AMERICAN JOURNAL OF PHYSIOLOGY
REGULATORY, INTEGRATIVE AND COMPARATIVE PHYSIOLOGY

Am J Physiol Regul Integr Comp Physiol 329: R245–R252, 2025.
First published June 26, 2025; doi:10.1152/ajpregu.00117.2025

SHORT REPORT

Integrative Physiology of Gut-Brain Communication

Midlife estradiol treatment reduces the firing rate of liver-related PVN neurons in ovariectomized high-fat diet-fed mice

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Abstract

Estrogen plays a critical role in the regulation of physiological functions, including metabolism, and its involvement in the regulation of insulin sensitivity and glucose homeostasis has major clinical relevance. Despite the importance of the brain-liver pathway in the regulation of glucose metabolism and that postmenopausal women have an increased risk of developing metabolic disorders, the effect of hormone therapy on hypothalamic neurons involved in the regulation of liver metabolism is not known. Here, we tested the hypothesis that in middle-aged, high-fat diet (HFD)-fed female mice, the excitability of liver-related neurons in the paraventricular nucleus (PVN) of the hypothalamus is increased, whereas estradiol treatment attenuates this increase. Mice fed with phytoestrogen-free control (low-fat diet) or HFD were ovariectomized, received a silastic capsule implant containing either estradiol or vehicle, and stayed on their respective diets. Estradiol treatment resulted in less fat mass and lower body weight. Liver-related neurons were identified with a retrograde, transsynaptic viral tracer, and patch-clamp recordings were conducted from identified neurons in the PVN. Our data show that the excitability of liver-related PVN neurons was increased in ovariectomized HFD mice compared with LFD-fed mice. In estradiol-treated HFD mice, the firing of liver-related PVN neurons was significantly reduced compared with vehicle-treated HFD mice, whereas in LFD mice, estradiol treatment did not alter the activity of liver-related PVN neurons. Our findings suggest that midlife estradiol treatment has beneficial effects on liver-related PVN neurons and thus may contribute to the improved metabolic status observed in estradiol-treated HFD mice.

NEW & NOTEWORTHY Menopause increases the risk of metabolic disorders, and despite the importance of the brain-liver pathway in the regulation of glucose homeostasis, the effect of estradiol treatment on liver-related neurons is not known. Our data show that in middle-aged, high-fat diet-fed, ovariectomized female mice, the excitability of liver-related neurons in the paraventricular nucleus is increased, whereas estradiol treatment attenuates this increase. These data suggest that midlife estradiol treatment is beneficial for the brain-liver pathway.

electrophysiology; estradiol; high-fat diet; liver-related PVN neurons; ovariectomy

INTRODUCTION

Estrogen plays an important role in the regulation of a variety of physiological functions, including energy metabolism. Indeed, its involvement in insulin sensitivity and glucose homeostasis has major clinical relevance (1). Sex differences in metabolic phenotype and protection from obesity-related metabolic and cardiovascular complications before menopause are recognized in both animal models and clinical subjects (2–4). In mice, high-fat diet (HFD) feeding increases body mass and adiposity in both sexes, leading to insulin resistance, glucose intolerance, and mild hyperglycemia with less marked hyperinsulinemia in females (5). These sex differences are not observed in ovariectomized animals (OVX) (6), suggesting that circulating ovarian hormones may have an important role in the maintenance of normal glucose homeostasis.

The liver, a key organ in the maintenance of glucose homeostasis, is regulated by both branches of the autonomic nervous system. In general, activation of sympathetic innervation promotes hepatic glucose production, whereas stimulation of the parasympathetic nerves of the liver decreases glucose production and increases glucose storage (7–9). Previous studies demonstrated that the paraventricular nucleus (PVN) of the hypothalamus, a key regulatory center

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Submitted 9 May 2025 / Revised 1 June 2025 / Accepted 17 June 2025

for neuroendocrine and autonomic functions, is largely involved in the regulation of glucose metabolism (10–12). Preautonomic PVN neurons, through direct and indirect connections to preganglionic neurons, modulate the activity of sympathetic and parasympathetic output and thus organ functions. Liver-related neurons were identified in the mouse PVN (13–15), and plasticity of the brain-liver pathway was observed in mouse models of metabolic diseases (16–19). It has been shown that liver-related PVN neurons are more active in male-leptin receptor-deficient *db/db* mice compared with lean mice (18), and more recently, in high-fat diet (HFD)-fed male mice (19). Despite that HFD feeding increases body mass and adiposity in both sexes and loss of circulating ovarian hormones has major effects on metabolism and glucose homeostasis, the cellular properties of liver-related neurons in female mice remain to be determined.

Here, we tested the hypothesis that in HFD-fed, middle-aged, ovariectomized female mice, the excitability of liver-related PVN neurons is increased, whereas systemic estradiol treatment attenuates this increase. Female C57BL/6J mice were fed with a phytoestrogen-free control (LFD) or HFD before ovariectomy (OVX). Following OVX, mice received either 17-β estradiol (E2) or vehicle (VEH, cholesterol) implants and stayed on their respective diets for an additional 8–10 wk. Liver-related PVN neurons were identified with a retrograde, transsynaptic viral tracer, and patch-clamp recordings were conducted to determine the activity of neurons in LFD and HFD mice receiving VEH and E2 treatment. We found that the excitability of liver-related PVN neurons was increased in ovariectomized HFD mice compared with LFD-fed mice. The firing of liver-related PVN neurons in E2-treated HFD mice was significantly reduced compared with the vehicle-treated HFD mice, whereas in LFD mice, estradiol treatment did not alter the activity of liver-related PVN neurons. Our findings suggest that midlife E2 treatment has beneficial effects on the activity of liver-related PVN neurons that may contribute to the improved metabolic status observed in estradiol-treated HFD mice.

MATERIALS AND METHODS

Animals

Female, 10-wk-old C57Bl/6J mice were purchased from the Jackson Laboratory (RRID:IMSR_JAX:000664). Mice were pair-housed in a room maintained at 20 ± 1°C on a 12-h light/12-h dark cycle, with ad libitum access to food and water except when specified. Experiments were conducted based on the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Tulane University's Institutional Animal Care and Use Committee.

Experimental Design

After 1 wk of acclimatization in the animal facility, mice were placed on a phytoestrogen-free control diet (low-fat diet or LFD, BioServ, No. F4031, 16% fat; 3.93 kcal/g). At ∼9.5 mo of age, mice were randomly divided into two groups and received a phytoestrogen-free high-fat diet (HFD, BioServ, No. F3282, 59% fat; 5.49 kcal/g) or were kept on the LFD for the remainder of the study (Fig. 1*A*). After ∼12 wk of HFD (12–13 mo of age), LFD and HFD mice underwent bilateral ovariectomy (OVX) and were implanted with subcutaneous capsules delivering either 17-β estradiol (E2) or cholesterol (vehicle, VEH).

Ovariectomy and Hormone Treatment

Mice were anesthetized with isoflurane and then shaved mid-dorsally on each side. The incision areas were cleaned with alcohol and betadine solution, and a 2-cm flank incision was performed at T12-L2 levels to perform ovariectomy (OVX), as described previously (20). Briefly, the ovary and surrounding fat were pulled out of the body cavity, a hemostat was placed at the boundary between the oviduct and uterus, and a nonabsorbable suture was placed just below the hemostat, next to the uterus. The ovary was dissected out with scissors above the ligature, and the uterus was returned to the abdomen. All incisions were closed in two layers with interrupted polydioxanone absorbable sutures. To determine the effect of E2 treatment, silastic capsule implants were prepared to deliver 17-β estradiol (2.5% E2) or cholesterol (vehicle) for the rest of the study. At the time of the OVX, capsules were implanted subcutaneously at the dorsal base of the neck. Animals were placed in a clean cage on a heating pad and allowed to recover from anesthesia before being returned to the vivarium. Mice were pair-housed with a diet and treatment-matched cage mate and maintained on their diets. To prevent scar tissue development, the implantation area was massaged weekly.

In a preliminary study conducted in a different set of ovariectomized mice ($n = 7$), we assessed the circulating estradiol levels produced by our silastic capsules. Animals were euthanized by decapitation while under anesthesia induced by ketamine (100 mg/kg) and xylazine (7 mg/kg), and trunk blood was collected. Uteri were removed and weighed to assess treatment efficacy. Blood was allowed to clot at room temperature for 90 min, and after centrifugation at 2,000 *g* for 15 min at room temperature, serum was collected. Estradiol levels were assayed using liquid chromatography mass spectrometry (LC-MS/MS) at the Mayo Clinic. This LC-MS/MS method for measuring estrogens (estradiol and estrone) can achieve an estradiol lower limit of quantitation of 3 pg/mL in as little as 100 μL serum. We established that the 2.5% E2 implants maintain mouse blood plasma estradiol levels within the high physiological range [68.75 ± 13.4 pg/mL E2 ($n = 4$) vs. 5.9 ± 3.65 pg/mL cholesterol ($n = 3$)]. In the current study, at the time of euthanasia, the uterus was dissected to confirm successful ovariectomies, and uterine weight was measured to validate estradiol administration [$F(1,35) = 32.69$, $P < 0.0001$] (Fig. 1*B*).

Metabolic Assessments

Body composition was determined with dual energy X-ray absorptiometry (DXA, InAlyzer2, Model M, Micro Photonics Inc., Allentown, PA) using 80 and 45 kV/0.85 mA in a fan beam configuration. DXA achieves a precision of <5% coefficient of variation (CV), with a standard deviation (SD) of <1%. It is calibrated with a reference phantom as described by the manufacturer, and the scan area was 14 × 21 cm, with

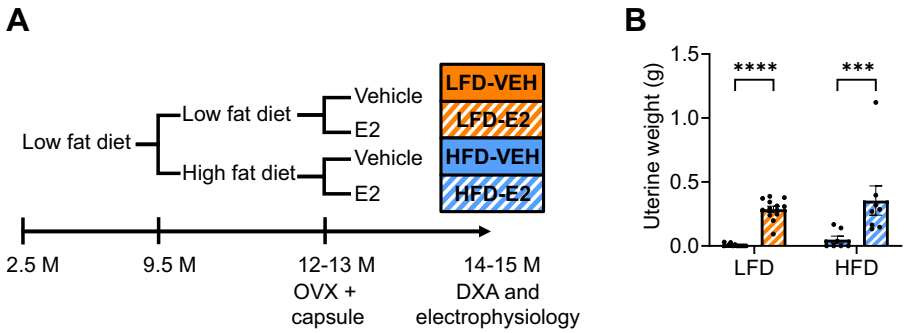

Figure 1. Experimental design and uterine weight. *A*: 11-wk-old female C57Bl/6J mice were fed a low-fat diet (LFD) for ~7 mo. Mice were exposed to a high-fat diet (HFD) or kept on the LFD for ~12 wk, then bilateral ovariectomy (OVX) was performed with subcutaneous implantation of capsules delivering either 17-β estradiol (E2) or cholesterol (vehicle, VEH). *B*: the weight of dissected uteri was determined after euthanasia by DXA to confirm successful OVX and E2 treatment. Circles represent individual mice. Data, represented as means ± SE, were analyzed by two-way ANOVA followed by uncorrected Fisher's LSD multiple comparisons. A significant effect of treatment was observed ***$P < 0.001$, ****$P < 0.0001$. LFD-VEH, solid orange bars; LFD-E2, striped orange bars; HFD-VEH, solid blue bars; HFD-E2, striped blue bars.

an image pixel pitch of 99 × 99 μm. Mice were maintained under anesthesia (isoflurane 2%) throughout the procedure.

Pseudorabies Virus Inoculation

Pseudorabies virus 152 (PRV-152; reports enhanced green fluorescence protein, supplied by NCRR CNNV Virus Center) was used to identify liver-related neurons, as described previously (14, 17, 19, 21). Briefly, under anesthesia, the liver was exposed, and three injections (2 μL/site) were made into the parenchyma of the left lobe. A drop of tissue adhesive (Vetbond, 3M) was used to seal each injection site to prevent the leakage of the virus. The animals were maintained in a biosafety level 2 facility for up to 120 h after inoculation for experiments in the PVN.

Brain Slices Preparation and Whole Cell Patch-Clamp Recordings

Acute brain slices were made, as described previously (17, 19, 22). Hypothalamic slices containing the PVN (300 μm) were made using a vibrating microtome. The slices were stored in a holding chamber at 34°C–36°C and then transferred to a recording chamber mounted on a fixed stage under an upright microscope (Nikon FN1).

Whole cell patch-clamp recordings with K-gluconate solution containing 0.1% biocytin were performed at 34°C–36°C from liver-related PVN neurons, as described previously (18, 19). In voltage clamp mode, spontaneous excitatory postsynaptic currents (sEPSCs) were determined at −60 mV, whereas the excitability of neurons was identified in current clamp mode. PVN neurons were initially hyperpolarized to −90 mV, and then depolarizing current steps were applied (1 s duration) to reveal the frequency of action potentials, as shown previously (18, 23–25). Synaptic currents and action potentials were analyzed offline using pCLAMP (Molecular Devices) or MiniAnalysis (Synaptosoft). Amplitude and area detection thresholds were set as three times the root mean square noise using MiniAnalysis. After the software-based automatic detection, the events were carefully reviewed and manually selected.

After recordings, the brain slices containing the neurons filled with biocytin (Sigma-Aldrich) were fixed in paraformaldehyde (4%) and stored at 4°C until visualization of recorded neurons with AMCA Avidin D (1:200), as described previously (17–19, 22). Images were taken with a confocal microscope (Nikon Ti2).

Statistical Analysis

Data are expressed as means ± SE. All statistical analyses were performed using Prism 10 (GraphPad). The effects of diet and treatment after OVX were evaluated using two-way ANOVA followed by an uncorrected Fisher's LSD post hoc test.

RESULTS

Overactivity of Liver-Related PVN Neurons Is Attenuated in Estradiol-Treated Middle-Aged, Ovariectomized, HFD Mice

To determine the effect of diet and estradiol treatment on body weight, the body composition of 14–15-mo-old E2 or VEH-treated LFD and HFD-fed mice was measured by DXA. As expected, HFD increased the body weight of mice (LFD-VEH: 33.5 ± 1.2 g, HFD-VEH: 41.5 ± 4.6 g, $n = 11$ and 8) [$F(1,34) = 8.960$, $P = 0.0051$], and estradiol treatment resulted in lower body weight (LFD-E2: 27.0 ± 0.6 g, HFD-E2: 32.7 ± 1.9 g, $n = 11$ and 8) [$F(1,34) = 11.16$, $P = 0.0020$] (Fig. 2); although the diet-by-treatment interaction was not significant [$F(1,34) = 0.2298$, $P = 0.6347$]. HFD feeding significantly increased fat mass (LFD-VEH: 11.3 ± 1.0 g, HFD-VEH: 18.2 ± 4.1 g) [$F(1,34) = 7.672$, $P = 0.0090$], whereas mice with E2 replacement had less fat (LFD-E2: 4.1 ± 0.3 g, HFD-E2: 8.0 ± 1.4 g) [$F(1,34) = 19.96$, $P < 0.0001$]; the interaction between diet and treatment on fat mass was not significant [$F(1,34) = 0.5819$, $P = 0.4508$]. Finally, ANOVA detected no significant effects of estradiol treatment for lean mass [$F(1,34) = 2.610$, $P = 0.1154$].

Previous findings in young adult males showed that the excitability of liver-related PVN neurons kept on HFD is increased (19); however, the firing activity of liver-related PVN neurons in female mice is not known. As a first step, we determined the baseline properties of liver-related PVN neurons in middle-aged, ovariectomized, LFD- and HFD-fed female mice. Figure 3 illustrates the

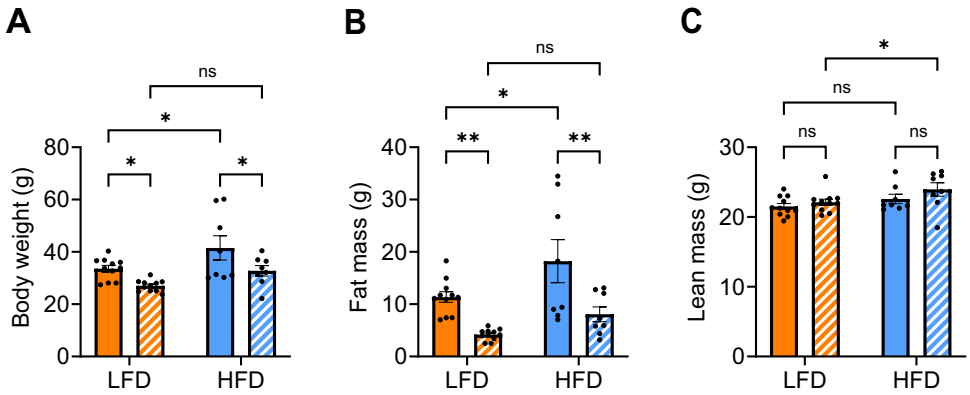

Figure 2. Body composition. Body composition was measured by DXA in anesthetized mice. *A*: body weight of mice following treatment with 17-β estradiol or vehicle. *B* and *C*: estradiol treatment reduced fat mass, whereas lean mass did not change. Data are represented as means ± SE. Circles represent individual mice. The effect of diet and treatment was analyzed by two-way ANOVA followed by uncorrected Fisher's LSD multiple comparisons. Significance: $*P < 0.05$, $**P < 0.01$; ns, not significant. LFD-VEH, solid orange bars; LFD-E2, striped orange bars; HFD-VEH, solid blue bars; HFD-E2, striped blue bars. E2, estradiol; HFD, high-fat diet; LFD, low-fat diet; VEH, vehicle.

location of recorded liver-related PVN neurons. HFD-VEH mice showed a trend toward a more depolarized membrane potential compared with LFD-VEH mice, although the difference in resting membrane potential was not significant (LFD-VEH: −49.3 ± 1.0 mV, $n = 25$ from 14 mice; HFD-VEH: −46.9 ± 1.9 mV, $n = 10$ from 6 mice) [$F(1,67) = 1.177$, $P = 0.2819$] and most neurons fired spontaneously (LFD-VEH: 17 out of 25; HFD-VEH: 7 out of 10). Interestingly, we found that HFD had a significant effect on the firing of liver-related PVN neurons, resulting in increased action potential frequency (Fig. 4, *A* and *B*). In LFD-VEH mice, the action potential frequency was 0.8 ± 0.3 Hz (ranged from 0.07 to 4.03 Hz, $n = 17$ from 11 mice), whereas in HFD-VEH mice, it was 3.8 ± 1.0 Hz (ranged from 0.02 to 7.4 Hz, $n = 7$ from 5 mice) [$F(1,49) = 11.37$, $P = 0.0015$; Fisher's LSD $P \leq 0.0001$] (Fig. 4*B*). Furthermore, the current-action potential frequency responses after a hyperpolarizing step followed by depolarizing current steps showed higher firing rates in HFD mice compared with LFD mice (simple linear regression, LFD-VEH: $R^2 = 0.61$, y-intercept = 2.13, $n = 18$ vs. HFD-VEH: $R^2 = 0.40$, y-intercept = 5.25, $n = 7$; $P < 0.0001$) (Fig. 4, *C* and *D*). There was no difference in the slope (LFD-VEH: 0.37 vs. HFD-VEH: 0.43; $P = 0.51$), indicating that the difference in firing frequency was maintained during the step protocol (0–30 pA).

Then, we determined the action potential frequency in liver-related neurons following E2 treatment in LFD and HFD-fed mice. Estradiol treatment had a significant effect on the firing rate of liver-related PVN neurons [$F(1,49) = 6.065$, $P = 0.0174$], and there was a significant diet-by-treatment interaction [$F(1,49) = 9.228$, $P = 0.0038$]. In HFD-E2 mice, the frequency of action potentials in liver-related PVN neurons was significantly lower compared with the HFD-VEH-treated mice (HFD-VEH: 3.8 ± 1.0 Hz, ranged from 0.02 to 7.4 Hz, $n = 7$ from 5 mice; vs. HFD-E2: 1.3 ± 0.3 Hz, ranged from 0.1 to 2.7 Hz, $n = 8$ from 6 mice) (Fisher's LSD $P = 0.002$) (Fig. 4). In contrast, in LFD mice, estradiol treatment did not change the firing of liver-related PVN neurons (LFD-VEH: 0.9 ± 0.3 Hz, ranged from 0.06 to 4.03 Hz, $n = 17$ from 11 mice vs. LFD-E2 mice: 1.1 ± 0.2 Hz, ranged from 0.03 to 3.98 Hz, $n = 21$ from 12 mice) (Fisher's LSD $P = 0.59$).

Next, we determined the excitatory synaptic regulation of liver-related PVN neurons. Neither diet nor estradiol had a significant effect on the frequency of sEPSCs [diet: $F(1,68) = 2.141$, $P = 0.1480$, treatment: $F(1,68) = 0.8321$, $P = 0.3649$]; and the interaction also was nonsignificant [$F(1,68) = 2.309$, $P = 0.1333$]. On the contrary, estradiol treatment altered the amplitude of sEPSCs in liver-related PVN neurons. In HFD-E2-treated mice, the amplitude of sEPSCs was larger compared with the vehicle-treated mice (HFD-VEH: 11.7 ± 1.2 pA,

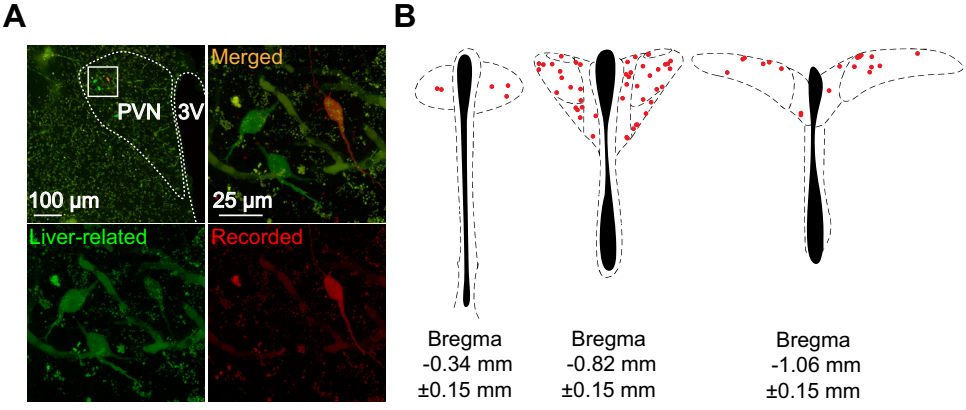

Figure 3. Identification and localization of recorded liver-related PVN neurons. *A*: a lower magnification confocal image shows the PVN, whereas the higher magnification boxed area illustrates a recorded (red), liver-related (green) PVN neuron. *B*: location of recorded liver-related PVN neurons. Red dots represent the approximate location of the recorded neurons. The schematic of the PVN map is based on Ref. 26. PVN, paraventricular nucleus of the hypothalamus; 3V: third ventricle.

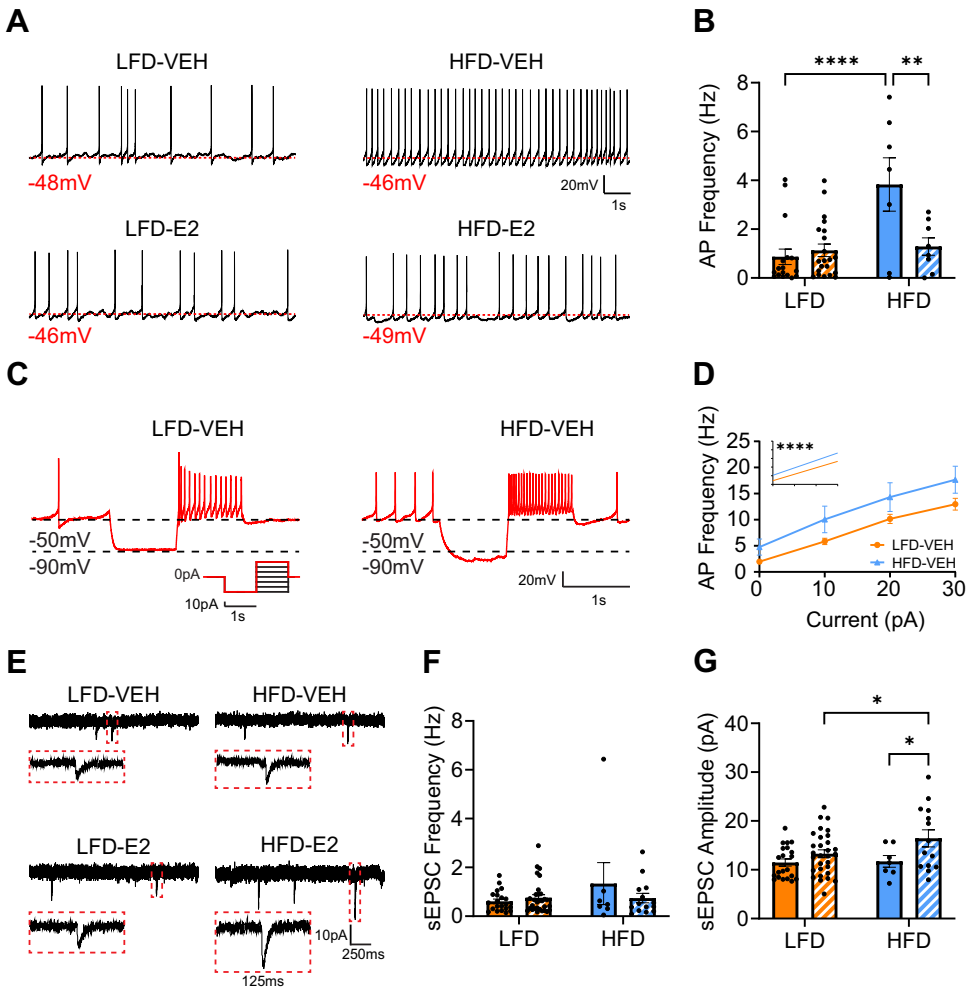

Figure 4. Overactivity of liver-related PVN neurons is attenuated in estradiol-treated middle-aged, ovariectomized, HFD mice. *A*: representative traces illustrate the firing rate of liver-related PVN neurons at baseline. *B*: bar graphs illustrate that the frequency of action potentials was significantly higher in HFD-VEH-fed mice compared with LFD-VEH mice, and estradiol treatment led to a decrease in firing rate in HFD-fed mice. *C*: representative traces illustrate the firing rate of liver-related PVN neurons. Neurons were hyperpolarized to ∼−90 mV, then depolarizing current steps were applied to determine the firing of liver-related neurons. *D*: current-action potential frequency curves and computed simple linear regression demonstrated that liver-related PVN neurons fire more in HFD mice. *E*: representative recordings of sEPSCs in liver-related PVN neurons. *F* and *G*: bar graphs illustrate that the frequency of sEPSCs (*F*) was similar among the groups, whereas the amplitude of sEPSCs (*G*) was larger in HFD-E2 mice compared with LFD-E2 and HFD-VEH mice. Circles represent individual neurons. Data were analyzed by two-way ANOVA followed by uncorrected Fisher's LSD multiple comparisons. Significance $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$. LFD-VEH, solid orange bars; LFD-E2, striped orange bars; HFD-VEH, solid blue bars; HFD-E2, striped blue bars. E2, estradiol; HFD, high-fat diet; LFD, low-fat diet; PVN, paraventricular nucleus; sEPSCs, spontaneous excitatory postsynaptic currents; VEH, vehicle.

ranged from 7.2 to 15.7 pA, $n = 7$ vs. HFD-E2: 16.4 ± 1.7 pA, ranged from 7.9 to 24.6 pA, $n = 14$) [$F(1,68) = 7.075$, $P = 0.0097$; Fisher's LSD $P = 0.027$]. In addition, the sEPSC amplitude in HFD-E2 mice was also larger compared with the LFD-E2 mice (LFD-E2: 13.3 ± 0.8 pA, $n = 30$ vs. HFD-E2: 16.4 ± 1.7 pA, $n = 14$) (Fisher's LSD $P = 0.035$) (Fig. 4, *E*–*G*); however, there was no significant interaction between the effects of diet and treatment [$F(1,68) = 1.401$, $P = 0.2406$].

DISCUSSION

In the present study, we investigated the effect of systemic estradiol treatment on PVN neuronal plasticity in middle-aged, ovariectomized mice with preexisting metabolic disorders. Our findings show that *1*) mice with E2 replacement had less fat mass and lower body weight than the vehicle-treated LFD and HFD mice, *2*) the excitability of liver-related PVN neurons is increased in HFD-VEH mice compared with LFD-VEH mice, and *3*) estradiol treatment attenuated the HFD-induced overactivity of liver-related PVN neurons. Our data, demonstrating overactivity of liver-related PVN neurons in middle-aged, ovariectomized female mice, together with previous findings in male mice (19), further suggest altered central autonomic circuitry, whereas estradiol treatment has a beneficial impact on PVN neurons within the brain-liver pathway.

The autonomic nervous system plays an important role in the maintenance of physiological functions, including metabolism. In general, increased activity of the sympathetic nervous system is acknowledged in the development and/or progression of metabolic diseases, including obesity and diabetes mellitus (27–29). Central nervous system networks modulate the activity of autonomic functions, and changes in the activity of organ-related neurons contribute to the increased or decreased autonomic outflow governing a particular organ. Within the hypothalamus, the PVN is known to integrate information and trigger the appropriate autonomic, neuroendocrine, and behavioral responses. Within the brain-liver pathway, liver-related neurons were identified in multiple areas of the brain, including the PVN (13, 15, 30, 31). In the case of glucose regulation, it has been shown that stimulation of PVN neurons leads to increased glucose levels through increased sympathetic outflow (11, 32), and our recent study demonstrated that a small subset of PVN neurons directly projects to liver-related sympathetic premotor neurons in the ventral brainstem (14). In general, liver-related PVN neurons were shown to express corticotropin-releasing hormone, oxytocin, and SIM1 (14, 15), and previous investigations showed altered neuronal activity in obese and diabetic mouse models. Synaptic regulation of

liver-related PVN neurons in adult male mice was established (17) along with changes in excitatory neurotransmission in streptozotocin-treated hyperglycemic male mice. Similarly, plasticity of liver-related neurons, including increased excitability of liver-related PVN neurons, was observed in male obese and diabetic *db/db* mice, as well as the existence of extrasynaptic, tonic inhibition (18).

More recently, the firing of liver-related PVN neurons, including sympathetic-related neurons, was revealed in male HFD-fed mice (19). Patch-clamp recordings showed that the excitability of liver-related PVN neurons is increased in male HFD-fed mice compared with mice on a control diet. Bath application of insulin resulted in suppression of the firing activity of liver-related PVN neurons (19). In contrast, a recent study by Martins Dos Santos et al. (31) found that insulin increases the activity of preparasympathetic liver-related PVN neurons, and insulin's effect is mTOR signaling dependent. Together, these findings not only suggest that HFD alters the brain-liver circuit but also point to autonomic branch-dependent responses to insulin. Our current data demonstrating that the excitability of liver-related PVN neurons is increased in middle-aged, ovariectomized, HFD-fed mice is aligned with previous findings in HFD-fed mice and in obese Zucker rats (33). These findings consistently show overactivity of PVN neurons in HFD animals; however, we must keep in mind that the previous studies used young adult male mice (18, 19), whereas our current data are from middle-aged females after loss of circulating ovarian hormones, and thus, different underlying mechanisms could be involved.

Menopause causes a decrease in circulating ovarian hormones and has been associated with an increased incidence of metabolic disorders, and preclinical studies in healthy animal models of menopause demonstrate that hormonal treatments prevent alteration of energy metabolism (34, 35). Consistent with the previous studies, we found that HFD feeding resulted in increased fat mass and body weight compared with LFD mice, whereas midlife estradiol treatment resulted in decreased fat mass and body weight in both groups. Furthermore, our patch-clamp recordings revealed that systemic estradiol treatment resulted in a lower action potential frequency in HFD-fed mice. The firing rate of liver-related PVN neurons after estradiol treatment was similar to the firing in LFD-fed mice. Previous findings showed that intrahypothalamic estradiol administration in female rats regulates glucose levels through the sympathetic nervous system (36), and estradiol replacement modulates activity of presympathetic PVN neurons (37). Lee et al. (37) used young OVX rats with short-term (days) estradiol treatment and found that estradiol affects gene expression density of potassium channel subunits, in particular Kv4.2, and it diminishes I_A current density in the dorsal cap subdivision of PVN. These findings suggest that estradiol has an inhibitory effect on I_A and is able to modulate the excitability of presympathetic PVN neurons. In addition, estradiol is known to affect phosphatidylinositol 3-kinase (PI3K) signaling (38), and we have shown previously that in liver-related PVN neurons, insulin modulates neurotransmitter release in a PI3K-dependent manner (17). Based on these findings, it is highly likely that in the PVN, estradiol and metabolic hormones (e.g., insulin) interact and modulate neuronal activity, although further detailed studies are required to establish this interaction in liver-related neurons.

Our data also revealed excitatory neurotransmission in the four groups. There was no significant difference in the sEPSC frequency between the groups; however, we found an increase in sEPSC amplitude in estradiol-treated HFD mice compared with vehicle-treated HFD mice. The increase in amplitude without a change in frequency and phasic current suggests postsynaptic changes. The input resistance was not different among the groups. Previously, glutamate receptor plasticity in ERβ-containing PVN neurons was shown in angiotensin II hypertension (39), and liver-related PVN neurons may be affected in a similar manner; however, determining the distribution of glutamate receptors and/or subunits would require additional, detailed investigations.

Our cellular findings show that systemic estradiol modulates hypothalamic neurons within the brain-liver pathway. Transsynaptic labeling with PRV-152 allows identification of liver-related PVN neurons; however, PRV does not differentiate between the branches of the autonomic nervous system, and our recordings were likely conducted from both presympathetic and preparasympathetic PVN neurons. In general, activation of hepatic sympathetic nerves increases hepatic glucose production and glycogenolysis, and stimulation of parasympathetic nerves leads to increased glucose storage and reduced hepatic glucose production (reviewed in Ref. 29). At the level of the PVN, preautonomic neurons could be differentiated based on their projection to sympathetic (VLM/VMM) or parasympathetic (DMV)-related brain areas, which allows PVN neurons to modulate sympathetic or parasympathetic output. Denervation studies combined with retrograde tracing also demonstrated a separation of presympathetic and preparasympathetic neurons at the level of the hypothalamus, including the PVN (40). Therefore, increased activity of PVN-VLM projecting liver-related neurons would ultimately lead to increased glucose production, whereas increased PVN-DMV activity would result in the opposite. At cellular levels, a recent study showed that insulin is able to modulate parasympathetic liver-related PVN neurons (31), whereas in one of our previous studies, we showed altered insulin sensitivity of VLM-projecting liver-related PVN neurons in high-fat diets (19). In our recordings, we did not differentiate between sympathetic and parasympathetic liver-related PVN neurons, and currently, we do not know whether estradiol modulates PVN neurons in an autonomic branch-dependent manner or if it has the same effect on all liver-related PVN neurons. Nevertheless, our study further suggests altered PVN neuronal activity in HFD mice and a potentially important role for estradiol in the regulation of the brain-liver pathway. On the contrary, due to the age difference (young adults vs. middle-aged) and potential influence of circulating hormones (male vs. female), further detailed studies are necessary to determine the plasticity of the brain-liver circuit in young females and in middle-aged males exposed to HFD in their midlife.

DATA AVAILABILITY

Datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

ACKNOWLEDGMENTS

We thank the Cardiometabolic Core and the Hormones and Behavior Core (J. Zha and I. Pires dos Santos) for the help, and Dr. Janet Ruscher of the Department of Psychology at Tulane University for expert statistical advice. Graphical Abstract created with a licensed version of BioRender.com.

GRANTS

The work was supported by NIH Grant P01AG071746.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

Andrea Zsombok is an editor of *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* and was not involved and did not have access to information regarding the peer-review process or final disposition of this article. An alternate editor oversaw the peer-review and decision-making process for this article.

AUTHOR CONTRIBUTIONS

J.M.D., L.A.S., and A.Z. conceived and designed research; A.J.R.M., L.D.D., C.M.D., G.L.W., S.K., V.F.d.S., M.J.M., and R.K.D. performed experiments; A.J.R.M., L.D.D., and C.M.D. analyzed data; L.D.D. and A.Z. interpreted results of experiments; A.J.R.M. and C.M.D. prepared figures; A.J.R.M. and A.Z. drafted manuscript; A.J.R.M., C.M.D., S.K., J.M.D., L.A.S., and A.Z. edited and revised manuscript; A.J.R.M., L.D.D., C.M.D., G.L.W., S.K., V.F.d.S., M.J.M., R.K.D., J.M.D., L.A.S., and A.Z. approved final version of manuscript.

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