



Cross-talk between metabolism and reproduction: the role of POMC and SF1 neurons

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Energy homeostasis and reproduction require tight coordination, but the mechanisms underlying their interaction are not fully understood. Two sets of hypothalamic neurons, namely pro-opiomelanocortin (POMC) neurons in the arcuate nucleus and steroidogenic factor-1 (SF1) neurons in the ventromedial hypothalamic nucleus, are emerging as critical nodes where metabolic and reproductive signals communicate. This view is supported by recent genetic studies showing that disruption of metabolic signals (e.g., leptin and insulin) or reproductive signals (e.g., estradiol) in these neurons leads to impaired regulation of both energy homeostasis and fertility. In this review, we will examine the potential mechanisms of neuronal communication between POMC, SF1, and gonadotropin-releasing hormone neurons in the regulation of metabolism and reproduction.

Keywords: hypothalamus, energy homeostasis, reproduction

INTRODUCTION

Since animals under metabolic stress must invest their energy in survival rather than reproduction, the reproductive axis has the capacity to respond to changes in caloric status. The hypothalamic signals driving the reproductive axis are suppressed when a mammal is in negative energy balance, whether that state is caused by inadequate food intake, excessive locomotor activity, or heavy thermoregulatory costs. Likewise, the energy demands of maintaining fertility and successful reproduction require increased food consumption and appropriate regulation of energy expenditure. Therefore, hypothalamic control of metabolism must be responsive to the reproductive state of the animal. However, despite the passage of 40 years since the discovery of gonadotropin-releasing hormone (GnRH; Schally et al., 1971), the afferent neuronal groups and pathways through which gonadal steroids and nutrient signals regulate GnRH release remain unresolved.

Much attention has focused on the role of hypothalamic neurons expressing kisspeptin in coordinating GnRH neuronal function and the physiological state of the animal. However, while the central role of kisspeptin in steroid feedback to the hypothalamus is clear, evidence of it conveying metabolic signals to the reproductive axis is equivocal. In addition, data suggest that other hypothalamic neurons also convey gonadal steroid input to GnRH circuitry, either directly or via the kisspeptin network.

Here we will discuss new findings resulting from genetically modifying pro-opiomelanocortin (POMC) and steroidogenic factor-1 (SF1) neurons of the hypothalamus. While primarily understood to function as metabolic regulators, these neurons are

emerging as critical nodes of communication that respond to both metabolic and reproductive cues and directly interact with reproductive circuitry. In particular, we will focus on their ability to transmit information gleaned from circulating factors, specifically leptin, insulin, and estradiol (E₂).

CANONICAL REPRODUCTIVE CIRCUITS

Hypothalamic GnRH neurons produce the final output of a complex neuronal system regulating fertility. Adult mammals possess a loose field of GnRH neurons stretching from the olfactory bulbs to the medial basal hypothalamus with a highly dense population of GnRH neurons within the preoptic area (POA) and adjacent to the organum vasculosum of the lamina terminalis (OVLT). The axons of GnRH neurons project to the median eminence (ME) where GnRH is secreted in pulses into the pituitary portal bloodstream. GnRH neurons can possess dendrites extending millimeters away from their cell bodies (Campbell et al., 2005; Cottrell et al., 2006), with the average extending over 550 μm (Roberts et al., 2006). Interestingly, many GnRH dendrites follow routes similar to those of their axons toward the ME. Dendrites of GnRH neurons frequently initiate action potentials due to their expression of voltage-gated sodium channels (Rhodes and Llinas, 2005). As a result of the morphology of these dendrites, highly proficient action potential initiation in the distal dendrites is possible even when synaptic potentials are quite small (Witkin and Silverman, 1985; Witkin et al., 1995). Distal portions of the GnRH dendrite, for instance segments located in the arcuate nucleus of the hypothalamus (ARC), thus provide synaptic input and can potentially

affect GnRH hormone secretion (Campbell and Suter, 2010; Zoli et al., 1998; Herbison, 2006).

A great deal of the recent focus on afferent control of GnRH secretion has been on kisspeptin neurons in the hypothalamus, based on the ability of mutations of the kisspeptin receptor to cause hypogonadotropic hypogonadism in humans and animal models (de Roux et al., 2003; Seminara et al., 2003). Kisspeptin exerts an extremely powerful and long-lasting depolarizing stimulus upon GnRH neurons (Gottsch et al., 2004; Han et al., 2005; Messenger et al., 2005; Pielecka-Fortuna et al., 2008) and plays a central role in the physiological regulation of GnRH release (Oakley et al., 2009; Ohkura et al., 2009). Confocal images have shown kisspeptin terminals in direct apposition to GnRH cell bodies (Clarkson and Herbison, 2006; Ramaswamy et al., 2008). In addition, in the rat and monkey, kisspeptin terminals form close contacts with GnRH terminals in the ME (Krajewski et al., 2005, 2010; Ciofi et al., 2006; Decourt et al., 2008; Lehman et al., 2010). Recent studies on the stimulatory effects of kisspeptin are consistent with actions at both the ME (d'Anglemont de Tassigny et al., 2008) and GnRH cell bodies in the POA (Pielecka-Fortuna et al., 2008; Moenter and Pielecka-Fortuna, 2010). Two populations of kisspeptin neurons exist in the hypothalamus; one is located in the anteroventral periventricular nucleus (AVPV; Smith et al., 2005), and the other is located in the ARC. Most ARC kisspeptin neurons co-express neurokinin B (NKB) and dynorphin (Dyn) in rat (Burke et al., 2006), mouse (Navarro et al., 2009), sheep (Goodman et al., 2007), goat (Wakabayashi et al., 2010), and possibly human (Rance, 2009) leading to these neurons acquiring the moniker of KNDy neurons (Cheng et al., 2010). Each of these three neuropeptides has been strongly implicated in the feedback regulation of GnRH neurons (Rance and Young, 1991; Sahu and Kalra, 1992; Rance and Bruce, 1994; Goodman et al., 2004; Foradori et al., 2005). Since the kisspeptin network is not the focus of this review, readers interested in this topic are referred to some excellent recent publications (Oakley et al., 2009; Navarro and Tena-Sempere, 2011).

POMC NEURONS LINK METABOLIC AND REPRODUCTIVE CIRCUITS

POMC NEURONS INNERVATE REPRODUCTIVE CIRCUITS

Besides kisspeptin neurons, other hypothalamic populations may also provide afferent signals on the hypothalamo-pituitary gonadal (HPG) axis to regulate reproduction. Particularly, recent evidence suggests that hypothalamic sites that regulate energy balance have important inputs to GnRH neurons. One example is POMC neurons in the ARC. POMC neurons have long been believed to be a primary central regulator of energy homeostasis (Huszar et al., 1997; Cone, 1999). The anorexigenic property of POMC neurons has been well established, as the deletion of POMC gene causes hyperphagia and obesity (Yaswen et al., 1999).

Evidence also suggests that POMC neurons innervate the reproductive circuits in the central nervous system (CNS). For example, POMC neurons make direct synaptic contact with GnRH neurons (Leranth et al., 1988; Thind and Goldsmith, 1988; Chen et al., 1989a). The major neurotransmitters released from POMC neurons include two POMC gene products: the anorectic peptide α -melanocyte-stimulating hormone (α -MSH) and the endogenous opioid β -endorphin (Cheung et al., 1997; Broberger et al., 1998; Hahn et al., 1998; Vrang et al., 1999). Fibers specifically

immunoreactive for β -endorphin have been identified within the immediate vicinity of GnRH neurons, and based upon electron microscopic evidence, the β -endorphin-immunoreactive terminals synapse on the GnRH neuron soma in the rat (Leranth et al., 1988; Chen et al., 1989b), sheep (Goodman et al., 2004), and monkey (Thind and Goldsmith, 1988). About 20–30% of POMC neurons in the ARC co-express estrogen receptor- α (ER α ; Lehman et al., 1993; de Souza et al., 2011; Xu et al., 2011), and an ER α -positive subpopulation of ARC POMC neurons has been shown to project to the POA, where GnRH neurons are concentrated (Simonian et al., 1999). Furthermore, GnRH neurons express receptors for β -endorphin. For example, μ -opioid receptors, have been identified in GnRH neurons of the guinea pig (Lagrange et al., 1995). In addition, δ -opioid receptors have been identified in mouse GnRH neuron-derived GT-1 cells and in a fraction of rat GnRH nerve terminals, including in the ME (Pimpinelli et al., 2006). Collectively, these findings indicate that POMC neurons are well positioned to provide synaptic inputs to GnRH neurons.

At the functional level, neurotransmitters released from POMC neurons have been shown to regulate the HPG axis. In both monkeys and rats, β -endorphin and other opioids exert inhibitory effects on GnRH and LH secretion (Bruni et al., 1977; Kinoshita et al., 1982; Gilbeau et al., 1985; Leadem and Kalra, 1985b,a; Wiesner et al., 1985; Leadem and Yagenova, 1987; Wardlaw and Ferin, 1990; Kalra and Kalra, 1996). This inhibitory effects appear to be tonic, as administration of naloxone, an opiate receptor antagonist, to rats increases LH production (Babu et al., 1987). Reducing the opioid inhibition also facilitates the production of the GnRH surge on proestrus (Lustig et al., 1988; Masotto et al., 1990; Hashimoto and Kimura, 1991; Zhang and Gallo, 2002). This inhibition by opioids can be interpreted as increased overall suppression of GnRH and LH release or as augmentation of steroid negative feedback. Indeed, opioid antagonism can also stimulate GnRH release independent of E₂ levels (Babu et al., 1987; Karahalios and Levine, 1988; Goodman et al., 1995). However, during the rat estrous cycle, β -endorphin levels fluctuate in the ARC, POA, and ME. The maximum levels are seen during diestrus while the lowest occurs on proestrus leading up to the LH surge (Gallo and Drouva, 1979), suggesting a role in negative feedback. While these studies strongly support an inhibitory effect of opioids on GnRH release, genetic evidence is less convincing. Mutant mice lacking the classical endogenous opioids (dynorphin, enkephalin, and endorphin; Konig et al., 1996; Rubinstein et al., 1996; Sharifi et al., 2001) as well as mutants of the three opioid receptors (Matthes et al., 1996; Sora et al., 1997; Roy et al., 1998; Simonin et al., 1998; Schuller et al., 1999; Zhu et al., 1999; Filliol et al., 2000) are fertile, although μ -opioid receptor-deficient mice display reduced spermatogenesis and impaired sexual function (Tian et al., 1997). Thus, at least when absent during the development of hypothalamic circuitry, opioid inhibition of GnRH release is not required for fertility.

In contrast, it has been demonstrated that α -MSH exerts an excitatory effect on the GnRH system, likely by acting on central melanocortin receptors (MCs; Celis, 1985; Backholer et al., 2009). A robust connection has recently been demonstrated between MCH neurons, which express MC4 receptors and receive direct input from POMC neurons, and GnRH neurons (Wu et al., 2009). Agouti-related peptide (AgRP), an endogenous receptor antagonist of MCs, decreases the magnitude of the LH surges in normally

fed rats (Watanobe et al., 1999; Schioth et al., 2001; Schioth and Watanobe, 2002), while anti-AgRP serum partially but significantly enlarges the LH surge (Schioth et al., 2001). Indeed, melanocortin 4 receptor (MC4R) deficient mice exhibit erectile dysfunction and changed sexual behavior in males secondary to obesity (Van der Ploeg et al., 2002) and reduced ovulation rates and fertility accompanied by increased follicular atresia by 6 months of age in females (Sandrock et al., 2009). Furthermore, *agouti* mice, which congenitally overproduce AgRP, have adult-onset infertility (Granhölm et al., 1986). Collectively, these findings point to α -MSH as a potential afferent signal to the HPG axis.

In addition to neuropeptides, POMC neurons release classical neurotransmitters GABA and glutamate (Hentges et al., 2004, 2009), both of which have been shown to regulate GnRH neurons (Kusano et al., 1995; Shepherd, 1996; Spergel et al., 1999; Sim et al., 2000; Sorra and Harris, 2000; Simonian and Herbison, 2001; DeFazio et al., 2002; Fiala et al., 2002; Han et al., 2002; Kuehl-Kovarik et al., 2002; Ottem et al., 2002; Moenter and DeFazio, 2005). In addition to direct synaptic inputs, POMC neurons may also influence GnRH neurons via kisspeptin intermediary neurons. Supporting this possibility, kisspeptin fibers have been shown in close apposition with ARC POMC neurons in ewes (Backholer et al., 2009, 2010), and double-label fluorescent immunohistochemistry showed that reciprocal connections exist between kisspeptin neurons and POMC neurons (Backholer et al., 2010).

The studies reviewed above emphasize the critical “choice” of POMC neurons to express β -endorphin or α -MSH. The former, possibly acting in concert with other opioids such as dynorphin (via its own kappa-opioid receptor; Navarro et al., 2009), is intimately involved in the negative feedback regulation of GnRH release. The latter, through activation of second-order metabolic circuitry, is involved in the gating of fertility during times of energy deprivation. The control of β -endorphin vs. α -MSH production by POMC neurons is an area of ongoing study (Wardlaw, 2011).

LEPTIN AND INSULIN ACT ON POMC NEURONS TO REGULATE BOTH REPRODUCTION AND ENERGY HOMEOSTASIS

Emerging evidence indicates that POMC neurons respond to metabolic cues to provide coordinated control of metabolism and reproduction. One example of these metabolic cues is leptin. Leptin is a circulating adiposity-related factor that informs the CNS regarding energy stores. Released by adipocytes when stored fat is plentiful, leptin acts in the hypothalamus to suppress body weight gain and to improve insulin sensitivity (Morton et al., 2003, 2005; Balthasar et al., 2004; Coppari et al., 2005; Dhillon et al., 2006; van de Wall et al., 2008). Mice lacking leptin or leptin receptors (LepRs) develop hyperphagic morbid obesity, insulin resistant diabetes, and hypothermia (Coleman, 1978). Leptin reduces food intake and body weight when administered to leptin-null mice (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995), and brain-specific deletion of LepRs leads to obesity (Cohen et al., 2001).

Leptin is also a prerequisite for pubertal development and successful reproduction. Humans and mice carrying leptin gene mutations fail to go through puberty, have low LH levels, and are infertile (Montague et al., 1997), and leptin administration, but not weight loss alone, allows pubertal progression and restores their

fertility (Barash et al., 1996; Chehab et al., 1996; Mounzih et al., 1997; Zioutopoulou et al., 2000). Leptin also overrides the fasting-induced suppression of LH secretion and fertility (Nagatani et al., 1998, 2000; Gonzalez et al., 1999; Kohsaka et al., 1999). In anorectic females and in athletes with extreme decreases in body adiposity, leptin can increase levels of luteinizing hormone (Licinio et al., 1998) and restore the menstrual cycle (Welt et al., 2004). Re-expression of LepRs in the brain of LepR-null mice restores fertility completely in males and partially in females (Kowalski et al., 2001; de Luca et al., 2005). In addition, AAV-induced expression of the LepR gene in the POA or ARC of LepR-null rats normalizes their estrous cycle length and increases GnRH concentrations in the hypothalamus (Keen-Rhinehart et al., 2005). Collectively, this evidence indicates that leptin, while primarily acting as a metabolic signal to maintain normal energy and glucose homeostasis, also plays essential roles in reproduction.

Insulin, another circulating factor related to adiposity, is also implicated in the coordinated control of metabolism and reproduction. Insulin levels in the circulation are proportional to adipose tissue in most mammals (Woods et al., 1979). Intracerebroventricular (icv) insulin administration results in a dose-dependent reduction in food intake and body weight (Woods et al., 1979), and neuron-specific deletion of insulin receptors (IRs) leads to increased body fat deposition (Bruning et al., 2000).

A variety of mouse models have demonstrated insulin's essential role in the central control of reproduction. For example, increased circulating levels of insulin during a hyperinsulinemic clamp stimulate LH secretion (Burcelin et al., 2003a). In addition, mice lacking IRs in the brain exhibit decreased spermatogenesis and follicular maturation, resulting in only 42–46% of matings successfully producing offspring (Bruning et al., 2000). The primary deficit in these mice was found to be a reduction in GnRH release and consequent reductions in pituitary gonadotropin secretion and gonadal function. In another study, expression of IRs in liver and pancreas alone was sufficient to maintain fertility in males, but females also required IR expression in the brain (Okamoto et al., 2004). Finally, a study of IR substrate 2 (IRS-2) knockout mice found that only 9% of IRS-2^{-/-} females and 89% of IRS-2^{-/-} males were fertile. IRS-2^{-/-} females showed a supranormal response to GnRH, consistent with hypothalamic hypogonadism (Burks et al., 2000). These studies show that, particularly in the female, IRs in the brain are required for fertility.

While it is clear that both leptin and insulin could signal the brain to coordinate energy status and reproductive demands, the exact brain sites where these signals are integrated remained unclear. GnRH neurons do not appear to be the direct target of these hormones. For example, double-labeling experiments using *in situ* hybridization and immunohistochemistry have shown few GnRH neurons, if any, to express LepRs in rats (Burcelin et al., 2003a) and monkeys (Finn et al., 1998). Insulin has a direct stimulatory effect on the output of GnRH in hypothalamic cells *in vitro* and *in vivo* (Kovacs et al., 2002; Burcelin et al., 2003b). However, mice lacking IRs specifically in GnRH neurons displayed normal pubertal timing and fertility (Divall et al., 2010), suggesting that the insulin responsiveness of GnRH neurons is low. While early reports suggested that 40% of kisspeptin mRNA-expressing cells in the ARC of mice express LepRs (Smith et al., 2006), other

laboratories have found fewer than 5% of kisspeptin neurons exhibit LepRs (Donato et al., 2011; Louis et al., 2011). The latter results appear to be borne out by the lack of a reproductive phenotype in mice with a targeted deletion of LepRs from kisspeptin neurons (Donato et al., 2011). Similarly, our preliminary data suggest that insulin sensing directly by kisspeptin neurons plays a minor role in mouse fertility (Qiu et al., 2011). Thus, leptin/insulin sensing outside of the dedicated reproductive circuitry is likely to play a role in their effects on reproduction.

POMC neurons are well positioned to be a direct target of leptin and insulin signals. POMC neurons express LepRs (Cheung et al., 1997; Elmquist et al., 1998; Baskin et al., 1999) and IRs (Benoit et al., 2002). LepRs in POMC neurons mediate a portion of leptin actions on energy homeostasis, as mice lacking LepRs specifically in POMC neurons are mildly obese and hyperleptinemic (Balthasar et al., 2004). Although deletion of IRs from POMC neurons does not affect body weight (Konner et al., 2007), simultaneous deletion of IRs and LepRs from POMC neurons produces more severe insulin resistance and diabetes than deletion of each individual receptor alone (Hill et al., 2010). Therefore, POMC neurons appear to be one important site where insulin and leptin signals interact to regulate energy and glucose homeostasis.

Our recent studies also pinpointed POMC neurons as a target of leptin/insulin actions important for fertility. We have reported that female mice lacking both leptin and IRs in POMC neurons (IR/LepR^{POMC}) exhibit lengthened reproductive cycles, follicular arrest, hyperandrogenemia, and infertility. These mice lack IRs and LepRs in POMC-expressing cells in the hypothalamus and pituitary corticotrophs and melanotrophs, but retain them in other cell types and tissues, such as liver and ovary (Hill et al., 2010).

These results were confirmed by an absence of altered IR and LepR expression in these tissues using qPCR. Despite the expression of POMC in corticotrophs, we found no alteration in corticosterone release. Pup numbers born to experimental females older than 4 months were significantly reduced (Hill et al., 2010). These females also showed a lengthened estrous cycle. In addition, the percentage of matings not producing a litter was higher for experimental females across all maternal ages. These reproductive deficits were not caused by abnormal prolactin or E₂ levels, and no pups born to IR/LepR^{POMC} dams died after birth. While hypothalamic GnRH gene expression was comparable among the groups, LH levels were significantly increased in IR/LepR^{POMC} females. Histological examination of their ovaries showed that double knockout females exhibited more degenerating follicles. Serum testosterone levels were significantly elevated in experimental females, accompanied by a significant elevation in the expression of ovarian 3 β -HSD I gene, which produces androstenedione. CYP17 gene expression was also slightly increased ($p = 0.0530$; Hill et al., 2010). Interestingly, males also exhibit reduced numbers of successful pairings with wild-type females despite an enthusiastic mounting response and increased testes weights (Figures 1A–C). In addition, a subset of males exhibited dramatically increased LH levels (Figure 1D) with normal FSH concentrations (data not shown). The heterogeneity in these mice may be due to their mixed strain background. Collectively, these results suggest that the absence of leptin and insulin signaling in POMC neurons may reduce the inhibitory opioid tone on GnRH neurons and cause basal LH levels to increase, disrupting reproductive function. Indeed, the absence of leptin and insulin signaling would both be expected to reduce β -endorphin production from its POMC precursor (Wardlaw, 2011).

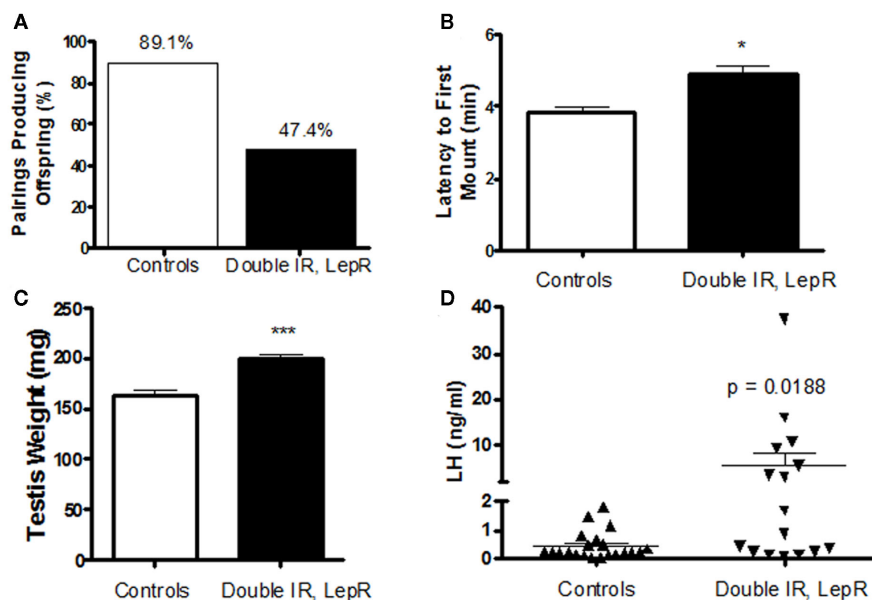


FIGURE 1 | Reproductive phenotype of 4-month-old IR, LepR^{POMC} mice.

(A) Male mice lacking insulin and leptin receptors in POMC neurons were paired with genetically normal control females and the number of pairings producing pups were quantified. (B) The amount of time before experimental and control males attempted to mount a novel control female was quantified.

Latency was scored during a 10-min period after male was introduced to the cage of a singly housed female in Proestrus under red lighting during the first half hour after lights out (1800 h). (C) Testes weights were measured at time of sacrifice. (D). Serum levels of luteinizing hormone were measured by RIA by the University of Virginia Ligand Assay and Analysis Core.

Further studies are required to determine whether β -endorphin production in these mice is reduced, and the signaling mechanisms involved.

ESTROGENS ACT ON POMC NEURONS TO REGULATE BOTH REPRODUCTION AND ENERGY HOMEOSTASIS

Steroid hormones such as E_2 exert potent feedback at both the neural and pituitary levels to regulate the HPG axis. During much of the female reproductive cycle, E_2 reduces the GnRH pulse amplitude (Sarkar and Fink, 1980; Caraty et al., 1989; Chongthammakun and Terasawa, 1993; Evans et al., 1994) and inhibits LH release via negative feedback actions on the hypothalamus as well as the pituitary gonadotropes (Shupnik et al., 1988; Shupnik, 1996). A similar mechanism appears to be at work in males via aromatization of testosterone to E_2 (Veldhuis and Dufau, 1987; Finkelstein et al., 1991a,b; Bagatell et al., 1994; Hayes et al., 2000; Schnorr et al., 2001). In the course of the later stage of the follicular phase, circulating E_2 levels rise resulting in a biphasic effect on GnRH secretion, causing a noticeable suppression of pulsatile GnRH and LH secretion, followed by an induction of a high amplitude GnRH surge supplementary to the LH surge, a process termed positive feedback (Crowder and Nett, 1984; Moenter et al., 1990). Lesion studies performed in the rat and hamster (Shander and Barraclough, 1980; Wiegand et al., 1980) have implicated anatomically distinct brain regions for the conveyance of the positive and negative feedback. These studies localized negative feedback to the ARC and ME and positive feedback to the POA and the suprachiasmatic nucleus.

Both positive and negative feedback regulation are primarily mediated by $ER\alpha$, but not by estrogen receptor- β ($ER\beta$). This is demonstrated by observations that global deletion of $ER\alpha$ in mice abolishes the positive and negative feedback responses, while mice with global $ER\beta$ deficiency appear to retain normal feedback responses (Couse et al., 1995, 2003; Wintermantel et al., 2006). However, the sites where estrogens act to regulate the HPG axis have not been fully revealed. Wintermantel et al. (2006) demonstrated that female mice lacking $ER\alpha$ only in the brain show blunted positive feedback and impaired fertility. While these findings identified brain $ER\alpha$ as the key mediator for the positive feedback, the exact $ER\alpha$ populations in the brain responsible for this regulation remain unknown. Singh et al. (2009) showed that deletion of $ER\alpha$ in the pituitary LH cells blunts E_2 -induced inhibition on LH secretion and results in infertility in female mice. Therefore, pituitary $ER\alpha$ is at least partly responsible for the negative feedback, but redundant $ER\alpha$ populations likely exist in the brain. Given that GnRH neurons do not appear to express $ER\alpha$ (Shivers et al., 1983), interneurons must exist to relay $E_2/ER\alpha$ signals on GnRH neurons.

Recent evidence suggests that POMC neurons may serve as interneurons mediating E_2 actions. First, POMC neurons co-express $ER\alpha$ (Miller et al., 1995; de Souza et al., 2011), suggesting that POMC neurons could be the direct target of estrogenic actions. E_2 treatment increases POMC expression (Hammer et al., 1994; Cheung and Hammer, 1997). β -endorphin positive fibers and opioid receptors in the POA also increase upon E_2 treatment (Hammer et al., 1994). In addition, E_2 regulates the excitability of POMC neurons. Using electron microscopy, Gao et al. (2007) reported that the number of excitatory synaptic inputs to ARC

POMC neurons rises as mice enter proestrus when E_2 levels are high. Further, central E_2 administration rapidly increases the excitatory synapses on POMC neurons, an effect that is also reflected by increased miniature excitatory postsynaptic current recorded from POMC neurons (Gao et al., 2007). Similarly, Malyala et al. (2008) reported that E_2 administration in hypothalamic slices increases excitability of POMC neurons by rapidly uncoupling $GABA_B$ receptors from their G-protein-gated inwardly rectifying K^+ channels. These studies demonstrated that E_2 directly acts on POMC neurons and regulates their cellular activity.

The physiological relevance of E_2 sensing by POMC neurons was further established by our recent findings in mice lacking $ER\alpha$ specifically in POMC neurons. First, we found that mutant females have a modest increase in plasma E_2 , raising the possibility of impaired negative feedback on the HGP axis (Xu et al., 2011). Thus, while E_2 replacement suppresses FSH and LH expression in the pituitary of ovariectomized (OVX) wild-type mice, this E_2 -induced suppression is blunted in OVX mutant mice (Xu et al., 2011). Interestingly, only 30% of female mice lacking $ER\alpha$ in POMC neurons successfully delivered pups (Xu et al., 2011). The averaged size of litters from these 30% mutant dams was significantly reduced compared to those from control dams (Xu et al., 2011). In addition, the mating time required for these mutant females to conceive was significantly increased (Xu et al., 2011). These findings indicate that $E_2/ER\alpha$ signals in POMC neurons are at least partially required to mediate the negative feedback regulation of the HPG axis and to maintain normal fertility.

Besides being a reproductive cue, estrogens also exert important anti-obesity effects in women (Flegal et al., 2002; Freedman et al., 2002; Carr, 2003) and female mammals (Drewett, 1973; Blaustein and Wade, 1976; Wallen et al., 2001; Rogers et al., 2009). E_2 reduces food intake and body adiposity and increase energy expenditure in animals and humans of both sexes through a hypothalamic mechanism (Dubuc, 1985; Wade et al., 1985; Hess et al., 1997; Heine et al., 2000). Effects of E_2 on energy balance are primarily mediated by $ER\alpha$, as women or female mice with mutations in the $ER\alpha$ gene display hyperadiposity (Heine et al., 2000; Okura et al., 2003), whereas $ER\beta$ -null mice have no body weight phenotype (Ohlsson et al., 2000). Interestingly, we recently found that a portion of anti-obesity effects of estrogens are mediated by $ER\alpha$ expressed by POMC neurons. For example, we found that female mice lacking $ER\alpha$ only in POMC neurons develop chronic hyperphagia and increased body weight (Xu et al., 2011). In addition, the leptin-induced suppression in food intake was blunted in female mice lacking $ER\alpha$ in POMC neurons (Xu et al., 2011). Collectively, these findings indicate that $E_2/ER\alpha$ signals within POMC neurons are not only important to mediate the negative feedback and maintain normal reproduction, but also are physiologically relevant in the regulation of feeding behavior. Therefore, E_2 signals within POMC neurons may coordinate the regulation of energy balance and reproductive demands.

SF1 NEURONS LINK METABOLIC AND REPRODUCTIVE CIRCUITS

SF1 NEURONS INNERVATE REPRODUCTIVE CIRCUITS

In addition to POMC neurons in the ARC, steroidogenic factor-1 (SF1) neurons, located in the ventromedial hypothalamic nucleus

(VMH) may serve as an important connection point relaying metabolic and reproductive cues.

The physiological relevance of VMH neurons to the regulation of body weight homeostasis is well recognized. Ikeda et al. (1995) discovered that a transcription factor, SF1, is expressed exclusively in the VMH neurons within the brain. SF1 neurons constitute the majority of VMH neurons (Stallings et al., 2002). During early development, SF1 is essential for the formation of the VMH architecture, as mice with embryonic deletion of SF1 gene do not form a VMH (Dellovade et al., 2000). These SF1 knockout mice develop massive obesity (Majdic et al., 2002). It is important to note that SF1 is also abundant in a number of endocrine organs, such as the pituitary gland, the adrenal gland and gonads (Zhao et al., 2001). Therefore, the obesity phenotype in global SF1 knockout mice may have been confounded by the dysfunctions of these tissues (Majdic et al., 2002). To circumvent this issue, a brain-specific SF1 knockout mouse line was generated to determine the role of VMH SF1 neurons in the context of body weight control (Kim et al., 2011). This brain-specific SF1 deletion also leads to disruption of the VMH structure and obesity in mice (Kim et al., 2011). Thus, these findings demonstrate that VMH neurons are physiologically important for the regulation of energy homeostasis.

VMH neurons may also regulate the central reproductive circuits. For example, the VMH has well defined projections to the medial central gray and periaqueductal gray regions that have been implicated in lordosis (Canteras et al., 1994). In addition, VMH neurons are also found to project to GnRH neurons (Boehm et al., 2005; Yoon et al., 2005). Further, numerous studies have suggested that VMH neurons regulate sexual behaviors (Blaustein and Feder, 1980; Rubin and Barfield, 1983a,b; Canteras et al., 1994; Mani et al., 1997; Sinchak et al., 2007). Mice with genetic ablation of VMH SF1 neurons show a significantly blunted lordosis quotient and receptivity (Kim et al., 2010). The role of the VMH in reproduction may be more extensive than just behavior. For example, the mutant female mice with genetic ablation of VMH SF1 neurons show severely irregular estrus cycles, and are infertile or subfertile (Kim et al., 2010). The impaired female fertility is likely due to defective ovulation, as demonstrated by decreased or absent corpora lutea in the ovary (Kim et al., 2010). Interestingly, exogenous administration of gonadotropins induced normal ovulation in these mice, demonstrating that the ovaries are functionally intact (Kim et al., 2010). Further, when the mutant females were stimulated with a synthetic GnRH agonist after priming, they exhibited markedly reduced LH secretion compared with wild-type littermates, arguing that disorganization in and around the VMH caused by SF1 ablation interferes with the GnRH priming process or gonadotrope LH capacity (Kim et al., 2010).

Collectively, these findings indicate that functional VMH SF1 neurons are required to maintain not only normal energy balance but also reproduction. Therefore, these SF1 neurons could serve as an important point of intersection for these two systems.

ESTROGENS ACT ON SF1 NEURONS TO REGULATE BOTH REPRODUCTION AND ENERGY HOMEOSTASIS

E_2 acts in the VMH to regulate energy balance. Abundant $ER\alpha$ is found in the ventrolateral subdivision of the VMH (Osterlund et al., 1998; Merchenthaler et al., 2004; Schlenker and

Hansen, 2006). To determine whether $ER\alpha$ in the VMH mediate the anti-obesity effects of estrogens, Musatov and colleagues used shRNA-mediated gene silencing approach to knock-down $ER\alpha$ in the VMH in female rodents. They found that VMH-specific $ER\alpha$ knock-down leads to obesity and metabolic syndrome primarily due to decreased energy expenditure (Musatov et al., 2007). We recently crossed $ER\alpha$ floxed mice (Feng et al., 2007) to SF1-Cre transgenic mice (Dhillon et al., 2006), which resulted in mice lacking $ER\alpha$ specifically in VMH SF1 neurons (Xu et al., 2011). This SF1-specific deletion of $ER\alpha$ results in modest body weight gain and hyperadiposity solely due to decreased energy expenditure in female mice (Xu et al., 2011). We further demonstrate that both basal metabolic rate and diet-induced thermogenesis in these mice are reduced, while the energy expenditure associated with physical activity is not altered (Xu et al., 2011). Interestingly, female mice lacking $ER\alpha$ in SF1 neurons also show increased visceral fat distribution, while the subcutaneous fat distribution is reduced (Xu et al., 2011). Consistent with this abdominal obesity, these mice predictably develop glucose intolerance (Xu et al., 2011). Collectively, our findings support a model in which E_2 acts on $ER\alpha$ in VMH SF1 neurons to stimulate energy expenditure and inhibit visceral fat expansion. As we also found that norepinephrine is decreased in mice lacking $ER\alpha$ in SF1 neurons (Xu et al., 2011), the effects of $E_2/ER\alpha$ in SF1 neurons on energy expenditure and fat distribution are presumably mediated by elevated sympathetic outflow.

The actions of E_2 in VMH neurons are also important for reproduction. For example, administration of E_2 in the VMH has been shown to modulate female sexual behaviors (Rubin and Barfield, 1983a). In addition, E_2 actions in the VMH may mediate induction of progesterone receptors in the VMH (McGinnis et al., 1981; Olster and Blaustein, 1989; Kalra, 1993; Moffatt et al., 1998), thereby permitting progesterone to reduce basal GnRH and LH release (Chappell et al., 1997). The role of VMH E_2 sensing in reproduction was further supported by our findings from mice lacking $ER\alpha$ only in SF1 neurons. These mutant female mice have irregular estrus cycles (Figure 2A). Further, most of mutant females (9 out of 10 mice tested) are infertile (Figure 2B). The only mutant dam that successfully conceived and delivered had smaller litter size than controls (Figure 2C). The impaired fertility in these females is likely due to anovulation, demonstrated by the lack of corpora lutea in the ovaries (Figure 2D). One caveat of this model is that $ER\alpha$ may also be deleted from SF1 cells in the pituitary, adrenal gland, and gonads, in addition to SF1 neurons in the VMH, which makes it difficult to attribute the fertility and ovarian phenotypes solely to the loss of $ER\alpha$ in SF1 neurons in the VMH. However, we did not find any significant reduction of $ER\alpha$ mRNA in the pituitary, adrenal gland, and ovaries from the mutant mice (Xu et al., 2011), which argues that the infertility/subfertility and anovulation phenotypes are likely due to the loss of $ER\alpha$ in VMH neurons. Interestingly, the brain-specific $ER\alpha$ knockout model shows the same fertility and ovarian phenotypes as in our mice lacking $ER\alpha$ in SF1 cells (Wintermantel et al., 2006), suggesting that $ER\alpha$ in the brain, such as in VMH SF1 neurons, is required to trigger ovulation in the ovaries. Collectively, these findings support a model in which adequate E_2 signaling in VMH SF1 neurons is required both for fertility and normal energy balance.

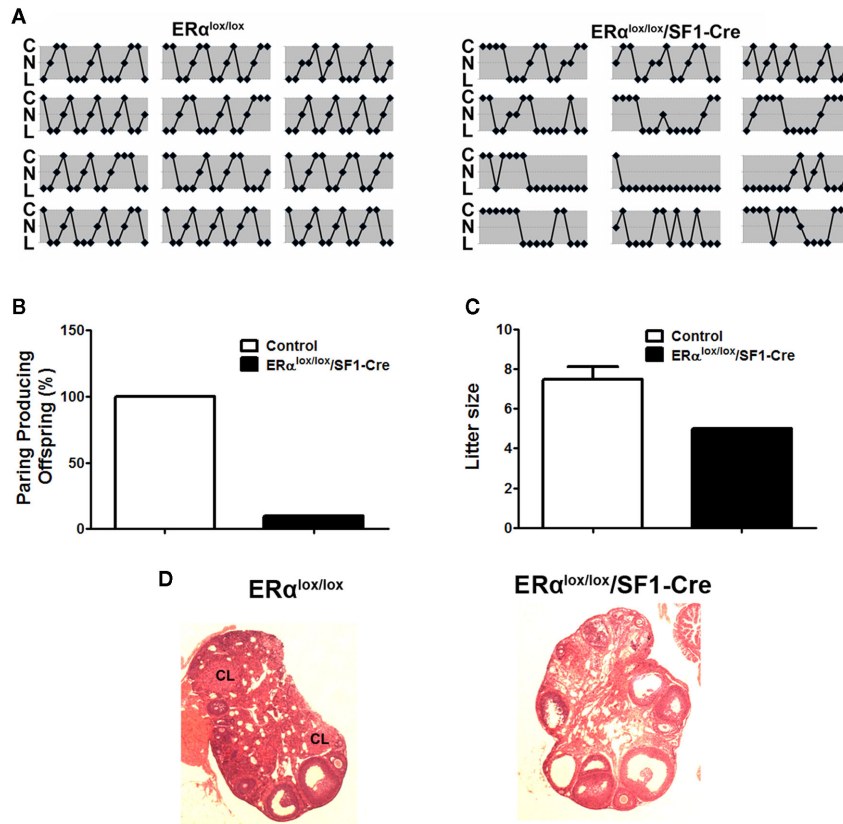


FIGURE 2 | Reproductive phenotype of female mice lacking ERα in SF1 neurons (ERα^{lox/lox}/SF1-Cre). (A) Estrus cycles from 12-week-old female mice lacking ERα in SF1 neurons and their wild-type controls were evaluated by vaginal cytology. Note: C, cornified cells (estrus); N, nucleated epithelial cells (proestrus); L, leukocytes (diestrus). (B) Female mice lacking ERα in SF1

neurons and their wild-type controls were paired with genetically normal control males and the numbers of pairings producing pups were quantified. (C) Litter size from dams that successfully delivered pups in (B). (D) H&E staining of ovaries from adult female mice. There was no corpus luteum in the ovary from mice lacking ERα in SF1 neurons.

CONCLUSION

While POMC and SF1 neurons have previously been thought to serve primarily in the control of food intake and energy balance, new genetic rodent models have highlighted their crucial roles in the maintenance of fertility. While additional work is needed to clarify the mechanisms by which these neuronal circuits modulate the GnRH system, these studies highlight the profound integration of reproductive and metabolic control. In particular, POMC and SF1 neurons are emerging as critical sites that both respond to circulating factors and directly interact with reproductive circuitry. Future studies of POMC and SF1 neurons may shed

light on the nature of positive and negative steroid feedback as well as integration of signals of adiposity in the control of the reproductive axis.

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