde (PFA). Brains were embedded in OCT and frozen in -80 ^{o}C after one overnight post fixation in 4% PFA and another overnight dehydration in 30% sucrose. Coronal sections were cut under cryostat (Vibratome) into 8 equal series at 18 μm.

Sections were first incubated for 40 min at 95 ^{o}C in 25 mM Tris–HCl (pH 8.5), 1 mM EDTA, and 0.05% SDS (Tris-EDTA-SDS) buffer for antigen retrieval and then blocked for 1 h in 10% BSA and 2% normal goat serum (NGS). Next, the sections were incubated overnight at 4 °C with primary antibody (ERα, 1:250, sc-8002, Santa Cruz). Following 3X 10 min washing in PBS,

sections were incubated with Fluorophore conjugated goat anti-mouse secondary antibody (Thermo Fisher Scientific) for 2 h at room temperature. After 2X 10 min washing in PBS, sections were incubated with DAPI, washed and coverslipped with Fluoromount-G.

The images were taken by DM1000 LED fluorescent microscope (Leica) or LSM780 confocal microscope (Zeiss). Confocal images that contain tiles and z-stacks were stitched and merged by maximum intensity projections using Zen Black (Zeiss). Cyan/magenta/yellow pseudo-colors were applied to all fluorescent images for color-friendly accessibility. Image processing was performed using the Leica Application Suite (Leica), Zen Black (Zeiss) and ImageJ (NIH). Quantification was performed using Cellprofiler software 3.1.8 (CellProfiler).

Statistics

Data are represented as mean ± SEM. Data with normal distribution and similar variance were analyzed for statistical significance using two-tailed, unpaired Student's t-tests. Paired data were analyzed by paired t-tests. Comparisons for more than two groups were analyzed by one-way ANOVA followed by post-hoc Tukey's analysis. Time course data were analyzed by two-way ANOVA followed by Sidak's multiple comparisons or repeated measures two-way ANOVA followed by Sidak's multiple comparisons for paired data. Significance was defined at a level of $P < 0.05$. Statistics were performed using GraphPad Prism 8 and RStudio.

Results

To determine if ERα MPA neurons are required for thermoregulation, we selectively ablated ERα neurons in adult female mice using AAV (adeno-associated virus) that expresses a genetically modified caspase 3 (Fig. 1.1A). AAV2-FLEX-Caspase 3 was delivered to the MPA of

Esr1Cre mice. Controls included wild-type mice receiving AAV encoding the Cre-dependent caspase 3 or *Esr1Cre* mice receiving AAV encoding a Cre-dependent GFP. Four weeks post-injection, *Esr1Cre* mice showed a >50% reduction in ERα immunoreactivity in the MPA compared to controls (Fig. 1.1B).

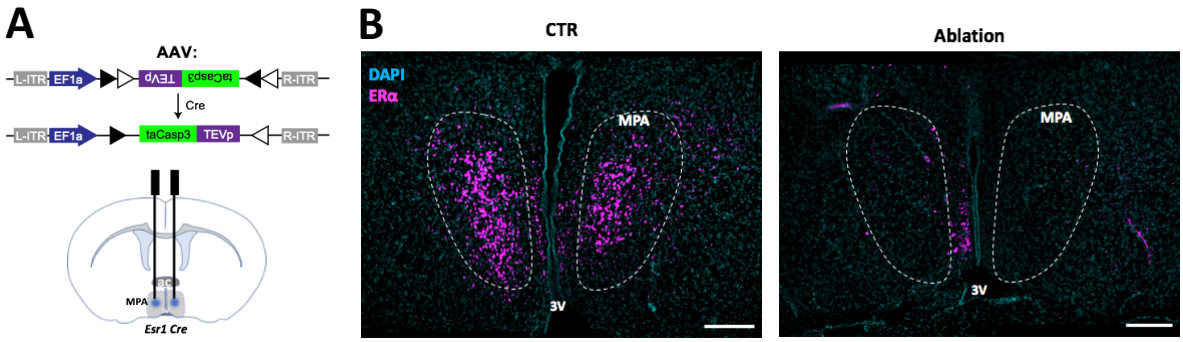

Figure 1.1: Method for ERα MPA neuron ablation. A. Schematic of the AAV encoding a Cre-dependent caspase delivered into the MPA of *Esr1Cre* mice to ablate Esr1-expressing cells. **B.** ERα immunoreactivity in the MPA of mice after AAV-mediated ablation of sr1-expressing cells (right) or controls (left), with a DAPI co-stain. 3v, third ventricle. Scale bar: 200 μm.

Ablation of ERα MPA neurons significantly increased core body temperature (Fig. 1.2A). The difference in core body temperature between *Esr1Cre* mice with ablated ERα MPA neurons and control mice could be seen during the light, dark, and 24 hour periods (Fig. 1.2B). The ablation had no effect on activity levels of the mice (Fig. 1.2C), indicating that the changes in core body temperature were not due to increased physical activity. The effect on core temperature indicates that ERα MPA neurons are critical for maintaining normal thermal homeostasis in female mice.

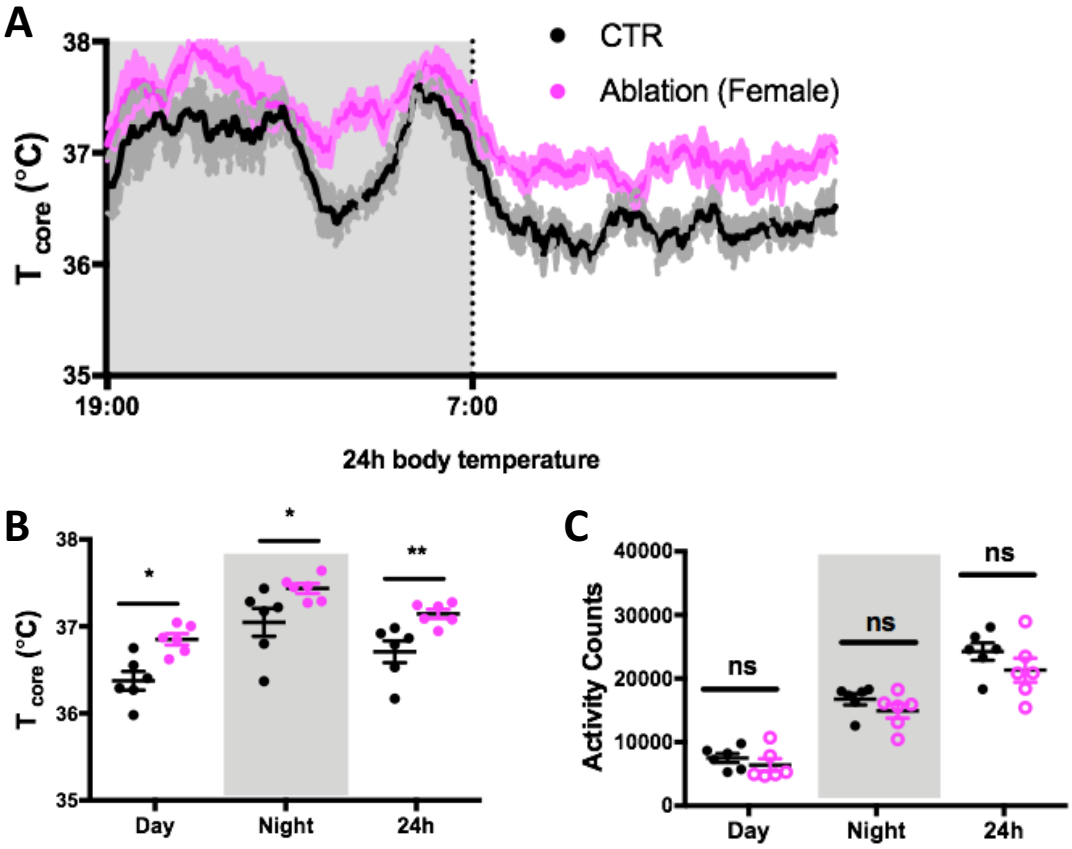

Figure 1.2: ERα neurons in the MPA are required for thermoregulation. A. Core body temperature over 24 hours, measured every 5 minutes for 3 days. Shading along curve denotes the standard error of mean. Control (black) n=8, ablated (pink) n=8 female mice. **B.** Average core body temperatures from panel C separated by day (7:00-19:00), night (19:00-7:00), and 24 hour periods. **C.** Average activity counts of mice from panel C measured every 5 minutes separated by day (7:00-19:00), night (19:00-7:00), and 24-hour period, ns=not significant $p>0.05$.

CHAPTER 2: ACTIVATION OF ERα NEURONS IN THE MPA DROP CORE BODY TEMPERATURE

Introduction

The effect of ablating ERα MPA neurons profoundly dropped core body temperature suggesting their necessity in maintaining homeostasis. The next step in our study was to activate the ERα MPA neurons to see if they are sufficient in driving the opposite effect. We used a viral construct to stereotaxically inject the DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) into the MPA genome of *Esr1Cre* female mice to express the human M3 muscarinic (hM3) receptor. Upon ligand activation a Gq signaling releases intracellular calcium stores and enhances neuronal excitation. Thus, neurons expressing hM3Dq treated with CNO are observed to have dramatically increased firing rates. We expect there to be a drop in core body temperature upon activation of ERα MPA neurons.

Materials and Methods

Animals

Wildtype (WT) C57BL/6J mice were purchased from Jackson Laboratories. Mice expressing the *Esr1 Cre* knock in allele (*Tm1.1(Cre)And*) was maintained on a C57BL/6 genetic background and bred in our colony at University of California, Los Angeles (UCLA). All mice were maintained under a 12:12 hour L/D schedule, and allowed to receive food and water ad libitum unless otherwise indicated. Mice were at 8-10 weeks old at the start of all the experiments.

Mouse Procedures

All studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. UCLA is AALAS accredited and the UCLA Institutional Animal Care and Use Committee (IACUC) approved all animal procedures. Mice were anaesthetized with isoflurane and received combinatorial analgesics (0.01mg/mL buprenorphine, 0.58mg/mL carprofen) pre- and post- any surgeries.

Stereotactic surgery

The pAAV-hSyn-DIO-hM3D(Gq)-mCherry purchased was Addgene plasmid # 44361 and # 65417 [14]. AAVs (150nL) were injected bilaterally under stereotaxis to the MPA (coordinates: AP 0.2 mm, ML ±0.35 mm, DV -5.3 mm from the surface of skull) of adult *Esr1*-Cre or WT mice. Unilateral photometry fiber was implanted using the coordinates above as for virus injections.

Temperature recording

A G2 eMitter (Starr Life Sciences) was implanted to the abdominal cavity and attached to the inside body wall of a mouse. Mice were single-housed in cages placed on top of ER4000 Energizer/Receivers. Nesting material was held constant to normalize behavioral temperature regulation. Gross movement and core body temperature were measured every 5 min using VitalView software (Starr Life Sciences). Tail skin temperature was monitored every 5 min using a Nano-T temperature logger (Star-Oddi). The logger was attached to the ventral surface and 1 cm from the base of the tail in a 3D-printed polylactic acid collar modified from Krajewski-Hall et al [12].

Chemogenetics

In DREADDs experiment, mice received i.p. injections of CNO (0.3 mg/kg, Sigma-Aldrich) or vehicle (saline, 0.15% DMSO) 3 h after the onset of the light phase. Saline and CNO were administered in the same mice alternatively as randomized cross design. Core and tail skin temperature were monitored continuously throughout the experiment. For extra control, the new DREADD ligand C21 (1mg/kg, Cayman Chemical Company) or vehicle (saline, 1% DMSO) was administered i.p. following the same experimental procedure as CNO injection. For intrahypothalamic injection, CNO was prepared at 2mM in aCSF. Before injection, the mice were connected to a 33G Stainless-Steel internal cannular (Plastic One) that was attached to 1 ul Hamilton Syringes through 40 cm non-compressive silicone tubing. CNO or vehicle (aCSF 3.4% of DMSO) was injected at 50nl/side in one minute. The mice were able to move freely during injection.

Infrared thermal images were captured using Industrial camera VarioCAM® HD head 800 (InfraTec infrared LLC) before (t0) CNO or vehicle injection, then 10 min, 20 min, 30 min, 50 min, 120 min and 240 min after injection. The infrared images were analyzed using software IRBIS3 (InfraTec infrared LLC). BAT skin temperature was the average temperature of a circular region above interscapular BAT and tail skin temperature was the average temperature of a 1 cm line along the tail starting at 1 cm from the base of the tail. Electrocardiogram (ECG) was recorded using ECGenie system (MouseSpecific Inc.).

Statistics

Data are represented as mean ± SEM. Data with normal distribution and similar variance were analyzed for statistical significance using two-tailed, unpaired Student's t-tests. Paired data

were analyzed by paired t-tests. Comparisons for more than two groups were analyzed by one-way ANOVA followed by post-hoc Tukey's analysis. Time course data were analyzed by two-way ANOVA followed by Sidak's multiple comparisons or repeated measures two-way ANOVA followed by Sidak's multiple comparisons for paired data. Significance was defined at a level of $P < 0.05$. Statistics were performed using GraphPad Prism 8 and RStudio.

Results

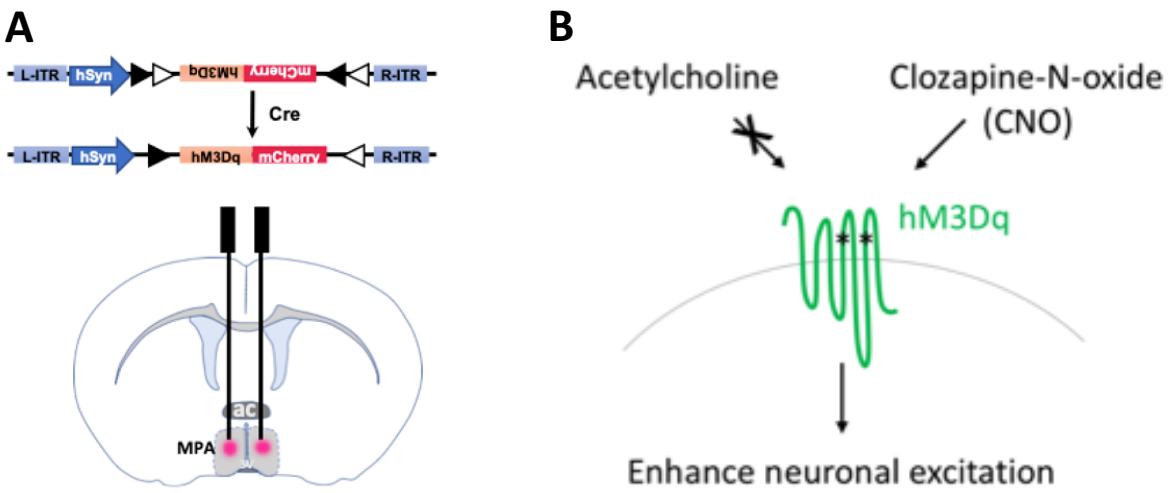

Figure 2.1: Method for ERα MPA neuron activation. A. Schematic of the AAV encoding a Cre-dependednt DREADD delivered into the MPA of *Esr1Cre* mice to ablate Esr1-expressing cells. **B.** Schematic of the DREADD, hM3Dq, being inactive to acetylcholine and activating a Gq coupled cascade when bound by CNO to activate the neuron.

We next investigated the effects of activating ERα MPA neurons by implementing chemogenetic DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). An AAV construct encoding the Cre-depended Gq-coupled DREADD receptor and an mCherry reporter were stereotaxically injected to the MPA of *Esr1Cre* or WT female mice (Fig. 2.1A). The hM3Dq (human M3 muscarinic receptor) is nonresponsive to acetylcholine ligand, but responds to clozapine-N-oxide (CNO), increasing the neuronal activity (Fig. 2.1B).

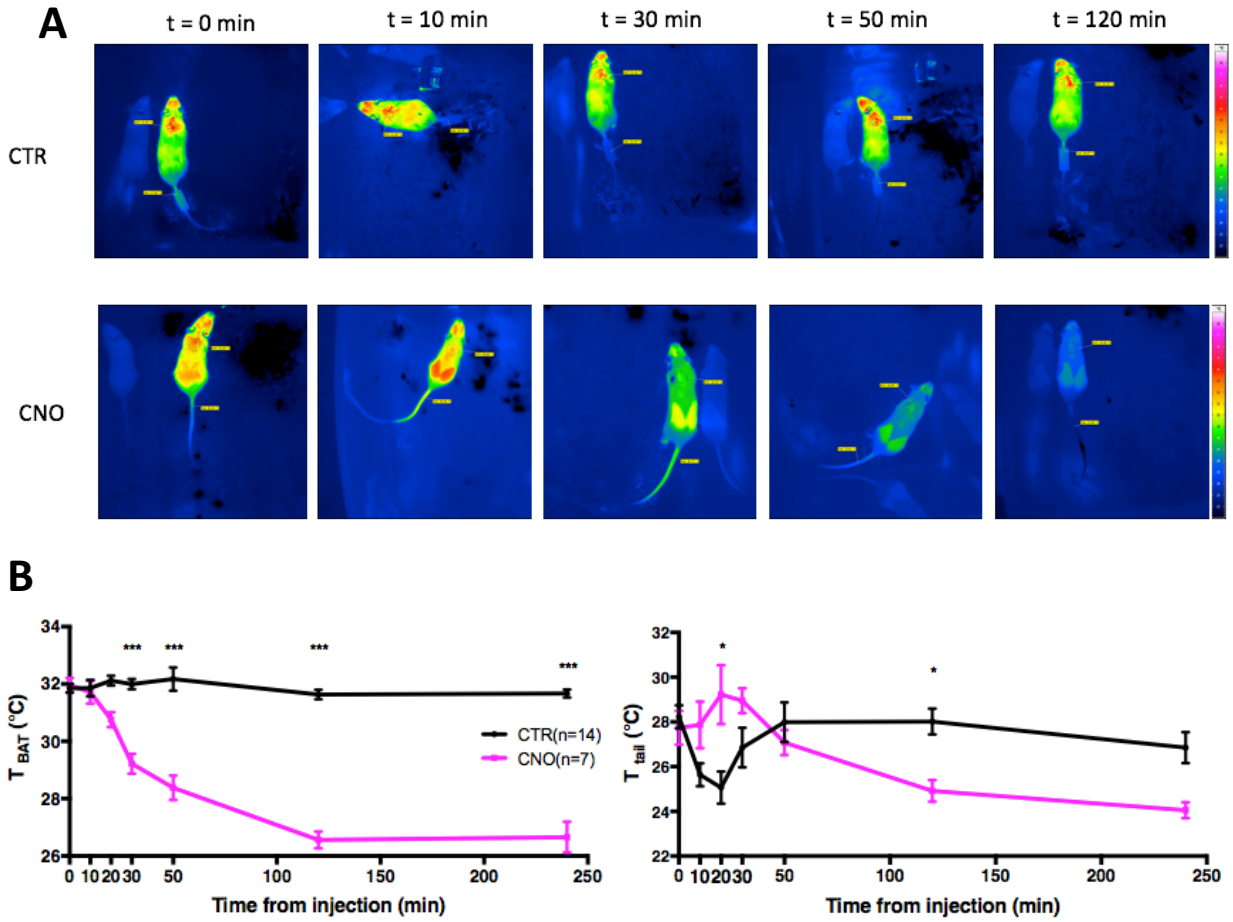

Figure 2.2: Thermal imaging of activated ERα MPA neurons. A. Representative infrared thermal images showing temperature after saline (CTR) or CNO injections in *Esr1Cre* female mice. **B.** Quantification of thermography images in BAT (left panel) and tail (right panel) regions before and after saline (CTR) or CNO injections in *Esr1Cre* female mice. *, p<0.05; **, p<0.01; ***, p<0.001.

Thermal homeostasis is maintained by the balance of heat production and heat dissipation. In mice, changes in heat production and dissipation are mediated by thermogenesis in the BAT and vasodilation or vasoconstriction at the tail skin. Changes in thermoregulation of BAT and tail skin temperature were analyzed based on infrared thermal imaging (Fig 2.2A) revealing that upon intraperitoneal administration of CNO, *Esr1Cre* mice exhibited a dramatic reduction in brown adipose tissue (BAT) region temperature up to 10°C lasting over 3 hours (Fig

2.2B, left). Along with a drop in BAT temperature, there was a fluctuation of tail temperature that initially rose, then fell in *Esr1Cre* mice upon administration of CNO (Fig 2.2B, right).

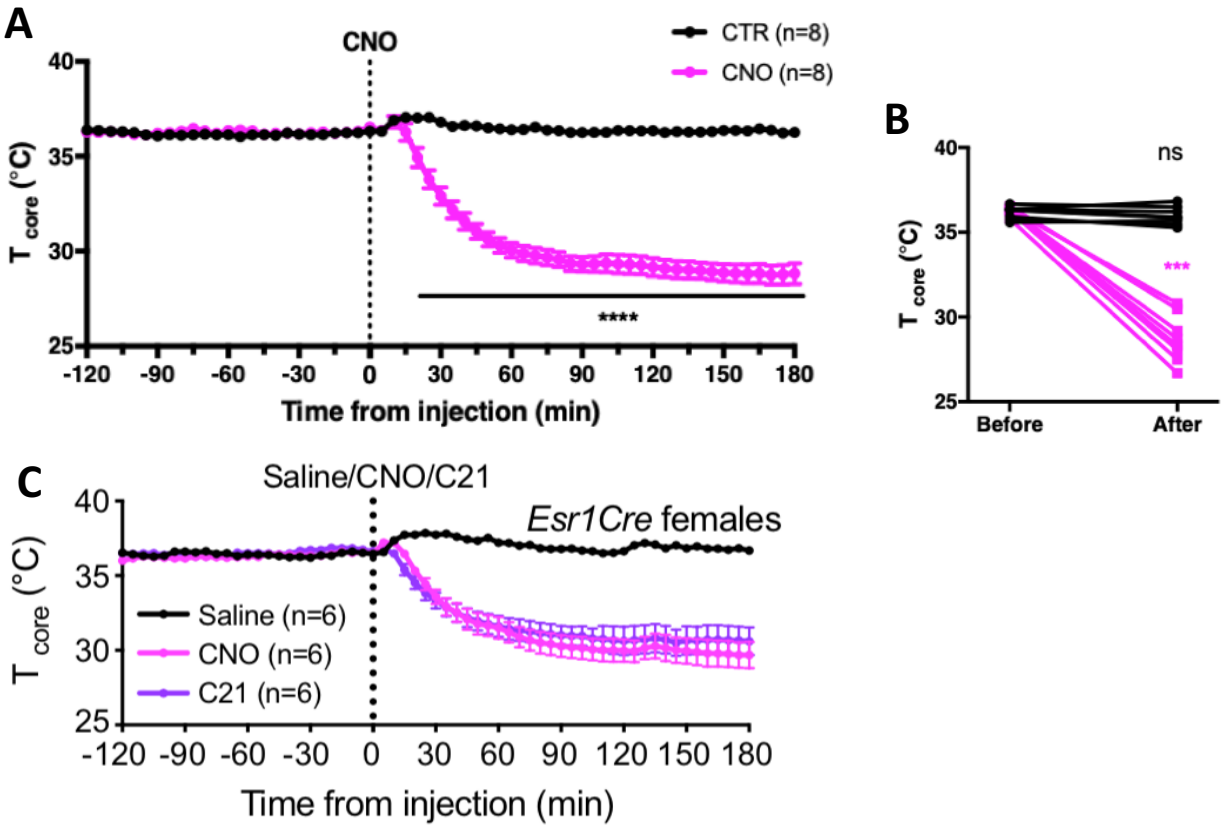

Figure 2.3: Activation of ERα neurons in the MPA drops core body temperature. A. Core body temperature measured every 5 minutes before and after injection (dotted line at x = 0) of saline (black) or CNO (pink) in *Esr1Cre* females previously injected with a Gq-coupled DREADD. **B.** Per-animal averages of core body temperature before (-120 min to 0 min) and after (120 min to 180 min) saline or CNO injection. **C.** Core body temperature before and after injection (dotted line at x = 0) of saline (black), CNO (pink), or C21 (purple) in *Esr1Cre* females previously injected with a Gq-coupled DREADD.

To further understand how ERα MPA neuronal activation affects mouse temperature equilibrium, we monitored the temperature of the body core and tail skin temperature simultaneously using thermal probes implanted intraperitoneally and a temperature logger attached near the ventral vein of the tail, respectively. Neuronal activation caused a rapid drop

in core body temperature (Fig. 2.3A). The drop in core body temperature cannot be attributed to CNO because CNO did not alter body temperature in WT mice. Additionally, administration of a different activating ligand, Compound 21 (C21), resulted in a similar reduction in body temperature in *Esr1Cre* female mice (Fig. 2.3C), so the effect is specific to activating ERα neurons.

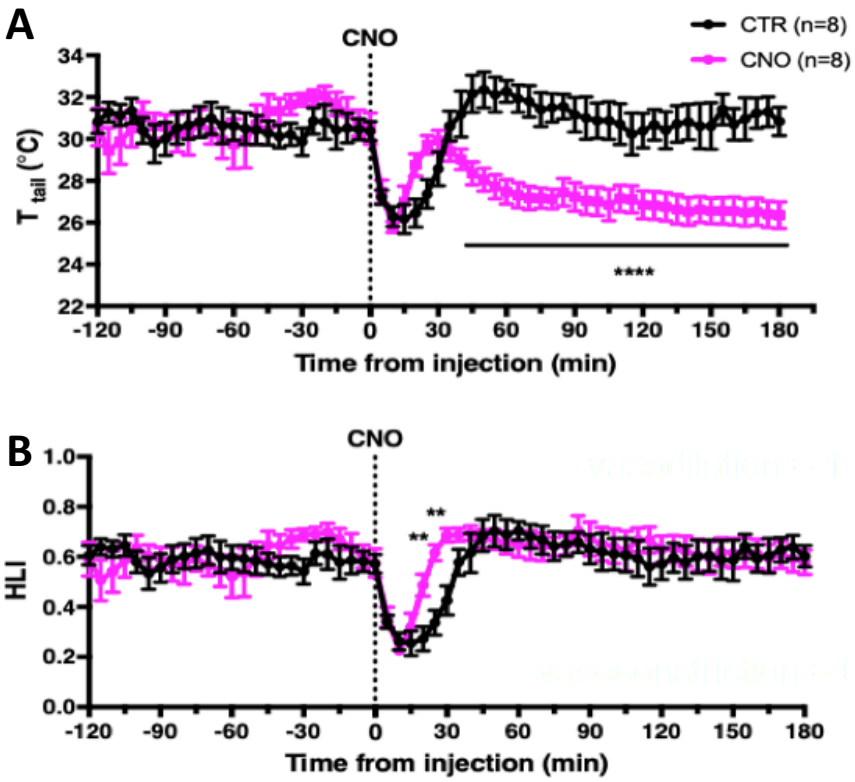

Figure 2.4: Activation of ERα neurons in the MPA is associated with heat dissipation through the tail. **A.** Tail temperature measured by attached tail thermo-logger before and after injection (dotted line at x = 0) of saline (CTR) and CNO in *Esr1Cre* female mice. **B.** Heat loss index (HLI) calculated by attached tail thermo-logger before and after injection (dotted line at x = 0) of saline (black) or CNO (pink) in *Esr1Cre* female mice.

The infrared thermal imaging revealed an increase in tail skin temperature 20 minutes after CNO injection whereas saline treatment was associated with a decrease in tail temperature due to handling stress (Fig. 2.2B, right). The same result was observed when

recording tail skin temperature using thermo-loggers attached to the tail (Fig. 2.4A). We analyzed this data to acquire a more direct readout for heat dissipation using the heat loss index (HLI): HLI = $(T_{skin} – T_{ambient})/(T_{core} – T_{ambient})$. HLI corrects for overall body cooling by comparing the temperature of the skin and core to the ambient temperature. A HLI approaching 0 is indicative of increased vasoconstriction, whereas HLI approaching 1 indicates increased vasodilation. HLI revealed that CNO injection elicited a profound increase in heat loss which temporally coincides with the initial decrease in core body temperature (Fig. 2.4B). Our results indicate that activating ERα MPA neurons initiate a rapid loss of heat via vasodilation of the tail, leading to a decreased core body temperature.

CHAPTER 3: ERα MPA NEURONS ARE REQUIRED FOR FASTING-INDUCED TORPOR

Introduction

We are interested in studying temperature regulation in mice and we initially found that chemogenetic activation of the ERα MPA neurons significantly drop core body temperature. Interestingly, this induced hypothermic state may reflect a naturally occurring behavior in rodents called torpor, indicating that these neurons may be regulating more than just temperature maintenance. Torpor is a state of extreme low metabolism and energy expenditure. It is often characterized by reduced body temperature, physical activity, cardiac function, brain activity, and basal metabolic rate [15-18]. The low metabolism and energy expenditure allows for animals to survive periods of low food intake. Torpor is different from other types of hibernation because it is not seasonal and is not voluntary. This is exciting because a torpor-like state can be induced from food deprivation, so we can manipulate the ERα MPA neurons to see if they play a contributing role in entering a torpor-like state. Because activation of ERα MPA neurons may reflect a torpor-like state, we suggest that ablation of ERα MPA neurons will lessen the torpor-effect in female mice.

Materials and Methods

Animals

Wildtype (WT) C57BL/6J mice were purchased from Jackson Laboratories. Mice expressing the *Esr1 Cre* knock in allele (*Tm1.1(Cre)And*) was maintained on a C57BL/6 genetic background and bred in our colony at University of California, Los Angeles (UCLA). All mice were

maintained under a 12:12 hour L/D schedule, and allowed to receive food and water ad libitum unless otherwise indicated. Mice were at 8-10 weeks old at the start of all the experiments.

Mouse Procedures

All studies were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. UCLA is AALAS accredited and the UCLA Institutional Animal Care and Use Committee (IACUC) approved all animal procedures. Mice were anaesthetized with isoflurane and received combinatorial analgesics (0.01mg/mL buprenorphine, 0.58mg/mL carprofen) pre- and post- any surgeries.

Stereotactic surgery

The pAAV-hSyn-DIO-hM3D(Gq)-mCherry purchased was Addgene plasmid # 44361 and # 65417 [14]. AAVs (150nl) were injected bilaterally under stereotaxis to the MPA (coordinates: AP 0.2 mm, ML ±0.35 mm, DV -5.3 mm from the surface of skull) of adult *Esr1*-Cre or WT mice. Unilateral photometry fiber was implanted using the coordinates above as for virus injections.

Temperature recording

A G2 eMitter (Starr Life Sciences) was implanted to the abdominal cavity and attached to the inside body wall of a mouse. Mice were single-housed in cages placed on top of ER4000 Energizer/Receivers. Nesting material was held constant to normalize behavioral temperature regulation. Gross movement and core body temperature were measured every 5 min using VitalView software (Starr Life Sciences).

Fasting-induced torpor

Mice were individually housed and core body temperature was monitored continuously as described above. Following baseline measurements (food and water ad libitum), mice were

placed in new cages and food was removed from the cages at 10 am. After 48 hours of fasting, the mice received their food back or were euthanized for perfusion. Mice are closely observed for symptoms of dehydration, sickness and immobility during fasting. For torpor induction during ERα ablation experiment, torpor bouts were defined when the core body temperature was equal to or below 31 ^{o}C.

Statistics

Data are represented as mean ± SEM. Data with normal distribution and similar variance were analyzed for statistical significance using two-tailed, unpaired Student's t-tests. Paired data were analyzed by paired t-tests. Comparisons for more than two groups were analyzed by one-way ANOVA followed by post-hoc Tukey's analysis. Time course data were analyzed by two-way ANOVA followed by Sidak's multiple comparisons or repeated measures two-way ANOVA followed by Sidak's multiple comparisons for paired data. Significance was defined at a level of $P < 0.05$. Statistics were performed using GraphPad Prism 8 and RStudio.

Results

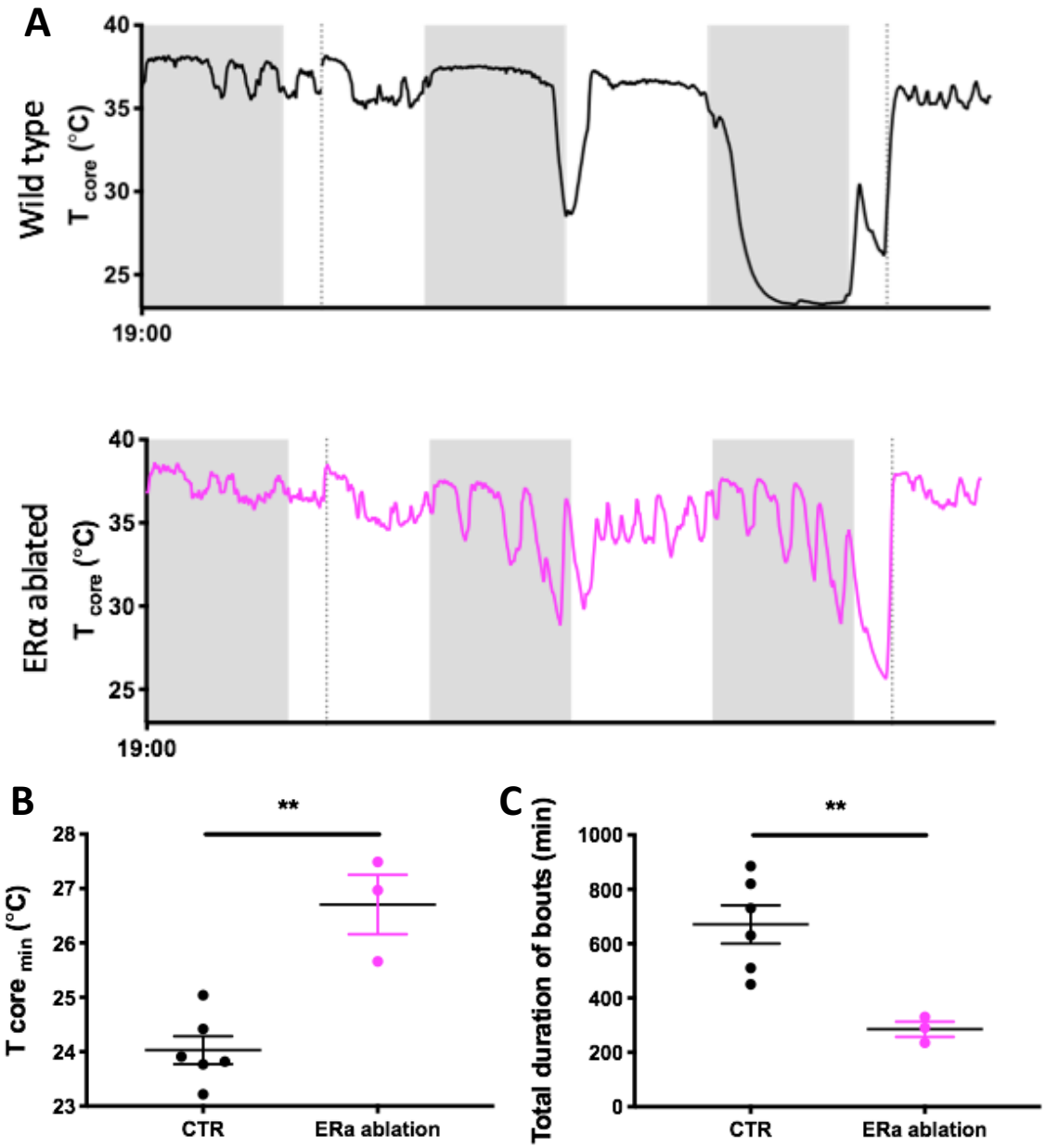

Figure 3.1: ERα MPA neurons are required for fasting-induced torpor. A. Representative core body temperature measured every 5 minutes over 3 days during fasting induced torpor in control (black) and ERα neuronal ablation (pink) mice. First vertical dotted line, begin fast; second vertical dotted line, end fast. **B.** Average minimum core body temperature over fasting periods. **C.** Average total duration spent in torpor-like bout over fasting period.

To determine if ERα MPA neurons are required for the induction of a torpor-like state, we selectively ablated ERα neurons in the MPA of female mice using an AAV that expresses a

genetically modified caspase 3 (Fig. 1.1A). AAV2-FLEX-Caspase 3 was delivered to the MPA of *Esr1Cre* mice. Controls included wild-type mice receiving AAV encoding the cre-dependent caspase 3 or *Esr1Cre* mice receiving AAV encoding a Cre-dependent GFP. Food deprivation for 48 hours induced bouts of deep hypothermia in mice with intact ERα MPA neurons (Fig. 3.1A, top). Ablation of the ERα MPA neurons led to a less dramatic torpor-like effect from food deprivation (Fig. 3.1A, bottom). Minimum core body temperature was evaluated and revealed that mice with fewer ERα MPA neurons had a higher minimum core body temperature compared to controls (Fig. 3.1B). Ablation of ERα MPA neurons also lead to a lower duration of time spent in a torpor-like state, determined by a core body temperature reaching 31°C or below (Fig. 3.1C). These studies suggest that ERα MPA neurons are necessary for fasting-induced torpor-like state.

DISCUSSION

Estrogens are recognized as modulators of temperature [19,20] and decreased levels of estrogen in menopausal and postmenopausal women is associated with hot flashes [21]. The medial preoptic area (MPA) of the hypothalamus is a region of the brain with many physiological functions including temperature regulation. While this region of the brain is rich in estrogen responsive neurons, it is unknown exactly how estrogen plays a role in the MPA's temperature regulation. Here we find that ERα MPA neurons are both sufficient and necessary to alter and maintain normal body temperature in female mice. ERα MPA neurons potentially regulate body temperature by decreasing core body temperature when chemogenetically activated with DREADDS. This drop in core body temperature is associated with increased tail skin vasodilation causing heat dissipation. DREADD-induced hypothermia is associated with a rapid change in tail skin vasodilation and consistent with previous studies showing the hypothalamic preoptic area regulates blood flow to the skin [23-25]. When core body temperature drops and heat dissipation decreases, BAT is not activated to increase thermogenesis, as seen by a drop in dorsal intrascapular temperature. This suggest a potential suppression of thermogenesis upon ERα MPA neuronal activation. Importantly, the increase in core body temperature was not associated by an increase in activity levels and therefore cannot be attributed to increased physical activity.

Complementary to neuronal activation studies, ERα MPA ablation disrupts normal thermoregulation in female mice. Ablated neurons increased core body temperature. This finding puts ERα MPA neurons in a broader light of thermoregulatory and metabolic responses with regard to fasting. By ERα MPA chemogenetic activation, we induced a torpor-like state,

characterized by dramatic reductions in body temperature and physical activity. These variables were replicated by a 48 hour fast. Ablation of ERα MPA neurons led to an attenuation of the torpor-like state in both minimum temperature and total duration.

Brain regions that coordinate hypometabolism and hypothermia are not well characterized, but are essential for understanding the physiology of torpor in rodents as well as giving insight into the etiology of hot flashes in women. Several studies have identified hypothalamic circuits involved in the regulation of body temperature. ERα MPA neurons project to other hypothalamic thermoregulatory regions including the DMH (dorsal medial hypothalamus), VMH (ventral medial hypothalamus), and ARC (arcuate nucleus) [25, 26]. Recent studies suggest that projections from the MPA to the DMH are essential for regulating thermogenesis [27]. Other findings in our lab have brought up the possibility that when ERα MPA neurons are activated, projections to the DMH may contribute to the suppression of BAT thermogenesis (data not shown). Additionally, ERα MPA neuronal activation may project to the ARC in regulating heat dissipation and decreased core body temperature [26, 28]. Further work would be needed to confirm these hypotheses, but the current findings suggest a potent role of ERα MPA neurons in coordinating thermoregulation.

Together, these studies identify ERα MPA neurons as a region of the thermoregulatory neural circuits that drives cooling, is sensitive to estrogen, and therefore may potentially play a role in the etiology of hot flashes, the most common complaint or perimenopausal and postmenopausal women.

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