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Commentary

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Activation of estrogen receptor α (ERα) in the brain prevents obesity as the result of increased energy expenditure and decreased food intake. While ERα is present on several neural populations, it is not clear how different regions of the brain mediate the weight-regulating effects of ERα activation. In this issue of the *JCI*, Xu and colleagues provide extensive evidence that ERα is abundant on neurons expressing single-minded–1 (SIM1) in the medial amygdala (MeA) and that loss of ERα in these cells enhances weight gain in both male and female mice, as the result of reduced physical activity. Moreover, focal deletion of ERα from the MeA recapitulated these alterations in energy homeostasis. Conversely, overexpression of ERα in the MeA partially prevented mice from diet-induced obesity, while chemogenetic activation of SIM1-expressing neurons in the MeA transiently promoted physical activity. The results of this study provide important insight into the regions of the brain that mediate ERα-dependent energy homeostasis.

Estrogen receptor α and body weight homeostasis

Decades of research have revealed that estrogen and its downstream target, estrogen receptor α (ERα, encoded by *ESR1*), are important mediators of body weight homeostasis (1–3). Multiple studies have demonstrated that activation of ERα by estrogen regulates food consumption, energy expenditure, and fat distribution (4–6). Importantly, these substantial alterations in energy balance are governed by neural mechanisms, as loss of ERα expression in the CNS impairs multiple facets of homeostatic equilibrium (7). Compared with control animals, mice with a CNS-specific ERα deficiency display increased body weight, adiposity, and visceral fat distribution as the result of hyperphagia, as well as decreased heat production and physical activity.

Previous studies have aimed to anatomically pinpoint and dissect specific functions of ERα in distinct regions of the brain. Deletion of *Esr1* in steroidogenic factor-1 (SF1) neurons of the ventromedial hypothalamic nucleus (VMH) increases weight gain in female mice due to decreased energy expenditure through a reduction of resting metabolic rate and thermogenesis but no differences in physical activity or energy intake (7). A model in which specific regions of the brain mediate the anabolic effects of ERα are further supported by the demonstration that deletion of *Esr1* from pro-opiomelanocortin (POMC) neurons of the arcuate nucleus of the hypothalamus (ARC) produces weight gain in female mice due to chronic hyperphagia (7). Regulation of food intake has been attributed to ERα expression in the preoptic anterior hypothalamus (POAH) (8), the dorsal raphe nucleus (DRN) (9), and the nucleus of the solitary tract (NTS) (10) of the hindbrain. While ERα expression in specific neurons and brain regions account for changes in heat production and food intake, the ERα-expressing neurons that alter physical activity have yet to be elucidated. In this issue, Xu et al. demonstrate that targeted deletion of *Ers1* from single-minded–1 (SIM1) neurons, which are abundantly expressed in the medial amygdala (MeA), causes hypoactivity and obesity in both male and female mice (11).

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Metabolic consequences of ERα deficiency in SIM1 neurons

Xu et al. explored potential areas of the brain that coexpress ERα and the transcription factor SIM1 by systematically quantifying overlap, and they deduced that the most abundant SIM1-expressing ERα neurons are located in the MeA (~80%) in both males and females (11). Interestingly, the number of SIM1-expressing ERα neurons in the MeA was higher in males (~7,000) compared with females (~4,000), a discrepancy that may account for disparate phenotypes between sexes. Although ERα-expressing SIM1 neurons were found in the paraventricular hypothalamic nucleus (PVN) and POAH, colocalization of SIM1 and ERα was minimal in these regions, compared with the MeA.

Having located the anatomical sites of intersectional SIM1 and ERα expression, Xu and colleagues selectively deleted *Esr1* from SIM1-expressing neurons (*Sim1-Cre Esr1*$^{fl/fl}$, referred to as SIM1-ERα-KO mice), which resulted in a multitude of metabolic consequences (11). When fed a standard chow diet, both female and male SIM1-ERα-KO mice exhibited late-onset obesity that was associated with increased fat mass as a result of reduced energy expenditure. Specifically, this fall in energy expenditure was ascribed to a robust decline in physical activity (assessed by ambulatory movements and rearing) that primarily occurred during the dark cycle: the subjective active period of these animals. Notably, the decreases in energy expenditure were subtle, producing weight gain over an appreciable time, which suggests that ERα signaling in SIM1 neurons may prevent

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age-associated obesity. Alternatively, as these animals are ERα deficient from the embryonic stage, the phenotypic effects may be tempered due to developmental compensation.

Nevertheless, SIM1-ERα-KO males were susceptible to diet-induced obesity, as these animals gained substantially more weight than controls when placed on a high-fat diet (HFD). Intriguingly, SIM1-ERα-KO females did not have the same predisposition for diet-induced obesity, as they had comparable weights to control animals. The drastic sex-dependent difference in overall number of ERα-expressing SIM1 neurons in the MeA may explain this phenotypic dimorphism. Alternatively, Xu et al. implicate that the female brain may comprise more resilient redundant pathways than their male counterparts. Reinforcing this view, multiple ERα-expressing sites in the brain, including the ARC and VMH, have been linked to energy-balance regulation in females (7); however, this ERα-expressing SIM1 population in the MeA is the lone population identified to this point that mediates body weight homeostasis in males.

A potential caveat of the results obtained from SIM1-ERα-KO mice is that the observed effects may be due to alteration of ERα-meditated genes that are also involved in cell signaling and neural communication (12). To address this issue, Xu and colleagues analyzed gene-expression profiles in the amygdala and found no marked changes in mRNA encoding the androgen receptor (AR), aromatase, glutamate decarboxylase 1 (GAD1), glutamate decarboxylase 2 (GAD2), glutaminase (GLS), glutamate-ammonia ligase (GLUL), melanocortin 4 receptor (MC4R), or nitric oxide synthase–1 (NOS1) in male or female SIM1-ERα-KO mice compared with controls (11). Furthermore, expression of hypothalamic genes associated with body weight regulation — such as those encoding agouti-related peptide (AgRP), leptin receptor, NOS1, neuropeptide Y (NPY), and POMC — were similar to controls. However, the possibility remains that undetected alternate gene-expression patterns and/or levels may affect energy balance in SIM1-ERα-KO mice. It should also be noted that deletion of *Esr1* from SIM1 neurons may be deleterious to endocrine function and/or chronic behavior; however, Xu et al. did not report any deficits in fertility between control and SIM1-ERα-KO dams. Moreover, Xu et al. employed a battery of tests and demonstrated that the hypoactivity observed in SIM1-ERα-KO mice is independent of anxiety, a state repeatedly assigned to neural circuits in the amygdala (13). Specifically, there was no difference in responses between SIM1-ERα-KO mice and control animals when tested in open field, light/dark, and elevated plus maze assays. Thus, the sharp decrease in locomotor activity observed in SIM1-ERα-KO animals is independent of a permanent anxiogenic condition.

The MeA mediates ERα-dependent effects on physical activity

The abundant compensatory mechanisms that are involved in the neural control of energy balance are a major limitation to genetic studies that rely on the developmental deletion of a particular allele (14–17). Furthermore, the gene of interest will be deleted in all cells that express the gene driving Cre expression. As SIM1-expressing neurons are present in multiple regions of the brain, the conclusions of Xu et al. based on SIM1-ERα-KO mice are limited in regard to the precise anatomical region of action. Xu et al. employed a viral-mediated delivery approach to further specify the region of the brain that mediates ERα-dependent effects on activity (11). The MeA of *Esr1*$^{fl/fl}$ mice was stereotaxically targeted with an adeno-associated virus expressing Cre-recombinase (AAV-Cre). Compared with control mice, selective removal of ERα from the MeA in adult animals resulted in a rapid, elevated weight gain on a standard chow diet and a dramatic body weight increase in response to an HFD due to considerable reductions in physical activity. In contrast, deletion of *Esr1* exclusively from the PVH did not affect physical activity, further supporting the role of MeA-specific ERα activity in the regulation of energy balance.

Together, these loss-of-function studies establish that ERα function in the MeA is required for energy homeostasis, but is ERα activation sufficient to maintain the balance in response to HFD? Xu et al., addressed this question by selectively overexpressing ERα in the MeA in mice harboring a Cre-activated human *ERS1* allele in the *Rosa26* locus (11). Following restricted AAV-Cre injection, overexpression of human ERα in the MeA partially protected mice from diet-induced obesity, compared with controls fed an HFD. While these results suggest that ERα induction in the MeA can limit diet-induced obesity, this approach will result in ERα expression at quantities that far exceed endogenous levels, as well as ectopic expression. A more physiologically relevant tactic would be to apply a Cre-dependent reactivation strategy that has been previously used to test sufficiency in models of energy balance (18, 19). Such an approach would result in endogenous reexpression of ERα only at sites that normally express this receptor.

Xu and colleagues also investigated the effects of the highly selective ERα agonist propyl pyrazole triol (PPT) on neural activity (11). In a SIM1 reporter mouse, PPT depolarized, increased the firing rate of, and decreased input resistance in the majority of SIM1 neurons in the MeA, while blocking action potential–dependent network activity. These effects were most likely mediated through an ERα-dependent mechanism, as few SIM1-expressing MeA neurons were responsive to PPT in SIM1-ERα-KO mice. Moreover, chemogenetic activation of SIM1-expressing MeA neurons with designer receptors exclusively activated by designer drugs technology (DREADD technology) (20, 21) resulted in acute stimulation that transiently heightened physical activity. Interestingly, the hM3Dq DREADD system that was employed has been demonstrated to signal for hours (14, 15, 20, 21); however, the behavioral effects in this model were short-lived. Xu et al. hypothesized that although SIM1-expressing MeA neurons can alter activity, they fail to regulate arousal and circadian control; therefore, activation during the light cycle led to transitory changes. It would be of value to repeat these experiments during the active period of these animals to further dissect the precise function of these neurons.

Conclusions and future directions

In humans, plasma levels of glucagon-like peptide–1 (GLP-1) strongly correlate with body fat mass in healthy adults (22). Treatment with GLP-1 receptor agonists leads

to weight loss in obese patients, particularly in those with diabetes (23). Furthermore, mice treated with a GLP-1–estrogen conjugate exhibit a greater weight reduction than those treated with GLP-1 alone, an approach that takes advantage of the weight loss effect of estrogen but avoids its carcinogenic qualities (24). Interestingly, GLP-1 receptors are densely expressed in the MeA (25). Xu and colleagues further identified a node through which GLP-1–estrogen exerts its therapeutic effects to combat obesity. Subcutaneous administration of GLP-1–estrogen increased expression of the estrogen target *Trim25* in both the amygdala and the hypothalamus, suggesting that ERα in the MeA contributes to the weight-regulating effects of this co-agonist conjugate. In support of this possibility, treatment of SIM1-ERα-KO mice with the GLP-1–estrogen conjugate was less effective at reducing body weight. Together, these results indicate that ERα-expressing SIM1 neurons in the MeA neurons may be critical for the weight loss–inducing properties of GLP-1–estrogen.

The work by Xu et al. provides further insight into the neuroanatomy involved in ERα-mediated obesity. To begin to address the neural circuitry through which ERα-expressing MeA neurons regulate changes in physical activity, this study demonstrates that the preponderance of SIM1-expressing MeA neurons are glutamatergic and these neurons send dense efferents to the red nucleus, as well as the dorsal and median raphe nuclei. Modern neuroscience tools have the ability to acutely and reversibly drive neural activity in animal models, allowing a more complete understanding of neuronal circuits involved in regulating specific phenotypes. Future studies should utilize these tools to trace inputs and outputs to ERα-expressing MeA neurons and elucidate unanswered inquiries, such as monosynaptic connectivity to and function of downstream regions on behavior (26). Moreover, these applications have potential to pinpoint the anatomical configuration of the circuitry involved in regulating phenotypes of interest, leading to putative therapeutic strategies.

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