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UNIVERSITY OF CALIFORNIA

Los Angeles

Estrogenic re-mapping of the metabolic response to an energy deficit

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor
of Philosophy in Molecular, Cellular, and Integrative Physiology

by

Paul Benjamin Vander

2026

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2026

ABSTRACT OF THE DISSERTATION

Estrogenic re-mapping of the metabolic response to an energy deficit

by

Paul Benjamin Vander

Doctor of Philosophy in Molecular, Cellular, and Integrative Physiology

University of California, Los Angeles, 2026

Professor Stephanie Correa, Chair

Endothermy is an adaptation that can be traced back hundreds of millions of years and is critical for nearly every aspect of mammalian biology. Despite the advantages associated with a stable internal temperature, endothermy is energetically expensive. To conserve energy when food is scarce, many species of mammals, including mice, have evolved the ability to reversibly enter a regulated state of hypothermia and hypometabolism, known as torpor. While torpor effectively lowers metabolic demands, it comes with significant reproductive tradeoffs. As a result, organisms have evolved sophisticated strategies to balance torpor and reproduction, thereby maximizing their fitness. In Chapter 1, I review the current knowledge of the signaling networks underlying the bidirectional relationship between torpor and reproduction. In summary, little is known about the cues that influence the reproductive system during torpor and their mechanism of action, although hypothalamic kisspeptin neurons are well-positioned to act as integrators. Conversely, gonadal steroids attenuate torpor in multiple species, yet the signaling mechanisms underlying their effect remain unknown. Promising candidates, including the preoptic area, exist, yet there have been no studies to directly test these

hypothesized targets. In Chapter 2, I confirm that circulating estrogens attenuate fasting-induced torpor in female mice and test the hypothesis that the preoptic area acts as a central mediator for their effects. In testing this hypothesis, I find that circulating estrogens alter the response properties of torpor-regulating neurons in the preoptic area yet require a peripheral signaling mechanism to drive torpor attenuation. Together, these works advance our understanding of the bidirectional relationship between torpor and reproduction in mammals and represent an important step towards the ultimate goal of manipulating these pathways to improve metabolic and reproductive health in humans.

The dissertation of Paul Benjamin Vander is approved.

Arthur P Arnold

Weizhe Hong

Christopher S Colwell

Stephanie Correa, Committee Chair

University of California, Los Angeles

2026

To Papa – you were my first scientific mentor, and I can't think you enough for the years of knowledge and wisdom you have shared with me. I strive every day to make you proud and carry on your example.

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VITA

EDUCATION

- 2020-
Present **University of California, Los Angeles (UCLA)**
PhD candidate – Molecular, Cellular & Integrative Physiology
GPA: 3.97/4.00
- 2015 –2019 **University of Michigan**, Ann Arbor, MI
Bachelor of Science – Biology
Overall GPA: 3.68/4.00; University Honors
Science GPA: 3.64/4.00

PUBLICATIONS

1. Camon C, Kip E, Lord R, Decourt C, Prescott M, Clarkson J, Sandoval NP, **Vander PB**, Van Veen JE, Prokai-Tatrai K, Correa SM, Campbell RE, Garratt M. Targeting Estrogen to the Brain via DHED does not seem to Protect Against Metabolic Dysfunction in Obese OVX Mice. *Endocrinology*. bqag082 (2026).
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SERVICE

2023-2025	CELL Scholars Mentor UCLA, https://bri.ucla.edu/outreach/cell-scholars/ <i>Mentored high school students learning to perform laboratory research. Met weekly with mentees to cultivate intellectual and technical skills to support their scientific training.</i>
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Chapter 1: Biological Mechanisms Balancing Torpor and Reproduction in Mammals

Abstract

Selective pressures have led to the development of innumerable unique strategies to maximize fitness – an organism's ability to survive and reproduce. One strategy is the ability to reversibly enter a regulated state of hypometabolism, known as torpor, to temporarily halt or slow important metabolic processes to conserve energy and avoid a lethal energy shortage. While torpor can be highly adaptive, it can also interfere with reproduction. As a result of this trade-off, organisms have developed adaptations that balance energy homeostasis needs with the metabolic demands of reproduction, thereby maximizing their fitness. In mammals, numerous studies have described a bidirectional relationship between torpor and reproduction, with reproductive state influencing the pattern and frequency of torpor use and, conversely, the reproductive axis undergoing dramatic changes during periods of frequent torpor use. Here, we review the current knowledge of the signaling networks underlying this bidirectional relationship, weigh hypotheses on unresolved questions in the field, and discuss the value and larger implications of these studies.

Introduction

Reproduction is a defining feature of life, and every organism has developed a unique and specialized strategy to maximize its fitness in a biosphere with finite resources. One strategy that has been adopted across a diverse range of biological taxa is the ability to enter a hypometabolic state to conserve energy^{1–4}. When employed by animals, this state is commonly referred to as “torpor”.

Torpor is a hypometabolic state where the resting metabolic rate of an animal is markedly reduced in a regulated and reversible manner. More specifically, resting metabolic rate is the rate at which adenosine triphosphate (ATP) is consumed in the absence of stress or physical movement. The most direct method to determine if an animal is in torpor is to measure metabolic rate, often achieved through indirect calorimetry, which measures respiratory gas exchange (O₂ consumption and CO₂ production). Other physiological parameters are also consistently altered during torpor, including core temperature⁵, subcutaneous temperature⁶, skin temperature⁷, heart rate⁸, blood pressure⁹, and synchronous brain activity¹⁰, as measured via electroencephalogram (EEG, which measures the electrical activity of the brain). Thus, in cases where metabolic measurements are not feasible or practical, these parameters can reliably indicate torpor.

The metabolic savings from torpor vary greatly from species to species¹¹ and are modulated by numerous internal and external factors^{12,13}. Yet their importance for survival is universally profound, as they enable mammals to live in harsh environments year-round. For example, arctic ground squirrels can lower their metabolic rate to less than 5% of typical levels during hibernation, allowing them to survive for months without food or water at sub-freezing ambient temperatures¹². While there is a tremendous degree of heterogeneity in how and when animals utilize torpor, there are three major categories of torpor utilization strategies amongst mammals: daily torpor, hibernation, and estivation^{11,14,15}. Daily torpor is an acute response to caloric deficit, with bouts that typically last <24 hours and are relatively “shallower” (lesser degree of metabolic suppression) than in hibernation¹¹. Metabolic rate during daily torpor varies greatly by

species (5-75% relative to baseline), with ~35% representing a rough average¹¹. Hibernation is a longer-term response that is timed seasonally, typically during winter when food availability, photoperiod, and ambient temperatures are at their lowest. Individual torpor bouts last several days, and the metabolic suppression is “deeper” than in daily torpor. In most cases, metabolic rate during hibernation is <10%, relative to baseline¹¹. Facultative hibernators enter and exit torpor in response to seasonal cues, such as ambient temperature and photoperiod, whereas obligate hibernators utilize an endogenous circannual clock that times torpor bouts appropriately, even in the absence of seasonal cues¹⁶. Estivation is similar to hibernation but is initiated during hot and dry periods and lacks a reduction in core body temperature due to higher ambient temperatures. However, this distinction can be blurred in species that enter torpor during the summer (“estivation”) and remain torpid through the winter (“hibernation”)¹⁵. It has been suggested that sleep is also a form of torpor¹⁷, based on its torpor-like features including a decrease in metabolic rate^{18–20}, heart rate²¹, core temperature²⁰, and synchronous brain activity²². However, the reduction in metabolic rate is far milder during sleep, and there is an increase in sleep following torpor^{23,24}, indicating that the functions of sleep and torpor are not redundant. Therefore, we will treat sleep and torpor as distinct states for the purposes of this review. We refer readers elsewhere for a more nuanced discussion of the evolutionary history and energetic relationship between sleep and torpor^{17,25,26}.

While torpor effectively lowers metabolic demands, it comes with significant reproductive tradeoffs. Many reproductive processes, such as fetal development, lactation, and

spermatogenesis, are energetically costly and are stopped or slowed during torpor^{27–29}. This could reflect either a regulated cessation to maximize the metabolic savings of torpor and/or a behavioral/metabolic incompatibility that precludes their continuation during torpor.

While torpor is a highly adaptive state that typically has net positive effects on fitness, its tradeoff with reproduction could reduce, cancel out, or even outweigh its benefits in certain contexts. As a result, organisms have evolved sophisticated strategies to balance torpor and reproduction. One example, common amongst hibernators, is to reproduce and enter torpor seasonally, following anticipated cycles of food availability and other environmental conditions. While the breadth of strategies employed across all mammals is tremendous and beyond the scope of this review, we emphasize that all of them require careful coordination between reproductive and metabolic systems, and there are significant consequences for an animal's fitness if the two systems are not coordinated effectively. There has been tremendous progress in understanding how these two systems communicate³⁰, yet our understanding remains incomplete, especially in the context of torpor. The purpose of this review is to summarize the current knowledge of how the reproductive system influences torpor usage and, conversely, how the reproductive system changes during periods of torpor use. We will also introduce fundamental questions that remain unanswered in the field, weigh hypotheses that address these questions, and discuss the larger implications of these studies.

In mammals, reproduction and fertility are regulated by the hypothalamus-pituitary-gonad axis (the “reproductive axis”), which has been reviewed previously^{31,32}. Simply, the pulsatile release of gonadotropin-releasing hormone (GnRH) from the hypothalamus into the anterior pituitary, via the hypophyseal portal system, stimulates the release of gonadotropins, namely luteinizing hormone (LH) and follicle-stimulating hormone (FSH), into the bloodstream. These hormones then stimulate gamete production and hormone release from the gonads. Hormones released from the gonads include steroid hormones, such as 17 β -estradiol, progesterone, and testosterone, as well as glycoprotein hormones, including activin and inhibin. Collectively, these gonadal hormones provide feedback to the hypothalamus and pituitary to regulate further GnRH and gonadotropin release. This feedback is primarily mediated by kisspeptin neurons – a pivotal group of cells that are regulated by gonadal hormones and non-reproductive signals, and stimulate GnRH release.

Influence of the reproductive axis on torpor usage

Reproductive hormones have a wide range of effects throughout the body, ensuring that physiology and behavior are aligned with the current reproductive state. Unsurprisingly, reproductive hormones strongly modulate torpor usage. While torpor and reproduction are not mutually exclusive^{33–39}, multiple studies of free-ranging animals have found that torpor use is reduced or entirely absent during critical reproductive periods^{35,36,38–42}. For example, little brown bats spend less time in daily torpor during pregnancy, suggesting a tradeoff between torpor and fetal development in this species³⁶. Here, we summarize what is known regarding the effects of reproductive hormones on torpor and the signaling

mechanisms, including potential neuroendocrine pathways, underlying these effects. More specifically, this section will highlight hormones whose primary functions concern the reproductive system, reserving discussion of other hormones, such as leptin and thyroid hormones, that are critical for reproductive function and influence torpor for later (see “Energy balance signals” below).

Hormones

Gonadal Hormones

Gonadal hormone receptors are widely distributed throughout the brain and body, and have extensive effects on behavior, physiology, and torpor. Loss of gonadal hormones via surgical gonadectomy delayed torpor cessation and increased the number of days spent in torpor in male and female Djungarian hamsters, which use daily torpor^{43,44}. Similar effects have been observed in laboratory mice, which also use daily torpor⁴⁵. These findings indicate that gonadal hormones have inhibitory effects on torpor, consistent with their function as signals of fertility and reproductive status. Here, we review the role of steroid hormones (e.g. estrogens, progestogens, and androgens) in regulating torpor, a phenomenon that has been widely studied (Table 1). Non-steroid gonadal hormones (e.g. activins, inhibins, and follistatin) are important for metabolism^{46–49}, yet they have been scarcely studied in the context of torpor and thus are not discussed here.

Estrogens

Estrogens are primarily synthesized by the ovaries and, to a lesser extent, the testes. In females, circulating estrogens are essential for the development of sex-typical traits.

During the reproductive period, males typically have lower levels of circulating estrogens compared to females^{50,51}. However, testosterone can be converted to 17 β -estradiol (E2), a potent estrogen, by the enzyme aromatase that is widely expressed throughout the body^{52,53}. Therefore, estrogen signaling is not a phenomenon exclusive to females, nor does it strictly support the development of female-typical traits. Among natural estrogens, E2 is typically the most abundant in circulation (although this may change during pregnancy or menopause) and is also the most potent^{54,55}. Multiple studies have tested the effects of E2 on torpor, with almost all finding that E2 potentially reduces torpor usage in both males and females^{13,45,56–58} (Table 1). E2 failed to have an effect in only one study (McAllan, 2012), which could be due to the shorter and more acute nature of the E2 treatment, species-specific differences, or study-specific variables such as housing conditions.

Progestogens

Progestogens are synthesized by the ovaries and testes. Progesterone (P4) is the major progestogen. Downstream metabolites of P4 are also weakly progestogenic, yet their physiological relevance is questioned and/or poorly understood. Non-gonadal sources of P4 also play critical roles in the reproductive axis^{59,60}, but generally do not contribute to the pool in circulation. Outside of the luteal phase, P4 levels are similar in males and females⁵¹, demonstrating that progestogen signaling is not a female-specific phenomenon. Accordingly, P4 has important functions in both sexes⁶¹. Multiple studies have tested the effects of P4 on torpor^{13,45,56,58} (Table 1), with the findings ranging from no effect to a mild inhibitory effect. Together, these studies suggest that the effects of P4

on torpor are mild and may vary based on the species, treatment method, and/or circulating E2 levels.

Androgens

Androgens are synthesized by the testes and, to a lesser extent, the ovaries. In males, androgens are essential for the development of sex-typical traits. Males typically have higher circulating androgen levels than females⁵¹. However, in females androgen receptors are widely expressed and have several biological functions⁶². Thus, androgens are not a male-specific class of hormone, nor do they exclusively promote male-specific traits. Excluding weak androgens whose physiological significance is unclear, the most abundant circulating androgen in males and females is testosterone (T)⁵¹ but the most potent is 5 α -dihydrotestosterone (DHT)^{63,64}. DHT is synthesized locally from T by 5 α -reductase, an enzyme expressed widely throughout the body in males and females. Notably, T can also be converted to E2 via aromatase and act via estrogen receptors. Thus, T could be considered a “pro-hormone” that becomes more active following enzymatic conversion to DHT or E2, although T itself does have androgenic effects^{63,64}. Multiple studies have tested the effects of androgens on torpor^{13,44,45,56,58,65} (Table 1), which demonstrate that androgens strongly inhibit torpor in both sexes in multiple species. Only one study failed to observe an effect of T on torpor (McAllan, 2012), which could be due to the shorter and more acute nature of the hormone treatment, species-specific differences, or study-specific conditions.

Non-gonadal hormones

Prolactin is widely recognized as a regulator of mammary tissue development and lactation and is primarily produced by the anterior pituitary. Prolactin potently reduced the frequency of torpor usage induced by short photoperiod and cold exposure in ovariectomized female Djungarian hamsters⁶⁶. However, the same prolactin treatment had no effect on torpor usage induced by cold exposure and food restriction under long photoperiod conditions⁶⁶. These results demonstrate that prolactin signaling decreases torpor usage in certain contexts, but this effect can be gated by an energy deficit and/or a long photoperiod.

Other non-gonadal circulating reproductive hormones include androgens and estrogens from non-gonadal organs, such as the adrenal glands^{67–69}, human chorionic gonadotropin, estriol, estetrol, and gonadotropins (namely LH and FSH). While the impact of these hormones from non-gonadal sources on torpor has not been evaluated, they are promising candidates that warrant further study based on their widespread receptor expression⁷⁰ and/or known effects on non-reproductive physiology^{69,71,72}.

Hormone signaling mechanisms

The effects of gonadal steroid hormones on torpor are well-described (Table 1), yet the cellular and molecular signaling mechanisms underlying these effects are yet to be well characterized. Here, we present hypothesized signaling mechanisms and weigh the evidence for each, focusing on those we consider the most promising.

Target tissues

Hypothalamus

The preoptic area (POA) is a brain region situated just anterior of the optic chiasm that controls several behaviors and homeostatic functions. Gonadal steroid receptors and aromatase are broadly expressed across multiple functional and transcriptomic cell types in the POA⁷³, and numerous studies have shown that local gonadal steroid signaling directly modulates the activity of POA neurons^{74–76}. Further, physiology and behavior are altered by local gonadal steroid signaling in the POA^{77,78}. A subset of POA neurons have been identified as central coordinators of torpor in laboratory mice^{79–81}. While the exact identity and properties of these neurons are an area of active research and debate^{82,83}, most evidence suggests that they are primarily glutamatergic and are directly sensitive to gonadal steroids. One study found that neurons marked by estrogen receptor alpha (ER α) expression are sufficient to induce a torpor-like state when activated, are more active during fasting-induced torpor, and are required for the full fasting-induced torpor response⁸¹. Another study detected ER α transcripts (*Esr1*) in POA neurons that are active during torpor⁷⁹. Additionally, ER α -expressing neurons in the POA widely co-express androgen receptor (AR)⁸⁴, aromatase⁸⁵, and, when estrogens are present, progesterone receptor (PR)⁸⁶, suggesting that these cells are capable of responding to multiple gonadal steroids. From these lines of evidence, we consider the POA the most promising candidate for mediating the effects of gonadal steroids on torpor use. This hypothesis remains untested, but is a major aim of our ongoing research.

Other candidates include the dorsomedial nucleus of the hypothalamus (DMH), arcuate nucleus of the hypothalamus (ARC), and suprachiasmatic nucleus (SCN), all of which

regulate torpor^{87–89}. In the DMH, activation of a subset of neurons is sufficient to promote torpor following caloric restriction⁸⁷, and, conversely, inactivating a different set of DMH neurons inhibits fasting-induced torpor⁸⁸. Early postnatal ablation of the ARC completely abolishes fasting-induced torpor in adulthood⁸⁹. Lesioning the SCN also alters torpor characteristics, with one study demonstrating a complete loss of torpor following lesion in adulthood⁹⁰, and others finding circadian and/or circannual disorganization of torpor bouts following lesion in adulthood^{91–93}. While current evidence supports the POA as the major central regulator of torpor, the DMH, ARC, and SCN all have the capacity to influence torpor and express gonadal steroid receptors^{94,95}, making them promising candidates. The effect of gonadal steroid signaling in these areas on torpor remains unevaluated, however.

Brainstem

While the field's attention has centered on the hypothalamus, multiple brainstem nuclei have also been implicated in the control of torpor, including the area postrema (AP)⁹⁶ and ventrolateral medulla (VLM)⁹⁷. Briefly, both AP GFRAL-expressing neurons and VLM catecholaminergic neurons are sufficient to induce a torpor-like state when activated^{96,97}. Further, fasting-induced torpor was inhibited when VLM catecholaminergic neurons were silenced⁹⁷. Both regions also respond to gonadal hormones^{98,99}, although their expression of gonadal hormone receptors is more limited compared to the hypothalamus^{94,95}. In summary, the brainstem is another auspicious candidate that remains unevaluated.

Peripheral metabolic organs

While the brain appears to be the master regulator of torpor, it integrates numerous cues from the periphery, some of which are modulated by gonadal steroids. For example, adipose-derived leptin inhibits torpor¹⁰⁰, and circulating estrogens influence leptin production via estrogen signaling in adipose tissue¹⁰¹. This could represent one potential mechanism underlying the effects of estrogens on torpor. Other untested targets include liver and muscle, which also influence metabolism in response to gonadal steroids^{102,103}.

Receptors

While gonadal steroids clearly modulate torpor (Table 1), it is not clear which receptor(s) mediate these effects. For example, there are multiple estrogen (ER α , ER β , GPER1) and progesterone (PR, mPR¹⁰⁴) receptors in mammals. Likewise, T has multiple possible signaling mechanisms: signaling directly via the androgen receptor (AR), undergoing conversion to DHT to activate AR more strongly, or undergoing conversion to E2 to signal via an estrogen receptor. Since there is only one known androgen receptor in mammals (AR), the effects of DHT on torpor are likely mediated by AR. However, further study using receptor-specific agonists and antagonists, as well as genetic knockouts, is necessary to validate this presumption and identify the specific receptor(s) that T, P4, and E2 signal through.

Summary and future directions

Gonadal steroids are the most well-studied reproductive hormones in the context of torpor, and they have overall inhibitory effects. Among these, T, DHT, and E2 appear to have the most potent effects on torpor, with P4 having milder and context- and/or species-

dependent effects. Prolactin strongly influences torpor use, indicating that the gonads are not the only source of torpor-modulating reproductive hormones. Other reproductive hormones remain understudied in the context of torpor, but warrant further attention given their important, yet often overlooked, roles in physiology and behavior. Further, the target tissue(s) and receptor(s) that mediate the effects of gonadal steroids on torpor remain poorly defined. Promising candidates, such as the POA, exist, but experimental data are needed to test these hypotheses.

Throughout this section, we have introduced and weighed the evidence for hypothesized target tissues individually, yet we emphasize that these hypotheses are not mutually exclusive. We believe that gonadal steroids likely influence torpor through a network of signaling mechanisms throughout the brain and body that jointly coordinate the animal's physiological state. Hypothesis-driven approaches based on individual candidates will be valuable to test the contributions of discrete signaling mechanisms within this network, but these should be complemented by hypothesis-free approaches to characterize the network more holistically and without bias.

Reproductive axis changes associated with periods of torpor use

The reproductive signaling axis can respond rapidly to both internal and external cues. For example, fasting, a cue that strongly induces torpor in many species, strongly suppresses the reproductive axis: reducing mating behavior and fertility¹⁰⁵, reproductive cycles¹⁰⁶, and disrupting fetal development¹⁰⁷. Further, histological examinations of the reproductive axis in hibernating animals have found evidence of a similar suppression

during the hibernation season: smaller pituitary and ovaries^{108,109}, ovaries without mature follicles and corpora lutea^{108–110}, and smaller and fewer interstitial (Leydig) cells of the testes^{110,111}. Free circulating reproductive hormone levels are also decreased during the hibernation season^{112–114}. While these studies demonstrate that the reproductive axis is typically suppressed during torpor, it should be noted that there are exceptions. Juvenile ground squirrels, for example, undergo sexual maturation during their first hibernation season despite months of hibernation, fasting, and dramatic weight loss³⁴.

We also note that changes in the reproductive axis are coincident with periods during which animals enter torpor frequently (periods of torpor use), but there is evidence that many changes are not strictly coupled to, or dependent on, torpor onset^{34,110,111}. This implies that most changes in the reproductive axis are initiated as part of a larger systemic response that is associated with, but not strictly dependent on, the occurrence of torpor. This topic remains an active area of research and discussion, however. In support of the hypothesis that torpor can directly modify reproductive processes, one study found that mammary explants from the pipistrelle bat, a seasonal hibernator that can enter torpor while lactating, exhibited decreased protein synthesis and, therefore, capacity to produce milk at 22°C (compared to 37°C)²⁸. These results suggest that these animals lack compensatory mechanisms to maintain adequate milk production when their core temperature is lowered during torpor, supporting the notion that torpor-related physiological changes can directly suppress reproductive function.

Energy balance signals

There are many energy balance signals involved in regulating the reproductive axis and torpor use – here, we focus on the signals that have not been reviewed previously and are well-studied in the context of torpor and reproduction. Previously reviewed signals include dietary lipids/fatty acids¹¹⁵ and adenosine²⁶. Signals that warrant further study include non-leptin adipokines, circulating glucose, insulin, and glucagon.

Leptin

Leptin is a hormone released by adipocytes that has well-established effects on many physiological processes, including torpor and reproduction. Leptin is released proportionally to the amount of lipids stored, and the abundance of leptin in the circulation is highly correlated with body fat percentage¹¹⁶. Thus, circulating leptin levels communicate the level of adipose energy reserves to the rest of the body. Leptin potentially reduces torpor use in mice^{100,117} and hamsters¹¹⁸ (Fig. 1). Knocking out the leptin receptor in the POA blocked the effects of leptin on fasting-induced torpor without affecting estrous cycles¹¹⁷, demonstrating a critical role for leptin receptor signaling in the POA and suggesting that leptin's effects on reproduction and torpor are mediated by separate pathways. Leptin's role in reproduction has been reviewed in detail previously¹¹⁹. Briefly, circulating leptin is necessary for reproductive function and stimulates the reproductive axis by modulating kisspeptin release in the hypothalamus (Fig. 1). Together, these results demonstrate that leptin potentially modulates torpor and the reproductive axis, aligning physiology and behavior with energy reserves.

Ghrelin

Ghrelin is a hormone released by the stomach that also has broad effects on physiology, including torpor and reproduction. Ghrelin release is increased in response to fasting and decreases following feeding^{120,121}, and is therefore thought to communicate gastrointestinal fuel status to the rest of the body¹²². In mice, exogenous ghrelin increases torpor use in response to fasting⁸⁹, and ghrelin-deficient mice utilize torpor less than controls in response to fasting¹²³, demonstrating that ghrelin is a torpor-promoting hormone, at least in mice. Ghrelin affects fasting-induced torpor through a Neuropeptide Y-dependent mechanism⁸⁹. Neuropeptide Y (NPY) is broadly expressed throughout the central and peripheral nervous system^{124,125} and is highly concentrated in the ARC¹²⁵, where it influences energy balance and food intake¹²⁶. NPY-expressing neurons in the ARC are sensitive to circulating ghrelin¹²⁷, raising the idea that ghrelin may exert its effects on torpor via these neurons, but this hypothesis has not been directly tested. Ghrelin's role in reproduction has been reviewed in detail^{128,129}. Generally, ghrelin suppresses reproduction through signaling at multiple levels of the reproductive axis (Fig. 1). Interestingly, NPY also potently regulates reproduction¹³⁰, raising the possibility that NPY neurons could mediate ghrelin's effects on both torpor and reproduction, but this idea remains untested. Together, these studies demonstrate that ghrelin is an important signal that aligns reproductive function and torpor use with energy availability.

Thyroid hormones

Thyroid hormones, secreted by the thyroid gland, are critical for regulating numerous physiological processes, including torpor and reproduction^{131,132}. Systemic treatment with the thyroid hormone triiodothyronine (T3) completely eliminates bouts of daily torpor in

Djungarian hamsters exposed to a short photoperiod¹³³. In the same study, pharmacologically inducing hypothyroidism had the opposite effect – increasing the frequency, depth, and duration of torpor bouts¹³³. In a separate study, T3 implants in the hypothalamus eliminated torpor bouts in Djungarian hamsters exposed to short photoperiod conditions, suggesting that T3 may exert its influence on torpor via the hypothalamus¹³⁴ (Fig. 1). The role of thyroid hormones in reproduction has been reviewed previously¹³¹. Briefly, both hypo- and hyper-thyroidism is associated with a reduction in reproductive function and fertility in both sexes. While the signaling mechanisms through which thyroid hormones exert their effects on the reproductive system are not fully understood, existing research has demonstrated extensive crosstalk between thyroid hormones and the reproductive system through numerous mechanisms^{135–137}, including signaling directly in the ovary (Fig. 1).

Seasonal cues

Photoperiod

Photoperiod is a variable that reliably tracks the time of year, particularly in the temperate zones, and has significant impacts on the body, including the reproductive axis and torpor. In multiple species, short photoperiod exposure strongly increases torpor use and is often used to induce torpor experimentally^{44,138}. Photoperiod also has well-established effects on reproductive function in animals that reproduce seasonally^{139,140}.

The effects of photoperiod on the body are primarily mediated by melatonin – a hormone that is released from the pineal gland at night, with the duration of its release

communicating photoperiod to the rest of the body¹⁴¹. Removal of the pineal gland decreases torpor frequency under short photoperiod conditions in *Peromyscus leucopus*, a nocturnal rodent that uses daily torpor¹³⁸. In this study, the effect of pinealectomy was rescued by melatonin treatment, demonstrating that melatonin inhibits torpor in this species and context. Melatonin consistently encodes photoperiod and thus may promote torpor in species that enter torpor during the summer, but this possibility has not been evaluated. The effects of melatonin on the reproductive axis have been reviewed previously¹⁴². Briefly, melatonin strongly suppresses the reproductive system in “long-day breeders,” including many rodent species, that reproduce in the summer but has the opposite effect in “short-day breeders,” such as sheep, that reproduce in the winter. Multiple hypothalamic regions, including the DMH, ARC, and median eminence, have been implicated in mediating the effects of melatonin on seasonal reproduction cycles¹⁴³. In conclusion, these data demonstrate that melatonin is an important signal that encodes photoperiod and aligns torpor use and reproduction to seasonal cycles.

Ambient temperature

Ambient temperature is a less reliable marker of the time of year, yet it remains an important environmental variable that can vary seasonally and broadly affects physiology and behavior, including torpor and reproduction. In mammals, ambient temperature sensing mechanisms have been reviewed previously^{144–146}. In short, ambient temperature is sensed by neuron fibers in the skin via temperature-sensitive ion channels^{144–146}. These neurons relay this thermal information to the hypothalamus via the spinal cord and lateral parabrachial nucleus.

Exposure to cold ambient temperature promotes torpor usage in multiple species^{147–150}. In addition, some studies find that ambient temperature modulates torpor depth and duration¹², although others find no effect¹⁵¹. This apparent contradiction could be due to species-specific differences and/or study-specific variables such as housing conditions. High (>30°C) ambient temperatures reduce reproductive success in laboratory mice¹⁵², but this phenotype is likely unrelated to torpor, since mice do not typically enter torpor at these temperatures. More likely, it may reflect the homeostatic challenge of heat stress, where maintaining body temperature within the homeostatic range is either not possible or requires an extreme energetic investment¹⁵³. In summary, ambient temperature influences torpor use in certain contexts and can impact reproductive success, but there is no evidence in mammals to suggest that these phenomena are related.

Summary and future directions

In summary, the reproductive axis is potently modulated by multiple torpor-related cues. While many of the signaling mechanisms remain incompletely understood, the hypothalamus is widely implicated as an integrator (Fig. 1). Within the hypothalamus, kisspeptin-expressing neurons (located in the ARC and anteroventral periventricular nucleus^{154,155}) and RFRP-3-expressing neurons (located in the DMH¹⁵⁶) are well-positioned to serve as downstream integrators and effectors. These neuronal populations are upstream of GnRH neurons and have opposing effects on GnRH release¹⁵⁷. Kisspeptin directly activates¹⁵⁸ whereas RFRP-3 (also known as the mammalian ortholog of gonadotropin-inhibiting hormone (GnIH)) suppresses GnRH neurons^{156,159}. Both

neuronal populations are responsive to non-reproductive stimuli, such as stress¹⁶⁰ and photoperiod^{161,162}. Multiple lines of evidence demonstrate that kisspeptin neurons respond to metabolic cues¹⁶³ but most evidence indicates that RFRP-3 neurons do not, based on their lack of response to leptin deficiency, diet-induced obesity, or fasting^{164,165}. Further study is needed, however, to directly test for a mechanistic link between kisspeptin/RFRP-3 expressing neurons and reproductive changes in response to torpor-related cues.

Discussion

While humans do not naturally enter torpor, the ability to enter torpor is shared by a phylogenetically diverse range of mammals⁴. This suggests that the regulatory mechanisms that control torpor may be conserved and influence aspects of energy expenditure even in species that do not naturally enter torpor. Early experimental evidence supports this assertion, as activating POA neurons in rats, which are homologous to torpor-inducing neurons in mice, induces a torpor-like hypometabolic state, even though rats do not naturally enter torpor^{80,166}. A study in non-human primates also found that activating POA neurons lowers core temperature, although this manipulation appears to also elicit cold-defense behaviors¹⁶⁷. Overall, these results broadly support the hypothesis that torpor regulatory mechanisms are conserved and could potentially be exploited to alter metabolism in species that do not naturally use torpor.

Due to the conserved nature of reproductive and metabolic systems across mammals, understanding how the reproductive system alters torpor use in non-human mammals will reveal novel insight into how reproductive state influences metabolism in humans. This knowledge will be critical for numerous areas of clinical practice, such as understanding and managing the metabolic changes associated with reproductive transition states, including puberty, menopause, pregnancy, the menstrual cycle, and endocrine disorders (e.g., PCOS). In addition, these insights could help clinicians manage metabolic changes associated with hormone treatments for other indications, such as contraception, fertility treatments, or gender-affirming care. Conversely, understanding how the reproductive axis changes during torpor, a state of hypometabolism and negative energy balance, will further our understanding of how reproductive physiology and fertility can be influenced by metabolic state in humans.

There is also growing interest in the formal possibility that a state of artificial torpor could be induced in humans to lower metabolic demands during ischemic incidents or deep space travel^{168,169}. The neuronal circuits that control torpor^{79–81,87,88,170} represent promising candidates for manipulation in humans. However, little attention has been paid to how these circuits could be influenced by, or influence, the reproductive state of the individual. Understanding how the reproductive axis and torpor circuits interact will be critical for ensuring the safety and efficacy of any artificial torpor-inducing treatments in humans.

More generally, effective communication between the metabolic and reproductive systems is critical for survival and reproductive success. However, in the face of rapidly changing habitats from human activity, many new challenges have arisen for mammals tasked with this delicate balancing act. Endocrine-disrupting chemicals, light pollution, global warming, and habitat loss all have the potential to disrupt or alter torpor use, thereby affecting survival and reproductive success. A mechanistic understanding of the biology underlying the balance between torpor and reproduction across species will be critical for ensuring that the disruptive effects of human activity on the ecology of torpor-utilizing species are minimized or eliminated.

In conclusion, understanding the mechanisms that regulate the balance between reproduction and energy balance is crucial for various areas of scientific research. Torpor is an extreme metabolic adaptation that significantly shifts this balance, and understanding its relationship with the reproductive axis will reveal broader truths about how organisms balance reproduction and energy expenditure to maximize their fitness. Finally, torpor is readily inducible and measurable in multiple species in both laboratory and free-ranging environments, making it feasible for study in a variety of contexts.

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Figure 1 was created using icons from BioRender. Vander, P. (2025)

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Figures and tables

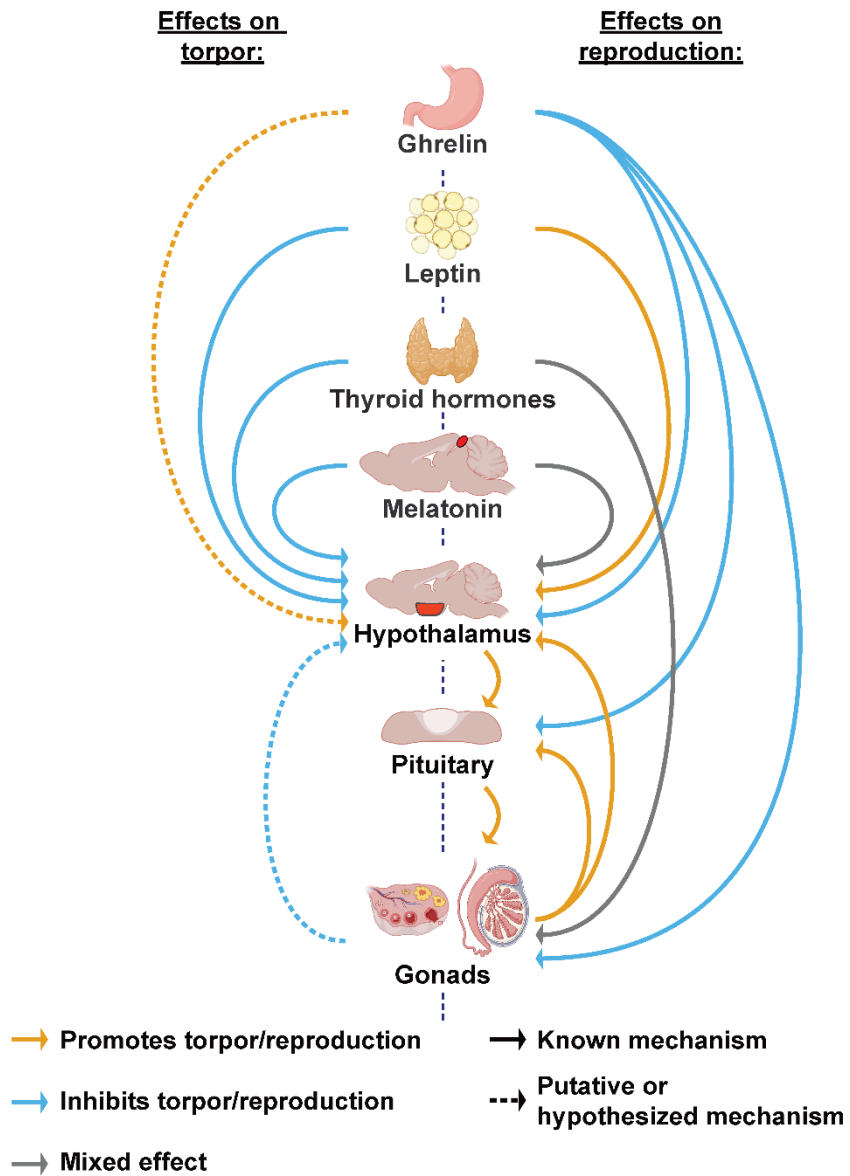


Figure 1: Neuroendocrinology of torpor and reproduction. Schematic depicting cues that regulate both torpor and reproduction and their known or putative/hypothesized signaling mechanisms.

Study	Species	Torpor type	Standard housing conditions	Conditions for torpor induction	Hormone treatments and sex/gonadal status	Treatment method	Dose	Resulting circulating hormone concentrations	Effect on torpor usage
Cintron-Colon, <i>et al.</i> 2019	<i>Mus musculus</i>	Daily torpor	22°C, 12:12 LD, ad lib food + water	Food restriction (100-50% of normal FI)	- E2, E2+P4, P4 (GDX F) - DHT (GDX M)	Bio-erodible pellet (21-day release)	E2: 0.5mg, P4: 10mg DHT: 5mg	Not measured	E2: ↓↓ P4: ↓ E2 + P4: ↓↓ DHT: ↓
Darrow, <i>et al.</i> 1988	<i>Cricetus cricetus</i>	Facultative hibernation	22°C, 16:8 LD, ad lib food + water	6°C, 16:8 or 10:14 LD	- E2 (GI + GDX F) - P4, E2+P4 (GDX F) - T (GDX F+M)	Silastic capsule	20mm capsule filled with crystalline hormone	Androgens: GDX M + T: 5.79ng/mL (0.08ng/mL w/ blank) GDX F + T: 0.96ng/mL (0.27ng/mL w/ blank) GI M (mature testes): 2.54ng/mL Estrogens: GDX F + E2 (or E2+P4): 236-951pg/mL (13pg/mL w/ blank) GI F (anestrous): 35pg/mL Progesterones: GDX F + P4 (or P4+E2): 3.7-9.4pg/mL (0.61pg/mL w/ blank) GI F (anestrous): 8.8pg/mL	T: ↓↓ (GDX M + F) E2: ↓ (GDX F, 10:14 LD) ↓↓ (GDX F, 16:8 LD) ↓↓ (GI F, 16:8 LD) P4: ↔ (GDX F, 10:14 LD) E2 + P4: ↓↓ (GDX F, 10:14 LD)
Mzilikazi, <i>et al.</i> 2002	<i>Saccostomus campestris</i>	Daily torpor	25°C, 14:10 LD, ad lib food + water	15°C, 10:14 LD, food restriction (70% of normal FI)	T (GDX M)	Silastic capsule	10mm capsule filled with crystalline hormone	Testosterone: GDX M + T: 6-8ng/mL (<1ng/mL w/ blank) GI M: 1.76ng/mL	T: ↓↓
Gao <i>et al.</i> 2007	<i>Mus musculus</i>	Daily torpor	20-25°C, 12:12 LD, ad lib food + water	No food (fasting)	E2 (GI ob/ob M and GI db/db M)	Silastic capsule	0.5cm capsule filled with crystalline hormone	Not measured	E2: ↓↓
McAllan <i>et al.</i> 2012	<i>Sminthopsis macroura</i>	Facultative hibernation	19°C, ad lib food + water	14:10 LD (hormone blockers) 10:14 LD (hormones)	Hormones: - E2, P4 (GI F) - T*, (GI M) Hormone blockers: - Tamoxifen, RU486 (GI F) - Flutamide (GI M)	Subcutaneous injection	E2: 0.15-0.3ng P4: 0.5-1ng T*: 2-4ng Tamoxifen: 0.06-0.12ng RU486: 1.4-2.8ng Flutamide: 3.8-7.6ng	Not measured	E2: ↔ Tamoxifen: ↔ P4: ↓ RU486: ↔ T*: ↔ Flutamide: ↔
Hall, <i>et al.</i> 1980	<i>Mesocricetus brandti</i>	Facultative hibernation	22°C, 14:10 LD (M), 16:8 LD (F), ad lib food + water	10°C	- E2 (GDX F+M, GI F) - P4 (GDX F+M) - T (GDX F+M, GI M+F) - DHT (GDX F+M)	Silastic capsule	5-20mm capsule filled with crystalline hormone	Testosterone: GDX M + T: 0.69-2.7ng/mL (<0.13ng/mL w/ blank) GI M (large testes): 2.3ng/mL GI M (small testes): <0.13ng/mL Estradiol: GDX F + E2: 93.3pg/mL (<10pg/mL w/ blank) GI F (estrus): 40.2pg/mL GI F (proestrus): 255.9pg/mL GI M (large testes): 20.2pg/mL Progesterone not measured	E2: ↓ ↓↓ (late hibernation season) P4: ↔ T: ↓↓ DHT: ↓↓
Ouarour, <i>et al.</i> 1991	<i>Phodopus sungorus</i>	Daily torpor	Temperature not reported, 16:8 LD, ad lib food + water	20°C, 10:14 LD	T, DHT (GI M)	Silastic capsule	10mm capsule filled with hormone	Not measured	T: ↓↓ DHT: ↓↓

Table 1: The effects of systemic gonadal steroid treatments on torpor.

Selected studies that have tested the effect of systemic gonadal steroid treatments on torpor. Abbreviations: FI = food intake, GI = gonad-intact, GDX = gonadectomized, LD =

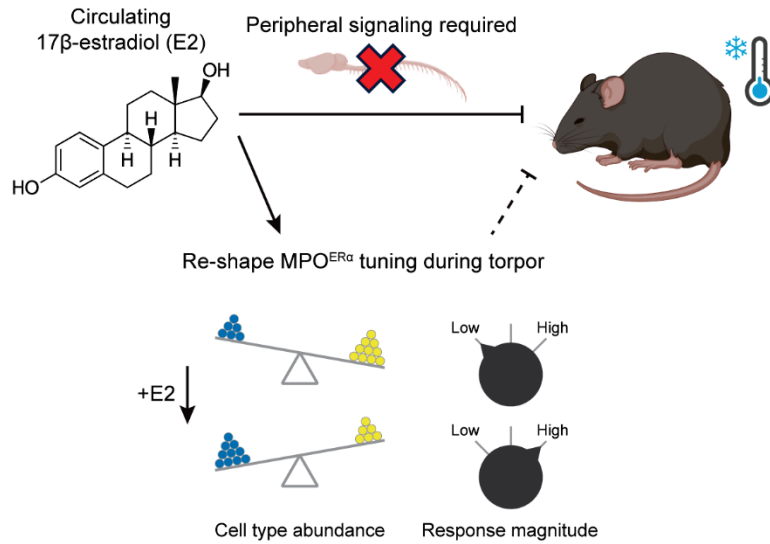
light-dark cycle (in hours), M = males, F = females. * Durateston, a long-acting formulation of conjugated testosterone (Testosterone propionate 6 mg/ml, Testosterone phenylpropionate 12 mg/ml, Testosterone isocaproate 12 mg/ml, Testosterone decanoate 20 mg/ml) was used in this study.

Chapter 2: Circulating estrogens attenuate torpor and alter the tuning of torpor-regulating medial preoptic area neurons in female mice

Abstract

In response to an energy deficit, mice can conserve energy by entering a hypometabolic state known as torpor. While torpor is adaptive, it has significant reproductive tradeoffs. Therefore, animals must carefully balance energy homeostasis and reproduction during an energy shortage. In support of this notion, we find that fasting-induced torpor use naturally varies across the estrous cycle, is increased by ovariectomy, and is decreased by estrogen treatment in female mice. Estrogen receptor alpha-expressing neurons in the medial preoptic area (MPO^{ERα} neurons) are central coordinators of torpor, leading us to hypothesize that estrogens attenuate torpor via this region. Using *in vivo* microendoscopic calcium imaging, we find that MPO^{ERα} neurons encode torpor, ambient temperature, and social states through heterogeneous cellular responses. Circulating estrogens selectively modify neuronal tuning during torpor, altering the abundance and increasing the response magnitude of torpor-tuned subpopulations. To directly test the sufficiency of the MPO pathway for torpor attenuation, we administered estrogen locally and observed a conditional effect, with attenuation in gonad-intact but not ovariectomized mice. To directly test necessity, we knocked out estrogen receptors alpha and beta in the MPO, which had no effect on torpor. Thus, we effectively excluded the MPO as a major direct mediator of estrogen's effects on torpor. Finally, we administered estrogen to the entire central nervous system, which had no effect on torpor. We conclude that circulating estrogens align torpor use with reproductive state in female mice through a mechanism that alters MPO^{ERα}-driven torpor control and requires peripheral estrogen signaling.

Graphical abstract



Introduction

Energy homeostasis and reproduction are inextricably linked. Reproductive transition states, such as puberty, pregnancy, and menopause, are associated with significant changes to energy expenditure and appetite^{171–176}. These changes are driven in large part by estrogens, which directly affect energy expenditure and appetite in humans and rodents^{177–179}. However, the signaling mechanisms underlying the effects of estrogens on energy homeostasis remain incompletely understood.

To conserve energy and maximize fitness, many organisms have evolved the ability to reversibly enter a regulated state of hypometabolism. In mice (*Mus musculus*), this state is called daily torpor (hereafter “torpor”) and is initiated in response to a caloric deficit.

Torpor involves several physiological changes, including decreases in core body temperature¹⁸⁰, heart rate⁸, locomotor activity⁷⁹, oxygen consumption¹⁸⁰, and synchronous brain activity, as measured via electroencephalogram^{22,181}.

While torpor is an adaptive state that effectively lowers metabolic demands, it comes with significant reproductive tradeoffs^{27–29,182}. As a result, animals must carefully weigh energy homeostasis and reproductive needs when faced with a caloric deficit. Accordingly, torpor use is reduced or absent during critical reproductive periods, such as pregnancy and lactation, in mammals^{182,40,35,38,36,39,42,41}. Circulating estrogens are known to attenuate fasting-induced torpor in multiple mammalian species, suggesting that they may coordinate reproductive state-dependent changes in torpor use^{56,13,183}. However, there are conflicting reports on the effects of estrogens on torpor in mice^{45,184,185}.

Numerous studies have identified the preoptic area (POA) as a central coordinator of torpor in mice^{79–81,186,187}. Discrete neuronal populations within the POA show increased activity during fasting-induced torpor and are sufficient to drive entry into a long-lasting torpor-like state following artificial activation. Conversely, silencing or ablating the same neurons in the POA compromises the animal's ability to fully express torpor when fasted. Together, these data definitively establish a causal relationship between torpor and neuronal activity in the POA. However, the POA is highly heterogeneous^{73,79,187} and how individual cell populations contribute to coordinating torpor and other homeostatic processes remains poorly understood.

Multiple lines of evidence indicate that torpor-coordinating neurons in the medial preoptic area (MPO) are directly sensitive to circulating estrogens. First, estrogen receptor alpha expressing neurons in the MPO (MPO^{ERα} neurons) are more active during fasting-induced torpor, are sufficient to induce a torpor-like state when activated, and are required for the full expression of fasting-induced torpor⁸¹. Second, *Esr1* transcripts (encoding ERα) were detected in POA neurons that are active during torpor⁷⁹. Together these data suggest that estrogen signaling within the MPO may directly mediate the effects of circulating estrogens on torpor.

Here, we tested the hypothesis that the MPO is a central mediator underlying the effects of circulating estrogens on torpor. First, we established that circulating estrogens attenuate torpor in female mice. Next, we demonstrated that circulating estrogens alter the tuning of MPO^{ERα} neurons, altering the relative abundance and increasing the response magnitude of torpor-tuned subpopulations. However, we found that estrogen signaling within the MPO is not fully sufficient or required for the effects of circulating estrogens on torpor, ruling it out as a major direct mediator. Finally, we showed that estrogen signaling throughout the central nervous system is not sufficient to attenuate torpor, indicating that peripheral signaling is required. In summary, circulating estrogens attenuate torpor and alter the responses of central torpor-regulating neurons, yet peripheral estrogen signaling is required for torpor attenuation, suggesting a multi-organ network for estrogenic control of torpor.

Results

Circulating estrogens attenuate torpor

Reproductive hormone levels, including circulating estrogens, fluctuate across the estrous cycle^{188,189}, leading us to the hypothesis that torpor use would also fluctuate accordingly. To test this, mice were fasted for 24 hours to induce torpor while core body temperature was monitored telemetrically (Fig. 1A). Estrous cycles were tracked via vaginal cytology, and trials (1-2 per mouse, 8-14 days apart) were categorized by stage at fast onset and offset. To assess if any order effects existed in our data, we included fast number (first vs second) as a factor in an additional analysis, and found no significant effect of fast number, nor an interaction with estrous cycle stage (Fig. S1A-C). When the trials were considered separately, the direction of the effects was largely consistent. Therefore, we concluded that any order effects were negligible, and pooled data from both trials for downstream analysis. Terminal uterine weight, which correlates with circulating estrogen levels¹⁹⁰ and increases during proestrus¹⁹¹, was measured to corroborate estrous cycle staging (Fig. S1D). Time in “deep torpor”, defined throughout as a core body temperature $<31^{\circ}\text{C}$, varied across estrous cycle stages and was significantly lower in the P→E group than in the M/D→D group (Fig. 1B). Because heavier mice exhibit a reduced propensity for torpor^{81,185,192}, we included body weight as an *a priori* covariate in this analysis. Here, starting body weight varied slightly between groups (Fig. S1E), but it was not a significant covariate, and the effect of estrous cycle remained after including it (Fig. 1C). Maximal hypothermic response (max ΔT) varied by estrous cycle stage and was higher in the P→E group compared to the M/D→D group (Fig. S1G). Temperature variability, as measured by standard deviation, also varied by estrous cycle stage and was lower in the P→E group compared to M/D→D, but mean hypothermic

response (mean ΔT), latency, and number of deep torpor bouts did not vary across estrous cycle stages (Fig. S1H-K). Bout duration and timing were also not significantly different across estrous cycle stages (Fig S1L). In summary, this experiment shows that torpor use varies across the estrous cycle and is lower in mice in the proestrus to estrus (P→E) stage, which is marked by an increase in sexual receptivity¹⁹³ and maximal circulating estrogen levels¹⁸⁹, compared to mice in the metestrus to diestrus (M/D→D) stage. This result led us to the preliminary hypothesis that circulating ovarian hormones attenuate torpor.

To test the effect of the ovaries on torpor, mice underwent ovariectomy (OVX) or sham operations (Sham) and were then fasted for 48 hours to induce torpor (Fig. 1D). Terminal uterine weight was lower in OVX animals, confirming that circulating estrogen levels were lowered (Fig. S2A). Time in deep torpor was higher in OVX animals (Fig. 1E). Due to increased weight gain post-surgery, body weight was higher in OVX animals, yet weight was not a statistically significant covariate, and the effect of OVX remained after its inclusion (Fig. 1F, S2B-C). Paradoxically, OVX animals lost more weight during the fasting period despite spending more time in deep torpor, although they remained heavier at the end of the fast (Fig. S2D-F). In OVX animals, mean ΔT was more negative, indicating a stronger hypothermic response, and temperature variability was higher, but max Δ , latency, bout number, bout timing, and bout duration were not significantly different (Fig. S2 G-L). In summary, ovariectomy led to an enhanced torpor response.

To ask whether circulating estrogens are sufficient to attenuate torpor, a new cohort of mice were ovariectomized, implanted with bio-erodible pellets containing 17 β -estradiol (E2) or vehicle and subsequently fasted for 48 hours to induce torpor (Fig. 1G). Uterine size was visibly larger in all E2-treated animals, confirming that circulating estrogen levels were effectively elevated (Fig. S2M). Time in deep torpor was greatly reduced in E2-treated animals (Fig. 1H). Body weight was equal between groups, weight was not a significant covariate, and its inclusion did not alter the effect of E2 treatment (Fig. 1I, S2N). E2-treated animals lost more weight during the fasting period, indicating higher energy expenditure during this timeframe (Fig. S2O). E2-treated animals had a smaller max ΔT , lower temperature variability, longer bout latency and shorter bout duration, but no change in mean ΔT , bout number, or bout timing (Fig S2P-U). Similar results were observed using chronic caloric restriction to induce torpor (Fig. S3A-M), with significant effects of E2 on time in deep torpor, max ΔT , and temperature variability, consistent with a previous study⁴⁵. In summary, these results demonstrate that circulating estrogens are sufficient to attenuate torpor.

Distinct MPO^{ER α} activity states encode torpor, ambient temperature, and social cues

To build a foundational understanding of how individual MPO^{ER α} neurons contribute to coordinating torpor, we performed *in vivo* microendoscopic calcium imaging across torpid and non-torpid states (Fig. 2A-E). Given the MPO's role in coordinating diverse homeostatic processes, including thermoregulatory responses to environmental temperature changes^{194–196} and innate social behaviors^{197–200}, we also recorded MPO^{ER α}

neurons during heat, cold, and a social interaction session to map cellular and population-level responses across stimuli.

Across torpid and non-torpid states, cellular responses were heterogeneous and their linear correlation with core body temperature (T-Core) was examined (Fig. 2F-H). Cells were grouped by the direction and significance of their correlation with T-Core, and correlation strength (r) and magnitude (slope) were examined within each subpopulation (Fig. 2I-L). Most cells were significantly correlated with T-Core (79%), either positively or negatively (42% and 37%, respectively). Functional subpopulations exhibited no significant spatial organization within the imaging field: subpopulations were anatomically intermingled, and spatial enrichment was not detected in any cell types across all sessions (Fig. S4A-B). Cross-registration of cells across recording days (Day 1: 15-26 hours from fast onset, Day 2: 39-54 hours) revealed day-to-day instability in cellular T-Core correlation (Fig. S4C-I). To assess whether this representational drift is functionally significant, we trained a linear regression model to predict T-Core from calcium activity, using each cell as a feature (Fig. S4J). Model performance, measured by Pearson correlation (r) between observed and predicted T-Core values, was significantly lower when models were trained and tested on separate days, compared to mixed-data controls. Therefore, data from each day were analyzed separately and subsequently pooled. Using principal component analysis (PCA) to visualize high-dimensional population dynamics as a 2-D projection, we found a striking separation by T-Core, suggesting that population-level calcium activity could predict T-Core (Fig. 2M). To test this, we used linear regression models to assess whether T-Core is encoded in the

calcium activity of MPO^{ERα} neurons (Fig. 2N). Model performance was high when trained on the full population or on isolated positively and negatively correlated subgroups but was significantly reduced when trained on only neutral cells or shuffled data (Fig. 2O). In summary, imaged MPO^{ERα} neurons display heterogeneous responses during torpor that encode core body temperature, lack spatial organization within the imaging window, and exhibit day-to-day instability.

To assess the role of MPO^{ERα} neurons in responding to ambient temperature changes, we recorded calcium activity during acute heating and cooling (Fig. S5A). Across ambient temperatures (T-Amb), we found heterogeneous cellular responses, which were grouped and analyzed based on their linear correlation with T-Amb (Fig. S5C-H). Functional subgroups were spatially mixed, and we found no evidence of significant anatomical organization in this context (Fig. S4A-B). PCA revealed a substantial separation across T-Amb values, and population encoding was formally assessed using linear regression models (Fig. S5I-J). Model performance was high when all cells, or isolated positive and negative subgroups, were included in the training set but was significantly reduced with only neutral cells or shuffled data (Fig. S5K). In summary, imaged MPO^{ERα} neurons exhibit heterogeneous cellular responses that encode ambient temperature during acute environmental heating and cooling and lack spatial organization within the imaging window.

To determine how MPO^{ERα} neurons coordinate thermoregulation across homeostatic contexts, we compared responses across torpor and heat/cold sessions. Only day 2

torpor data were considered here, because it was collected on the same day as heat/cold data. Among neurons significantly correlated with both T-Core and T-Amb, tuning direction was largely conserved (54/81 cells), though a substantial subset displayed opposite tuning (27/81 cells) (Fig. S5L). Among cells not tuned to both T-Core and T-Amb, most were tuned to one (38/41 cells), and only a very small fraction were tuned to neither (3/41 cells). To determine if the functional classification of neurons in one context predicted their responses in the other, we grouped cells by T-Core identity and evaluated their T-Amb tuning, and vice versa (Fig. S5M-N). T-Core tuning direction was largely preserved in cells grouped by T-Amb identity, though the z-scored ΔF values were smaller, indicating a weaker relationship. Conversely, grouping cells by T-Core identity weakened T-Amb tuning and altered its direction. Together, these results demonstrate that imaged $\text{MPO}^{\text{ER}\alpha}$ neurons encode T-Core and T-Amb through distinct, but overlapping, activity states.

To assess the role of $\text{MPO}^{\text{ER}\alpha}$ neurons in innate social behavior, we recorded calcium activity during an interaction session with a novel male conspecific (Fig. S6A). Neuronal responses were heterogeneous, persisted following male removal, and strongly discriminated between the pre-male baseline and interaction session (Fig. S6B-J). Functional cell groups were spatially intermingled, and we found no evidence of significant anatomical organization within the imaging window (Fig. S4A-B).

Next, we compared social and torpor responses (Fig. S6K-M). Neurons whose calcium activity positively correlated with T-Core (less calcium activity during torpor) had higher

calcium activity when a social partner was present, while neurons negatively correlated with T-Core (more calcium activity during torpor) showed little response. Reciprocally, the calcium activity of social activated neurons decreased during torpor, while social suppressed neurons showed an increase. These results suggest that $\text{MPO}^{\text{ER}\alpha}$ neurons encode torpor and social stimuli via distinct and largely opposite activity states. We also compared social and ambient temperature responses (Fig. S6N-P). Social-activated neurons were more active during heat and cold compared to baseline. Conversely, social-suppressed neurons were less active during both heat and cold. Across T-Amb populations, social response was largely positive, driven by a larger fraction of social-activated neurons in this dataset. These results demonstrate that $\text{MPO}^{\text{ER}\alpha}$ neurons encode T-Amb and social information through distinct but overlapping activity states. In summary, $\text{MPO}^{\text{ER}\alpha}$ neurons show coordinated tuning in distinct contexts, spanning a continuum of shared, overlapping, and opposite activity states across stimuli (Fig. S6Q).

Circulating estrogens selectively alter the tuning of $\text{MPO}^{\text{ER}\alpha}$ neurons during torpor

Next, we tested whether circulating estrogens alter $\text{MPO}^{\text{ER}\alpha}$ responses during torpor, a mechanism that could, at least in part, underlie their torpor-attenuating effects. Using the same mice described above, we ovariectomized and treated each with either vehicle or E2 before repeating the imaging sessions.

Vehicle and E2 groups differed in sampled temperature ranges and frequencies (Fig. 3A), which could alter the effective statistical power of our analysis. To ensure any differences detected were biological and not an artifact of uneven sampling, we performed *a priori*

analyses on both the full dataset (“observed”, Fig. 3B-G) and a downsampled dataset in which the sampling range and frequency were equalized (“downsampled”, Fig. 3H-M). In both treatment groups, we observed heterogeneous cellular responses (Fig. 3B, 3H), which were grouped by T-Core correlation, as before (Fig. 3C-D, 3I-J). Across both datasets, E2 treatment significantly altered functional cell type proportions. Specifically, the abundance of positively correlated cells was significantly increased in the downsampled but not observed dataset and negatively correlated cells were significantly less abundant in the observed dataset and showed a trend (adjusted $p = 0.08$) towards a reduction in the downsampled dataset. In both datasets and cell types, the strength of the correlation between calcium activity and T-Core was not significantly altered (Fig. 3E, 3K). However, the slope was higher in the E2 group in both cell types and datasets (Fig. 3F, 3L). Using estimated spiking activity inferred through deconvolution, rather than z-scored ΔF , as the primary activity metric yielded qualitatively similar results, suggesting that these findings are robust to the choice of activity metric (Fig. S7A-C). Model performance decoding T-Core from the full population was reduced in the E2-treated group in the observed, but not the downsampled, dataset, suggesting this difference is an artifact of uneven sampling. Together, these findings indicate that, among sampled $\text{MPO}^{\text{ER}\alpha}$ neurons, E2 alters the relative abundance and increases the response magnitude of torpor-tuned subpopulations, without compromising the fidelity of population-level T-Core encoding.

During heating and cooling sessions, neuronal responses were heterogeneous and encoded ambient temperature, as before, but no differences were detected between

vehicle and E2 treatment groups (Fig. S7D-I). T-Core tuning during heat/cold sessions was not altered, demonstrating that MPO^{ERα} neuronal responses to local temperature were not broadly altered by E2 (Fig. S7J-M). Social responses were also heterogeneous and encoded social partner presence, as before (Fig. S7N-S). Notably, social activated cells showed a trend towards stronger activation in E2-treated animals, consistent with previous reports that E2 increases MPO neuronal gain to male-associated cues²⁰⁰, although this effect was not statistically significant here.

In summary, circulating E2 significantly influences the tuning of MPO^{ERα} neurons during torpor, but not in other contexts assessed here.

Estrogen signaling in the MPO conditionally attenuates torpor

To test whether local estrogen signaling in the MPO is sufficient to recapitulate the torpor-attenuating effect of circulating estrogens, we administered E2 or vehicle directly to the MPO immediately before fasting for 48 hours to induce torpor (Fig. 4A-C), in mice with (gonad-intact) or without (OVX) ovaries. To confirm that microinjections into the MPO did not leak into circulation, we measured uterine weight 1 day after an injection that was conducted after the experimental trials were complete. In both gonad-intact and OVX animals, uterine weight was not significantly increased by E2 to the MPO, confirming an absence of substantial leakage to the periphery (Fig. S8D, M). For gonad-intact animals, E2 treatment significantly decreased the amount of time spent in deep torpor when body weight, a significant covariate in this experiment, was included (Fig. 4D-F). There was no difference in starting body weight between groups (Fig. S8E). To check for potential order

effects, fast number was included as a factor in an additional analysis, which revealed a significant effect of fast number, possibly due to body weight differences, but no interaction with E2 treatment (Fig. S8A-C). Further, when the trials were considered separately, the direction of the effect of E2 treatment was consistent. Therefore, we concluded that order effects had a negligible interaction with E2 treatment and proceeded to analyze the pooled dataset. Other measures of torpor response were not significantly altered with E2 treatment except for bout duration, which was shortened with E2 (Fig. S8F-L). In ovariectomized animals, baseline body weight did not differ between groups and, surprisingly, we detected no differences between E2 and vehicle treatment in any measure of the torpor response (Fig. 4G-I, S8N-U).

We hypothesized that this conditional effect could be caused by estrogen insensitivity in the MPO following ovariectomy. To test this, we quantitatively compared ER α protein expression in the MPO of ovariectomized (OVX) and sham-operated controls (Sham) (Fig. S9A-C). Mean pixel intensity and ER α + cell counts were similar between OVX and Sham animals, suggesting that OVX does not alter the ability of the MPO to sense local estrogens. Alternatively, this conditional effect may stem from the acute nature of the intra-MPO treatment. If torpor attenuation requires chronic estrogen signaling, as with the pellet used for systemic treatment, gonad-intact mice, unlike ovariectomized mice, may benefit from baseline priming by endogenous estrogens. To test if E2 introduced at fasting onset is sufficient to attenuate torpor, we treated ovariectomized animals with acute subcutaneous injections of estradiol benzoate (EB), rather than the chronic slow-release pellets used previously. Terminal uterine weight, measured 3-7 days after injection, was

significantly increased by all EB doses tested here, except 0.25 μ g which showed a very strong trend ($p = 0.052$), validating that circulating estrogen levels were effectively increased (Fig. S9G). Acute EB treatment significantly reduced the amount of time in deep torpor, an effect that was statistically significant compared to vehicle at all doses tested here (Fig. S9D-F). Body weight was not significantly different between groups (Fig. S9H). EB treatment weakened mean ΔT and lowered temperature variability, as indicated by significant differences between mice treated with 4 μ g EB compared to vehicle, but had no effect on weight lost, max ΔT , bout latency, bout number, bout duration, or bout timing (Fig. S9I-O). These results demonstrate that acute systemic, but not MPO-specific, estrogen treatment delivered at fasting onset is sufficient to attenuate torpor in ovariectomized animals.

In summary, we conclude that local estrogen signaling in the MPO can attenuate torpor, but it is not sufficient to fully recapitulate the effects of circulating estrogens. Further, there is an undetermined mechanism that gates this effect in ovariectomized animals without reducing ER α expression in the MPO or suppressing systemic responsiveness to acute E2.

Estrogen receptors in the MPO are not required for the effects of circulating estrogens on torpor

To test whether estrogen signaling in the MPO is required for the effects of circulating estrogens on torpor, we knocked out ER α , the most highly expressed estrogen receptor in the MPO^{79,201,202}, locally via stereotaxic injection (Fig. 5A). Post-hoc histology validated

that viral expression was targeted to the MPO (Fig. 5B) and immunofluorescence confirmed that the number of ER α + cells was significantly reduced in the MPO, but not in the neighboring BST (Fig. 5C-D, S10A-B). In the gonad-intact state, there was no difference in body weight or any measure of fasting-induced torpor (Fig 5E-G, S10C-J), suggesting that ER α in the MPO is not required for the effect of endogenous estrogens on torpor. Since ER α in the preoptic area regulates ovarian function and estrogen production^{203,204}, we considered that our manipulation could be altering the reproductive system and endogenous estrogen levels. Although estrous cyclicity was not significantly altered between groups (Fig. S10K–L), we sought to strictly control circulating hormone levels in a second trial by treating ovariectomized animals with vehicle or E2. Terminal uterine weights showed a marked increase in E2-treated animals, confirming that circulating estrogen levels were increased (Fig. S10P). There were no differences in body weight between groups in this experiment (Fig. S10Q). Across both control and KO animals, E2 treatment reduced deep torpor duration and increased weight loss, with no main effect of KO or E2-KO interaction (Fig. S10M-O, R). Together, these experiments demonstrate that ER α in the MPO is not required for the effects of circulating estrogens on torpor.

Estrogen receptor beta (ER β , gene *Esr2*) is also expressed in the MPO and could contribute to mediating the effects of circulating estrogens on torpor. To test this possibility, we knocked out both ER α and ER β in the MPO (Fig. 5H). Post-hoc histology validated that viral expression was localized in the MPO (Fig. 5I). Fluorescent immunostaining for ER α and RNAscope *in situ* hybridization for *Esr2* confirmed that the

number of ER α ⁺ and *Esr2*⁺ cells was significantly reduced in the MPO, but not in the neighboring BST (Fig. 5J-M, S11A-C). In the gonad-intact state, there was no difference in body weight or any measure of torpor (Fig. 5N-P, S11D-K). While the estrous cycle of MPO ER $\alpha\beta$ -KO animals was not significantly altered (Fig. S11L-M), we proceeded to ovariectomize and treat all animals with vehicle or E2 to more tightly control the reproductive state, as before. Uterine weights were higher in E2-treated animals, validating the efficacy of the estrogen treatment (Fig. S11Q). There were no differences in body weight between groups (Fig. S11R). Across both control and KO animals, E2 treatment reduced deep torpor duration and increased weight loss, with no main effect of KO or E2-KO interaction (Fig. S11N-P, S). From these experiments, we conclude that, collectively, ER α and ER β in the MPO are not required for the effects of circulating estrogens on torpor.

Estrogen signaling in the central nervous system is not sufficient to attenuate torpor

To broaden our scope and test if estrogen signaling throughout the central nervous system is sufficient to attenuate torpor, we chronically administered E2 to the cerebrospinal fluid via a cannula and osmotic mini-pump assembly targeted to the lateral ventricle (Fig. 6A-B). Efficacy of E2 treatment was confirmed by an increase in hypothalamic progesterone receptor (PR) expression, a well-established biomarker for central estrogen signaling^{205–207} (Fig. 6C-D). Compartmental specificity of the E2 treatment was assessed by measuring terminal uterine weight (Fig. S12A). Although uterine weight was higher in E2-treated animals, values in this experiment (20-26 mg)

remained consistently lower than those observed in gonad-intact animals across this study (40-155 mg; Fig. S1D, S2A, S8D), indicating that circulating estrogen levels were sub-physiological and unlikely to have a significant influence on the results. Comparing vehicle- and E2-treated animals, we did not detect differences in body weight or any measure of torpor (Fig. 6E-G, S12C-I). Given the insufficiency of this treatment to recapitulate the effects of systemic E2 treatment, we conclude that circulating estrogens require peripheral signaling to attenuate torpor.

Discussion

The findings presented here demonstrate that circulating estrogens alter the response properties of torpor-coordinating MPO^{ERα} neurons yet require a peripheral signaling mechanism to drive torpor attenuation. While it remains unclear whether estrogens organize these central and peripheral effects in parallel or in sequence, these findings suggest that estrogenic control of torpor relies on a multi-organ network, consistent with the body-wide effects of circulating estrogens in coordinating other behavioral and physiological changes^{31,208}.

Systemically, circulating estrogens attenuate torpor, a finding that is supported by multiple experiments presented here. First, we observed natural variation in torpor use across the estrous cycle, with the lowest use when fast onset occurred during proestrus. Although reports conflict on when circulating estrogen levels peak²⁰⁹, most evidence points to maximal levels during proestrus^{189,210}, a consensus that is supported by our finding that proestrus mice exhibited the highest uterine weight. Next, we utilized ovariectomy, which

revealed a net attenuating effect of the ovaries on fasting-induced torpor. Finally, estrogen treatment confirmed that circulating estrogens were sufficient to attenuate torpor. Together, this combination of naturalistic observations, hormone depletion, and targeted replacement provides robust, converging evidence that estrogens attenuate torpor and is consistent with the existing literature in mice^{45,184} and other species^{13,56,183}. However, it contrasts with recent results from Marshall et al. that report an effect of estrogen treatment in the opposite direction¹⁸⁵. One plausible explanation is the gonadal status of the mice: here we use ovariectomized mice to isolate the effects of exogenous estrogens, whereas Marshall et al. evaluated only gonad-intact animals. In summary, we conclude that the isolated effect of circulating estrogens on torpor is attenuation, and future work should clarify whether intact gonads alter or reverse the direction of this phenomenon.

Centrally, MPO^{ERα} neurons are estrogen-sensitive coordinators of torpor⁸¹. Here, we report three key findings that provide deeper insight into their function: 1) they heterogeneously encode internal and external variables through related activity states, 2) circulating estrogens selectively alter their tuning during torpor, yet 3) local signaling in the MPO does not directly mediate the effects of circulating estrogens on torpor.

Torpor and social stimuli are represented by inverse activity states in which cellular responses are mostly opposite, while torpor and ambient temperature are encoded by distinct, yet overlapping, states in which many, but not all, cells respond similarly. These data suggest that torpor and social behavior, as measured here, have an antagonistic relationship and are coordinated by opposing mechanisms. In contrast, torpor and

thermoregulation during acute heat/cold are likely coordinated by a combination of shared and context-specific mechanisms. While fever was not tested here, these results are consistent with a previous study that found preoptic neurons exhibit heterogeneous and partially overlapping responses to torpor and pyrogenic lipopolysaccharide treatment¹⁸⁷.

Circulating estrogens alter the abundance of functional MPO^{ERα} subpopulations by decreasing the abundance of torpor-active MPO^{ERα} cells and/or increasing the abundance of torpor-inactive MPO^{ERα} cells. Both possibilities align with torpor attenuation and suggest that estrogens alter the net output signal from MPO^{ERα} neurons. Circulating estrogens also increase the response magnitude of both torpor-active and torpor-inactive MPO^{ERα} neurons. This suggests two non-mutually exclusive possibilities: that estrogens 1) increase neuronal responses to local brain or body temperature and/or 2) desensitize downstream effector circuits, thereby requiring a stronger MPO^{ERα} neuronal signal to drive equivalent changes in core body temperature during torpor. While we cannot rule out an increase in responsivity to local temperature, it is unlikely the primary driver, as E2 treatment did not affect core body temperature tuning during heat and cold challenges. In summary, these results point to a model where circulating estrogens attenuate torpor by diminishing both the torpor-promoting signal from MPO^{ERα} neurons and possibly the gain of downstream effector circuits. However, because tests of sufficiency and necessity ruled out the MPO as a major direct mediator for the physiological effects of circulating estrogens on torpor, we conclude that direct estrogen signaling in MPO^{ERα} neurons is insufficient and dispensable for estrogenic attenuation of torpor. Therefore, signaling

outside the MPO must be necessary for any functionally significant consequences of the E2-induced shift in MPO^{ERα} tuning.

In testing the necessity of estrogen signaling in the MPO for torpor attenuation, our results again conflict with Marshall et al., who report a decrease in torpor expression following ERα knockdown in the MPO of gonad-intact female mice¹⁸⁵. Notably, Marshall et al. did not assess or account for potential disruptions to the endogenous reproductive system, raising the possibility that their ERα knockdown could have caused compensatory changes in ovarian estrogen production, which is regulated by ERα in the preoptic area^{203,204}. Here, we monitored estrous cycles to assess baseline reproductive status and tested all MPO ERα-KO animals in a second trial following ovariectomy to rule out secondary endocrine disruptions. Therefore, we conclude that ERα in the MPO is dispensable for the effects of circulating estrogens on torpor and suggest that further work is necessary to clarify the role of ERα in non-MPO preoptic subregions in regulating torpor and/or reproductive physiology.

Peripherally, estrogen signaling is required to attenuate torpor, given our finding that estrogen administration throughout the central nervous system is insufficient. Now, it becomes important to identify peripheral signaling targets that could mediate the effects of estrogens on torpor. Auspicious candidates implicated in the estrogenic control of energy homeostasis include adipose tissue^{100,101}, skeletal muscle¹⁰³, and the liver¹⁰². It should be noted that our data do not rule out a mechanism that relies on both central and

peripheral estrogen signaling to attenuate torpor, and a multi-organ network model must be considered for future investigation.

Reproductive and metabolic systems are highly conserved across mammals, and the effects of estrogen withdrawal and replacement on metabolic health are mirrored in humans and rodent models^{174,175,179,211,212}. While humans do not naturally enter torpor, phylogenetic and experimental evidence supports the hypothesis that the regulatory mechanisms of torpor are highly conserved and regulate aspects of energy expenditure even in strictly homeothermic species^{4,80,166,167}. In conclusion, these findings advance our basic understanding of metabolic pathways in mice and represent an important step towards the ultimate goal of understanding how these pathways could be selectively manipulated in humans to promote metabolic health.

Resource availability

Lead contact

Requests for further information and resources should be directed to and will be fulfilled by the lead contact, Stephanie Correa (stephaniecorrea@ucla.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

Code used to process calcium imaging videos can be found at https://github.com/pbvander/PV_minian. Code used to further analyze the output from this initial processing step can be found at

https://github.com/pbvander/minian_output_analysis. Other original code used here can be found at https://github.com/pbvander/Code_for_Vander_2026.

The full calcium imaging dataset is available on Dryad (doi.org/10.5061/dryad.mw6m906d3). All other datasets are available by email request.

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Icons from BioRender were used in the Graphical Abstract and Figures 2, 4, and 6. Vander, P. (2026) <https://biorender.com/lh948f9>.

Author contributions

Conceptualization + Funding Acquisition: SC, PV. Investigation: PV, JP, AA, MU, AV. Methodology: PV, SC, WH, FS, LG. Resources: PV, SC, FS, WH. Software + Visualization + Writing – original draft: PV. Writing – review & editing: SC, PV, FS, LG.

Declaration of interests

The authors declare no competing interests.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Google Gemini to improve readability and conciseness throughout the manuscript. All AI outputs were reviewed by the authors and edited as necessary. The authors take full responsibility for the final version of the published article.

Methods

Experimental model and study participant details

Experimental subjects

Adult female mice (*Mus Musculus*) in this study were bred and/or housed in approved facilities at UCLA. Female was defined as shorter anogenital distance at weaning (21 days of age) and the presence of ovaries and/or uterus at >8 weeks of age. Female mice were used exclusively for this study based on their higher propensity for fasting-induced torpor⁴⁵, higher levels of circulating estrogens¹⁸⁹, and higher expression of estrogen receptors in the MPO⁸¹. For experiments involving stereotaxic surgery, mice were 8-12 weeks of age at the start of the experiment. For all other experiments, mice were 7-9

weeks of age at the start of the experiment. C57BL/6J mice (JAX stock no. 000664) were used for all experiments where transgenic animals were not required. Transgenic strains used: *Esr1-Cre* ($Esr1^{tm2.1(cre)And}/J$, JAX stock no. 017913), *Esr1 flox* ($Esr1^{tm1Sakh}$, JAX MGI ID 4459300), *Esr2 flox* (C57BL/6N- $Esr2^{tm1Ksk}/J$, JAX stock no. 032174), *Nkx2.1-Cre* (C57BL/6J-Tg(Nkx2-1-cre)2Sand/J, JAX stock no. 008661). All mice were maintained under a constant 12:12 L/D schedule at room temperature (~22-24 °C), with *ad lib* access to food (Mouse Diet 9F, LabDiet) and water. All mice were singly housed following surgery and throughout the experimental period.

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.

Method details

Surgical procedures

Mice were anaesthetized with 3% isoflurane (maintained within a range of 2-3% during surgery), and the incision site was shaved and sterilized with alternating swabs of betadine and 70% ethanol. Internal incisions in the abdominal body wall were closed with absorbable suture (4-0 Vicryl), and skin incisions were closed with non-absorbable suture (4-0 Ethilon) or 9 mm wound clips. Triple antibiotic ointment was applied on top of the closed incision after surgery and later re-applied in the event of local inflammation during recovery. Buprenorphine (0.074 mg/kg) and carprofen (7.11 mg/kg) were administered

via intra-peritoneal (IP) injection for analgesia immediately following surgery and again 12-24 hours after surgery. For GRIN lens implant procedure, carprofen was administered one hour before surgery, instead of following surgery, to proactively prevent inflammation. Mice were given at least two weeks to recover following stereotaxic surgery, at least one week to recover after surgeries that involved an incision in the abdominal body wall (ovariectomy, temperature probe implant), and at least two days after minor surgeries that involved an incision only in the skin (bio-erodible pellet implant).

Stereotaxic procedures

Mice were prepared for surgery as described above. Under anesthesia, mice were positioned in a stereotaxic frame (Kopf, Model 1900), and an incision in the skin above the skull was made. The periosteum was removed with a sterile cotton-tipped swab. The skull was leveled, and small holes were drilled through the skull and dura at the desired coordinate (MPO: AP -0.1 or 0, ML +/-0.4, VL: AP -0.5, ML 1) For experiments involving cranial implants (GRIN lens for calcium imaging, cannula for E2 delivery to MPO or VL), the skull was scored with a scalpel and cleaned with 3% hydrogen peroxide to improve glue binding. For procedures with AAV injections, a fine glass needle was lowered to the MPO (DV -5.1 for calcium imaging experiment, DV -5.3 for all others) and the virus was infused at 5 nL/second to each hemisphere. For calcium imaging experiments, AAV-FLEX-GCaMP8m stocks were diluted to a final concentration of 1/3 in artificial cerebrospinal fluid (aCSF, 3525 Tocris) and 300 nL was injected into the right hemisphere. For all other experiments, 150 nL was injected to each hemisphere. Following injection, the needle was left in place for 10 minutes before removing. For calcium imaging

experiments, a 0.6 mm-diameter x 7.3 mm-long GRIN lens (1050-004597, Inscopix) was lowered to DV -4.9 following AAV injection. Lenses were routinely re-used and were cleaned with acetone, 1% tergazyme, then 70% ethanol between uses. All lenses were closely inspected before use. The lens was lowered slowly over 30 minutes, pausing for 1 minute every 1 mm, to minimize tissue damage. No tunneling or aspirating of brain tissue was performed. For cannulas aimed at the MPO, a 4 mm-long, 26 Gauge guide cannula (C235GS, P1 Technologies) was implanted at DV -3.1, and a dummy injector cannula (C235DCS, P1 Technologies) with a 2 mm projection was inserted to reach DV -5.1. A dust cap was then secured on top of the guide/dummy assembly to prevent contamination. For cannulas aimed at the VL, a 3 mm-long, 30 Gauge cannula (Brain infusion kit 3, Alzet) was implanted at DV -2.5. All implants (GRIN lens or cannula) were secured to the skull with cyanoacrylate glue (Gorilla Super Glue Gel). After glue was in place, an accelerator (Loctite SF 7452) was directly applied to the glue to rapidly cure it. For VL cannulas, glue alone was used, and the white plastic tab atop the cannula was left in place, as the mechanical force required to remove it could dislodge the cannula, in our experience. For GRIN lenses and MPO cannulas, a layer of dental cement (Contemporary Ortho-Jet, Lang Dental) was added directly on top of the glue. For calcium imaging experiments opaque black dental cement was used to prevent light contamination. To protect the exposed GRIN lens between implant and baseplate attachment, a small plastic cap from a 0.1 mL PCR tube was cemented in place covering the lens. The incision over the skull was closed with suture following stereotaxic procedures with no implant and VL cannula implants. For MPO cannulas, the edges of the incision were anchored alongside the implant with dental cement. For GRIN lens

implants, GLUture was used to fill the space between the skin and dental cement. Mice were given at least two weeks to recover following stereotaxic surgery before starting any experimental data collection.

Baseplate attachment

For calcium imaging experiments, a metal baseplate was attached above the GRIN lens at least two weeks after AAV injection and GRIN lens implant. Under anaesthesia, the plastic cap covering the lens was carefully removed by drilling away the cement holding it in place. Next, the Miniscope was secured in the baseplate, attached to the stereotaxic frame, and positioned above the GRIN lens so that fluorescent cells in the MPO were visible. The baseplate was then secured to the existing cement layer covering the skull using a layer of cyanoacrylate glue, followed by a layer of black dental cement. Finally, a black plastic cap was secured in the baseplate to protect the lens from dust and light.

Temperature probe implantation

Mice were prepared for surgery as described above. An incision was made through the skin and abdominal body wall on the ventral flank of the animal, and the temperature probe was inserted into the abdominal cavity. CubeWorks TS100 probes were used for the first estrogen treatment experiment (Fig. 1G-I, S2M-U), and STARR Life Science G2 E-Mitter probes were used for all other experiments.

Ovariectomy

Mice were prepared for surgery as described above. An incision was made through the skin and abdominal body wall on the dorsal flank of the animal. The gonadal fat pad was retrieved with forceps, and the ovary was removed using a high-temperature cauterizer. The same procedure was repeated on the other side of the animal. Control “sham” surgeries were performed in the same manner, minus the cauterization step to remove the ovaries.

Bio-erodible pellet implantation

Pellets were implanted 1-2 weeks after ovariectomy. Mice were prepared for surgery as described above. A small incision was made in the intrascapular skin on the back parallel to the mouse’s body. Blunt dissection was used to separate the skin from subcutaneous tissue, and a bio-erodible pellet containing 17 β -estradiol (E2) or vehicle (E-121/C-111, Innovative Research of America) was gently inserted into the subcutaneous space. Pellets contained 0.5 mg E2 and had a 21-day release duration.

Osmotic mini-pump preparation and implantation

Two mini-pumps were used for each animal. First, all animals were given an aCSF-filled pump as a control when the cannula was implanted. 2 weeks later, this control pump was replaced with a treatment pump filled either with aCSF (vehicle) or an (E2) solution. Mini-pumps were prepared according to the manufacturer’s instructions. Briefly, an osmotic mini-pump (Model 1002, Alzet) was filled and connected to the cannula (Brain infusion kit 3, Alzet) with the polyethylene tubing provided in the kit (inner diameter = 0.03 inches). The tubing was cut to ~3 cm in length and attached to the cannula on one end and the

flow moderator on the other. This assembly was filled with aCSF using a 21 Gauge needle attached to an extra length of catheter tubing. The mini-pump was weighed, filled with aCSF, then weighed again to verify that it had been filled completely. The flow moderator was then inserted and the pump was primed by submerging in sterile saline at 37 °C for 2-24 hours. During the cannula implantation procedure (described in the “stereotaxic procedures” section above), a subcutaneous pocket was made along the midline of the animal's back using a blunt-tip hemostat just before the cannula was lowered into the brain. The pump and tubing assembly was gently guided into this subcutaneous pocket as the cannula was positioned over the skull.

Two weeks after the implantation of the cannula and the first pump, treatment began by swapping the original control pump for a new treatment-filled pump. The treatment pump was prepared as described above, but without the tubing. To swap the pumps, a small incision was made perpendicular to the skin over the tubing, just above where the tubing meets the flow moderator. The old pump was carefully retrieved and removed from the flow moderator-tubing-cannula assembly, and the new pump was attached. The new pump was then replaced into the subcutaneous pocket, and the incision was closed with suture.

Estradiol benzoate (EB) preparation and treatment

Estradiol benzoate (Sigma E8515) was dissolved in DMSO (Sigma D8418) to make a 30 mg/mL stock solution. This stock solution was then diluted 1:100 in corn oil and mixed thoroughly to make a working stock (final concentration 300 µg/mL EB + 1% DMSO in

corn oil). This working stock was further diluted in vehicle (1% DMSO in corn oil) to prepare lower concentrations. Mice were treated with EB by subcutaneously injecting 40 μ L of the EB solution at the start of the fasting period.

E2 delivery to MPO

Water-soluble E2 (Sigma E4389) was dissolved in aCSF (Tocris 3525) to make a 1 μ g/150 nL stock solution. The potency of the water-soluble E2 preparation (45 mg/g E2) was accounted for, so the concentration stated above represents the amount of E2 in solution. As a vehicle control, cyclodextrin (Sigma C0926) was dissolved in aCSF to match the amount present in the E2 solution (21.22 μ g / 150 nL). The vehicle or E2 solution was delivered to the MPO of awake mice just before fasting. Briefly, the mouse was restrained, the dust cap and dummy cannula were removed, and a 33 Gauge injector cannula (P1 Tech C235IS) with a 2 mm projection (reaching DV -5.1) was fully inserted into the guide cannula. 150 nL was injected at 5 nL/sec to each hemisphere. The injector cannula was left in place for 10 minutes after the injection was finished. The mouse remained restrained throughout this procedure to avoid twisting or biting the injection tubing. Gonad-intact animals were treated in a counterbalanced, two-trial design, with at least 1 week between trials, while ovariectomized animals were evaluated in a single-trial design. This modification was necessary for ovariectomized animals because they exhibited rapid weight gain, leading to minimal torpor use in later trials. The order of injections (E2 or vehicle) was randomized for gonad-intact animals, and 9/12 mice received both injections. 1 mouse only received E2 injection due to their guide cannula detaching from the skull between the first and second trial, 1 mouse received both injections but data

from the vehicle trial (second trial) was excluded because its water bottle leaked and flooded the cage during fasting, and 1 mouse received both injections, but data from the E2 trial (first trial) was excluded because it was co-treated with Methyl-Piperidinopyrazole (MPP) at that time. Terminal infusions for each animal were administered at least 1 week after the conclusion of experimental trials and 1 day before euthanasia and tissue collection.

Estrous cycle staging

Vaginal lavage samples were collected daily between 0 and 4 hours after lights on (Zeitgeber time/ZT 0 – ZT 4. Briefly, samples were collected by flushing the vaginal canal with saline ~12 times, or until the saline became visibly clouded (whichever occurred first). The sample was then collected in an untreated plastic 96-well plate and covered to reduce evaporation. Within 48 hours, samples were triturated to resuspend any cells present, smeared onto a glass microscope slide, and imaged with a light microscope. No staining was performed. Stages were determined manually by a single experimenter, based on the relative proportions of cell types present in the sample, as described previously²¹³. Each stage was assigned to a 24-hour period which started at ZT 12 the previous day and ended at ZT 11.99 on the day of vaginal lavage, following the “behavioral stage assignment” criteria described previously²¹⁴. Core body temperature was used as a secondary method to confirm estrous cycle stage, as core body temperature reliably changes during the estrus stage²¹⁵. For the experiment testing the effects of the estrous cycle on torpor, each mouse was fasted twice, and trials were grouped by the stage of the estrous cycle at the beginning and end of the fast. The estrous

cycle of each mouse was tracked for 8-14 days before the first fast, and for another 8-14 days between fasts. Any trial where the estrous cycle of the mouse was irregular, ambiguous, or absent leading up to the fasting period was excluded. Every mouse was fasted in a different stage between the first and second trial (duplicates were excluded). After experimental trials were complete, estrous cycle continued to be tracked daily until they were euthanized at least 2 days later.

Telemetric core body temperature measurement

Core body temperature was passively sampled at an interval of 1 minute (calcium imaging experiments) or 5 minutes (all other experiments). For CubeWorks probes, core body temperature was recorded using CubiSens Gateway and TS100 GUI software. For STARR Life Sciences probes, core body temperature and gross locomotor activity were recorded using ER4000 or ER4500 energizer receivers and VitalView (v6.0 or v5.1) software.

Fasting-induced torpor

3 hours after lights on (ZT 3), mice were weighed and transferred to a fresh cage with water, but no food. Nesting material was constant between all animals. At the end of the fasting period, mice were weighed again, and *ad lib* food access was restored.

“Deep torpor” was defined as core body temperature < 31 °C. “Max ΔT ” (maximal hypothermic response) was defined as the difference between minimum temperature during fasting and the preceding 48-hour baseline mean. “Mean ΔT ” (mean hypothermic

response) was defined as the difference between the mean temperature during fasting and the preceding 48-hour baseline mean. “Latency” was defined as the time between the onset of fasting and the first deep torpor bout. Deep torpor bouts were defined as continuous periods with core body temperature $< 31\text{ }^{\circ}\text{C}$. Bout onset and offset were defined as the first timepoints below and above $31\text{ }^{\circ}\text{C}$, respectively, and timing analyses compared the circadian timing of each bout's midpoint (Zeitgeber Time/ZT halfway between onset and offset).

Calorie restriction induced torpor

1-2 weeks after ovariectomy, but before E2 treatment, food intake was measured for all mice across 72 hours to establish a baseline for caloric intake. 4 days after estrogen treatment, caloric restriction began. Between 8.5-14.5 hours after lights on (ZT 8.5-14.5), mice were provided with a daily food ration that was a specific percentage of their baseline caloric intake (100% for 2 days, then 60% for 4 days, then 50% for 6 days). Some E2-treated animals had leftover food following days when the food ration was 100% of baseline caloric intake, which was discarded before providing the next ration. On days where the food ration was $<100\%$ of baseline caloric intake, every animal ate all the food provided.

Calcium imaging – data acquisition

Before any experimental data collection, mice were habituated to the head-mounted Miniscopes by attaching a 3D-printed “dummy” Miniscope (weight- and shape- matched) for 1 hour each day for 3 consecutive days. During experimental recordings, the

Miniscope was attached to a non-motorized commutator (CMTR-2-NM-UCLA-NT, NeuroTek), which allowed the mouse to freely move without twisting the cable. The commutator was then attached to a Miniscope DAQ v3.3, and Miniscope DAQ Software v1.10 was used to record data, stop/start recordings, and set parameters. For all recording sessions, data from a full-color camera that was aimed at the cage and connected to the same computer was captured at 30 FPS in parallel to Miniscope data. These videos were used for manual behavior annotations. All Miniscope recordings were acquired at 15 FPS, and LED intensity and detector gain were set so that pixel values spanned the dynamic range of the sensor without oversaturating pixels. LED values were kept below 8 to avoid bleaching or heating the brain. EWL values, which determine the focal plane of the Miniscope, were set manually to maximize the number of cells in focus. LED, gain, and EWL values varied across individuals and between pre- and post-OVX recordings but were consistent within consecutive days of recording for each animal. Just before each Miniscope attachment, the GRIN lens was cleaned with a cotton-tipped applicator soaked in 70% ethanol. Following Miniscope attachment, mice were left undisturbed for at least 15 minutes before beginning any recordings. Mice had *ad lib* access to water throughout all recording sessions and had *ad lib* access to food outside of fasting periods. Individual recording sessions were stopped and started manually without disturbing the animal. USB power to the Miniscope was disconnected between recording sessions to minimize heat generation.

Fasting-induced torpor:

Mice were weighed and transferred to a fresh cage with water but no food 3 hours after lights on. Nesting material was present and constant between animals. ~14.5 hours after fasting began, the Miniscope was attached to begin day 1 torpor recordings. 10-minute recording sessions were completed approximately every 60 minutes from 15-26 hours of fasting. After the final recording was completed, the Miniscope was detached. Later, ~38.5 hours after fasting began, the Miniscope was re-attached for the second day of recording. 10-minute recording sessions were completed approximately every 60 minutes from 39-44 hours of fasting. At 45 hours of fasting, a “re-feeding” session was completed. During this session, 10 minutes of data were recorded while the mouse was still fasting, then animals were re-fed by an experimenter dropping a single food pellet into the bottom of the cage. This recording session continued uninterrupted for 10 more minutes, or until their core body temperature had reached 35 °C for at least one minute (whichever occurred later). After this recording session ended, mice were given *ad lib* food. Recording days with little to no incidence of torpor (minimum T-Core > 33 °C and less than 10 minutes < 34 °C) were excluded (total 2 days from 2 mice).

Heat/cold exposure:

>2 hours after the “re-feeding” session had ended, ambient temperature was manipulated by changing the air temperature within a thermal chamber. First, a 5-minute baseline was recorded at room temperature (22-23 °C), then the chamber was warmed to 37 °C over 15-20 minutes and kept at that temperature for 30 minutes. Miniscope recordings continued uninterrupted through this period. Miniscope recordings were then stopped, and the chamber was rapidly cooled back to room temperature by setting the chamber to

22 °C and opening the door to allow room temperature air to flow in. After the chamber cooled to 24 °C (10-15 minutes), the chamber door was closed, and mice were allowed to recover for at least 30 minutes. After the recovery period, another 5-minute baseline period was recorded before cooling the chamber to 5 °C over 45-55 minutes and keeping it at that temperature for 30 minutes. Ambient temperature within the chamber was monitored in real time via a digital thermometer every 3 minutes. Ambient temperature data was later up-sampled via linear interpolation to a sampling frequency of every 1 minute (to match core body temperature sampling frequency). Recording sessions with technical issues (Miniscope failure during heat, water bottle dripping onto lens, or the Miniscope becoming loose in baseplate) were excluded from analysis (total 3 sessions from 3 mice).

Male social partner:

>1 hour after the cold exposure session had ended, mice were given a male social stimulus. First, a 5-minute baseline period was recorded, then a gonad-intact, sexually naïve adult male mouse was added to the cage. 10 minutes later, the male was removed and recording continued for another 5 minutes. Miniscope recording was uninterrupted throughout. 1 recording session from 1 mouse was excluded due to Miniscope becoming loose in the baseplate earlier that recording day.

Calcium imaging – data analysis

Miniscope videos from the same day (no Miniscope detachment between recordings) were concatenated and processed using the pre-processing, initialization, and CNMF

modules from MiniAn²¹⁶, and motion correction from NormCorre²¹⁷ (see https://github.com/pbvander/PV_minian for further details on code implementation). Pilot experiments revealed that NormCorre removed motion artifacts from our recordings much more reliably than MiniAn's motion correction module. In MiniAn, putative cells were identified using a seed-based approach and filtered primarily by signal-to-noise ratio, with noise defined as high-frequency signals above 0.9 Hz. Putative cells with ratios <1.2 were excluded. After processing in MiniAn was completed, all outputs were manually checked in CScreener (<https://github.com/hongw-lab/CScreener>). Putative cells that did not have any visible dynamic calcium activity (spatially compact changes in cellular signal that were distinct from background) in the raw motion-corrected video were removed at this step. To register individual cells across recording days, we used CellReg²¹⁸. Fields of view were rigidly aligned, and cell pairs with a spatial correlation >0.3 were considered the same cell. Every putative cell pair was manually verified for accuracy and no false positives were identified.

The cellular calcium activity data were then aligned with other data streams (core body temperature, ambient temperature, social partner presence) using the timestamps generated by the acquisition software for each frame. For downstream analysis, we defined F as the "YrA" variable from MiniAn, which represents the detrended + demixed GCaMP signal for each cell. To normalize data across conditions, z-scored ΔF values, defined as $(F - F_0) / SD(F_0)$, were calculated for each condition, where F_0 equals the mean of all F values across the baseline period for each recording type. For spiking rate analysis, the "S" variable from MiniAn was used, which represents the deconvolved spike

signal for each cell, derived from a 2nd-order autoregressive model fit to the YrA variable. Lastly, the data were averaged into 1-minute bins to match the sampling frequency of core and ambient temperature. For “torpor” recordings (all recordings during the fasting period and the re-feeding recording session), baseline was considered time points where core body temperature was $> 35^{\circ}\text{C}$. For heat and cold exposure, baseline was considered the 5-minute period before the chamber was heated or cooled. For male social stimulus, baseline was considered the 5-minute period before the male was added to the cage. For comparisons between vehicle and E2 treated animals, uneven sampling across core body temperatures (due to the influence of E2 on core body temperature during fasting) was corrected by random downsampling. First, we calculated the number of observed timepoints across 1°C bins of core body temperature for each group. We then downsampled whichever group had more observed timepoints in each bin by randomly excluding timepoints until groups were equal. This process was repeated 500 times to reduce variability of random downsampling, and we used the mean of all numeric metrics, such as correlation coefficients, and the mode of all categorical metrics, such as correlation type/significance, across iterations for downstream analysis.

Single cell tuning analysis:

Functional cell types for torpor and heat/cold analyses were defined based on the linear correlation between z-scored ΔF values and T-Core/T-Amb, as described previously²¹⁹. The observed Pearson correlation coefficient (r) for each cell was compared to a null distribution of 1,000 r values calculated from the same neuronal data, but with randomly shuffled temperature labels. Cells where the un-shuffled value fell within the top 2.5% or

bottom 2.5% of shuffled values were considered “positive” or “negative” correlated, respectively. For social tuning analysis, an ROC-based approach was used, as described previously²²⁰. Briefly, a range of binary thresholds, spanning the minimum and maximum z-scored ΔF values for each neuron, were used to decode a binary event vector (presence or absence of male social partner), yielding an ROC curve from the true positive and false positive rate at each threshold. Only data from pre-male baseline and the interaction session were considered here, since neuronal responses persisted after male removal. The area under the ROC curve (AUROC) was computed and compared to a null distribution of 1,000 AUROC values computed from the same neuronal data, but with randomly shuffled labels. Cells where the un-shuffled AUROC value fell within the top 2.5% or bottom 2.5% of shuffled values were considered “activated” or “suppressed” by the male social partner, respectively. The “ROC score” was calculated as $2 * |AUROC - 0.5|$, which represents the absolute predictive power of the cell, scaled from 0 – 1 (0 = no predictive power above chance, 1 = total predictive power).

Population encoding analysis:

For torpor and heat/cold analyses, a linear regression model was used to decode temperature values based on z-scored ΔF values from a group of cells. Briefly, a 5-fold cross-validation approach was used. First, the data were split into 5 chunks (stratified by temperature to ensure that diverse values were included in both training and test sets), and a linear regression model was trained on 4/5 chunks and then tested using the remaining 1 chunk. The Pearson correlation coefficient between the observed and predicted temperature data in the test set was averaged across all 5 iterations and served

as the evaluation metric for this analysis. For male social stimulus, logistic regression was used to decode a binary event vector (male social partner present or absent) based on z-scored ΔF values from a population of cells. Only data from pre-male baseline and the interaction session were considered here, since neuronal responses persisted after male removal. As described above, a 5-fold cross-validation approach was used, with data stratified by male social stimulus presence/absence. From the probability outputs of the logistic regression model, a range of binary thresholds from 0-1 were used to build an ROC curve predicting male social stimulus absence/presence, and the area under the ROC curve (AUROC) was used as the evaluation metric for this analysis.

Principal component analysis (PCA):

Principal components were calculated from z-scored ΔF values of an entire population of cells. Since z-scored ΔF values are already centered and scaled, no further centering or scaling of data was done prior to running the PCA.

Spatial enrichment test:

Cells not significantly correlated or responsive within a recording session were excluded from this analysis. First, the centroid of each weighted cell footprint was calculated. Then, for each recording session and cell type the mean distance between all centroid pairs was calculated and compared to a null distribution of 1,000 distance values calculated from the same data but with randomly shuffled cell type labels. The rank of the observed data within the null distribution, divided by the number of iterations (1,000) was

considered the p-value for this analysis, and p-values were corrected for multiple cell type and session comparisons via the Holm method.

Perfusion and tissue collection

Mice were euthanized with a lethal dose of pentobarbital (180 mg/kg), injected IP. First, the uterus was dissected and weighed. Next, the animal was transcardially perfused with ice-cold RNase-free (DEPC-treated) PBS for 4-5 minutes, then 4% paraformaldehyde (PFA) for 5 minutes. After perfusion, the brain was carefully dissected and post-fixed in 4% PFA overnight at 4 °C, then switched to a 30% sucrose solution and incubated at 4 °C for 2 days for cryoprotection. Finally, the brains were embedded in OCT medium and stored at -80 °C.

Cryosectioning

Brain tissue was cut into coronal sections on a cryostat (temperature between -13 and -21 °C) at 20-30 µm thickness. Sections were collected in DEPC-treated 1% neutral buffered formalin (NBF) in phosphate-buffered saline (PBS) solution to protect against RNase activity. Sections were then transferred to a cryoprotectant solution (30% sucrose w/v, 1% polyvinyl-pyrrolidone (PVP-40) w/v, 30% ethylene glycol w/v in DEPC-treated PBS) and stored at -80 °C.

Immunofluorescence

Sectioned brain tissue was mounted onto glass Superfrost Plus slides (Fisher Scientific 12-550-15) in preparation for staining. For immunostaining to detect estrogen receptor

alpha (ER α), antigen retrieval was performed: first slides were dehydrated by incubating in 70%, then 100% ethanol for five minutes each then allowed to dry for 30 minutes. Next slides were incubated in antigen retrieval buffer (25 mM Tris-HCl pH 8.5, 1 mM Ethylenediamine Tetraacetate (EDTA), 0.05% w/v sodium dodecyl sulfate (SDS) in PBS) at 95 °C for 40 minutes. Lastly, they were allowed to cool for 20 minutes at room temperature. No antigen retrieval was performed for immunostaining to detect progesterone receptor (PR). Next, sections were incubated for 30 minutes at room temperature in 10% v/v normal goat serum (ThermoFisher 01-6201 or abcam ab7481) + 0.2% v/v Triton-100 (Fisher Scientific BP151) in PBS to block non-specific binding sites in the tissue. Next, sections were incubated overnight at 4 °C in 1% v/v normal goat serum + 0.02% v/v Triton-100 + primary antibody in PBS. Final concentrations for each primary antibody: 0.4% v/v for ER α antibody (Santa Cruz Biotechnology, sc-8005), 0.1% v/v for PR antibody (abcam ab101688). Next, slides were washed in PBS shaking at 100 rpm three times to remove unbound antibody. Next, slides were incubated for 2 hours at room temperature in 1% v/v normal goat serum + 0.02% v/v Triton-100 + 0.2% v/v secondary antibody (Jackson ImmunoResearch 115-606-003 for ER α immunostaining, Jackson ImmunoResearch 111-606-003 for PR immunostaining) in PBS. Slides were washed once with PBS shaking at 100 rpm, then incubated at room temperature for 15 minutes in 0.1% DAPI (ThermoFisher D1306) in PBS. After 3 more 10-minute washes in PBS shaking at 100 rpm, a glass coverslip was placed covering all the sections and sealed in place with Fluoromount-G (ThermoFisher 00-4958-02). Slides were kept at 4 °C and protected from light before imaging.

RNAscope in situ hybridization

Single-molecule fluorescent *in situ* hybridization was used to label *Esr2* according to manufacturer instructions in the RNAscope Multiplex Fluorescent Detection Kit version 2 (Advanced Cell Diagnostics 323100), with minor modifications. Briefly, sections were mounted onto glass Superfrost Plus slides (Fisher Scientific 12-550-15), baked for 30 minutes at 65 °C, fixed in 10% neutral buffered formalin (Millipore Sigma HT501128) in PBS for 20 minutes at 4 °C, dehydrated with 3-minute incubations in 50%, 70%, 95%, then 100% ethanol at room temperature, air dried for 20 minutes at room temperature, treated with RNAscope hydrogen peroxide (Advanced Cell Diagnostics 3223345) for 10 minutes at room temperature, heated to 95 °C for 5 minutes in RNAscope target retrieval reagent (Advanced Cell Diagnostics 322000), then treated with RNAscope protease III (Advanced Cell Diagnostics 322337) for 17 minutes at 40 °C. Next, probes targeting *Esr2* (Advanced Cell Diagnostics 316121-C3) were added to hybridize with their mRNA target (2 hours at 40 °C). Following probe hybridization, the signal was amplified via 15-30 minute incubations in Amp 1, Amp 2, and Amp 3 reagents at 40 °C, developed using horseradish peroxidase (15 minutes at 40 °C), and fluorescently labeled using Opal 690 (Akoya Biosciences FP1497001KT, 0.25% v/v final concentration) for 30 minutes at 40 °C. Finally, the sections were counterstained with RNAscope DAPI for 30 seconds at room temperature, covered with a glass coverslip, and sealed using ProLong Gold Antifade mounting media (ThermoFisher P36930). Slides were kept at 4 °C and protected from light before imaging.

Microscopy

An upright light microscope was used for vaginal cytology imaging. A Nikon ECLIPSE Ti2 widefield inverted fluorescence microscope was used for all other microscopy. For quantitative analysis of intensity or cell counts, images were acquired using a 20X objective. For qualitative analysis of stereotaxic targeting (virus/cannula/lens location), images were acquired using a 4X objective.

Image quantification

CellProfiler analysis (Sham vs OVX ER α expression):

Single-frame images from each side of the preoptic area were acquired and segmented into nuclei, based on the DAPI signal, using the Otsu method with three classes and a global threshold strategy. For each putative nucleus, the mean pixel intensity of the ER α signal was measured and classified as ER α - or ER α +, using a global threshold that was set to the background signal intensity of each image.

QUINT spatial quantification (ER α knockout validation and PR expression):

Multi-frame stitched images containing both hemispheres of the preoptic area and mediobasal hypothalamus were rigidly aligned to the Allen Brain Atlas in QuickNII, using anatomical features, visualized by DAPI stain, as landmarks. Next, non-rigid transformations were applied to the alignment in VisuAlign. The immunoreactivity signal (ER α or PR) was then segmented into putative nuclei in Ilastik. Briefly, we used the pixel classification module, followed by the object classification module, with manual annotations to distinguish positively labeled cells from background signal, and non-specific signal. Finally, the segmentation and alignment data were analyzed with Nutil.

For ER α knockout validation in the MPO ER α -KO experiment, spatial quantification data were excluded for 5/22 mice (n=1 control, 4 KO mice) due to staining artifacts from incomplete perfusion. Their temperature data were retained, however, as MPO viral targeting and reduced ER α expression could be confirmed by a qualitative visual inspection.

RNAscope quantification (ER β knockout validation):

Esr2⁺ cells from one hemisphere were manually counted in the MPO and BST using the Cell Counter plugin in ImageJ. Both regions were defined based on the Allen Brain Atlas. Each cell nucleus, labeled by the DAPI counterstain, was considered and marked *Esr2*⁺ if it met all of the following criteria: 1) the nucleus is round in shape and in focus (irregular shapes or out-of-focus nuclei were not considered), 2) 7 or more individual puncta are visible within the nucleus OR one or more extraordinarily bright puncta was visible within the nucleus (indicating overlapping puncta). All counts were performed by a single experimenter.

Quantification and statistical analysis

Statistical details, including n, what n represents, and the test used, can be found in the figure legends. Further details, including exact p-values can be found in Sheet S1. We recommend using the openxlsx package in R to programmatically access source data, since navigating the many tabs of Sheet S1 in Excel is difficult.

All statistical analysis was performed in R (version 4.3.2). For independent between-subjects comparisons, the `t_test()` and `anova_test()` functions from `rstatix` (version 0.7.2) were used. For analyses with a mix of within-subjects and between-subjects comparisons, a linear mixed effects model (`lme()` function from `nlme` version 3.1-163) was used for normally distributed data, while a generalized linear mixed effects model (`glmer()` function from `lme4` version 2.0-6) was used for non-normally distributed data. To accommodate the non-independence of repeated observations within the same subject, animal ID was included as a random effect in the model (intercept-only; 1 | mouse_id). `lme()` models were analyzed using `anova()` from the base R stats package, and `glmer()` models were analyzed using `Anova()` function from `car` version 3.1-2. For analyses with >2 groups, pair-wise comparisons were conditionally performed following a main effect among all groups. The Holm method was used to adjust p-values for multiple comparisons. For all figures, ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

Additional Resources

Full protocols are available upon email request to the lead contact.

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-Estrogen Receptor alpha antibody	Santa Cruz Biotechnology	Cat# sc-8005; RRID:AB_627556

Anti-Progesterone Receptor antibody [SP42]	Abcam	Cat# ab101688; RRID:AB_10715248
Anti-mouse secondary antibody (Alexa Fluor® 647 AffiniPure® F(ab') ₂ Fragment Goat Anti-Mouse IgG (H+L))	Jackson ImmunoReserch	Cat# 115-606-003; RRID:AB_2338921
Anti-rabbit secondary antibody (Alexa Fluor® 647 AffiniPure® F(ab') ₂ Fragment Goat Anti-Rabbit IgG (H+L))	Jackson ImmunoReserch	Cat# 111-606-003; RRID:AB_2338079
Bacterial and virus strains		
AAV1-pGP-Syn-FLEX-jGCaMP8m-WPRE (1.7 * 10 ¹³ GC/mL)	Addgene (gift from GENIE Project). Zhang <i>et al.</i> ²²¹	Cat# 162378-AAV1; RRID:Addgene_162378
rAAV2/hysn-eGFP (6.1*10 ¹² virus molecules/mL)	UNC Vector Core	N/A
rAAV2/hysn-GFP-Cre (2.5*10 ¹² virus molecules/mL)	UNC Vector Core	N/A
Chemicals, peptides, and recombinant proteins		
aCSF	Tocris	Cat# 3525
17β-estradiol (E2) pellets (0.5mg, 21-day release)	Innovative Research of America	Cat# E-121
Placebo (Vehicle) for 17β-estradiol (E2) pellets (0.5mg, 21-day release)	Innovative Research of America	Cat# C-111
17β-Estradiol (cyclodextrin encapsulated for water solubility)	Millipore Sigma	Cat# E4389
Cyclodextrin (control for water-soluble 17β-Estradiol)	Millipore Sigma	Cat# C0926
Estradiol benzoate (EB)	Millipore Sigma	Cat# E8515
DMSO	Millipore Sigma	Cat# D8418
Corn oil	Fisher Scientific	Cat# S25271
Normal goat serum	Abcam	Cat# ab7481
Normal goat serum	ThermoFisher	Cat# 01-6201
DAPI (4',6-diamidino-2-phenylindole, dihydrochloride)	ThermoFisher	Cat# D1306
RNAscope kit (RNAscope Multiplex Fluorescent Detection Kit version 2)	Advanced Cell Diagnostics	Cat# 323100
RNAscope Multiplex Fluorescent Detection Reagents v2	Advanced Cell Diagnostics	Cat# 323110
RNAscope Probe Diluent	Advanced Cell Diagnostics	Cat# 300041

<i>Esr2</i> RNAscope probe (“Mm-Esr2-C3”)	Advanced Cell Diagnostics	Cat# 316121-C3
RNAscope Target Retrieval reagent (10x)	Advanced Cell Diagnostics	Cat# 322000
Fluorophores for RNAscope (Opal 690)	Akoya Biosciences	Cat# FP1497001KT
RNAscope DAPI	Advanced Cell Diagnostics	Cat# 320858
RNAscope Wash Buffer (50x)	Advanced Cell Diagnostics	Cat# 320058
RNAscope Hydrogen Peroxide	Advanced Cell Diagnostics	Cat# 3223345
RNAscope Protease III	Advanced Cell Diagnostics	Cat# 322337
Formalin Solution, neutral buffered 10%	Millipore Sigma	Cat# HT501128
UltraPure 20x SSC Buffer	ThermoFisher	Cat# 15557036
Cyanoacrylate glue (Gorilla Super Glue Gel)	Gorilla	N/A
Dental cement powder (Contemporary Ortho-Jet)	Lang Dental	Cat# 1520BLK
Dental cement liquid (Contemporary Ortho-Jet)	Lang Dental	Cat# 1503BLK
GLUture	Zoetis	Cat# 10014489
Fluoromount-G	ThermoFisher	Cat# 00-4958-02
ProLong Gold Antifade mounting media	ThermoFisher	Cat# P36930
Deposited data		
Calcium imaging data	Dryad	doi.org/10.5061/dryad.mw6m906d3
Experimental models: Organisms/strains		
C57BL/6J mice	The Jackson Laboratory	Strain #:000664; RRID:IMSR_JAX:000664
<i>Esr1-Cre</i> mice (<i>Esr1</i> ^{tm2.1(cre)And} /J)	The Jackson Laboratory	Strain #:017913; RRID:IMSR_JAX:017913
<i>Esr1 flox</i> mice (<i>Esr1</i> ^{tm1Sakh} , JAX MGI ID 4459300)	N/A	JAX MGI ID 4459300
<i>Esr2 flox</i> mice (C57BL/6N- <i>Esr2</i> ^{tm1Ksk} /J)	The Jackson Laboratory	Strain #:032174; RRID:IMSR_JAX:032174
<i>Nkx2.1-Cre</i> mice (C57BL/6J-Tg(<i>Nkx2-1-cre</i>)2Sand/J)	The Jackson Laboratory	Strain #:008661; RRID:IMSR_JAX:008661
Software and algorithms		
VitalView (v5.1, 6.0)	STARR Life Sciences	N/A

CubiSens TS100 GUI	CubeWorks	N/A
Miniscope DAQ Software v1.10	https://github.com/Aharoni-Lab/Miniscope-DAQ-QT-Software	N/A
R (v4.3.2)	https://www.r-project.org/	N/A
ImageJ	https://fiji.sc/	N/A
CellProfiler	https://cellprofiler.org/	N/A
QuickNii	https://www.nitrc.org/projects/quicknii	N/A
VisuAlign	https://www.nitrc.org/projects/visualign/	N/A
Ilastik	https://www.ilastik.org/	N/A
Nutil	https://www.nitrc.org/projects/nutil	N/A
Code to process calcium imaging videos	https://github.com/pbvander/PV_minian	N/A
Code to analyze output from calcium imaging video processing	https://github.com/pbvander/minian_output_analysis	N/A
MATLAB (R2023a)	MathWorks	N/A
Other		
GRIN lens (0.6 mm diameter x 7.3 mm length)	Inscopix	Cat# 1050-004597
Core body temperature probes (G2 E-Mitter)	STARR Life Sciences	N/A
Core body temperature probes (TS100)	CubeWorks	N/A
Telemetry pads (ER-4000/ER-4500)	STARR Life Sciences	N/A
Cubisens Gateway	CubeWorks	N/A
Miniscope V4.4	Open ephys	Cat# OEPS-7407
Miniscope V4 base plate (variant 2)	Open ephys	Cat# OEPS-7416
Miniscope DAQ v3.3	Open ephys	Cat# OEPS-7431
Miniscope -> commutator coaxial cable	NeuroTek	Cat# COAX-CBL-3FT-NT
Commutator -> DAQ coaxial cable	DigiKey	Cat# 744-1329-ND
DAQ -> Computer cable	Open ephys	Cat# OEPS-5110

Miniscope V4 dummy (3D printed)	https://github.com/Aharoni-Lab/Miniscope-v4/blob/master/Miniscope-v4-Body-Parts/Parts%20v4_2/Miniscope_v4_Dummy.step	N/A
Commutator for Miniscope	NeuroTek	Cat# CMTR-2-NM-UCLA-NT
Superfrost Plus slides	Fisher Scientific	Cat# 12-550-15

Main Figures

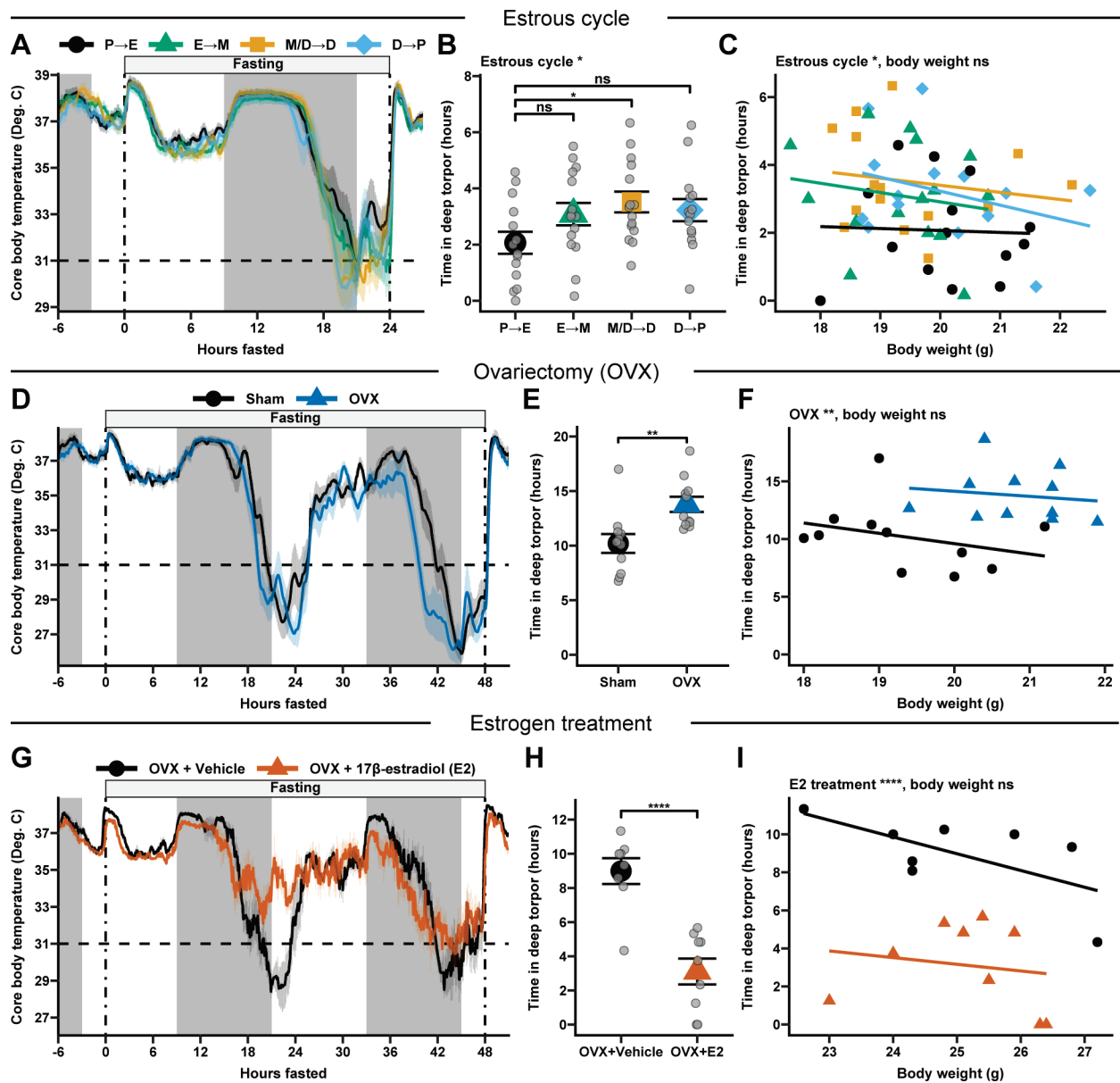


Figure 1: Circulating estrogens attenuate torpor

A, D, G) Core body temperature during fasting-induced torpor. Dark rectangles indicate lights off in the vivarium in all figures.

B, E, F) Time in deep torpor (core body temperature <31 °C).

C, F, I) Time in deep torpor, with starting body weight as a covariate.

All data are presented as mean $\pm$ SEM. A-C: n=14-15 trials per group, collected from 39 total mice (1-2 trials per mouse). D-F: n=11 mice per group. G-I: n=8-9 mice per group. B, C: linear mixed effects model (Holm correction for multiple post-hoc comparisons). E, H: unpaired t-test. F, I: ANCOVA. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

See also Fig. S1-S3

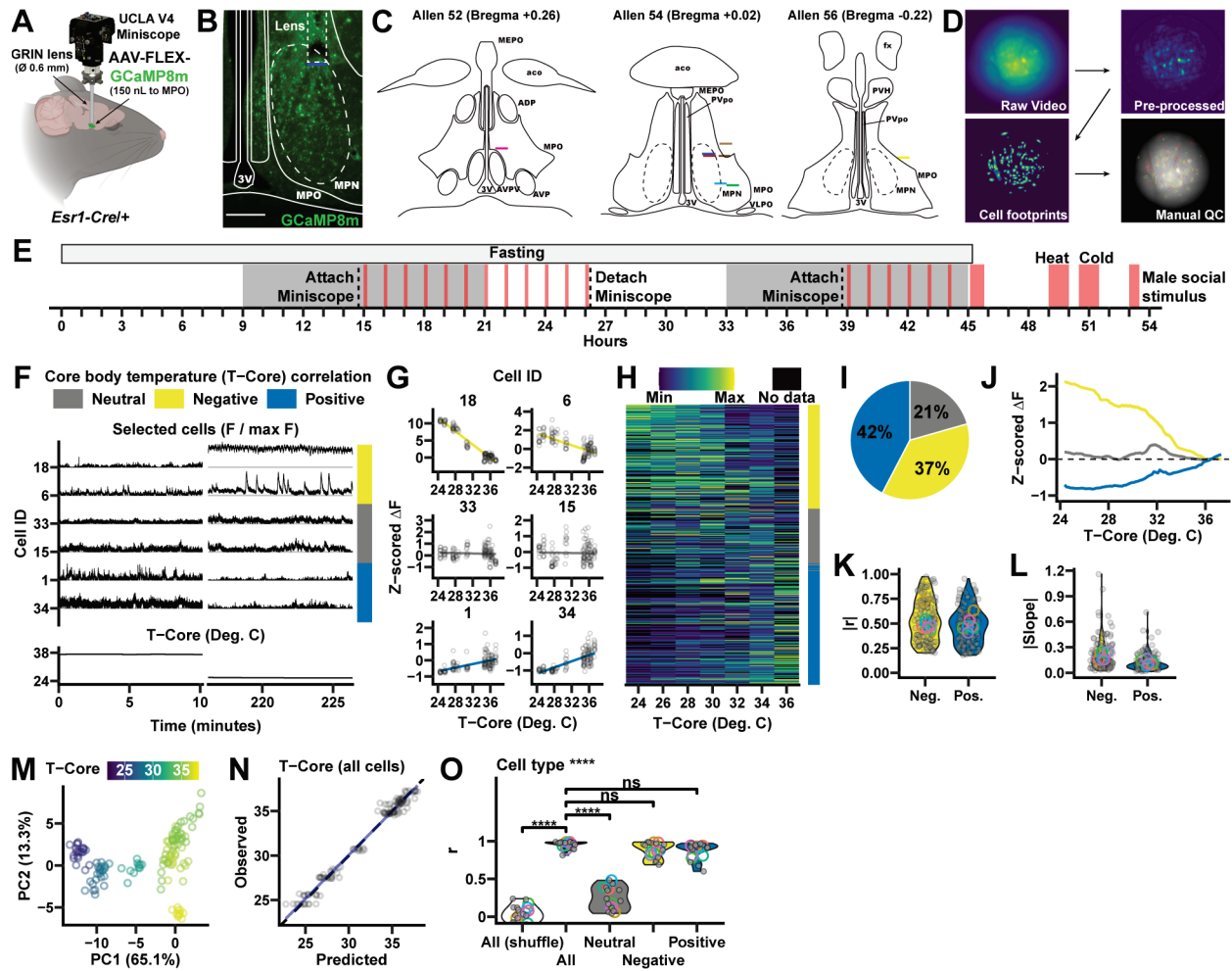


Figure 2: Heterogeneous cellular responses within the MPO^{ERα} population encode core body temperature during torpor

A) Schematic depicting experimental set-up for *in vivo* microendoscopic calcium imaging of MPO^{ERα} neurons.

B) Selected brain section with GCaMP8m expression in the MPO and GRIN lens aimed at the MPO. Scale bar 200 μ m.

C) Schematic depicting stereotaxic targeting of GRIN lens for each animal.

D) Schematic overview of the processing pipeline for Miniscope videos.

E) Experimental timeline. Red rectangles indicate imaging sessions.

- F) Scaled calcium activity data for selected cells across two 10-minute imaging sessions between 15-19 hours of fasting (top) and core body temperature (bottom).
- G) Z-scored and temporally binned data for the same set of selected cells in panel F across all 11 10-minute imaging sessions between 15-26 hours of fasting.
- H) Heatmap of responses from all cells and all mice across T-Core values (normalized to minimum and maximum values for each cell). Each row represents a single cell, and rows are sorted by Pearson's correlation coefficient with T-Core. Labels to the right of the heatmap indicate the T-Core correlation type of each cell.
- I) Fractional abundance of each functional cell type across all mice.
- J) Z-scored ΔF activity for each functional cell type population across T-Core values (smoothed by a moving average window of 3 °C).
- K) Strength of Z-scored ΔF – T-Core correlation (absolute value of Pearson's correlation coefficient, $|r|$) for individual cells.
- L) Magnitude of Z-scored ΔF – T-Core correlation (absolute value of slope) for individual cells.
- M) Representative principal component analysis of all cells from day 1 recordings (15-26 hours fasting) for one mouse.
- N) Predicted T-Core from a linear model trained on all cells (same input data as panel M).
- O) Pearson's correlation coefficient (r) between observed and predicted T-Core values using linear regression models trained on all cells, shuffled data, or just one functional cell type.

Data in F is shown as raw F values, with each cell trace normalized to its maximum value across the entire recording day. Data in J is presented as mean $\pm$ SEM, using a moving average to smooth across T-Core values (window width = 3 °C). Data in K-L: small grey dots represent individual cells, larger colored dots represent per-animal means. Data in M: individual data points represent a single 1-minute timepoint. Data in N: individual data points in grey, black dashed line represents the line of identity ($y = x$), blue line indicates the linear regression fit. Data in O: small grey dots represent individual days of recording (1-2 per mouse), larger colored dots represent per-animal means. H-L: $n=399$ cells from 7 total mice. O: $n=13$ days of recording from 7 total mice (2 days from each mouse except 1 mouse, from which 1 day was excluded due to lack of torpor). O: linear mixed effects model, Holm correction for multiple post-hoc comparisons. ns $p \geq 0.05$, **** $p < 0.0001$.

See also Fig. S4-6

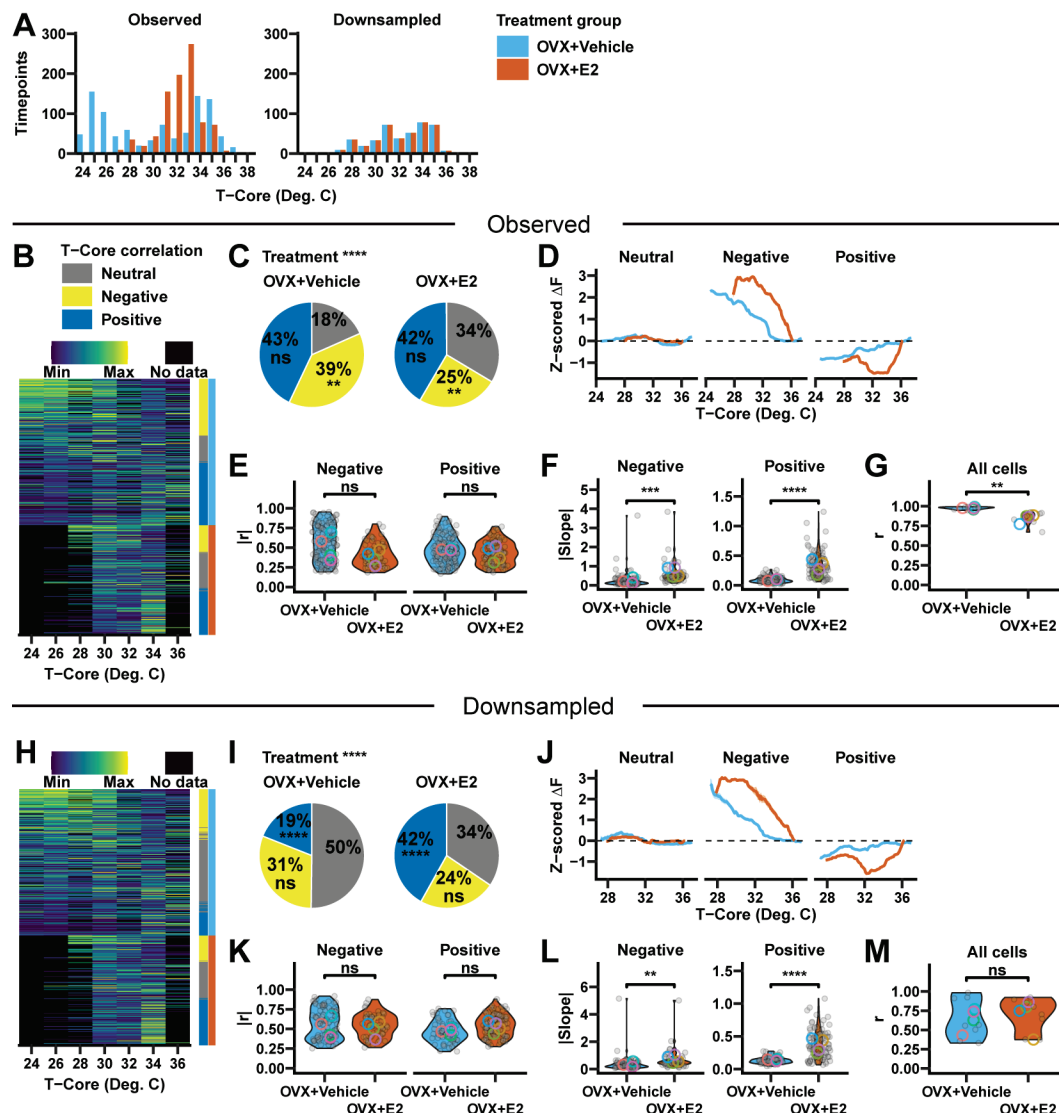


Figure 3: Circulating estrogens alter the tuning of MPO^{ERα} neurons during torpor

A) Number of 1-minute timepoints across T-Core values by group. Left: observed distribution. Right: distribution following downsampling procedure.

B-G) Analysis of the full observed dataset

H-M) Analysis of the downsampled data to equalize sampling frequency and range between groups.

B, H) Heatmap of responses from all cells and all mice across T-Core values (normalized to minimum and maximum values for each cell). Each row represents a single cell, sorted by its Pearson's correlation coefficient with T-Core. Labels to the right of the heatmap indicate the T-Core correlation direction of each cell and its treatment group. Data in B and H are identical except for the row order and the T-Core correlation labels, which changed as a result of the downsampling procedure. Representative data from a single downsampling iteration is shown in H.

C, I) Fractional abundance of each functional cell type across all mice.

D, J) Z-scored ΔF activity for each functional cell type population across T-Core values (smoothed by a moving average window of 3 °C). Representative data from a single downsampling iteration is shown in J.

E, K) Strength of Z-scored ΔF – T-Core correlation (absolute value of Pearson's correlation coefficient, $|r|$) for individual cells.

F, L) Magnitude of Z-scored ΔF – T-Core correlation (absolute value of slope) for individual cells.

G, M) Pearson's correlation coefficient (r) between observed and predicted T-Core values using linear regression models trained on all cells.

Data in A is presented as the sum across all animals. Data in D, J: mean $\pm$ SEM, using a moving average to smooth across T-Core values (window width = 3 °C). Data in E-F, K-L: small grey dots represent individual cells, larger colored dots represent per-animal means. Data in G, M: small grey dots represent individual days of recording (2 per mouse), larger colored dots represent per-animal means. B-F, H-L: $n=534$ cells (305

vehicle, 229 E2) from 8 total mice (4 vehicle, 4 E2). G, M: n=16 days of recording (8 vehicle, 8 E2) from 8 total mice (2 days of recording per mouse). C, I: Chi-squared test. Following a main effect of treatment, post-hoc tests for each cell type were performed by comparing the abundance of the target cell type across treatment groups while non-target cell types were pooled into a single category. E, G, K, M: linear mixed effects model. F, L: Gamma generalized linear mixed effects model with a log link function. C, E-F, I, K-L: Holm correction for multiple cell type comparisons. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

See also Fig. S7

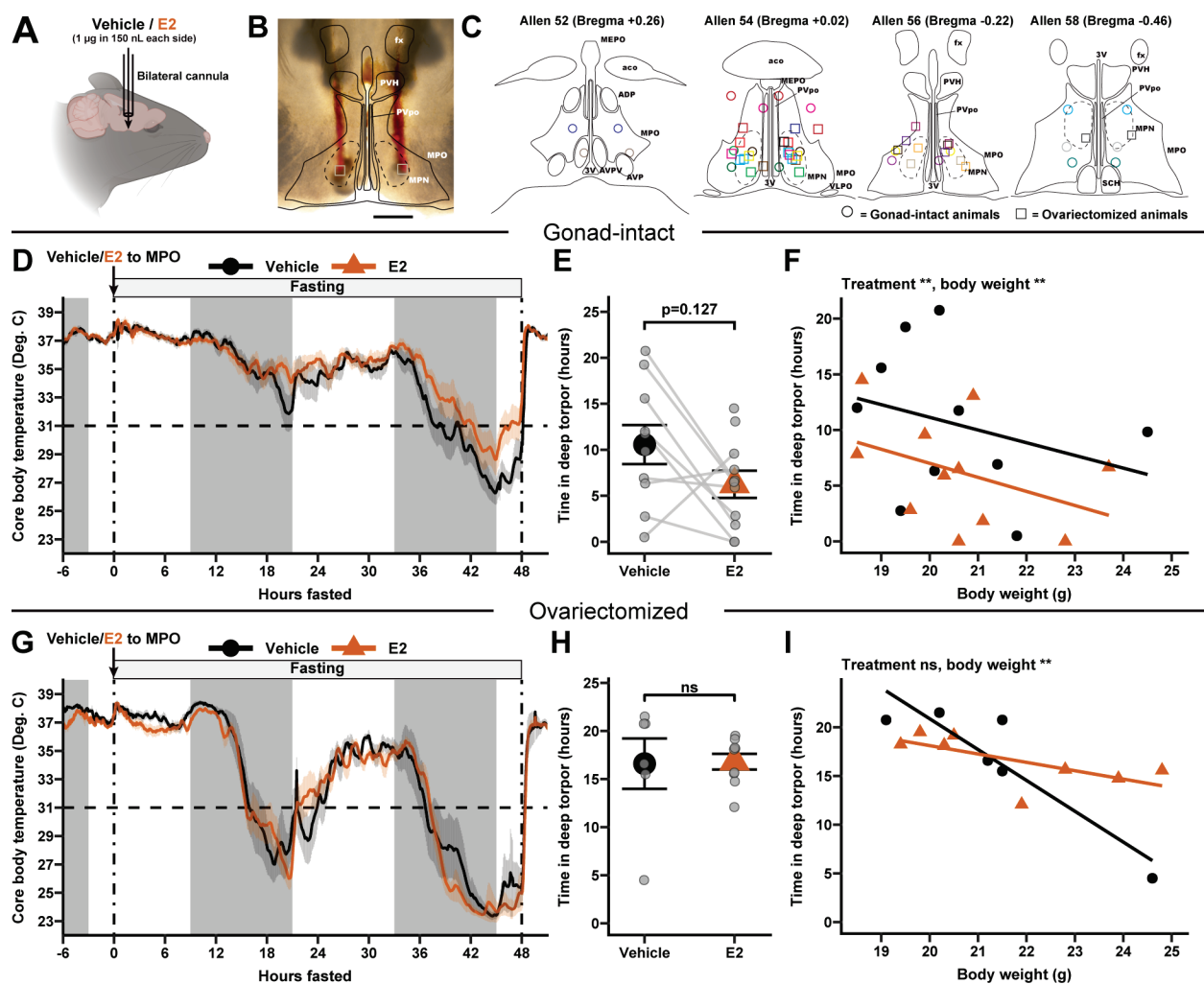


Figure 4: Estrogen signaling in the MPO attenuates torpor in gonad-intact, but not ovariectomized, female mice

A) Schematic depicting experimental set-up for injecting 17 β -estradiol (E2) or vehicle directly to the MPO.

B) Selected brain section showing cannula implantation site. Scale bar: 500 µm.

C) Schematic depicting stereotaxic targeting of cannula for each animal.

D, G) Core body temperature during fasting-induced torpor. Arrow depicts timing of injection to MPO (hours fasted = 0).

E, H) Time in deep torpor (core body temperature $<31^{\circ}\text{C}$).

F, I) Time in deep torpor, with starting body weight as a covariate.

All data are presented as mean $\pm$ SEM. Gray lines in E connect repeated measures from individual mice across trials. D-F: $n=10-11$ trials per group, collected from 12 total mice (1-2 trials per mouse). G-I: $n=6$ vehicle, 9 E2 mice. E, F: Linear mixed effects model.

H: unpaired t-test. I: ANCOVA. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$.

See also Fig. S8-S9

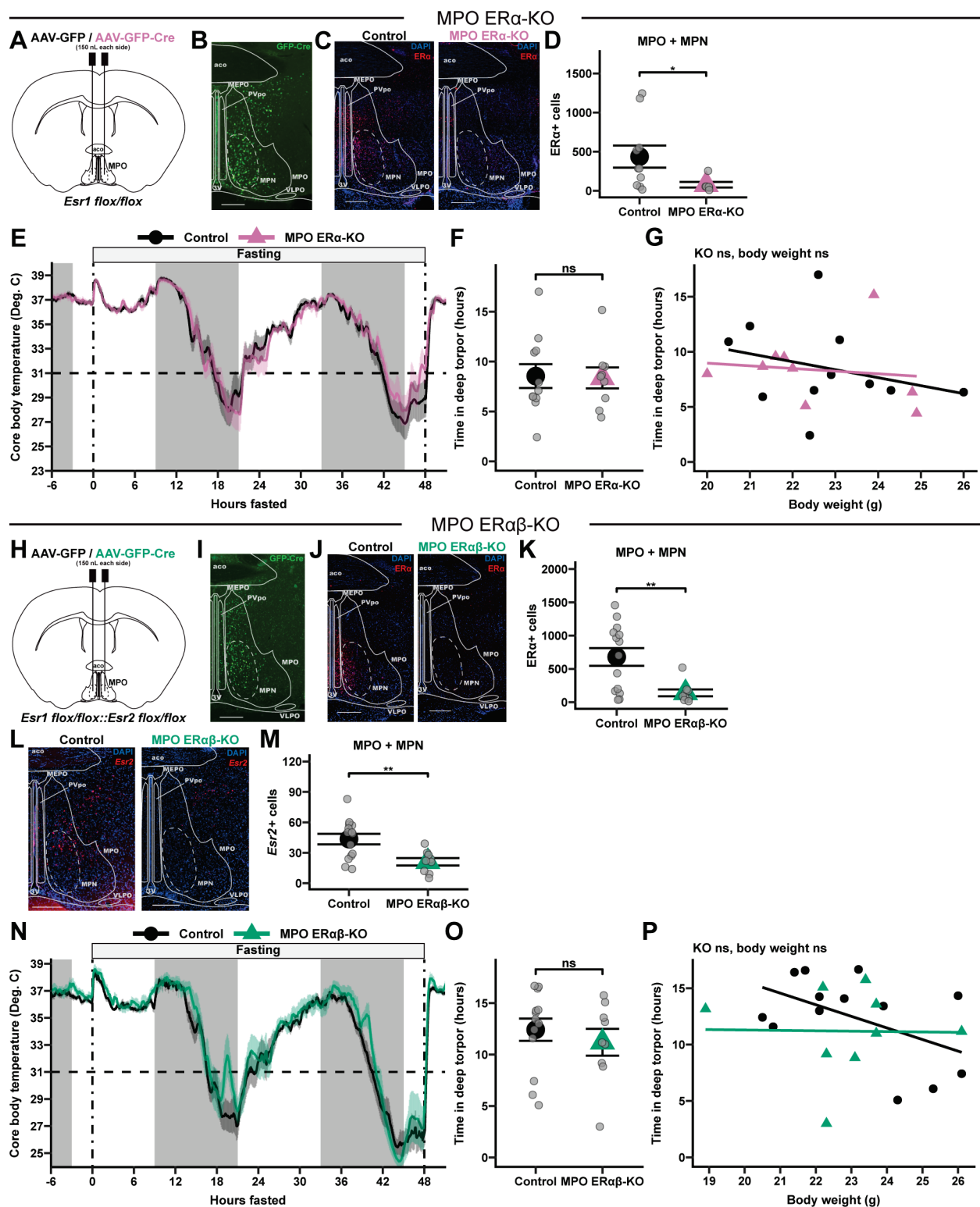


Figure 5: Estrogen receptors alpha (ER α) and beta (ER β) in the MPO are not required for the effects of endogenous E2 on torpor in gonad-intact female mice

A, H) Schematic depicting strategy to knock out estrogen receptors in the MPO

B, I) Selected brain section with GFP-Cre expression. Scale bar: 250 μ m.

C, J) Selected brain sections stained for DAPI and ER α . Scale bar: 250 μ m.

D, K) Quantification of ER α + cells detected in the MPO + MPN in control and KO animals.

E, N) Core body temperature during fasting-induced torpor

F, O) Time in deep torpor (core body temperature <31 °C).

G, P) Time in deep torpor, with starting body weight as a covariate.

L) Selected brain sections stained for DAPI and estrogen receptor beta mRNA (*Esr2*).
Scale bar: 250 μ m.

M) Quantification of *Esr2* cells detected in the MPO + MPN in control and KO animals.

All data are presented as mean +/- SEM. D: n=10 control, 6 KO mice. E-G: n=11 control, 9 KO mice. K, M: n=14 control, 9 KO mice. N-P: n=13 control, 9 KO mice. D, F, K, M, O: unpaired t-test. G, P: ANCOVA. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$

See also Fig. S10-S11

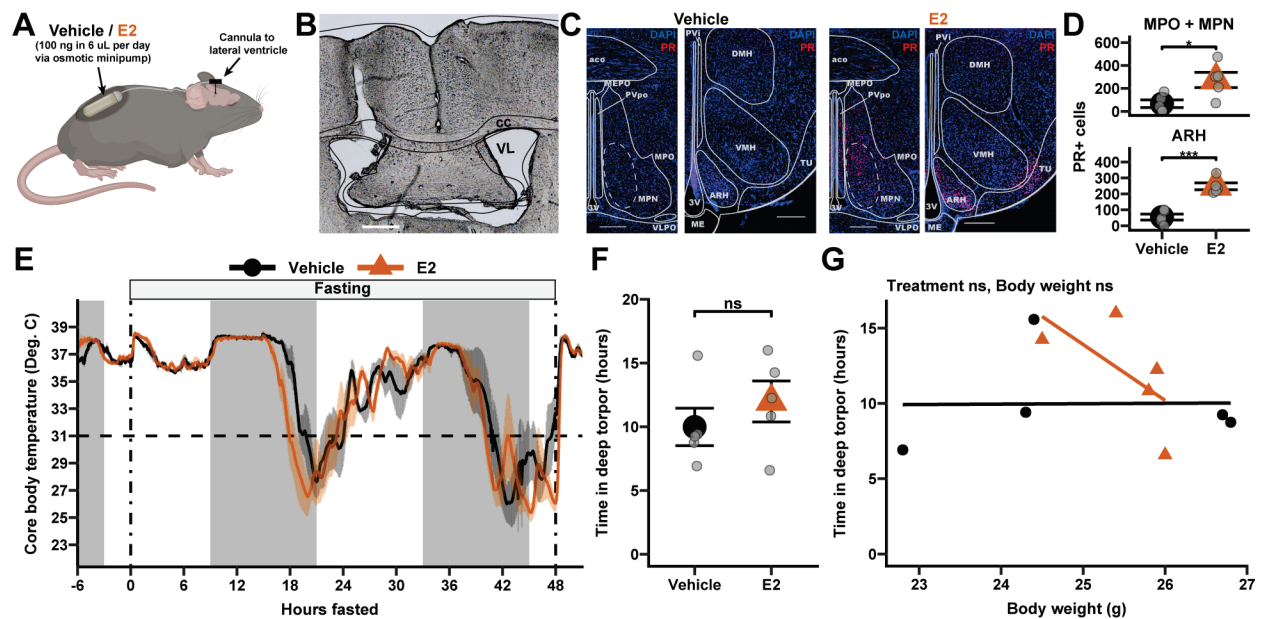


Figure 6: Estrogen signaling in the central nervous system is not sufficient to attenuate torpor in ovariectomized female mice

A) Schematic depicting experimental set-up to chronically deliver E2 to the whole central nervous system via the lateral ventricle (VL).

B) Selected brain section showing cannula implantation site. Scale bar 500 μ m.

C) Selected brain sections stained for DAPI and progesterone receptor (PR). Scale bar 250 μ m.

D) Summary of number of PR-expressing cells in select estrogen-sensitive brain regions.

E) Core body temperature during fasting-induced torpor

F) Time in deep torpor (core body temperature <31 $^{\circ}$ C).

G) Time in deep torpor, with starting body weight as a covariate.

All data are presented as mean $\pm$ SEM. D-G: n=5 mice per group. D: unpaired t-test with Holm correction for multiple comparisons. F: unpaired t-test. G: ANCOVA. ns $p \geq 0.05$, * $p < 0.05$, *** $p < 0.001$.

See also Fig. S12

Supplementary figures

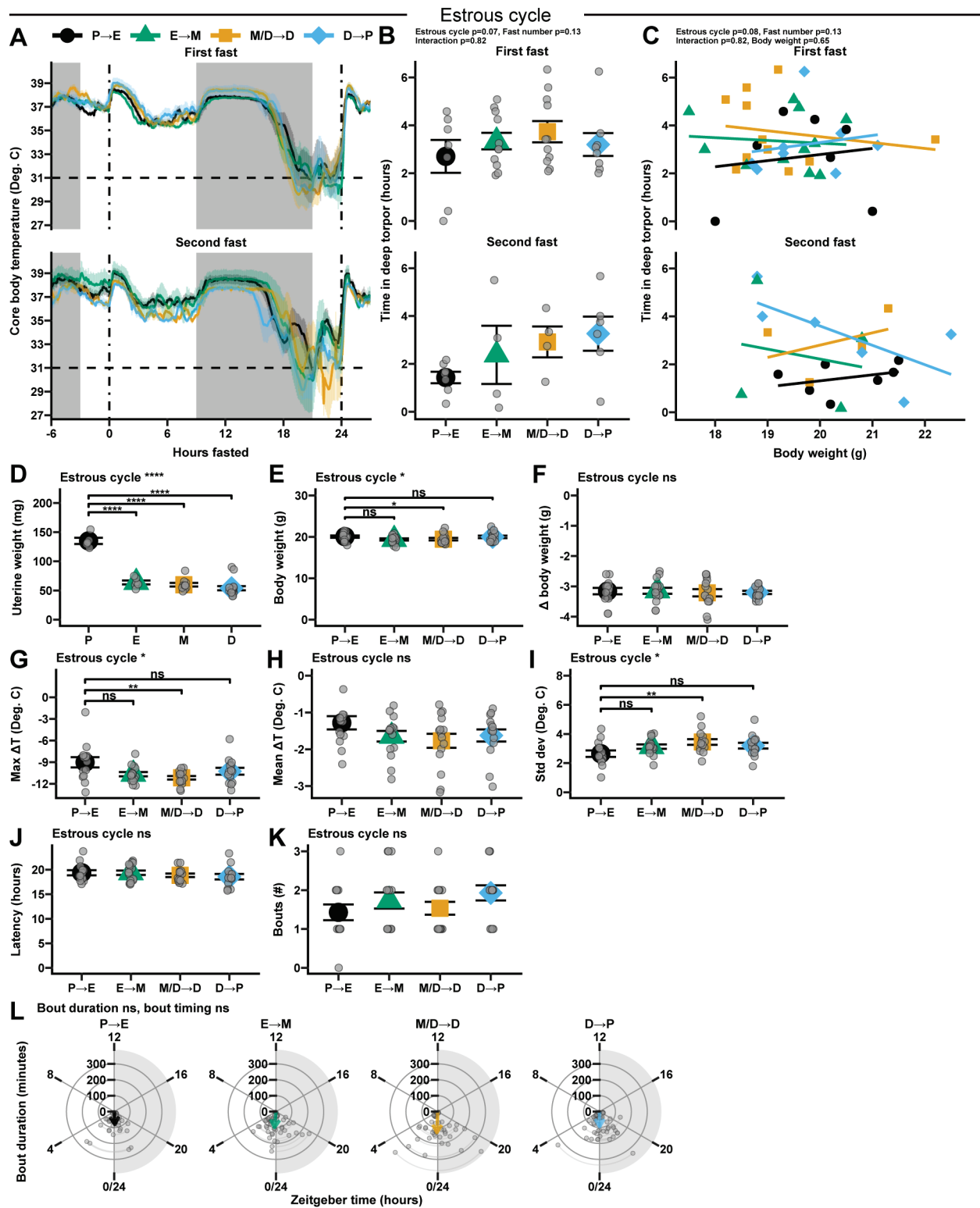


Figure S1 (related to Figure 1): Additional characterization of the effects of the estrous cycle on torpor

A-C) Data from Fig. 1A-C, separated by trial.

A) Core body temperature during fasting-induced torpor.

B) Time in deep torpor (core body temperature $<31^{\circ}\text{C}$).

C) Time in deep torpor, with starting body weight as a covariate.

D) Uterine weight measured at the end of the experiment

E) Body weight measured immediately before each fasting trial

F) Change in body weight across the fasting period

G) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)

H) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)

I) Temperature variability for each trial (standard deviation of temperature during fasting)

J) Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)

K) Number of deep torpor bouts per trial

L) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center of the circle represents the “mean bout”. The length of the arrow represents the mean

duration of all bouts, and the angle of the arrow represents the mean temporal midpoint of all bouts.

All data are shown as mean $\pm$ SEM. A-C, E-K: $n=14-15$ trials per group, collected from 39 total mice (1-2 trials per mouse). D: $n=5$ P, $n=6$ E, $n=10$ M, $n=16$ D mice. L: $n=20$ P $\rightarrow$ E, 26 E $\rightarrow$ M, 23 M/D $\rightarrow$ D, 27 D $\rightarrow$ P bouts, from a total of 38 mice (1 mouse had no bouts). B-C, E-L: linear mixed effects model (Holm correction for multiple comparisons). D: ANOVA for main effect, unpaired t-test for post-hoc pairwise comparisons (Holm correction for multiple comparisons). ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

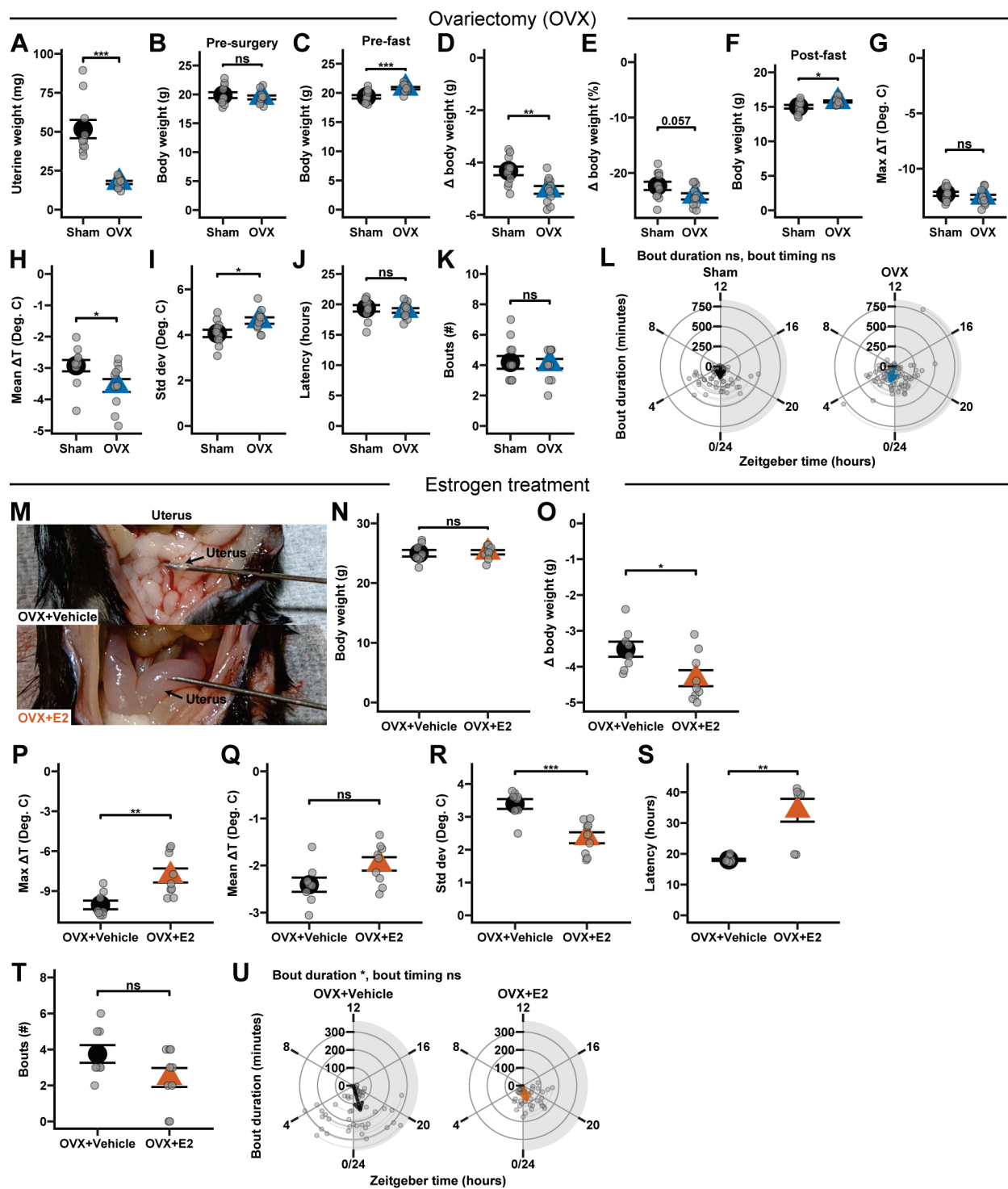


Figure S2 (related to Figure 1): Additional characterization of the effects of ovariectomy and estrogen treatment on torpor

A) Uterine weight measured at the end of the experiment

B) Body weight measured immediately before the ovariectomy procedure

C, N) Body weight measured immediately before fasting

D-E, O) Change in body weight across the fasting period. D and O show the change as an absolute value, while E shows the change relative to pre-fasting weight.

F) Body weight measured at the end of the 48-hour fasting period

G, P) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)

H, Q) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)

I, R) Temperature variability for each trial (standard deviation of temperature during fasting)

J, S) Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)

K, T) Number of deep torpor bouts per trial

L, U) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center of the circle represents the “mean bout”. The length of the arrow represents the mean duration of all bouts, and the angle of the arrow represents the mean temporal midpoint of all bouts.

M) Selected image showing uterine morphology in vehicle- and E2-treated mice.

All data are shown as mean $\pm$ SEM. A-K: n=11 mice per group. L: n=45-46 bouts per group from 22 total mice (each mouse had at least one bout). N-T: n=8-9 mice per group. U: n=30 vehicle, 22 E2 bouts from 15 total mice (2 E2 mice had no bouts). A-K, N-T: unpaired t-test. L, U: linear mixed effects model. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

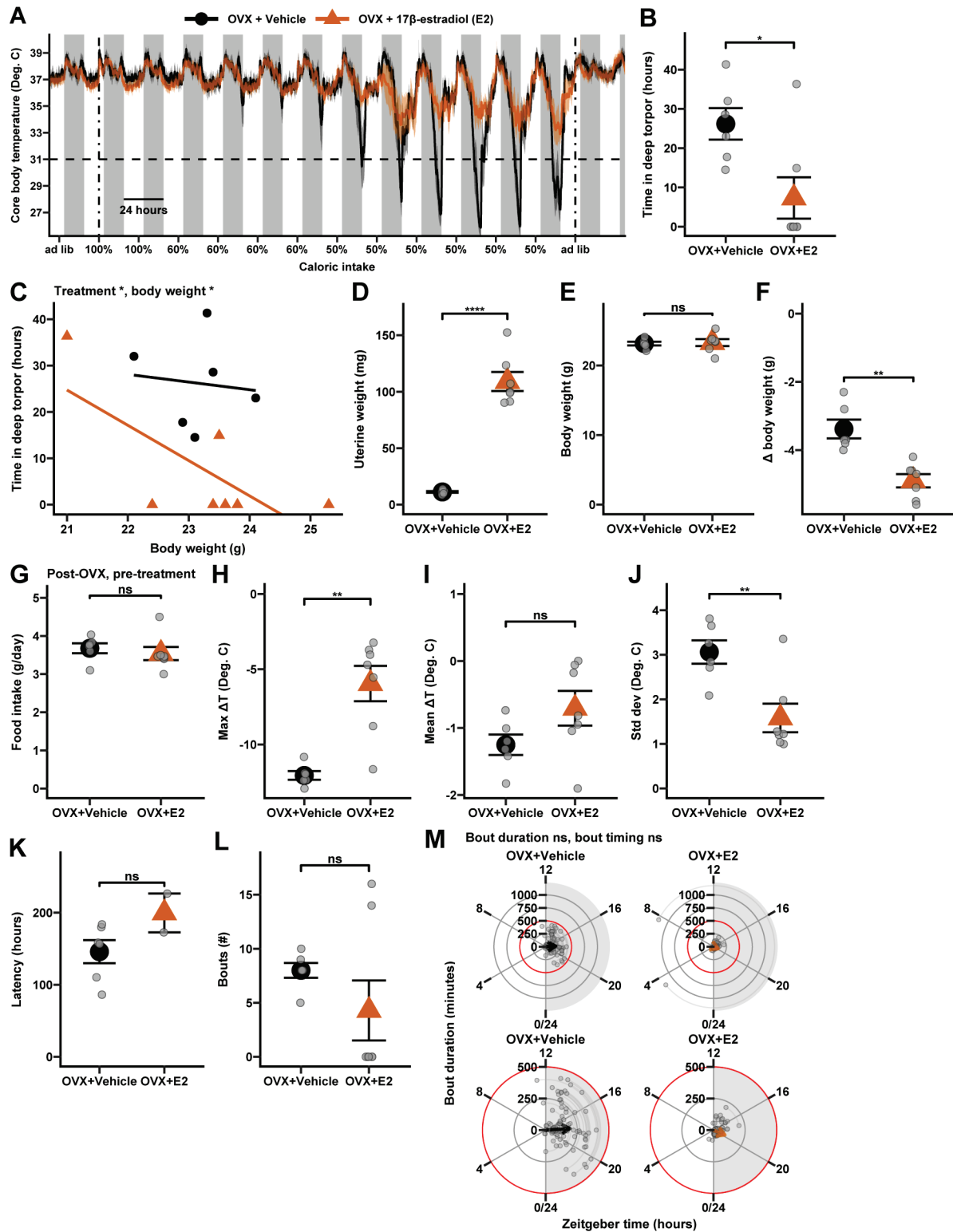


Figure S3 (related to Figure 1): Estrogen treatment attenuates torpor induced by calorie restriction

- A) Core body temperature during calorie restriction-induced torpor.
- B) Time in deep torpor (core body temperature $<31^{\circ}\text{C}$).
- C) Time in deep torpor, with starting body weight as a covariate.
- D) Uterine weight measured at the end of the experiment
- E) Body weight measured immediately before fasting
- F) Change in body weight across the fasting period.
- G) Food intake measured after ovariectomy (OVX), but before vehicle/E2 treatment.
- H) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)
- I) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)
- J) Temperature variability for each trial (standard deviation of temperature during fasting)
- K) Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)
- L) Number of deep torpor bouts per trial
- M) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center of the circle represents the “mean bout”. The length of the arrow represents the mean duration of all bouts, and the angle of the arrow represents the mean temporal midpoint

of all bouts. Top: plots showing full data range. Bottom: zoomed view of the same data, marked by a red circle in the top graph.

All data are shown as mean $\pm$ SEM. A-L: n=6-7 mice per group. M: n=48 vehicle, 30 E2 bouts from 8 total mice (5 E2 mice had no bouts). B, D-L: unpaired t-test. C: ANCOVA. M: linear mixed effects model. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

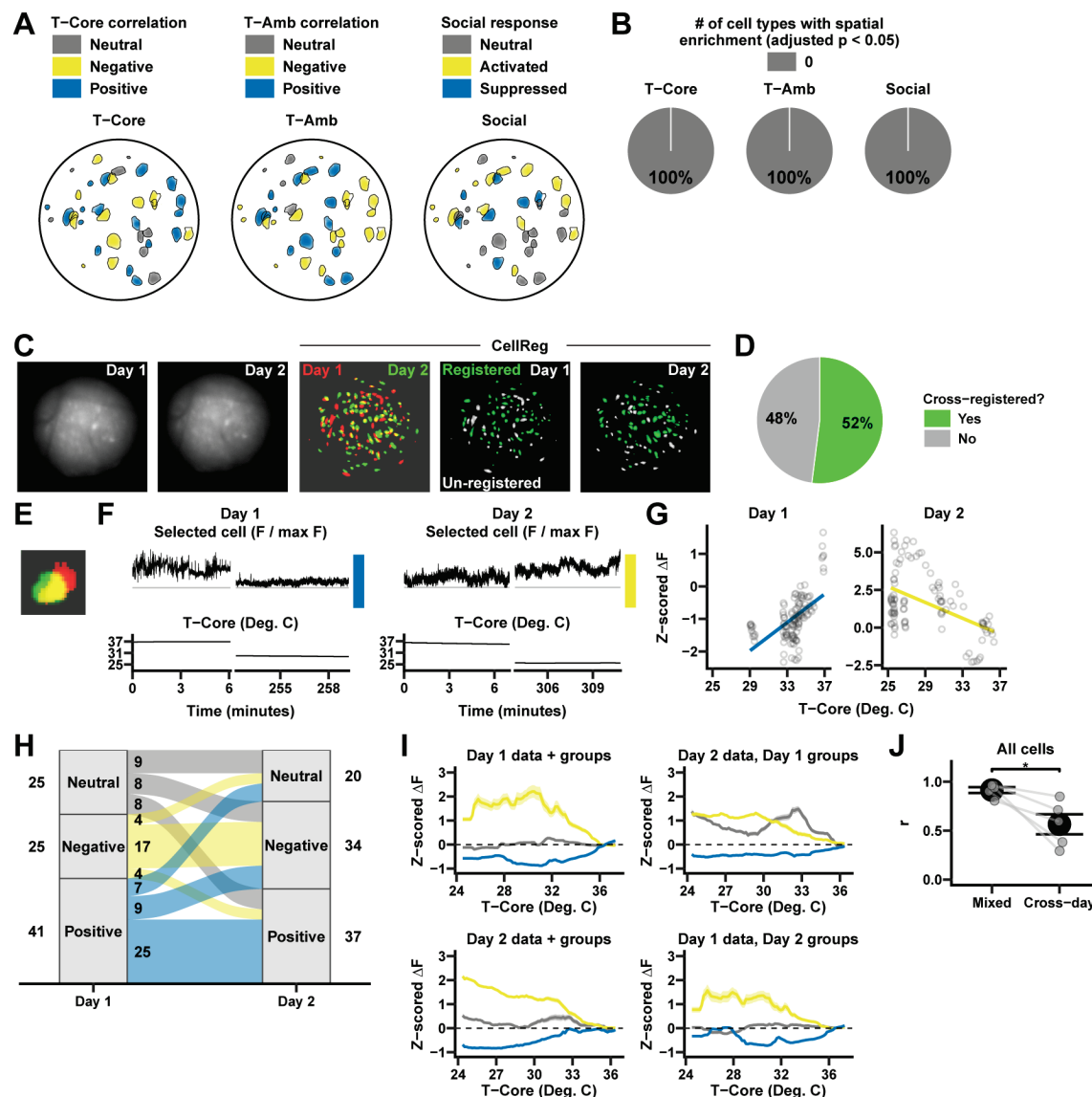


Figure S4 (related to Figure 2): Anatomically intermingled MPO^{ERα} subgroups undergo representational drift across days

A) Representative field of view from a single mouse on one day of recording across stimuli.

B) Spatial enrichment test results for all recording days.

- C) Schematic overview of the cross-registration procedure used to map cells across days of recording.
- D) Cellular cross-registration efficiency across all recordings.
- E) Spatial footprint of a single cell mapped across days (day 1 footprint in red, day 2 footprint in green).
- F) Scaled calcium activity data (top) and core body temperature (bottom) for the cell shown in panel D.
- G) Z-scored ΔF for the cell shown in panels E and F across all imaging sessions on day 1 and day 2.
- H) Group identity of all cross-registered cells on day 1 and day 2.
- I) Calcium activity across T-Core during torpor sessions for each day. Left: cell groups are defined by their correlation with T-Core during that day's recording. Right: cell groups are defined by their correlation with T-Core on the opposite day.
- J) Pearson's correlation coefficient between observed and predicted T-Core values using linear regression models trained on all cells, comparing mixed and day-separated train-test splits.

Data in I and J are shown as mean $\pm$ SEM. * $p < 0.05$. B: T-Core $n=12$ recording days from 7 mice (1-2 days per mouse, with 2 total days excluded for lack of torpor), T-Amb $n=5$ recording days from 5 mice, Social $n=6$ recording days from 6 mice. H-I: $n=91$ cells from 10 recording days (5 mice, 2 days per mouse). J: $n=5$ mice. B: Permutation test with Holm correction for multiple comparisons (see methods). J: paired t-test. * $p < 0.05$.

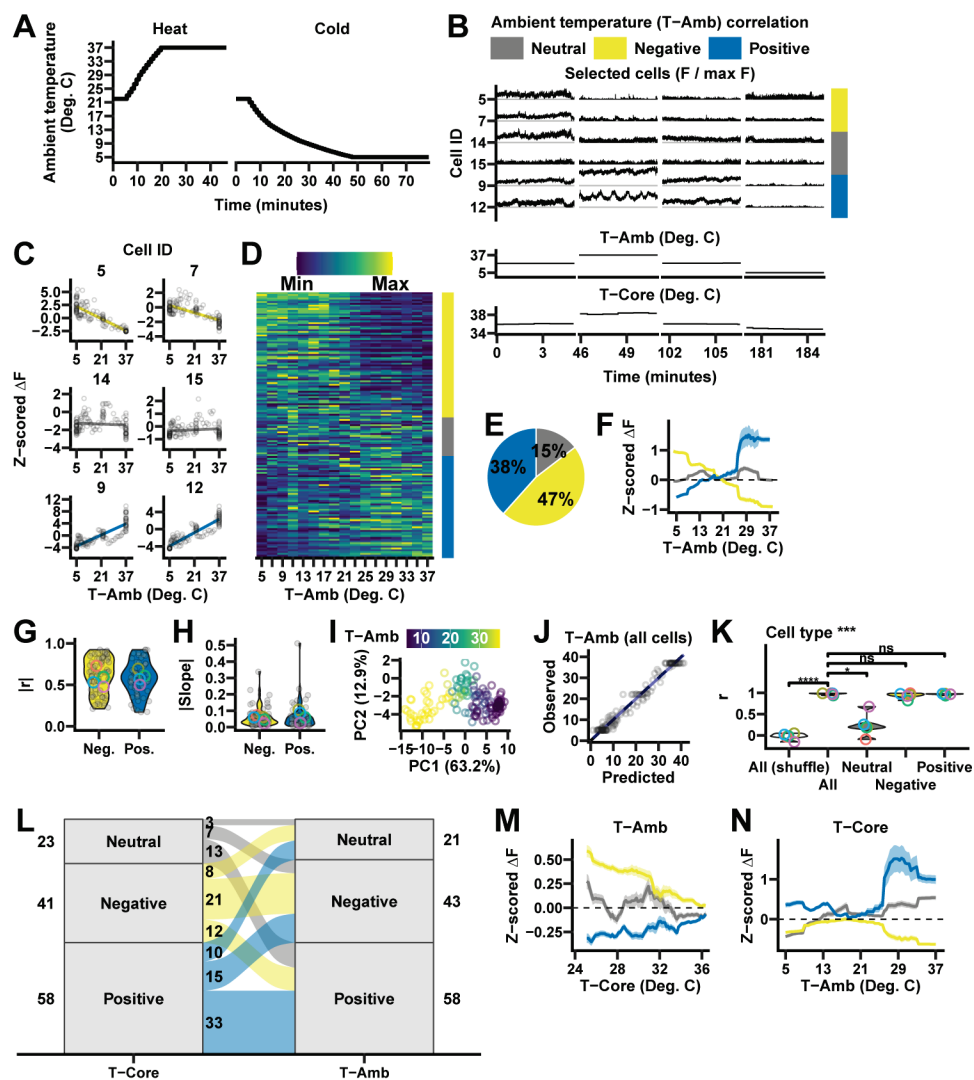


Figure S5 (related to Figure 3): MPO^{ERα} neurons encode ambient temperature through heterogeneous cellular responses that partially overlap with torpor representations

A) Ambient temperature during the heating and cooling sessions (representative data from a single mouse).

B) Scaled calcium activity data for selected cells in the first and last 5 minutes of heat and cold imaging sessions (top), ambient temperature (T-Amb, middle), and core body temperature (T-Core, bottom).

- C) Z-scored and temporally binned data for the same set of selected cells in panel B across the entire heat and cold imaging sessions.
- D) Heatmap of responses from all cells and all mice across T-Amb values (normalized to minimum and maximum values for each cell). Each row represents a single cell, and rows are sorted by Pearson's correlation coefficient with T-Amb. Labels to the right of the heatmap indicate the T-Amb correlation type of each cell.
- E) Fractional abundance of each functional cell type across all mice.
- F) Z-scored ΔF activity for each functional cell type across T-Amb values (smoothed by a moving average window of 8 °C).
- G) Strength of Z-scored ΔF – T-Amb correlation (absolute value of Pearson's correlation coefficient, $|r|$) for individual cells.
- H) Magnitude of Z-scored ΔF – T-Core correlation (absolute value of slope) for individual cells.
- I) Representative principal component analysis of all cells from heat and cold sessions for one mouse.
- J) Predicted T-Amb from a linear model trained on all cells (same input data as panel I).
- K) Pearson's correlation coefficient (r) between observed and predicted T-Core values using linear regression models trained on all cells, shuffled data, or just one functional cell type.
- L) Group identity of individual cells across torpor (T-Core) and heat/cold (T-Amb) sessions.
- M) Calcium activity across T-Core during torpor sessions for cell groups defined by their correlation with T-Amb during heat/cold sessions.

N) Converse of M. Calcium activity across T-Amb during heat/cold for cell groups defined by their correlation with T-Core during torpor sessions.

Data in B is shown as raw F values, with each cell trace normalized to its maximum value across each recording session. Data in F is presented as mean $\pm$ SEM, using a moving average to smooth across T-Core values (window width = 8 °C). Data in G-H: small grey dots represent individual cells, larger colored dots represent per-animal means. Data in J: individual data points in grey, black dashed line represents the line of identity ($y = x$), blue line indicates the linear regression fit. Data in K: each colored dot represents per-animal means. D-H: n=151 cells from 5 mice. K: n=5 mice per group. L-N: n=122 cells from 4 mice. K: Linear mixed effects model. Holm correction for multiple post-hoc pairwise comparisons. ns $p \geq 0.05$, * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

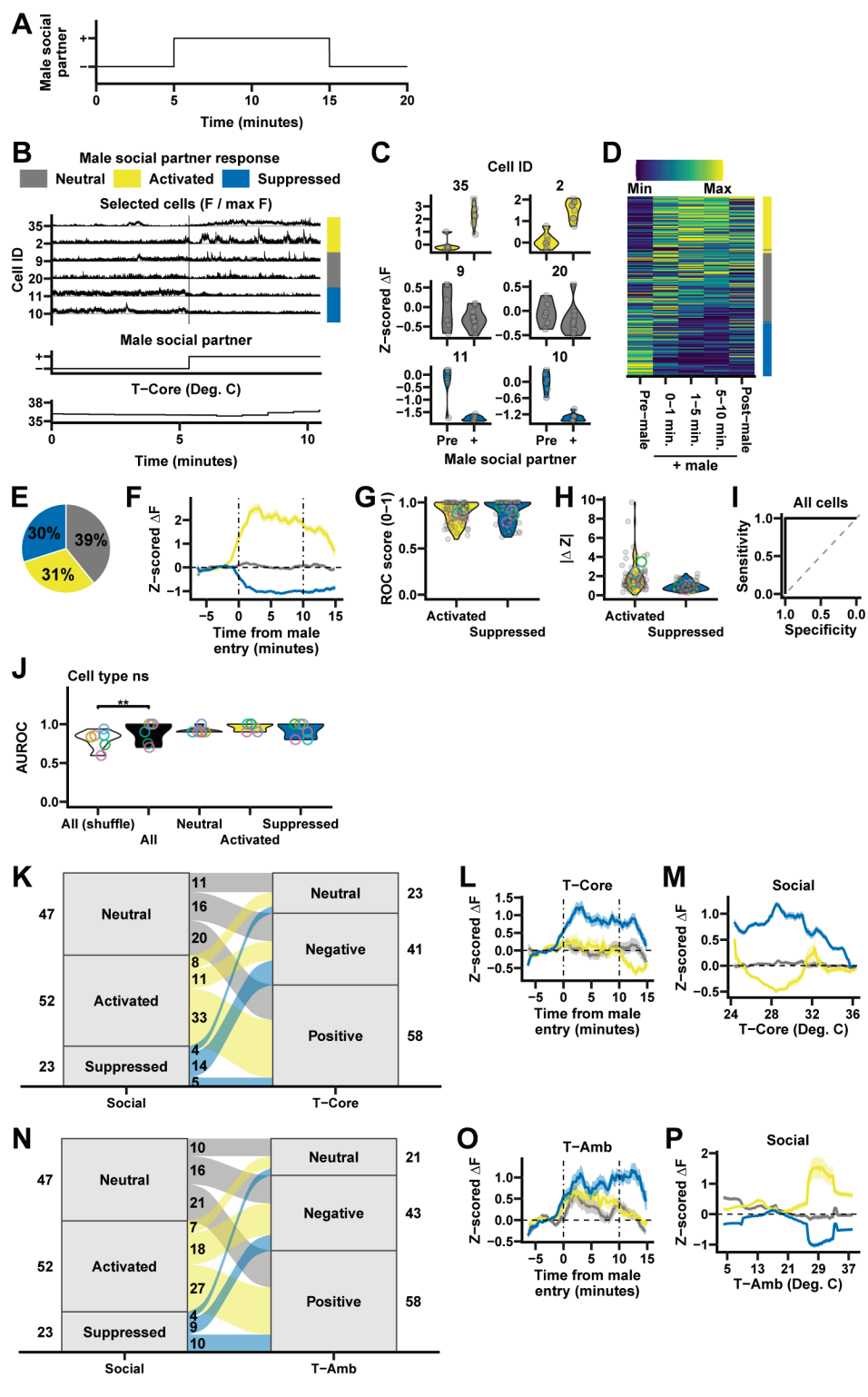


Figure S6 (related to Figure 3): Heterogeneous $MPO^{ER\alpha}$ responses encode a male social partner through an activity state that is inverse to torpor

- A) Schematic representation of timing for social recording sessions.
- B) Scaled calcium activity data for selected cells in the first 10 minutes of the social recording sessions (top), male social partner presence (middle), and core body temperature (T-Core, bottom).
- C) Z-scored and temporally binned data for the pre-male baseline and social interaction periods (same cells as panel B).
- D) Heatmap of responses from all cells and all mice across the social session (normalized to minimum and maximum values for each cell). Each row represents a single cell, and rows are sorted by AUROC value discriminating between the pre-male baseline and male social interaction period. Labels to the right of the heatmap indicate the response type of each cell.
- E) Fractional abundance of each functional cell type across all mice.
- F) Z-scored ΔF activity for each functional cell type across the social session (smoothed by a moving average window of 2 minutes). Vertical dashed lines at 0 and 10 represent male entry and male removal, respectively.
- G) Discriminability between pre-male baseline and male social interaction period ($2 * |0.5 - \text{AUROC}|$) for each cell.
- H) Response magnitude (difference in mean Z-scored ΔF) between pre-male baseline and male social interaction period for each cell.
- I) Representative ROC curve from a logistic regression model trained to decode pre-male baseline and social interaction period on all cells from one mouse.
- J) AUROC for logistic regression models trained on all cells, shuffled data, or just one functional cell type.

- K) Group identity of individual cells across social and torpor (T-Core) sessions.
- L) Calcium activity across social sessions for cell groups defined by their correlation with T-Core during torpor sessions.
- M) Converse of L. Calcium activity across T-Core during torpor sessions for cell groups defined by their response type during social sessions.
- N) Group identity of individual cells across social and heat/cold (T-Amb) sessions.
- O) Calcium activity across social sessions for cell groups defined by their correlation with T-Amb during heat/cold sessions.
- P) Converse of O. Calcium activity across T-Amb during heat/cold sessions for cell groups defined by their response type during social sessions.
- Q) Heatmap showing tuning across torpor, heat/cold, and social sessions. Each row is a cell, and rows are sorted by T-Core correlation values. Only cells with data for all stimuli are shown. T-Core/T-Amb: color represents r . Social: color represents $2 * (0.5 - \text{AUROC})$.

Data in B is shown as raw F values, with each cell trace normalized to its maximum value across each recording session. Data in F is presented as mean $\pm$ SEM, using a moving average to smooth across T-Core values (window width = 8 °C). Data in G-H: small grey dots represent individual cells, larger colored dots represent per-animal means. Data in J: each colored dot represents per-animal means. D-H: $n=194$ cells from 6 mice. J: $n=6$ mice per group. K-Q: $n=122$ cells from 4 mice. J: Linear mixed effects model. ns $p \geq 0.05$, ** $p < 0.01$.

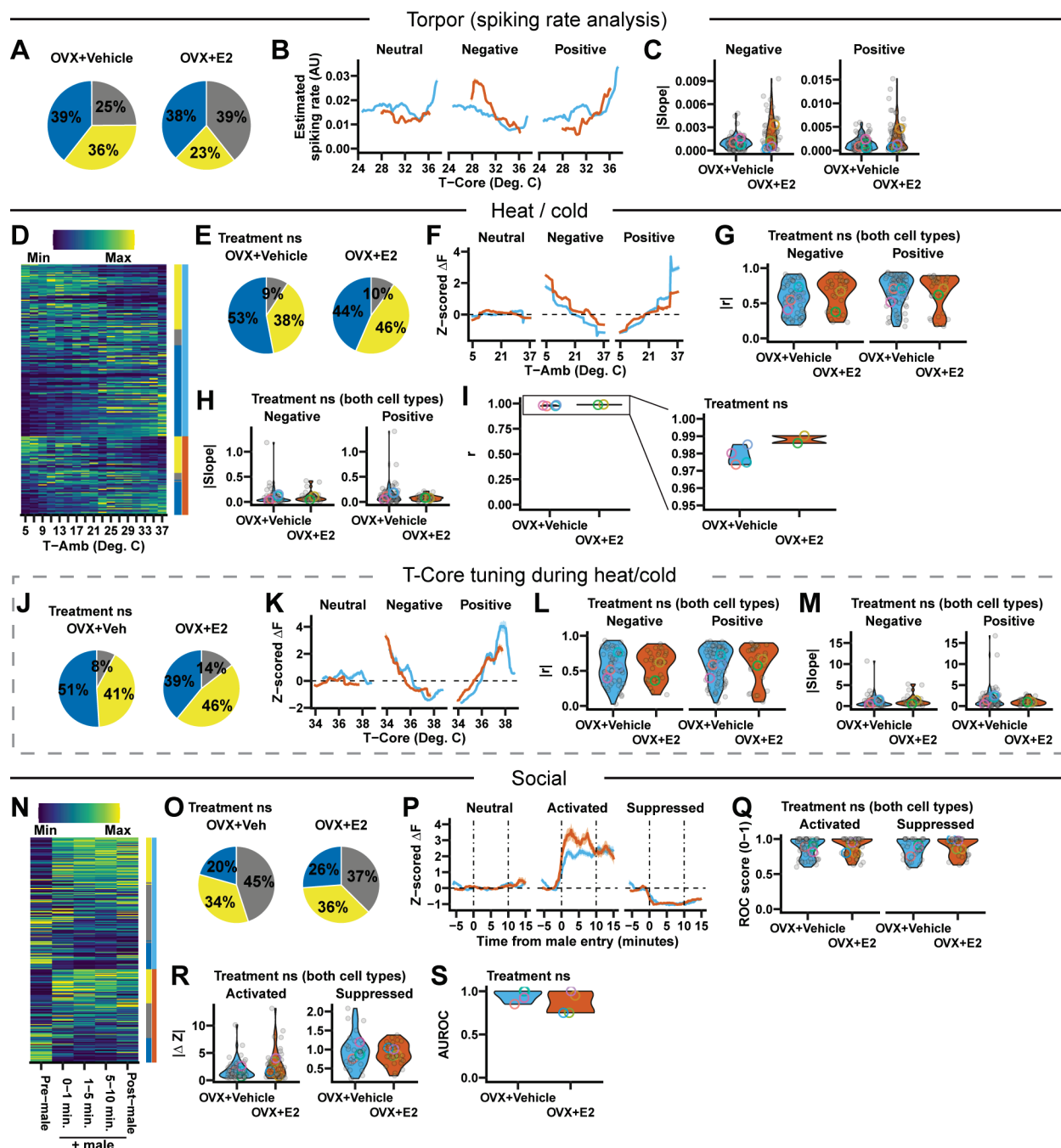


Figure S7 (related to Figure 3): Circulating E2 does not significantly affect MPO^{ER α} neuronal responses to ambient temperature or a male social partner

A-C) Re-analysis of torpor data using estimated spiking activity inferred through deconvolution, as opposed to z-scored ΔF as the primary activity metric.

- A) Fractional abundance of each functional cell type across all mice.
- B) Estimated spiking rate for each functional cell type population across T-Core values (smoothed by a moving average window of 3 °C).
- C) Magnitude of estimated spiking activity – T-Core correlation (absolute value of slope) for individual cells.
- D-I) Ambient temperature tuning during heat and cold sessions
- D) Heatmap of responses from all cells and all mice across T-Amb values (normalized to minimum and maximum values for each cell). Each row represents a single cell, and rows are sorted by treatment group and Pearson's correlation coefficient with T-Amb. Labels to the right of the heatmap indicate the treatment group and T-Amb correlation type of each cell
- E) Fractional abundance of each functional cell type across all mice.
- F) Z-scored ΔF activity for each functional cell type across T-Amb values (smoothed by a moving average window of 8 °C).
- G) Strength of Z-scored ΔF – T-Amb correlation (absolute value of Pearson's correlation coefficient, $|r|$) for individual cells.
- H) Magnitude of Z-scored ΔF – T-Amb correlation (absolute value of slope) for individual cells.
- I) Pearson's correlation coefficient (r) between observed and predicted T-Amb values using linear regression models trained on all cells.
- J-M) Core body temperature tuning during heat and cold sessions
- J) Fractional abundance of each functional cell type across all mice.

- K) Z-scored ΔF activity for each functional cell type across T-Core values (smoothed by a moving average window of 0.5 °C).
- L) Strength of Z-scored ΔF – T-Core correlation (absolute value of Pearson's correlation coefficient, $|r|$) for individual cells.
- M) Magnitude of Z-scored ΔF – T-Core correlation (absolute value of slope) for individual cells.
- N-S) Social partner tuning
- N) Heatmap of responses from all cells and all mice across the social session (normalized to minimum and maximum values for each cell). Each row represents a single cell, and rows are sorted by treatment group and then AUROC value discriminating between the pre-male baseline and male social interaction period. Labels to the right of the heatmap indicate the treatment group and response type of each cell.
- O) Fractional abundance of each functional cell type across all mice.
- P) Z-scored ΔF activity for each functional cell type across the social session (smoothed by a moving average window of 2 minutes). Vertical dashed lines at 0 and 10 represent male entry and male removal, respectively.
- Q) Discriminability between pre-male baseline and male social interaction period ($2 * |0.5 - \text{AUROC}|$) for each cell.
- R) Response magnitude (difference in mean Z-scored ΔF) between pre-male baseline and male social interaction period for each cell.
- S) AUROC for logistic regression models trained on all cells.

Data in B, F, K, P: mean $\pm$ SEM, using a moving average to smooth (B: window width = 3 °C, F: window width = 8 °C, K: window width = 0.5 °C, P: window width = 2 minutes). Data in G-H, L-M, Q-R: small grey dots represent individual cells, larger colored dots represent per-animal means. Data in I, S: colored dots represent per-animal means. A-C: n=534 cells (305 vehicle, 229 E2) from 8 total mice (4 vehicle, 4 E2). D-H, J-M: n=220 cells (151 vehicle, 69 E2) from 6 mice (4 vehicle, 2 E2). I: n=6 mice (4 vehicle, 2 E2). N-R: n=258 cells (151 vehicle, 107 E2) from 8 mice (4 vehicle, 4 E2). S: n=8 mice (4 vehicle, 4 E2). E, J, O: chi-squared test. G, L, Q: linear mixed effects model. H, M, R: Gamma generalized linear mixed effects model with a log link function. I, S: unpaired t-test. G-H, L-M, Q-R: Holm correction for multiple cell type comparisons. ns $p \geq 0.05$.

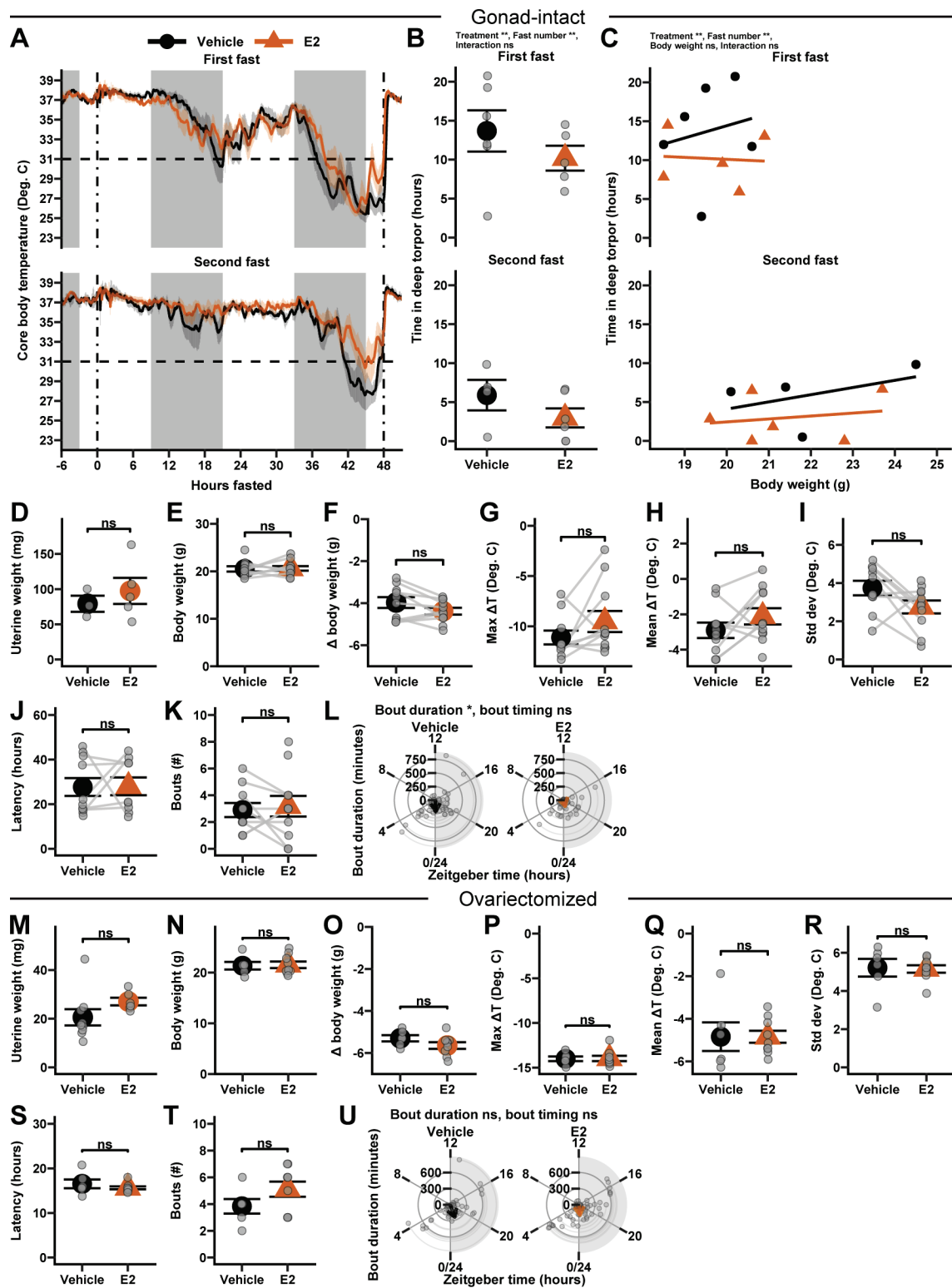


Figure S8 (related to Figure 4): Additional characterization of the effects of estrogen signaling in the MPO on torpor

A-C) Data from Fig. 4D-F, separated by trial.

A) Core temperature during fasting-induced torpor.

B) Time in deep torpor (core body temperature $<31^{\circ}\text{C}$)

C) Time in deep torpor, with start body weight as a covariate

D, M) Uterine weight measured at the end of the experiment

E, N) Body weight measured immediately before each fasting trial

F, O) Change in body weight across the fasting period

G, P) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)

H, Q) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)

I, R) Temperature variability for each trial (standard deviation of temperature during fasting)

J, S) Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)

K, T) Number of deep torpor bouts per trial

L, U) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center

of the circle represents the “mean bout”. The length of the arrow represents the mean duration of all bouts, and the angle of the arrow represents the mean temporal midpoint of all bouts.

All data are shown as mean $\pm$ SEM. E-K: Gray lines connect repeated measures from individual mice across trials. A-C: n=6 first fast vehicle, n=5 first fast E2, n=4 second fast vehicle, n=6 second fast E2 mice. D: n=3 vehicle, 5 E2 mice. E-K: n=10-11 trials per group, collected from 12 total mice (1-2 trials per mouse). L: n=29 vehicle, 35 E2 bouts from 19 trials and 11 mice (2 E2 trials had no bouts). M = n=9 vehicle, 6 E2 mice. N-T: n=6 vehicle, 9 E2 mice. U: n=24 vehicle, 46 E2 bouts from 15 mice (all mice had at least one bout). B: ANOVA. C: ANCOVA. D: unpaired t-test. E-L, U: linear mixed effects model. M-T: unpaired t-test. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$

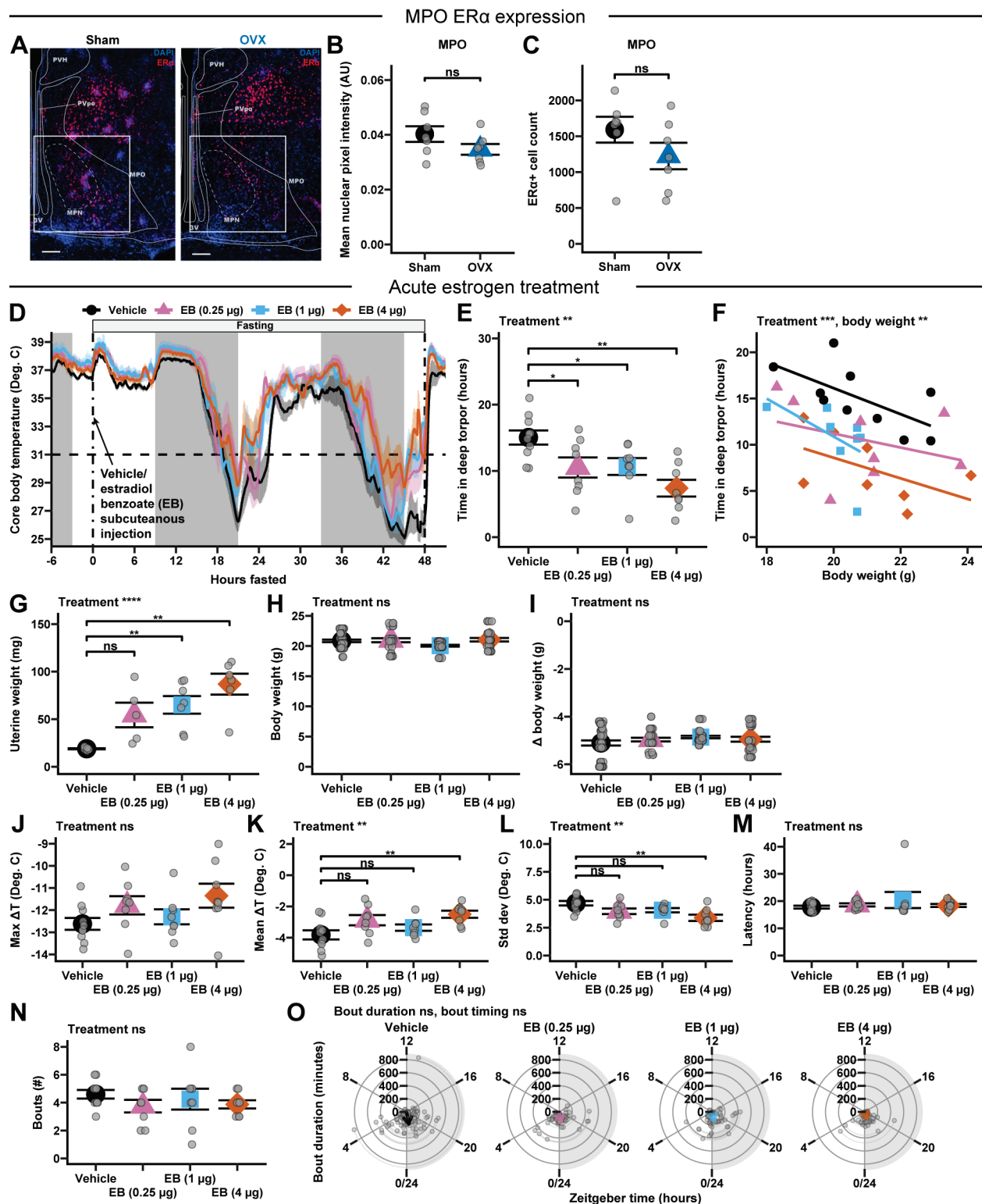


Figure S9 (related to Figure 4): MPO ER α expression and responsiveness to acute estrogen treatment are preserved following ovariectomy

A) Selected brain sections stained for DAPI and estrogen receptor alpha (ER α). White rectangle indicates region of interest that was considered for quantitative analysis. Scale bar 100 μ m.

B) Mean pixel intensity of ER α signal intensity for all nuclei identified in the region of interest (POA) per mouse.

C) Number of ER α + nuclei identified in the region of interest (POA) per mouse.

D) Core temperature during fasting-induced torpor. Arrow indicates timing of subcutaneous injection (hours fasted = 0).

E) Time in deep torpor (core body temperature <31 °C)

F) Time in deep torpor, with start body weight as a covariate

G) Uterine weight measured at the end of the experiment (3-7 days after estrogen injection)

H) Body weight measured immediately before each fasting trial

I) Change in body weight across the fasting period

J) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)

K) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)

L) Temperature variability for each trial (standard deviation of temperature during fasting)

M Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)

N) Number of deep torpor bouts per mouse

O) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center of the circle represents the “mean bout”. The length of the arrow represents the mean duration of all bouts, and the angle of the arrow represents the mean temporal midpoint of all bouts.

All data are shown as mean $\pm$ SEM. B-C: n=7 mice per group. D-F, H-N: n=10 vehicle, 8 EB (0.25 μ g), 8 EB (1 μ g), 8 EB (4 μ g), 7 EB (12 μ g) mice. G: n=7 vehicle, 5 EB (0.25 μ g), 7 EB (1 μ g), 6 EB (4 μ g), 7 EB (12 μ g) mice. O: n=46 vehicle, 30 EB (0.25 μ g), 35 EB (1 μ g), 31 EB (4 μ g), 31 EB (12 μ g) bouts from 41 mice (all mice had at least one bout). E, G-N: ANOVA with post-hoc t-tests (Holm correction) following significant main effect. F: ANCOVA. O: Linear mixed effects model. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

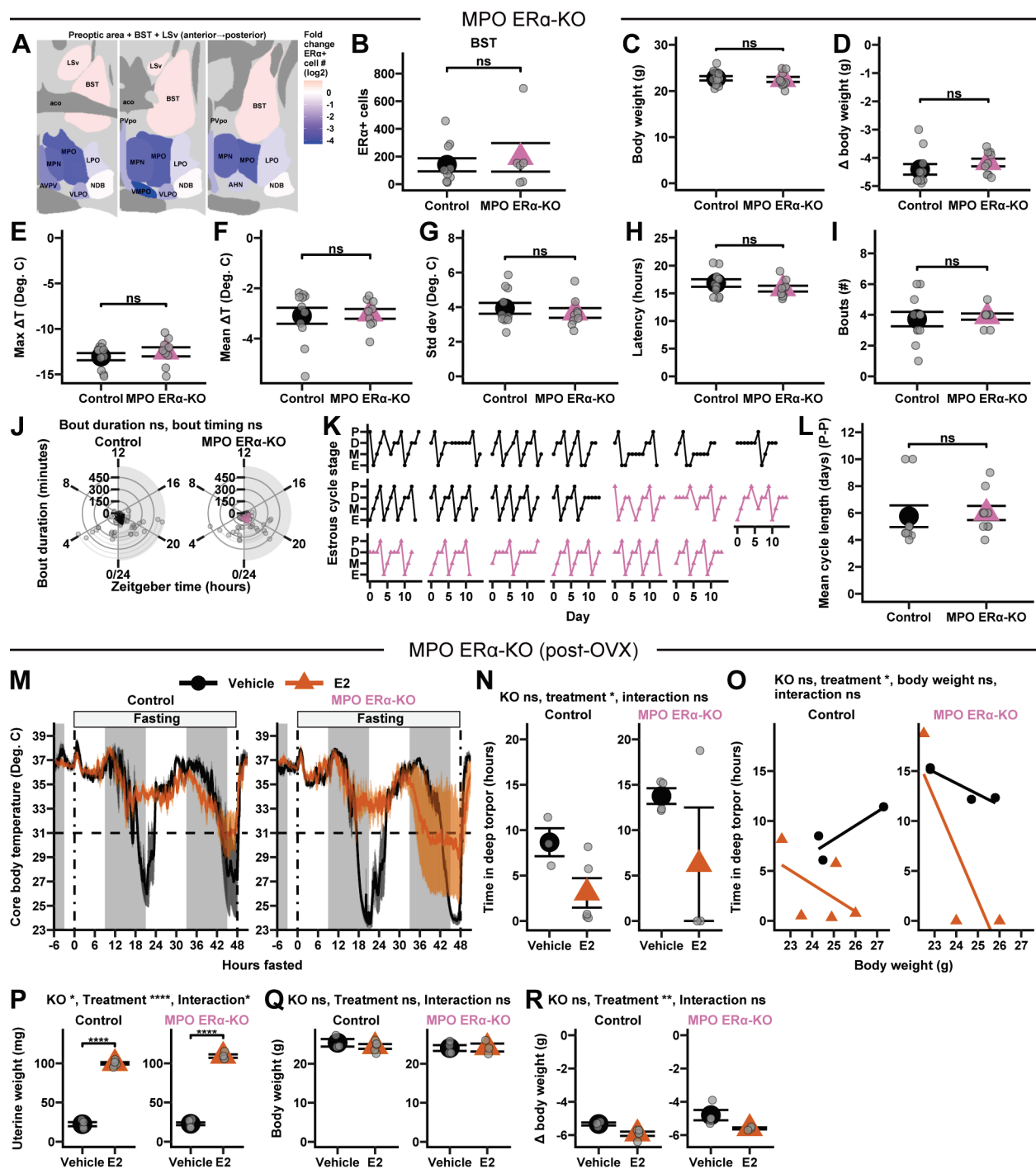


Figure S10 (related to Figure 5): ER α in the MPO is not required for the effects of exogenous estrogens on torpor

- A) Schematic summary of the change in ER α + cells detected across brain areas between control and KO animals.
- B) Quantification of ER α + cells detected in the BST in control and KO animals.
- C, Q) Body weight measured immediately before each fasting trial.
- D, R) Change in body weight across the fasting period
- E) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)
- F) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)
- G) Temperature variability for each trial (standard deviation of temperature during fasting)
- H) Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)
- I) Number of deep torpor bouts per mouse
- J) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center of the circle represents the “mean bout”. The length of the arrow represents the mean duration of all bouts, and the angle of the arrow represents the mean temporal midpoint of all bouts.
- K) Estrous cycle for each mouse before any fasting trials
- L) Mean cycle length for each mouse (days between P stages).

- M) Core body temperature during fasting-induced torpor
- N) Time in deep torpor (core body temperature $<31^{\circ}\text{C}$).
- O) Time in deep torpor, with starting body weight as a covariate.
- P) Uterine weights measured at the end of the experiment

All data are presented as mean $\pm$ SEM. A-B: n=10 control, 6 KO mice. C-I, K: n=11 control, 9 KO mice. J: n=41 control, 35 KO bouts from 20 mice (all mice had at least one bout). L: n=9 control, 9 KO mice (2 control mice did not complete a full P-P cycle). M-R: n=3 control vehicle, 5 control E2, 4 KO vehicle, 3 KO E2 mice. B-I, L: unpaired t-test. J: linear mixed effects model. N, P-R: ANOVA, O: ANCOVA. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

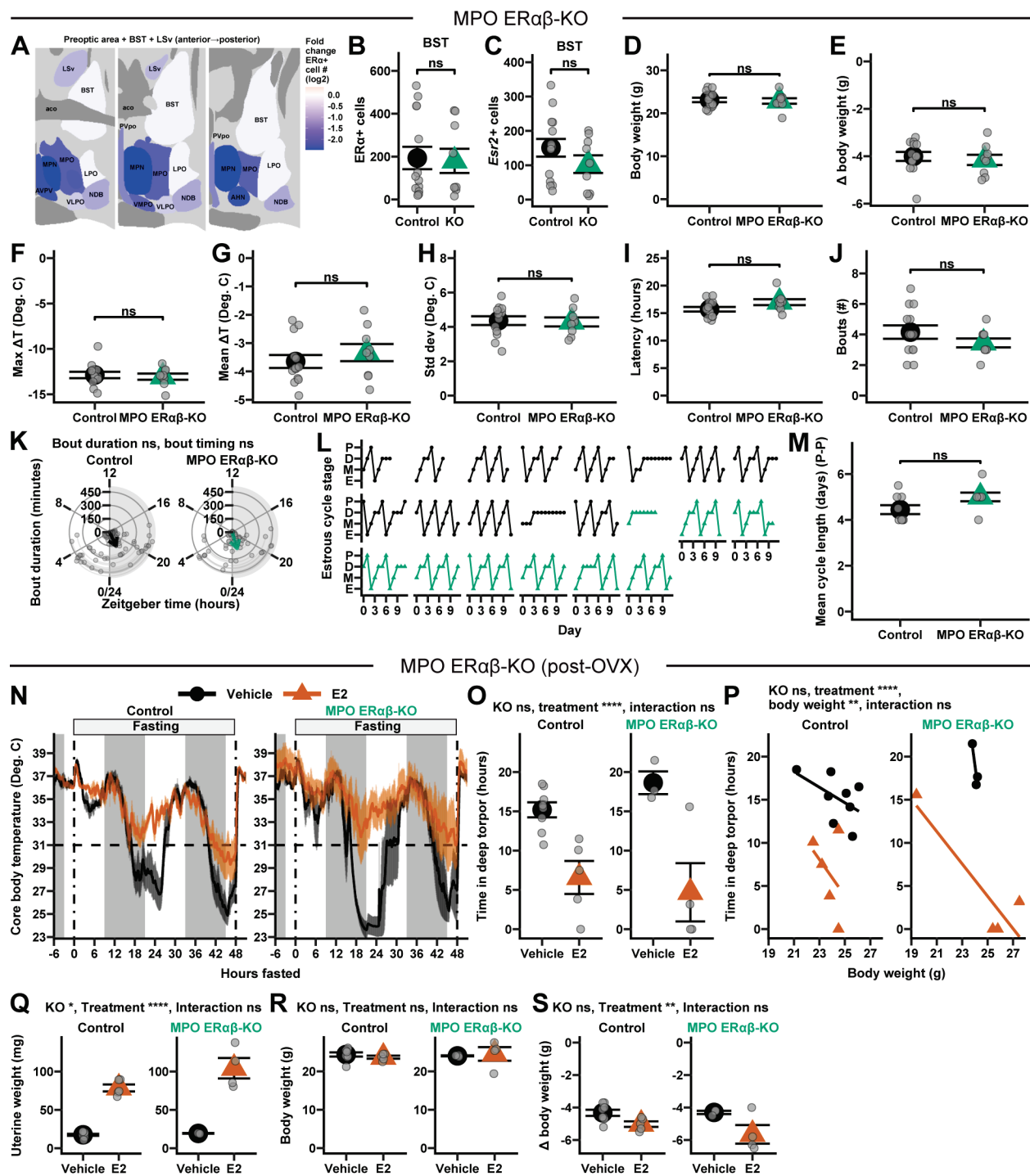


Figure S11 (related to Figure 5): ERα and ERβ in the MPO are collectively not required for the effects of exogenous estrogens on torpor

- A) Schematic summary of the change in ER α + cells detected across brain areas between control and KO animals.
- B) Quantification of ER α + cells detected in the BST in control and KO animals.
- C) Quantification of *Esr2*+ cells detected in the BST in control and KO animals.
- D, R) Body weight measured immediately before each fasting trial.
- E, S) Change in body weight across the fasting period
- F) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)
- G) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)
- H) Temperature variability for each trial (standard deviation of temperature during fasting)
- I) Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)
- J) Number of deep torpor bouts per mouse
- K) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center of the circle represents the “mean bout”. The length of the arrow represents the mean duration of all bouts, and the angle of the arrow represents the mean temporal midpoint of all bouts.
- L) Estrous cycle for each mouse before any fasting trials

M) Mean cycle length for each mouse (days between P stages).

N) Core body temperature during fasting-induced torpor

O) Time in deep torpor (core body temperature $<31^{\circ}\text{C}$).

P) Time in deep torpor, with starting body weight as a covariate.

Q) Uterine weights measured at the end of the experiment

All data are presented as mean $\pm$ SEM. A-C: n=14 control, 9 KO mice. D-J, L: n=13 control, 9 KO mice. K: n=54 control, 31 KO bouts from 22 mice (all mice had at least one bout). M: n=9 control, 8 KO mice (4 control mice and 1 KO mouse did not complete a full P-P cycle). N-S: n=8 control vehicle, 5 control E2, 3 KO vehicle, 4 KO E2 mice. B-J, M: unpaired t-test. K: linear mixed effects model. O, Q-S: ANOVA, P: ANCOVA. ns $p \geq 0.05$, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

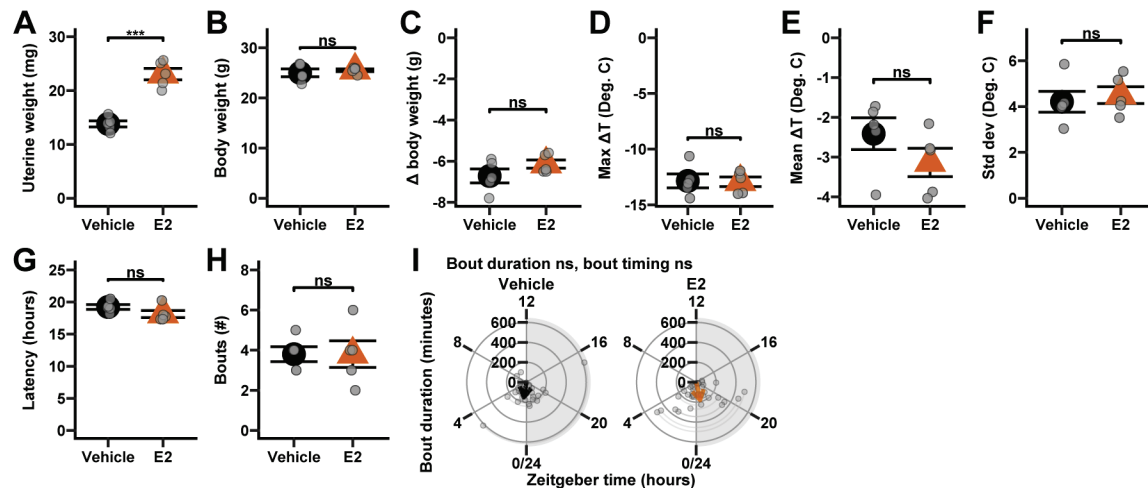


Figure S12 (related to Figure 6): Additional characterization of the effects of estrogen signaling in the central nervous system on torpor

A) Uterine weights measured at the end of the experiment.

B) Body weight measured immediately before each fasting trial.

C) Change in body weight across the fasting period

D) Maximal hypothermic response for each trial (minimum temperature during fasting – mean temperature pre-fasting)

E) Mean hypothermic response for each trial (mean temperature during fasting – mean temperature pre-fasting)

F) Temperature variability for each trial (standard deviation of temperature during fasting)

G) Latency to enter deep torpor (time between start of fasting and start of first deep torpor bout)

H) Number of deep torpor bouts per mouse

I) Raleigh plot for bouts of deep torpor across the fasting period. Each point represents the start and end of a single bout of deep torpor. The line connecting each pair of points represents the bout itself. Distance from the center of the circle represents the duration of the bout, and the angle within the circle represents the start and stop time of the bout, transformed to Zeitgeber time (time relative to lights on). The colored arrow in the center of the circle represents the “mean bout”. The length of the arrow represents the mean duration of all bouts, and the angle of the arrow represents the mean temporal midpoint of all bouts.

All data are presented as mean $\pm$ SEM. A-H: $n=5$ mice per group. I: $n=19$ bouts per group from 10 mice (all mice had at least one bout). A-H: unpaired t-test. I: linear mixed effects model. ns $p \geq 0.05$, *** $p < 0.001$.

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