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Running the Female Power Grid Across Lifespan Through Brain Estrogen Signaling

Holly A. Ingraham, **Candice B. Herber**, **William C. Krause**

Department of Cellular and Molecular Pharmacology, School of Medicine, Mission Bay, University of California, San Francisco, California, USA;

Abstract

The role of central estrogen in cognitive, metabolic, and reproductive health has long fascinated the lay public and scientists alike. In the last two decades, insight into estrogen signaling in the brain and its impact on female physiology is beginning to catch up with the vast information already established for its actions on peripheral tissues. Using newer methods to manipulate estrogen signaling in hormone-sensitive brain regions, neuroscientists are now identifying the molecular pathways and neuronal subtypes required for controlling sex-dependent energy allocation. However, the immense cellular complexity of these hormone-sensitive brain regions makes it clear that more research is needed to fully appreciate how estrogen modulates neural circuits to regulate physiological and behavioral end points. Such insight is essential for understanding how natural or drug-induced hormone fluctuations across lifespan affect women's health.

Keywords

female physiology; central estrogen signaling; hypothalamus; ventromedial hypothalamus; arcuate nucleus; sex-dependent neurocircuits; reproduction; menopause; estrogen receptor alpha; brain-bone connection; women's health

THE FEMALE BRAIN: WAITING FOR A MOLECULAR FRAMEWORK

It has been decades since the first three-dimensional (3D) atomic structures were solved for nuclear hormone receptors, including those that bind sex steroids. Among the major sex steroids, estrogen has emerged as the primary driver of female physiology across different life stages, beginning at birth and ending with its near depletion in the postmenopausal period. To put this in perspective, a quick search for estrogen in PubMed as of 2021 yields well over 250,000 citations. Estrogen is perhaps best studied for its potent effects on reproductive and breast tissues. This latter focus arose primarily because of the proliferative effects of estrogen, especially in hormone-sensitive breast cancer. Despite the known benefits of estrogen on multiple organs, including the brain, findings from the Nurses'

Holly.Ingraham@ucsf.edu .

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Health Study done nearly 30 years ago, linking hormone replacement therapy and cancer, albeit small, stood out as a pivotal moment in women's health that dramatically changed how estrogen is viewed (1). Rather than altogether rejecting estrogen therapies, we assert that identifying hormone-responsive pathways and neurocircuits in the brain will likely uncover new strategies to exploit estrogen's benefits (and minimize the adverse effects).

Before delving into emerging data outlining how central estrogen functions in the female brain, it is worth remembering that estrogen levels in females are constantly changing during different life stages, including puberty, menses, pregnancy, lactation, and menopause (Figure 1a). Here, we focus on the estrogen-sensitive neural circuits and signaling pathways that modulate physiological responses. These pathways enable females to modulate their homeostatic systems and intrinsic behaviors to eventually enable appropriately cued social behaviors. Rather than addressing how estrogen influences cognition or sex-specific social behaviors, we focus on the molecular events that allow central estrogen in the medial basal hypothalamus (MBH) to control female physiology. Similarly, we do not review the role of sex chromosomes in the brain, which can be found elsewhere (2). Instead, we concentrate on how gonadal- and locally generated estrogen affects innate and adaptive responses. Much remains to be discovered as to how central estrogen coordinates neurocircuits to control physiology across lifespan. It is hoped that highlighting current progress in this field will inspire others to help chart the complex molecular blueprint of the female brain.

STEROIDS IN PRENATAL DEVELOPMENT: SCULPTING CIRCUITS AND BEHAVIOR

Over decades, neuroendocrine-behavioral research suggests that estrogen acts early in the embryonic male brain as a primary driver of masculinization, particularly in rodents. Key regions targeted by estrogens include subcortical regions that are classified as sexually dimorphic, as their molecular signatures diverge based on genetic sex. Unless otherwise noted, the terms sex-differences or sex-specific refer to chromosomal sex assignment as XX (female) or XY (male), rather than gender assignments including genetic and trans-XX males, trans-XY females, or non-binary individuals (Johns Hopkins Medical School of Medicine; https://www.hopkinsmedicine.org/news/articles/glossary-of-terms-1).

The dominant and early effects of estrogens on organizing the male rodent brain are thought to be in place a few weeks after birth, concomitant with a surge in testicular androgen biosynthesis (3). Moving into the brain freely, testosterone is then converted locally by a single enzymatic step via aromatase (P450arom) that is encoded by the gene *CYP19A1.* However, the notion that local estrogen biosynthesis alone establishes male behavior is not set in stone—active debate still lingers as to the importance of early estrogens on male behavior, especially in humans. In rodents, postnatal estrogen treatment does generate male-specific behaviors, and hypothalamic regions important for sexual behaviors have high *Cyp19a1* expression, implying that estrogen is enriched locally in these brain regions (4). Although manipulating the *Cyp19a1* gene in all (5) or discrete brain regions alters some sex-specific behaviors in both male and female mice (6), others find that masculine behaviors depend on the X-linked androgen receptor (AR, NR3C4). For example, the global

knockout of AR leads to deficits in both sexual (mounting) and aggressive behaviors in male mice (7). Treatment with dihydrotestosterone (DHT), the only androgen unable to be converted to estradiol, successfully rescues sexual behavioral deficits after deleting estrogen receptor alpha (ERα, NR3A1; encoded by *Esr1*) in male mice, but not when AR is deleted. Eliminating both ERα and estrogen receptor beta (ERβ, NR3A2; encoded by *Esr2*) reduces male sexual behaviors (8). Interestingly, while genetic ablation of ERα in excitatory or inhibitory neurons using *Vglut2-Cre* or *Vgat-Cre* impairs female fertility (9), aggressive behaviors in male mice are diminished only after ablating ERα in inhibitory GABAergic neurons (10). Collectively, these findings suggest that both estrogen and androgen signaling are needed to achieve the entire repertoire of sexual behaviors. Aside from the behavioral changes, others have shown that excess androgens, specifically intracerebroventricular infusion of DHT into female mice, results in long-term metabolic changes, including insulin resistance (11). In humans, others have argued strongly that androgens are the driving force for masculinizing the male brain based on the fact that 46XY individuals with complete androgen insensitivity identify with the female gender and are sexually attracted to males (12). Although extremely rare, 46XY males with mutations in either *CYP19A1* or *ESR1* who cannot make or respond to estrogen, respectively, self-identify only with the male gender and present clinically with tall stature due to incomplete closure of epiphyseal growth plates in long bones (3, 13, 14). In-depth reviews covering the role of estrogen signaling in male behavior and physiology can be found elsewhere (3, 15).

Any organizational effects of estrogen on the female brain are delayed from those of males owing to the simple fact that the female ovary is thought to remain quiescent, producing few if any steroids in the fetus until the peripubertal stage in mice. For rats, a surge in estrogen begins in both sexes beginning at postnatal day 8, and in humans, a small peak occurs in females during early infancy (16). The presence of alpha fetal protein in female rodents is proposed to occlude estrogen from crossing the blood–brain barrier (BBB) (17). Delayed steroidogenesis in the ovary can be attributed to the sexually dimorphic expression of steroidogenic factor 1 (SF-1, NR5A1), which remains low in the embryonic ovary compared to elevated expression in somatic cells of the testis (18). The thorny issue of how sex steroids molecularly organize hormone-responsive circuits in the MBH to generate sex differences remains unresolved. Studies find that estrogen increases spine density and alters synaptic structure and function (19). Indeed, estradiol benzoate treatment of Purkinje cells cultured from brains lacking *Cyp19a1* will reverse lower spine number and dendritic growth (19). Estrogen is also required for the cyclic increase in termini and functional connections in ventral lateral region of the ventromedial hypothalamus (VMHvl) neurons involved in female sexual behavior (20). Data from nonhuman primates suggest that working memory and spine density of pyramidal neurons improve with estradiol treatment (21). Collectively, these data might suggest that cycling hormone levels or the timing of steroid production contribute to sex differences noted in the human brain (22, 23).

GENERATING ESTROGENS IN GONADS AND THE BRAIN

To appreciate how steroids are generated and why their levels are tightly regulated during late embryonic development, it is necessary to discuss another nuclear receptor transcription factor, SF-1. Broadly speaking, SF-1 is essential for the steroidogenesis and biosynthesis

of estrogen. Early in development, SF-1 is highly restricted to the bipotential gonad and adrenal gland (24), the anterior pituitary (25), and the primordial MBH (26). As the bipotential gonad and reproductive tracts become morphologically distinct in males and females, expression of SF-1 increases in the testis compared to that of the ovary (18). Essentially, this sex-specific enrichment in the testes helps ensure proper male reproductive differentiation with steroid production peaking just before birth. After birth, steroid production ramps up in females for a short window and then subsides in both sexes until hypothalamic signals awaken a new wave of gonadal steroidogenesis at puberty (16).

SF-1 is a critical factor in regulating a host of steroidogenic enzymes in the adrenal gland and gonads. SF-1 fulfills this role by controlling the rate-limiting first step that initiates the conversion of cholesterol into pregnenolone by cholesterol side-chain cleavage (P450scc, *Cyp11A1*). When deficient in humans, adrenal insufficiency and impaired gonadal development occur (27). The 21-carbon progesterone steroid generated by p450scc is further modified by designated hydrolases and dehydrogenases to generate endogenous androgens, including androstenedione, testosterone, dehydroepiandrosterone (DHEA), and DHT. As mentioned above, only the first three of these can undergo conversion to estradiol, the most potent of three endogenous estrogens that bind with high affinity to both ERs (Figure 1b). The closest relative of SF-1, liver receptor homolog-1, is also important for generating gonadal steroids, as reviewed in Reference 28.

Despite its pivotal role in producing sex steroids, SF-1 does not adopt the classical features of steroid hormone receptors. Instead, it is constitutively nuclear and functions as a monomer binding with a high affinity to an estrogen response element half-site. SF-1 also fails to bind cholesterol or any other steroid-like molecule. Instead, high-resolution atomic X-ray crystal structures revealed that the rodent and human SF-1 bind to phospholipids with the C16/C18 acyl chains nestled into the large hydrophobic ligand-binding pocket (29, 30). Later, Blind et al. (31) showed that the highest-affinity phospholipid ligand for SF-1 is phosphatidylinositol (3,4,5)-trisphosphate, inducing a conformational change in residues commonly mutated in patients with disorders of sexual development (31, 32). In addition, posttranslational modifications impact SF-1 activity, including phosphorylation (33) and sumoylation, with the latter acting to restrain testosterone production and Leydig cell proliferation in male mice (34). SF-1 also regulates other key genes in male sex determination, including the Y-linked male sex-determining gene, *Sry*, and the polypeptide anti-Müllerian hormone that destroys the developing female reproductive tract (18, 35).

Unlike the rare *ESR1* or *CYP19A1* mutations, *NR5A1* heterozygous mutations account for a substantial percentage of 46XY disorders of sexual development (36) that can mimic the female phenotype found in SF-1 null mice; these mutant mice ultimately die due to adrenal and gonadal dysgenesis (37). Gene dosage effects are also apparent in mice by the impaired gonadal and adrenal development exhibited by *Sf-1* heterozygotes (38). In the brain, SF-1 serves as a defining marker for the ventromedial hypothalamus (VMH, VMN) in the MBH. There, SF-1 appears uncoupled from steroidogenesis and instead is critical for the organization of the VMH. In mice lacking *Sf-1*, a notable expansion of surrounding GABAergic neurons occurs together with increased *Nkx2-1* VMH precursors and loss of projections to the bed nucleus of the stria terminalis (BNST) and the amygdala

(39, 40), suggesting that transcriptional targets of SF-1 mediate normal circuitry between the hypothalamus and limbic structures in the telencephalon. Lineage tracing of SF-1 shows that it appears in the earliest born neurons to populate the VMH proper, specifically in the VMHvl (41). The central nervous system (CNS)–restricted pattern of SF-1 expression to the VMH was successfully leveraged by Elmquist and colleagues (42) to genetically manipulate gene expression of the leptin receptor (*LepR*) gene in the VMH via a Cre-recombinase (Cre) genetic strategy. Since then, scores of studies have used the *Sf-1-Cre* to manipulate gene expression in all major VMH subregions. Embryonic expression of SF-1 in the VMH anlagen ensures that targeted alleles will be successfully recombined in most but not all VMHvl neurons, ranging from 50% to 80% penetrance depending on the targeted allele and the lab (43, 44). As *Sf-1-Cre* is also active in peripheral endocrine tissues, it becomes essential to confirm with follow-up studies that phenotypes originate in the brain. A more in-depth discussion of the VMH in coordinating female physiology is presented below.

Hormone-sensitive neurocircuits can also be influenced by the local generation of estrogen due to regional aromatase expression that often colocalizes with ERs (Figure 1b). It was shown that aromatase is highly enriched in the male and female brain, specifically in the BNST, VMHvl, medial amygdala (MeA), and preoptic area (POA), using the *Cyp19a1* promoter fused to the placental alkaline phosphatase-1 tracer (4). These regions are highly enriched for ERα and implicated in behaviors that influence reproductive success, including male aggression, female lordosis, pheromone responses, and maternal behaviors. That these *Cyp19a1*-enriched regions reasonably reflect estrogen production has yet to be shown, as few studies have actually measured local estradiol (E2) levels. Newer data found that eliminating aromatase in *Nestin-Cre;Arom*$^{fl/fl}$ male mice reduced brain E2 by ~30% as measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) (5). All sexual behaviors were restored in *Nestin-Cre;Arom*$^{fl/fl}$ gonadectomized (GDX) male mice after replenishing with both testosterone and E2. Testosterone alone only partially restored these behaviors, underscoring the importance of local estrogen production in generating behavioral outputs. Another study found that in aged, GDX marmosets, E2 was lowered in the periphery but not in the hypothalamus after prolonged exposure to the aromatase inhibitor, letrozole; the doses used were similar to those used in breast cancer survivors (45). The only facet affected by this chronic treatment was increased female facial temperatures after a thermal challenge, suggesting that sex-specific thermosensitive neurons are sensitive to local estrogen. In both men and women, positron emission tomography and the aromatase radiotracer [^{11}C]vorozole revealed differences in aromatase availability that correlated with lower self-control and obesity (46). Further efforts that couple accurate E2 measurements with either *Cyp19a1* gene manipulations or pharmacological inhibition of aromatase activity are needed to fully define how regional E2 synthesis impacts brain function.

WHICH ESTROGEN RECEPTOR PREDOMINATES IN THE FEMALE BRAIN?

Evolutionary protein reconstruction supports the idea that the original ancestral steroid receptor that evolved hundreds of millions ago is most similar to ERα and ERβ based on ligand-binding domain (LBD) structures (47). Both of these nuclear receptors are highly enriched in subcortical and cortical brain regions, often exhibiting nonoverlapping patterns. As a side note, these estrogen sensors reside primarily in the nucleus in a ligand-independent

manner in contrast to other steroid hormone receptors (Figure 2). Using an ERβ fused to red fluorescent protein reporter, reciprocal expression patterns of ERα in the arcuate nucleus (ARC) and VMHvl and ERβ in the paraventricular hypothalamic nucleus (PVN) can be observed (48). Other nuclei, including the medial preoptic area (MPOA), MeA, and BNST, show expression of both receptors in the rat brain (49). Functional comparisons of ERα and ERβ null mice demonstrate a requirement for ERα to maintain metabolic and body weight homeostasis in females and males (50–53), which is mirrored by *Cyp19a1* knockout mice. Weight gain in ERα and aromatase loss-of-function mutants results from decreased energy expenditure, defined as the energy necessary to support basic cellular and organ function, thermoregulation, digestion, and energy expended during physical activity, as discussed below.

Expression of the third estrogen receptor, a G protein–coupled transmembrane estrogen receptor (GPER, GPR30), is reported in the rodent brain but remains understudied compared to ERs (54). That the synthetic ligand GC-1 (Tespria) of GPER reversed some but not all hypometabolic phenotypes and reduced markers of inflammation in obese, ovariectomized females suggest that pharmacological activation of GPER1 partially mitigates the loss of ovarian hormones. Interestingly, hyperphagia, bone loss, and hypoactivity associated with estrogen depletion were unaffected by GC-1 treatment (55). It will be interesting to determine whether the molecularly distinct transduction pathway of GPER regulates unique physiological end points or perhaps functions in non-neuronal brain populations, such as microglia.

Although molecular details of central estrogen signaling are scant compared to those published for peripheral tissues, it is reasonable to assume that ERs function through a classic ligand-dependent model, with endogenous or synthetic ligands inducing conformational changes in the helical bundle of the LBD that then triggers coactivator recruitment (56). Both ERs form homodimers and are recruited to the classical palindromic two half-sites. A big question about the brain is whether there are similar or distinct targets compared to the wealth of data already published for immortalized cultured cells. Several groups have already compiled differentially expressed genes after genetic deletion of ERα and ERβ or after ovariectomy and estrogen treatment (57, 58) and readily identified well-known ERα targets, such as *Greb1* and *Pgr* (59). However, scores of new potential gene targets have also emerged, many of which are key transducers of neuropeptide signaling, dopamine signaling (*Slc6a3*), prolactin signaling (*Prlr*), and more.

Obtaining a comprehensive integrative omics map of ERα targets in the VMHvl, BNST, ARC, and other regions faces two substantial technical hurdles. First, immortalized cell lines that reflect the neuronal complexity of estrogen-responsive neuron clusters do not exist; second, standard chromatin immunoprecipitation (ChIP) methods are simply not feasible, requiring a minimum of 5–10 million cells. Tollkuhn's group (60) successfully adapted CUT&RUN technology (61) to define ERα targets in subcortical brain regions in just 400,000 nuclei—this method designed to be used on a small number of cells revealed some surprising findings. They report that most neuronal ERα targets are distinct from those identified in peripheral tissues or immortalized cells. Moreover, in contrast to ChIP-seq data sets obtained from breast cancer cell lines (62), where ERα cooperates with the

forkhead box protein FoxA1 (63), these new CUT&RUN data obtained from the brain reveal that ERα and nuclear factor I X-type are recruited to genes associated with sex-biased psychiatric disorders (60). Defining ERβ and progesterone receptor (PR) brain targets via this method will ultimately generate a comprehensive molecular framework to understand central hormone action.

OPTIMIZING FERTILITY IN FEMALES

The energetic costs of mammalian reproduction require intricate coordination with metabolic homeostasis. Both under- and overweight deviations affect the onset of fertility and maintenance of the reproductive cycle in females, as reviewed in 64. Furthermore, cyclical changes in the reproductive endocrine axis that support the ovulatory cycle also modulate the balance between energy storage and energy utilization. For females, estrogen production begins at puberty to ensure coordination of egg maturation and release with egg implantation. After puberty, cyclic changes in hormones follow a stereotypic pattern with estrogen exerting positive-feedback regulation resulting in the ovulatory surge. This preovulatory rise in estrogen is coupled with significant physiological and behavioral changes, as Slonaker documented nearly 100 years ago (65). In hindsight, these early descriptive studies set up the hypothesis that intrinsic changes in female physiology direct major adaptive responses in locomotor activity and ingestive behaviors beginning at puberty. Similar behavioral changes were not observed in male rats. Moreover, the periodic bursts of activity dissipate, eventually evolving into a sedentary, inactive, and infertile state in both ovariectomized (OVX) and aged female rats. Aside from the logistics of egg production and fertilization requirements, a host of metabolic and behavioral changes are needed to capitalize on the potential fertilization of oocytes. These changes are all directed by central estrogen signaling.

Studies published over the last decade have begun to provide anatomical and mechanistic insights into the programs that coordinate reproductive physiology, energy homeostasis, and behavioral changes to maximize reproductive success. Understanding these physiological processes will likely impact many facets of human medicine, from understanding how the reproductive system ages with time, how the central circuits are affected by natural or drug-induced menopause, and what pathways might be tapped in the future to circumvent the use of estrogen in hormone replacement therapy. Below, we detail the current work outlining the effects of estrogen in two MBH regions, the ARC and VMHvl.

NEURONAL DIVERSITY AND PRIVILEGED LOCATION OF THE ARCUATE NUCLEUS

Historically, the ARC was characterized simply as a ball of cells near the third ventricle that controlled pituitary cell size. Nearly a century later, researchers discovered that the ARC is a critical sensor of nutrient and hormonal signals and directs a host of physiological responses in metabolism and reproduction (66). ARC development begins in the mouse at embryonic day (E) 10 (67). This process will eventually give rise to at least eight distinct neuron subtypes in the adult rodent, as annotated by the expression of signature neuropeptides or neurotransmitters, including Agouti-related peptide/neuropeptide Y (AgRP/NPY), pro-

opiomelanocortin (POMC), kisspeptin (Kiss1/Tac2), kisspeptin and neurokinin B dynorphin (KnDy), somatostatin (SST), growth hormone–releasing hormone (GHRH), tyrosine hydroxylase/dopamine transporter, and cocaine and amphetamine-regulated transcript (CART) neurons (68). All eight of these major neuron subtypes are expressed in both male and female rodents. Recent single-cell RNA-sequencing (scRNA-seq) of the ARC supports further subdivision of these major cell types, underscoring the increasing cellular complexity within this small hypothalamic region (68–70). The developmental pathways contributing to the birth and development of POMC neurons are the most detailed, as reviewed elsewhere (67). Lineage-tracing and fate-mapping experiments determined that ARC *Pomc*-expressing progenitor cells further develop into NPY, GHRH, and KISS1-expressing neurons (71). For this latter cell type, recent studies find that ARC^{Kiss1} neurons exhibit enriched expression of *Esr1*, *Sox14*, *NR5a2*, and *Ar* (68), and are detected as early as E13.5 (72).

The ARC resides in an anatomically favorable location in the MBH, just lateral to the third ventricle, where circulating hormones and nutrients can easily pass through the BBB (73, 74). Indeed, some ARC neurons are positioned outside the BBB, with axons projecting through the median eminence, allowing them to sense circulating nutrient cues from blood or cerebral spinal fluid (73, 74). The ARC is also positioned adjacent to tanycytes that line the ventral region of the third ventricle dorsal to the median eminence. These specialized elongated cells with neuron-like processes make synaptic-like contacts with blood, cerebral spinal fluid, and hypothalamic neurons to transmit information about circulating nutrients to effector neurons controlling aspects of energy utilization (75, 76). Studies demonstrate that activated tanycytes induce hyperphagia in rodents in the fed state and alter ARC^{NPY} or ARC^{POMC} neuron function (77). Interestingly, sex-dependent metabolic phenotypes have emerged after genetic manipulation of tanycytes, consistent with the fact that tanycytes themselves are estrogen-sensitive (78). Recently it was shown that a conditional knockout of tanycytes using the *Rax-Cre;ERT2* allele crossed to the *Eno2-IsI-DTA* allele resulted in increased visceral adiposity and insulin insensitivity in male, but not female mice (79). The juxtaposition of the ARC to these nutrient-sensing cells may facilitate the plasticity needed to coordinate sex-specific energy balance with hormonal fluctuations.

ESTROGEN SIGNALING AS AN ENERGY SENSOR IN THE ARCUATE NUCLEUS

Currently, most neuronal subtypes in the ARC are directly or indirectly sensitive to estrogen signaling. The ARC expresses *Cyp19a1*, albeit at lower levels than other regions, such as the VMH (80), with higher levels observed in males. It is unclear whether this sex difference leads to elevated local estrogen levels in the male ARC. However, both negative feedback in the hypothalamus-pituitary-gonadal axis (HPG) axis and maintenance of ARC^{TH} (dopaminergic) neurons appear compromised in the global aromatase knockout (5, 81). Bulk RNA-seq of the mouse ARC demonstrates that estrogen signaling regulates genes associated with inflammation, cell signaling, and neurotransmission (58). Newer scRNA-seq data report that each neuronal cluster in the ARC, as defined by their signature neuropeptide/neurotransmitter, expresses differing ERα levels, presumably to tune their output responses (68–70). This fine-tuning would allow $ARC^{ERα}$-responsive neurons to control energy

homeostasis and optimize reproductive fitness by changing their sensitivity to oscillating estrogen levels during the estrous cycle. As estrogen levels fluctuate during the estrous cycle, so do energetic outputs such as food intake. For example, high circulating estrogen levels at the apex of follicular maturation and impending ovulation would redirect energetic resources toward activities associated with mate seeking rather than food consumption (43, 44, 82). The molecular details of ERα actions in $ARC^{ER\alpha}$ neurons have yet to be fully elucidated. Two downstream ERα targets in the ARC (*Kiss1*, *Tac2*) are regulated by direct recruitment to estrogen response elements and indirect mechanisms, possibly by tethering to DNA via cofactor recruitment (58). Nongenomic, fast-acting membrane-associated ERα activity is also implicated, especially in POMC and AgRP/NPY neurons that exhibit an influx of calcium in response to estrogen (83). Regardless of the exact molecular mechanism, sensing and responding to estrogen signaling allows specialized neurons in the ARC to sense available resources and elicit a physiological response (84). Newer experimental approaches have helped solidify concepts initially proposed over 40 years ago on the role of central estrogen signaling in female reproduction (85). Below, we summarize current literature on how ARC neurons deconstruct estrogenic cues to regulate energy homeostasis and discuss new wrinkles that broaden our views on how central estrogen affects food intake and modulates hormonal fluctuations during the reproductive cycle.

Despite the essential role of the ARC in controlling food consumption, it is less certain how $ARC^{ER\alpha}$ signaling regulates this process. Conflicting evidence either supports or questions direct control of hunger and satiety pathways by $ARC^{ER\alpha}$ neurons. Estrogen signaling in the ARC is proposed to impact food intake by directly modulating communication between ARC^{POMC} and ARC^{AgRP} neurons or between ARC^{Kiss1} and $ARC^{POMC/AgRP}$ neurons. Approximately 20–30% of $ARC^{POMC/CART}$ anorexigenic neurons that signal satiety also express ERα (43) and synapse onto neighboring orexigenic AgRP/NPY neurons (86). These neurons have reciprocal projections back onto POMC neurons presumably for coordinating responses to hunger and satiety cues. Consistent with this idea, genetic deletion of ERα in ARC^{POMC} neurons using the *POMC-Cre* driver (*POMC-Cre;Esr1*$^{fl/fl}$) elevates *Vglut2* transcripts and the spontaneous firing rate of POMC neurons (83). This increase in spontaneous firing rate enhances the release of the excitatory neurotransmitter glutamate and/or the inhibitory neurotransmitter β-endorphin onto neighboring NPY neurons (83). Within ARC^{NPY} neurons, estrogen upregulates *Opmr1* (encoding the μ-opioid receptor) and sensitizes responses to β-endorphin released from neighboring ARC^{POMC} neurons, resulting in a decrease in food intake (83). Estradiol also blunts the orexigenic effects (or hyperphagia) when NPY, but not AgRP, is injected intracerebroventricularly in OVX rats (87). In light of these results, it was unexpected to find that ERα is completely excluded from AgRP/NPY neurons, implying indirect regulation by estrogen, possibly in the liver (88) or by other ERα-positive ARC neurons.

Although food intake increases in *POMC-Cre;Esr1*$^{fl/fl}$ mice, where estrogen signaling would be blunted in the ARC (43), two studies from our group that abolished all or most estrogen signaling in the ARC failed to find any overt phenotype in food intake during the dark cycle. Indeed, eliminating ERα in the ARC in *Nkx2-1Cre;Esr1*$^{fl/fl}$ during development (59) or by stereotaxic delivery of AAV-Cre to the ARC of *Esr1*$^{fl/fl}$ adult mice (57) showed no overt

changes in food intake in female mice. Observed differences from these studies could arise from institutional differences in assay parameters and data collection (89) or result from the Cre drivers used. A further complication arises from the expanded expression of *POMC-Cre* in ARC and non-ARC neurons during development (67, 71). These data open the possibility that ERα signaling in the ARC is not the only driver of estrogen-regulated food intake in female mice. Other estrogen-sensitive brain regions, including the nucleus tractus solitarius and the dorsal raphe nucleus, also express *POMC* and could easily contribute to the anorexigenic behavior in female rodents (90, 91).

At a cellular level, ERα-sensitive ARC^{Kiss1} neurons excite POMC neurons by three mechanisms: (*a*) through a sodium/calcium exchanger (92), (*b*) their regulation of fast-acting glutamatergic synaptic input to POMC and AgRP/NPY neurons (93), and (*c*) their sensitivity to hunger cues by upregulating the growth hormone secretagogue receptor, GHS-R (94). Interestingly, chemogenetic activation of ARC^{Kiss1} neurons using AAV-DIO-hM3Dq driven by the *Kiss1-Cre* driver failed to alter feeding during the dark cycle (95). In contrast, silencing ARC^{Kiss1} neurons by Cre-dependent expression of tetanus toxin shifted the circadian rhythm of food intake in female mice from the dark to light phase (96). Clearly, additional studies are needed to understand how estrogen signaling in the ARC and elsewhere successfully resets the drive for food to meet reproductive demands.

KISSPEPTIN NEURONS: ESTROGEN SIGNALING AND REPRODUCTION

Estrogen controls different facets of reproduction by modulating positive and negative feedback from the ovary to the brain using the HPG axis. Arguably, the most well-studied of all estrogen-sensitive ARC neurons are ARC^{Kiss1} neurons. Neurons marked by *Kiss1* expression are also sprinkled in the anteroventral periventricular (AVPV) nucleus ($AVPV^{Kiss1}$), the medial amygdala (MeA^{Kiss1}), and the preoptic area (POA^{Kiss1}). Kiss1 signaling plays a critical role in driving the onset of puberty, as shown by disorders of puberty in mice and humans harboring genetic mutations in either KISS1 or its cognate receptor, G protein–coupled receptor 54 (GPCR54, KISS1R) (97–100). As nearly all ARC^{Kiss1} neurons express ERα (59, 101), they are well positioned to sense and respond to oscillating estrogen levels for controlling pulsatile gonadotropic-releasing hormone (GnRH) (102), which then initiates a chain of events beginning with the pituitary secretion of gonadotropins [follicle-stimulating hormone (FSH) and luteinizing hormone (LH)] and ending with egg maturation and ovarian steroid production. Ovarian estrogen subsequently feeds back to the brain to either generate a surge in hormone levels for ovulation (positive feedback) or maintain constant hormone levels for follicular development and implantation (negative feedback) (Figure 3).

Decades of research aimed at unraveling the complex mechanisms exerted by estrogen on the HPG axis suggest that estrogen signaling through ERα in $AVPV^{Kiss1}$ and ARC^{Kiss1} neurons, but not in GnRH neurons, contributes to positive and negative feedback on the HPG (102). As reviewed in Reference 103, positive feedback occurs for a short time. In rodents, this regulatory event requires a critical threshold of circulating estrogen to increase the spontaneous firing of GnRH neurons and generate the LH surge. This period of positive feedback is characterized by increased expression of kisspeptin from $AVPV^{Kiss1}$ neurons in

rodent brain slices. Ex vivo brain slice recordings of AVPV^{Kiss1} neurons marked by a green fluorescent protein reporter demonstrate that estrogen increases neuron excitability and burst generation in males and females (104), suggesting that positive feedback mechanisms are conserved between sexes. To better understand positive feedback, ERα was conditionally knocked out using the *Vgat-Cre* (GABA) or *Vglut2-Cre* (Glut) for eliminating estrogen signaling in GABAergic and glutamatergic neurons, respectively. Loss of ERα signaling in glutamatergic neurons completely abolished the positive feedback loop (9). Consistent with these data, ERα signaling in all Kiss1 neurons (and other cells) is essential for positive feedback based on the finding that E2 treatment no longer elicited the expected LH surge in mouse models designed to test negative and positive feedback (105). A newer study from Moenter and colleagues (106) points to the importance of AVPV^{Kiss1} neurons in positive feedback (Figure 3) by showing that CRISPR-Cas9 gene editing in these neurons, but not ARC^{Kiss1} neurons, dampened the LH surge generation without affecting the estrous cycle.

Along with positive feedback, estrogen exerts negative feedback on the HPG axis to facilitate the proper maturation of the ovulatory Graafian follicle and maintain constant GnRH pulses (107). Negative feedback disappears after conditionally deleting ERα in most adult neurons using the tamoxifen-inducible *CaMKIIa-Cre*, resulting in high circulating LH and FSH levels (108). However, deleting ERα in just Kiss1 neurons (*Kiss1-Cre;Esr1$^{fl/fl}$*) suggests that while estrogen signaling in ARC^{Kiss1} neurons is critical for restraining pubertal onset owing to suppression of *Kiss1* by ERα (98, 108), it appears dispensable for negative feedback (105). Further, when ERα is knocked down in ARC^{Kiss1} neurons using CRISPR-Cas9, estrous cycles are partially disrupted, but surprisingly the number of LH pulses and levels of LH remain unchanged (106). Consistent with this report, we found that negative feedback in mice is not dependent on estrogen signaling in the ARC. Indeed, deleting ERα by stereotaxic delivery of adeno-associated virus (AAV)-Cre to the ARC of adult female *Esr1$^{fl/fl}$* mice failed to increase uterine weights or elevate LH and circulating E2 (59), suggesting that facets of negative feedback are driven by estrogen signaling in other hypothalamic or nonhypothalamic nuclei (Figure 3). On that note, the pituitary may also be a critical site of negative feedback based on high LH levels in mouse models that delete ERα using an *alphaGSU-Cre* driver (109). Recently, chemogenetic activation of posterodorsal medial amygdala (MePD^{Kiss1}) neurons in female mice led to an LH surge that involves activation of neurokinin B signaling (110). Collectively, these studies suggest that understanding the precise role of estrogen in negative feedback during the female reproductive cycle will continue to evolve.

KISSPEPTIN NEURONS LINKING REPRODUCTION TO SKELETAL PHYSIOLOGY

Women suffering from a critically low body mass index, anorexia, or morbid obesity often suffer from hypogonadism, infertility, and bone disorders. As mentioned above, ERα-expressing ARC^{Kiss1} neurons are developmentally derived from POMC-expressing precursors and are molecularly competent to sense a variety of nutrients (111) owing to their regulation by orexigenic AgRP neurons (112). Stimulating AgRP neurons by starvation or optogenetic activation inhibits ARC^{Kiss1} neurons and results in infertility and

impaired bone mass (112, 113). Recently our lab and others' identified a female-specific node in the ARC that normally restrains high bone mass, strengthening the relationship between central estrogen signaling and energy allocation (59, 114). Using complementary approaches to conditionally delete ERα in ARC^{Kiss1} neurons, we showed that in both genetic (*Nkx2-1Cre;Esr1*$^{fl/fl}$ and *Kiss1Cre;Esr1*$^{fl/fl}$) and AAV-Cre-directed knockout mouse models, a marked sex-dependent increase in volumetric bone mass resulted (~500% increase) that was accompanied by higher mechanical strength as shown in 3-point bending and L5 vertebrae crush assays. Changes in bone mass were sex specific, occurring in female mice only. No compensatory changes were observed in circulating pituitary hormones, including FSH, a known skeletal effector (59, 115). Importantly, E2, as measured by LC-MS/MS, could not account for the bone phenotype in all three models. The high bone mass phenotype persisted in OVX and aged females (59). These data infer that central estrogen signaling in the brain directly or indirectly places a break on prepubertal bone growth in female rodents (Figure 4). The exact connection between prepubertal longitudinal bone growth, growth hormone, and estrogen signaling is still being worked out. Conditional deletion of ERα in POMC neurons was also found to modestly increase estrogen-sensitive cortical bone mass in females (114), possibly reflecting the fact that ARC^{Kiss1} and ARC^{POMC} neurons share a common developmental precursor (71).

Another subset in the ARC worth considering is a dorsal medial cluster expressing *Slc6a3*, which is upregulated by estrogen. ARC^{Kiss1} neurons are thought to inhibit dopaminergic neurons, resulting in an estrogen-dependent release of prolactin (Prl) (116). It will be interesting to determine how these estrogen-responsive ARC modules (ARC^{Kiss1} and ARC^{Slc6a3}) coordinate female bone and energy metabolism, especially during pregnancy. Currently, the limited efficacy of *Slc6a3-Cre* in the hypothalamus (117) makes it difficult to adequately assess the role of ERα in these $ARC^{Slc6a3/ERα}$ neurons. In summary, we speculate that when needed, estrogen-sensitive ARC^{Kiss1} neurons limit bone formation, allowing for the reallocation of energetic outputs during the preovulatory period or late stages of pregnancy. These normal restraints are gone once this circuit is severed and high bone mass forms (118). The existence of this brain-to-bone node in ARC^{Kiss1} neurons raises an obvious question: Is this increase in bone formation achieved by a neuron- or hormone-based mechanism?

THE VMHvl: A DIVERSE CLUSTER OF ESTROGEN-RESPONSIVE NEURONS

The VMH consists of distinct modules—defined by their cytoarchitecture, gene expression, and function—that control multiple aspects of behavior and physiology in both males and females. Structure-function studies of the VMH collectively support a division in which the dorsome-dial/central VMH subnucleus operates independently of biological sex to regulate energy and glucose homeostasis (119–122) as well as fear and anxiety-like behavior (123–126). In contrast, the VMHvl, defined in part by ERα expression, controls male- and female-specific behaviors (social and asocial), maintains energy expenditure in females, and participates in glucose homeostasis (43, 44, 127) (Figure 5). Functional genetic tools and single-cell transcriptomics are beginning to clarify the regulatory roles of ERα signaling by providing a mechanistic framework for estrogen's modulation of physiological and behavioral responses across reproductive life stages.

The VMHvl exhibits several structural and molecular differences between males and females. Among these are male-specific increases in VMHvl volume, resulting from larger cell body size and greater numbers of axonal and dendritic spines (128). However, the VMHvl input/output connectivity is dynamically modulated by changes in circulating steroid hormone levels, including estrogens. Females express more ERα in the VMHvl than males, and this difference is established postnatally (44, 129–131). Further, female-biased ERα expression in the VMHvl corresponds with the emergence of sex-specific DNA methylation patterns in the *Esr1* locus (132). Female VMHvl neurons also express higher levels of *Pgr* [encoding the PR, NR3C3 (133)]. While VMHvl^{PR} neurons are necessary for male-specific aggressive behavior and optimize specific reproductive behaviors in male and female mice, ablating PR-expressing VMHvl neurons fails to influence body weight maintenance in either sex (133). The absence of an overt metabolic phenotype is somewhat remarkable, as nearly all (>90%) of the VMHvl^{PR} neurons, as counted bilaterally, coexpress ERα. Thus, their loss would significantly reduce the number of ERα-positive neurons and implies that the functional segregation of VMHvl$^{PR/ERα}$ and VMHvl^{ERα} populations must stem from other molecular or neuroanatomical facets.

Projections from the PR-expressing VMHvl neurons exhibit sex differences. Female VMHvl^{PR} neurons densely innervate the AVPV, a brain region enriched for both ERα and *Kiss1* and one critical for female-specific reproductive behaviors, as discussed above (20, 133). This sex difference is unlikely to result from more VMHvl^{PR} neurons in females, as other target regions, such as the MPOA and the periaqueductal gray, do not exhibit a sex bias in VMHvl^{PR} projection densities. It is worth noting that a brain-wide analysis of VMHvl^{ERα} neurons failed to identify any notable sex differences in the afferent or efferent projections (134). As more granular approaches are used to precisely map projections from molecularly distinct VMHvl^{ERα} subsets, the influence of sex and hormone status on VMHvl^{ERα} projections might become more evident.

Sex differences between male and female gene expression programs in the VMHvl appear to depend almost exclusively on gonadal hormones rather than sex chromosomes (135). Indeed, new evidence from scRNA-seq data of VMHvl neurons supports a primary role for gonads in establishing molecular and organizational sex differences. Two separate studies identified a female subset of VMHvl neurons, marked by Reprimo (*Rprm*) and at least one male-specific subset marked by Prodynorphin (*Pdyn*) (136, 137). Although *Rprm* expression is nearly absent in males, paradoxically, its expression in females is independent of circulating estrogens or functional ERα in the VMHvl. Additionally, sex chromosomes are not involved in the sex-specific repression of *Rprm* in males, as this female-enriched transcript is extinguished in XX female mice harboring male gonads. Thus, the male-specific repression of *Rprm* expression may arise from early organizational changes in response to gonadal hormones. Furthermore, male-biased *Pdyn* expression is also hormone dependent, though in this case, ongoing testicular hormone production is required for its male-biased expression in the VMHvl. Finally, the sex-biased gene expression arising from testes-dependent hormones in the VMHvl has been observed for other subcortical brain regions, including the periventricular nucleus, POA, the ventral premammillary nucleus, the amygdala, and the BNST (135). Together, these data underscore the role of steroid hormones

rather than sex chromosomes in the organization and maintenance of sex differences in neuropeptide signaling and other neuroeffectors.

Given the variety of reproductive and metabolic functions attributed to VMHvl^{ERα} neurons, it is unsurprising that scRNA-seq profiling and targeted functional manipulations would reveal significant molecular heterogeneity among VMHvl^{ERα} neurons. Indeed, molecular, functional, and anatomical differences have been identified among the hundreds of ERα^{+} neurons present in the VMHvl, with the caveat that these signatures depend on the positioning of neurons with a 3D space (134, 136, 138). In some cases, a clear functional distinction can be made within this heterogeneity. For example, more medially located VMHvl^{ERα} neurons are active during episodes of female-intruder aggression. In contrast, ERα neurons on the lateral edge of the VMHvl appear to be engaged during reproductive behaviors (138). However, a clear-cut, simple correlation between different molecular clusters with either functional output or anatomical projections has failed to emerge (136). The lack of a simple one-to-one ratio between molecular, anatomical, and functional features suggests that distinct modules within heterogeneous VMHvl^{ERα} neurons driving behavioral and physiological subroutines combine to elicit optimal output for a given set of metabolic, reproductive, or social cues.

ESTROGEN CONTROLS MODULES OF ENERGY EXPENDITURE IN VMHvl NEURONS

Several metabolic dysregulations observed in ERα null mice are recapitulated by conditional ERα knockout in the CNS and peripheral nervous system using Nestin-Cre (43). Both male and female *Nestin-Cre;Esr1$^{fl/fl}$* mice exhibit increased body weight and visceral fat mass. Similar to ERα null mice, the obesogenic phenotype in these conditional knockout animals is accompanied by decreased energy expenditure, including a drop in spontaneous physical activity. However, unlike ERα null mice, *Nestin-Cre;Esr1$^{fl/fl}$* mice are hyperphagic; whether their increased food intake results from a behavioral change or simply reflects an increase in body weight remains to be determined. The obesogenic phenotype in both global ERα knockout and *Nestin-Cre;Esr1$^{fl/fl}$* mice occurs with sexual maturity, suggesting that gonadal hormones must maintain and activate CNS circuits that regulate energy balance.

Through a variety of targeted ERα knockdown and knockout studies as well as chemogenetic manipulations, the VMHvl has emerged as a critical estrogen-sensitive node responsible for controlling female energy expenditure, thus relegating the regulation of food intake to ERα-expressing neurons in other brain regions. Manipulating rodent VMHvl^{ERα} neurons by various techniques reveals that this cluster controls female energy expenditure by regulating spontaneous physical activity and brown adipose tissue thermogenesis (43, 44, 137, 139, 140). By contrast, MeA^{ERα} neurons are essential for normal physical activity levels in both sexes (141), illustrating that central control of energy expenditure by ERα is not limited to female-specific modules, as found for the VMHvl.

Two conditional ERα knockout models, neither of which fully eliminates ERα in the VMHvl (43, 44), revealed an interesting functional dichotomy for VMHvl^{ERα} neurons. Initially, Xu et al. (43) utilized *Sf-1-Cre* to ablate ERα expression specifically from the

VMH while sparing its expression in the rest of the CNS (recall that all neurons in the VMH lineage transiently express SF-1). Notably, ERα expression persists in a subset (~50%) of VMHvl neurons in these *Sf-1-Cre;Esr1*$^{fl/fl}$ mice. Increased visceral adiposity specifically in females mirrors the visceral adiposity seen in the global ERα null mouse models (52), along with underlying hypometabolism resulting from impaired thermogenic capacity. However, spontaneous physical activity was unaffected in this model. In addition, female *Sf-1-Cre;Esr1*$^{fl/fl}$ knockout mice are infertile. Correa and colleagues (44) used a different approach to indirectly target ERα expression in the VMHvl after eliminating the homeobox transcription factor Nkx2-1, which is critical for early embryonic hypothalamic development, including the VMH. Using the same *Sf-1-Cre* driver, *Sf-1-Cre;Nkx2-1*$^{fl/fl}$ mutant females were missing ~26% of the VMHvl^{ERα} neurons. Similar to *Sf-1-Cre;Esr1*$^{fl/fl}$, these *Sf-1-Cre;Nkx2-1*$^{fl/fl}$ mice were not hyperphagic. However, the neurons lost in *Sf-1-Cre;Nkx2-1*$^{fl/fl}$ mice appeared to be functionally distinct from those affected in the *Sf-1-Cre;Esr1*$^{fl/fl}$ mice, since neither female fertility nor brown adipose tissue thermogenic capacity was affected. Instead, weight gain in *Sf-1-Cre;Nkx2-1*$^{fl/fl}$ females resulted from a reduction in spontaneous physical activity. That discrete populations of VMHvl^{ERα} neurons may contribute to distinct aspects of female energy expenditure are supported by more recent scRNA-seq profiling. Recall that *Rprm* expression marks a female-specific subset of VMHvl^{ERα} neurons, and targeted knockdown of this transcript by delivering small interfering RNA (siRNA) to the VMHvl specifically affected female thermogenesis but not physical activity levels (136, 137). Efforts to further parse VMHvl molecular signatures combined with physiological/behavioral studies will undoubtedly identify new functional subsets within VMHvl^{ERα} neurons.

MECHANISMS OF ERα-DEPENDENT REGULATION OF ENERGY HOMEOSTASIS

Despite ample evidence that ERα in VMHvl neurons is necessary to maintain normal energy expenditure levels in female mice, identifying direct, estrogen-sensitive target gene(s) responsible for these functional outputs has been lacking. Krause et al. (57) recently showed that ligand-activated ERα is recruited to the melanocortin-4 receptor gene (*Mc4r*) to upregulate its expression. This study also answers a long-standing question: How does the preovulatory estrogen surge drive behaviors, such as physical activity, to capitalize on periods of peak reproductive capacity? Using chemogenetics and CRISPR-mediated activation, we discovered that estrogen co-opts MC4R signaling in ~200 VMHvl^{ERα} neurons to drive spontaneous activity during female sexual receptivity without affecting thermogenesis or food intake (Figure 6). Together with the fact that estrogen regulates the activity of ARC^{POMC} neurons, which generate the endogenous ligand for MC4R (83, 84), these findings reinforce the partnership between estrogen and melanocortin signaling and further illustrate how reproductive and metabolic signals converge to satisfy the reproductive drive for females. That VMHvl^{MC4R} neurons project to “speed” and arousal centers in the hippocampal region and hindbrain offers the first hint as to how estrogen acting in the VMHvl causes a flurry of physical activity.

Defining the molecular consequences of ERα in the VMHvl and other brain regions is further complicated because an ERα variant lacking a functional DNA binding domain can rescue some but not all metabolic phenotypes of ERα null mice (53). Others report that the benefits of estrogens on metabolism depend solely on the activation function 2 in the LBD, inferring that coregulators rather than the DNA binding domain recruit ERα to target genes (142). As these models manipulate ERα globally, it is unclear whether these mechanisms are operational in the VMHvl. In addition, ERα has been shown to influence signal transduction cascades directly. Multiple studies have linked high E2 (natural or pharmacological) with phosphatidyl inositol-3 kinase (PI3K) and AMPK signaling pathways in the VMHvl (53, 57, 127, 140), which in turn modulates synaptic plasticity (143). Inhibiting PI3K signaling largely recapitulates phenotypes observed following the loss of estrogen signaling or ERα neurons in the VMH (43, 44) and prevents the estrogen-dependent excitement of $VMHvl^{ERα}$ neurons (127). In addition, brain-derived neurotrophic factor (BDNF), an upstream activator of PI3K, is expressed in the VMH and particularly enriched in the VMHvl (144). VMH-targeted *Bdnf* knockout does not impact energy expenditure or physical activity (145). Still, conditional knockout of glutamatergic components downstream of BDNF impairs neuronal excitability and glucose homeostasis, specifically in female mice (146). Such nongenomic pathways may underlie estrogen's ability to rapidly alter VMHvl membrane conductance and sensitivity to excitatory neurotransmitters (147).

In characterizing the impact of $VMHvl^{ERα}$ neurons on female metabolism, it is worth considering the potential contribution of the tuberal nucleus (TU) that lies just outside the lateral edges of the shell encasing the VMH proper (Figure 5). Anatomically, TU and $VMHvl^{ERα}$ neurons are often difficult to disentangle, especially in the more posterior regions, when the TU encroaches on the lateral edge of the VMHvl. In fact, in rats, it has been suggested that TU neurons express ERα and project to many of the same target brain regions as VMHvl neurons (148). As a result, ERα-expressing neurons in the VMHvl and TU are sometimes considered a single unit (134). However, other lines of evidence argue that the TU and VMHvl are molecularly and functionally distinct. TU neurons downregulate Nkx2-1 in the postnatal period (44, 149), which continues to be enriched in the VMHvl. TU neurons are also overwhelmingly GABAergic (150), while VMHvl neurons are glutamatergic (41, 121, 149). Moreover, targeting ERα neurons using the *Vglut2-Cre* ablates all ERα in the VMHvl, whereas targeting ERα with *Vgat-Cre* leaves it fully intact in the VMHvl (10). This study establishes that TU neurons do not contribute to the pool of ERα-expressing neurons in the VMHvl region. Finally, TU and VMHvl neurons differentially affect food intake and activity. Whereas stimulating or ablating somatostatin-expressing TU neurons drives or reduces food intake, respectively, while leaving energy expenditure unchanged (151), $VMHvl^{ERα}$ neurons affect spontaneous activity and energy expenditure without changing food intake (44, 57, 137). In conclusion, VMHvl and TU neurons appear to represent two distinct neuronal populations that should be considered as separate anatomical and functional entities.

OTHER ESTROGEN-RESPONSIVE BRAIN REGIONS YET TO BE FULLY EXPLORED

The diverse functions of central estrogen signaling in two regions of the MBH, the ARC and VMHvl, are now beginning to come into sharp focus. With newer technologies, we have come to appreciate the diversity of neuronal subtypes within these two well-studied MBH nuclei. Following the molecular and functional annotation of hormone-responsive neurons, it becomes crucial to ask how estrogen modulates afferent/efferent projections and neuronal plasticity and how the quick pace of neurotransmission is influenced by the slower time course inherent in classic genomic signaling. More effort is needed to gain the level of clarity that has been brought to bear on well-studied cortical regions, such as the hippocampus. In addition, other brain regions are sure to contribute to sex differences in energy homeostasis and deserve further study. Among the hormone-sensitive brain regions worth considering, two of the most tantalizing sexually dimorphic regions include the preoptic area of the hypothalamus, the MPOA, and the central amygdala region, the MeA. Although these two brain regions have not yet received the same attention given to the ARC and VMHvl, three studies in the last year established that excitatory and inhibitory neurons in the MPOA and periventricular nucleus, some of which are estrogen responsive, control core body temperature (152–154). It remains to be determined whether all or just some of these thermoregulatory neurons are directly modulated by estrogen, perhaps accounting for the higher core body temperature observed in C57BL/6 female mice that is also hormone dependent (155). On a similar note, most studies seeking to classify the molecular properties of neurons and circuits that modulate female physiology have used intact or OVX adult female mice. On occasion, aged intact female mice are included. Casting a broader net to ask how estrogen-responsive neurons in the ARC or VMHvl directly or indirectly affect the late stages of pregnancy or lactation when estrogen surges or drops, respectively, will be of interest.

Finally, while much of this review is focused on estrogen signaling in neuronal function, another understudied but exciting area is the role of estrogen in central neuroinflammatory responses and metabolism, as reviewed in Reference 156. Related to this discussion, others propose that estradiol protects the female brain from inflammation by maintaining the integrity of the BBB (157). Depleting neuron-derived E2 in the forebrain by targeting *Cyp19a1* increased neuroinflammation and damage and resulted in cognitive deficits in the Barnes test in a two-vessel occlusion model for generating global ischemia (158). Dietary excess easily triggers the morphological hallmarks of microgliosis in the MBH before overt obesity, but only in male mice, as reviewed elsewhere (159). This sex difference begs the question as to whether estrogen signaling in adjacent MBH neurons or microglia confers protection in females. On that note, neuroinflammatory gene markers were found depressed in microglia harvested from female brains. However, when transplanted into a focal cerebral ischemia model, their protective effects do not depend on circulating sex steroids (160, 161). Future efforts in this research area are highly relevant to understanding the onset of neurodegenerative diseases as females age.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The identification of ERα-dependent changes in gene expression and circuit architecture is clarifying the impact of hormone signaling across cell types and timescales. Ultimately, it becomes important to know if estrogen-responsive brain nodes discovered in animal models will translate to women's health. Two life conditions are worth considering in this context: (*a*) natural hormone fluctuations across lifespan that affect health ranging from metabolism to mental disorders and (*b*) frequently prescribed drugs that profoundly change hormone levels. An example of the first condition is human menopause, which often leads to metabolic decline, including decreased lean mass, increased visceral adiposity, and poor blood glucose regulation (162); every one of these symptoms mimics phenotypes in female rodents lacking central estrogen signaling. The widening time gap between the age of menopause onset and today's extended longevity in women exacerbates health issues and raises a fundamental question: How can postmenopausal women achieve healthy aging in an estrogen-depleted state? This concern is further compounded for younger women subjected to premature menopause induced by hormone therapy. Examples of the second condition that involve drug therapies are plentiful. While widely used today for minimizing postmenopausal symptoms and for feminizing hormone treatment in transgender medicine, estrogen replacement therapy is not without risks. Oral contraceptives taken by millions of women override hormonal fluctuations during the menstrual cycle, masking the normal ebb and flow of estrogen on brain function.

Additionally, to prevent the reoccurrence of ER-positive breast cancer, millions of survivors are urged to take antihormonal therapy for 5–10 years. Commonly prescribed drugs include the selective ER modulator tamoxifen or aromatase inhibitors, such as anastrozole, exemestane, and letrozole. In addition, newer selective ER degraders have recently emerged that block estrogen signaling and induce degradation of ERα (56). Given that some of these drugs pass readily through the BBB, knowing how they regulate hormone-sensitive brain regions could ultimately address unwanted side effects, thereby improving premature termination of these cancer therapies. To date, only a handful of studies have addressed this issue, and more basic research in this understudied area is needed.

In the future, investigators should consider incorporating experimental parameters that better address the notable sex differences in male and female rodents. For example, using thermoneutral housing temperatures in female mice can provide a much more robust experimental model that more closely mimics the human lifestyle (163). Alternatively, leveraging new techniques, such as CRISPR-based gene editing to retool the classic lab rat model would allow one to leverage their reliable and robust physiological responses, especially when assessing reproduction. Finally, to fully appreciate how central estrogen signaling elicits appropriate metabolic and behavioral responses, the field must also work out how the slow course of hormone action effectively modulates neurotransmission. In meeting these challenges, our research community will continue to actively reshape the science of hormone action in the brain.

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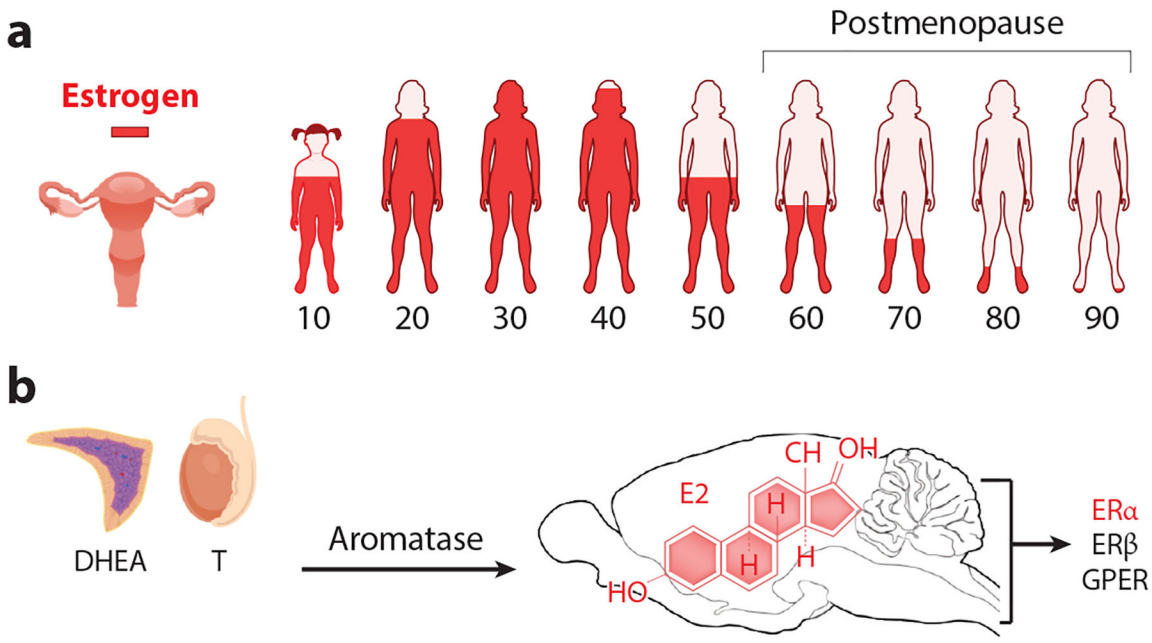


Figure 1.

Estrogen signaling in females and the medial basal hypothalamus. (*a*) Levels of ovarian estrogen during lifespan in women with significant depletion beginning before menopause (~47 years old). (*b*) Sources of androgens from the testes or adrenals can be converted to estradiol in the gonads, tissues, or in specific brain regions expressing *Cyp19A1*. E2 binds three estrogen receptors, all of which are found in the brain. Abbreviations: DHEA, dehydroepiandrosterone; E2, estradiol; ERα, estrogen receptor alpha; ERβ, estrogen receptor beta; GPER, G protein–coupled transmembrane estrogen receptor.

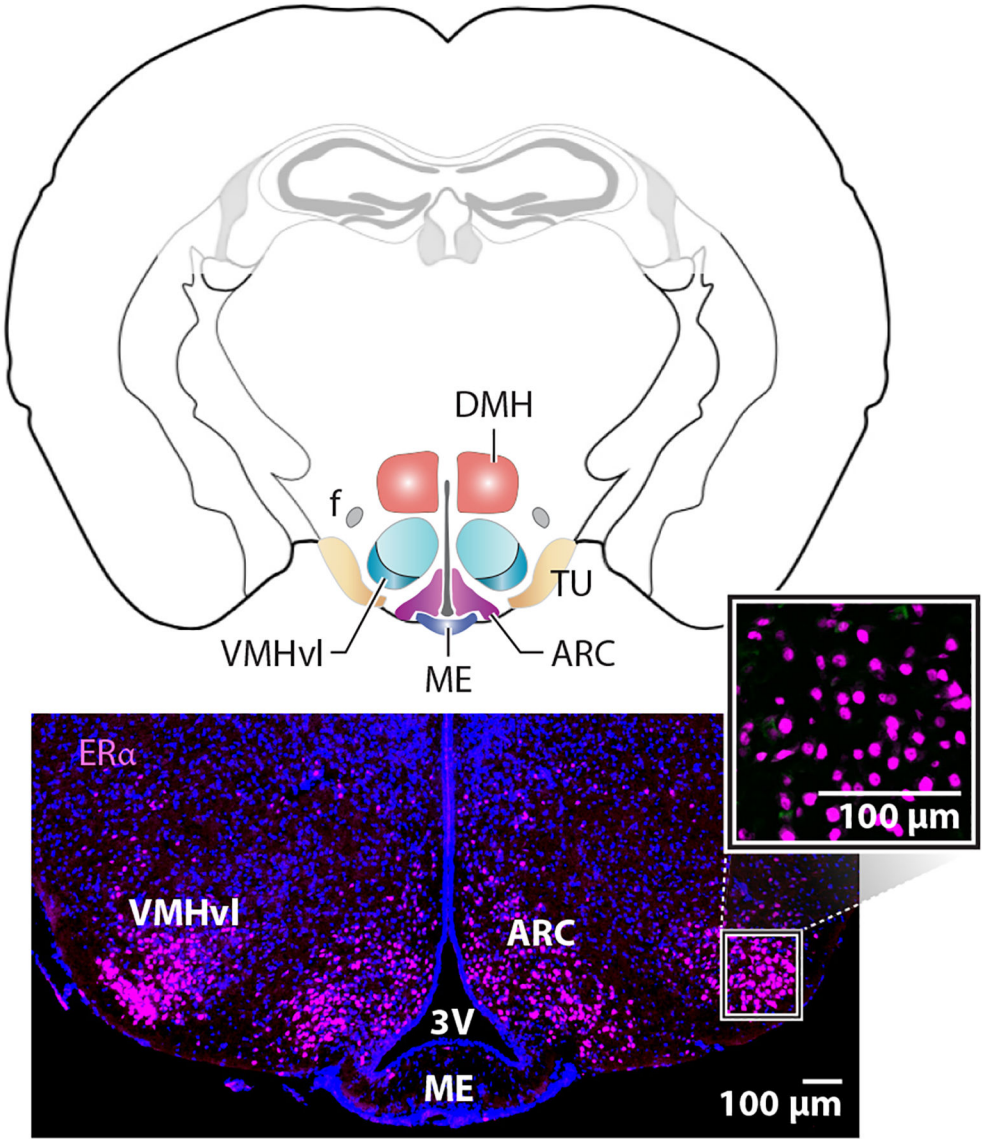


Figure 2.

Enriched expression of estrogen receptor alpha (ERα) in the female medial basal hypothalamus is independent of gonadal hormones. Coronal mouse brain schematic with colored regions of interest, including the ventral lateral region of the ventromedial hypothalamus (VMHvl), the arcuate nucleus (ARC), and the tuberal nucleus (TU). Other landmarks include the fornix (f), dorsal medial hypothalamus (DMH), median eminence (ME), and third ventricle (3V). Lower panels show the expression of ERα in the VMHvl and ARC. The higher magnification panel shows nuclear staining at basal hormone levels or in estrogen-depleted ovariectomized females.

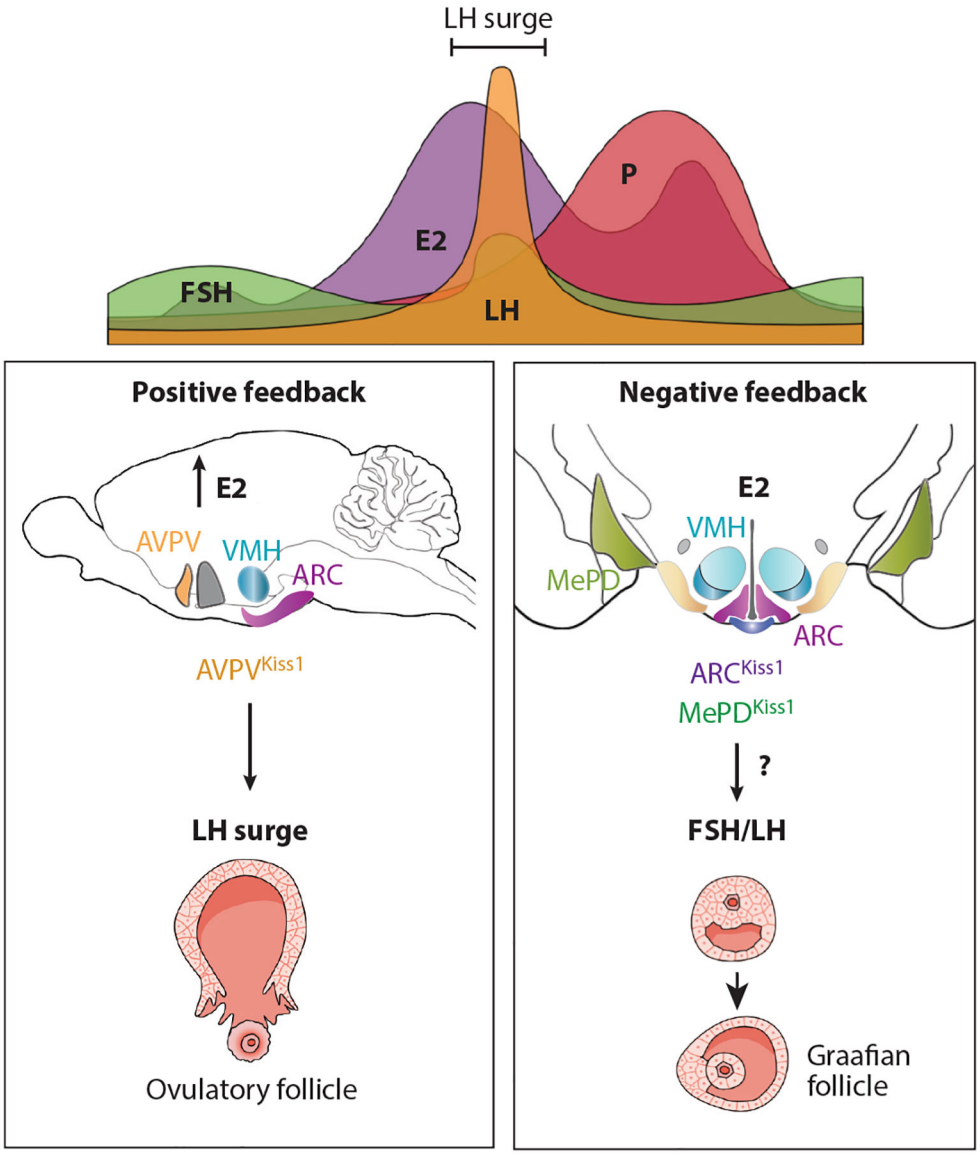


Figure 3.

Neurons in the female reproductive cycle. The top panel shows a typical cycle of hormone fluctuations with E2 levels peaking as LH surges to then trigger ovulation. Collective work, including specific targeting of $AVPV^{Kiss1}$ neurons, shows that this population is critical for generating an LH surge during high E2 levels for positive feedback (*left lower box*). Estrogen signaling in ARC^{Kiss1} neurons is important for maximizing the number of continuous estrous cycles: estrus, diestrus, and proestrus. The number of LH pulses or levels of LH are unchanged after deleting ERα in the ARC or ARC^{Kiss1} neurons. Thus, other estrogen-sensitive brain regions, such as the MeA, must contribute to negative feedback when E2 and LH are low (*right lower box*). Abbreviations: ARC, arcuate nucleus; AVPV, anteroventral periventricular (nucleus); E2, estradiol; ERα, estrogen receptor alpha; FSH, follicle-stimulating hormone; Kiss, kisspeptin; LH, luteinizing hormone; MeA, medial

amygdala; MePD, posterodorsal medial amygdala; P, progesterone; VMH, ventromedial hypothalamus.

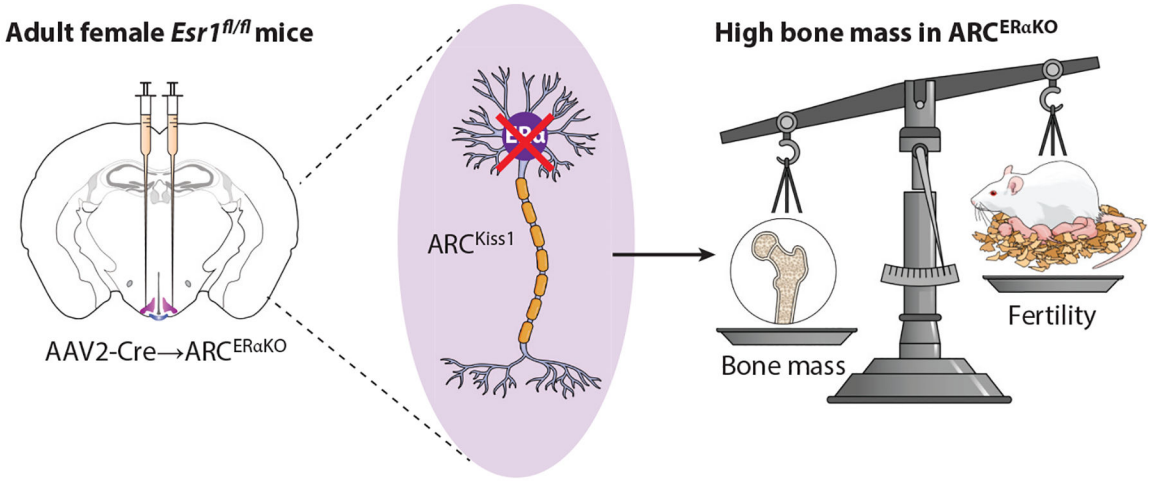
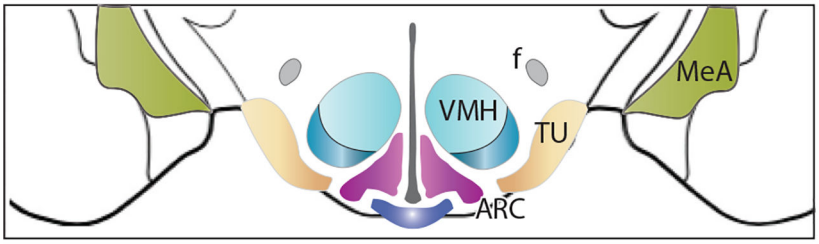


Figure 4.

Estrogen signaling in female ARC^{Kiss1} neurons restrains bone. Scheme showing stereotaxic elimination of *Esr1*$^{fl/fl}$ in the ARC. Other genetic methods have also been used and show a significant increase in bone mass or high bone mass phenotype with drop infertility. The underlying molecular mechanism for this brain-to-bone connection remains to be determined. Abbreviations: AAV, adeno-associated virus; ARC, arcuate nucleus; Kiss, kisspeptin; KO, knockout; VMHvl, ventral lateral region of the ventromedial hypothalamus.



	Lineage markers	ERα⁺ subtypes	Exitatory or inhibitory	ERα⁺ function in females
ARC	*Dlx* *Otp* *Nkx2-1* *Tbx3*	*Kiss1* *Th/Slc6a3* *Pomc/Cart* *Sst* *Ghrh*	GLUT GABA	Reproduction Bone metabolism Energy balance
VMHvl	*Nkx2-1* *Sf-1*	*Sst* *Tac1* *Rprm* *Mc4r*	GLUT	Reproduction Physical activity Thermogenesis Glucose sensing
MeA	*Dbx1* *Sim1* *Foxp2* *Nkx2-1*	*Kiss1*	GABA (MePD) GLUT (MePV)	Reproduction Physical activity
TU	*Nkx2-1* *Otp* *Dlx*	*Sst* (ERα⁻)	GABA	Food intake

Figure 5.

Diversity in estrogen-responsive ARC and the VMHvl neurons. Schematized coronal section through the MBH brain region. Table summarizing lineage markers, defined ERα neuron subtypes, excitatory (GLUT) or inhibitory (GABA) neurotransmitter type, and functional changes in female mice following regional knockout of ERα for the ARC, VMHvl, MeA, and TU. All four areas share *Nkx2-1* as a common developmental marker, and as such, Nkx2-1-Cre will eliminate the expression of genes in these and other brain nuclei. Note that *Pomc*/*Cart* neurons include both excitatory and inhibitory subtypes. Abbreviations: ARC, arcuate nucleus; *Cart*, cocaine and amphetamine-regulated transcript; ERα, estrogen receptor alpha; f, fornix; GABA, gamma-aminobutyric acid; *Ghrh*, growth hormone–releasing hormone; GLUT, glutamate; *Kiss*, kisspeptin; MBH, medial basal hypothalamus; *Mc4r*, melanocortin-4 receptor; MeA, medial amygdala; MePD, posterodorsal medial amygdala; MePV, posteroventral medial amygdala; *Pomc*, proopiomelanocortin; *Rprm*,

Reprimo; *Sst*, somatostatin; *Th*, tyrosine hydroxylase; TU, tuberal nucleus; VMHvl, ventral lateral region of the ventromedial hypothalamus.

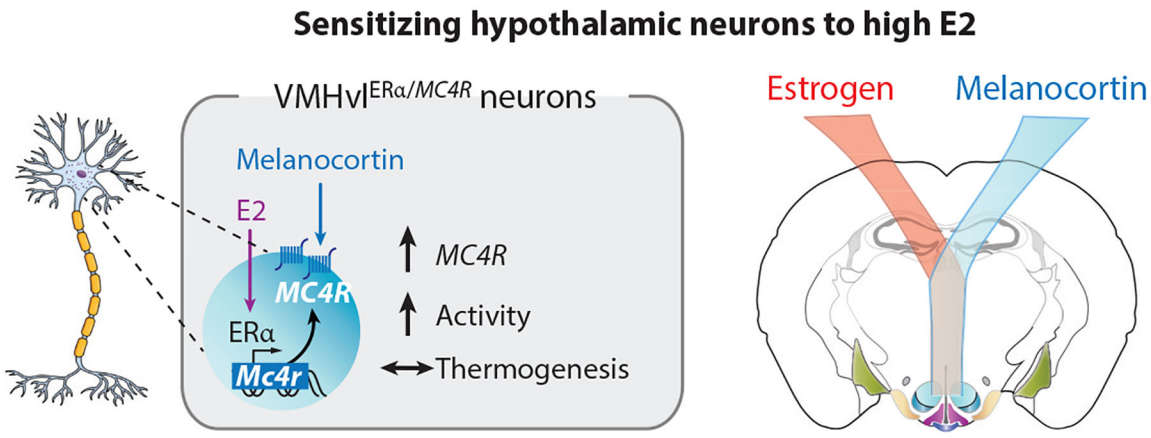


Figure 6.

Estrogen signaling in female VMHvl$^{ERα/MC4R}$ neurons promotes locomotor activity. High estrogen levels during proestrus or after E2 treatment increase *MC4R* expression in about 200 VMHvl neurons, which, when stimulated, promote physical activity. This molecular pathway demonstrates how the estrogen receptor can sensitize adaptive behaviors to prioritize energy utilization in service of reproductive fitness. Abbreviations: E2, estradiol; ERα, estrogen receptor α; *MC4R*, melanocortin-4 receptor; VMHvl, ventral lateral region of the ventromedial hypothalamus.