

Supplementary Material

Endocannabinoid Signaling at Hypothalamic Steroidogenic Factor-1/Proopiomelanocortin Synapses is Sex- and Diet-Sensitive

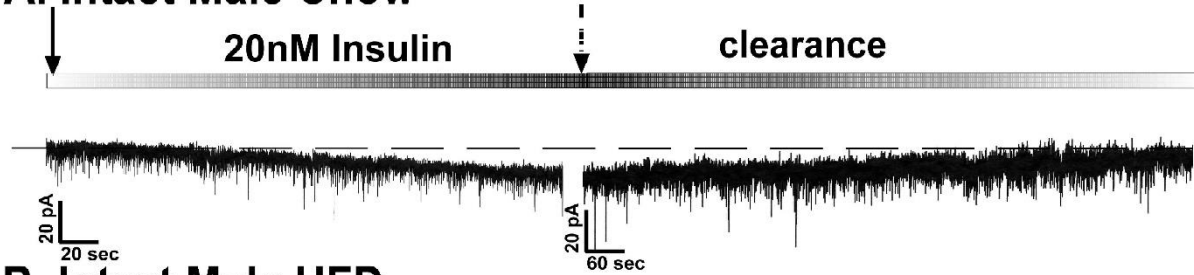
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*** Correspondence:**

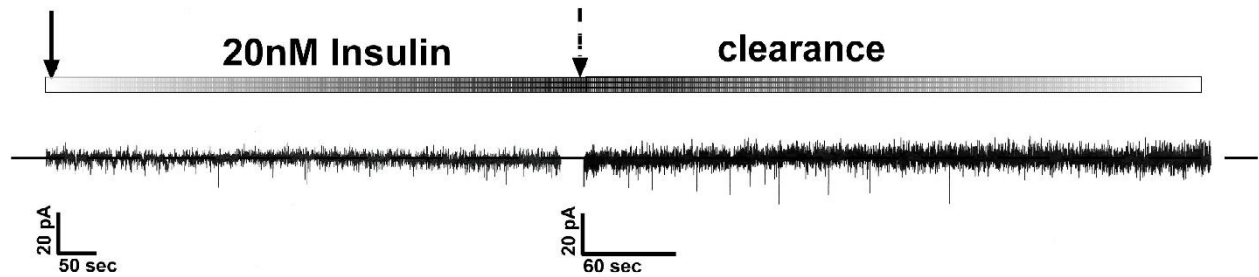
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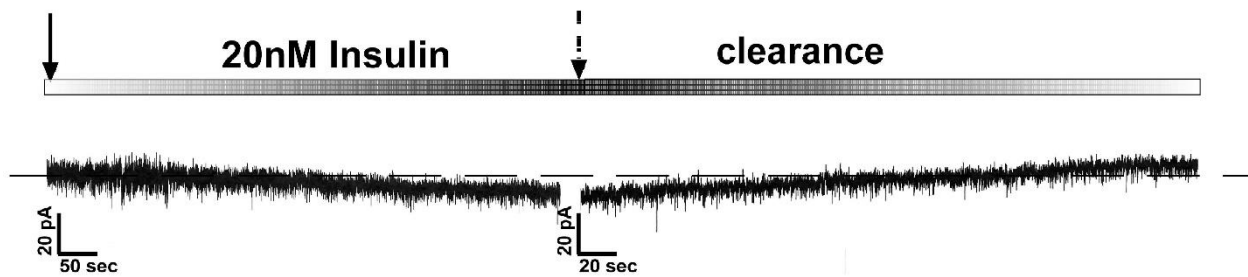
A. Intact Male Chow



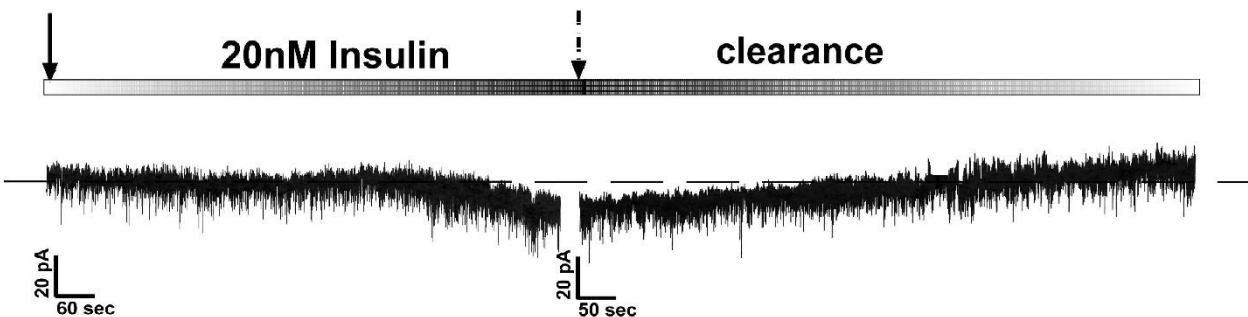
B. Intact Male HFD



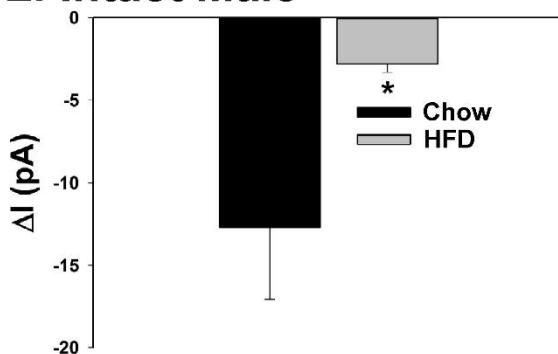
C. Periovarian Female Chow



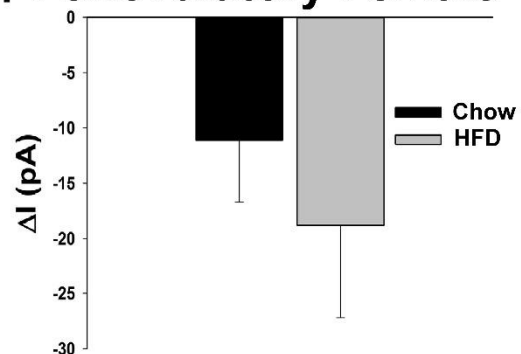
D. Periovarian Female HFD



E. Intact Male



F. Periovarian Female



Supplementary Figure 1 Chronic exposure to a HFD sex dependently attenuates the insulin-induced activation of TRPC5 channels in ARC POMC neurons. Membrane current traces of identified POMC neurons obtained from grain-based chow-fed males (**A**; n=6)), and periovulatory females (**C**; n=4) that illustrate the ability of insulin (20 nM) to cause an inward current. Membrane current traces from other identified POMC neurons taken from HFD-fed males (**B**; n=6), and periovulatory females (**D**; n=5), that illustrate how the HFD largely abolishes the insulin-induced inward current in the former but not the latter. Concurrently, the composite bar graphs (**E**) and (**F**) show the dramatic reduction of the insulin-induced inward current caused by the HFD in male but not periovulatory female animals. Bars represent means and vertical lines 1 S.E.M. of the inward current measured in POMC neurons from animals fed either a grain-based chow (open columns) or HFD (black columns). * = $P < 0.05$, Student's t-test.

Male	% Cells with eEPSCs	Female	% Cells with eEPSCs
Chow	54/72 (75%)	Chow	25/34 (74%)
HFD	11/16 (69%)	HFD	2/10 (20%)*

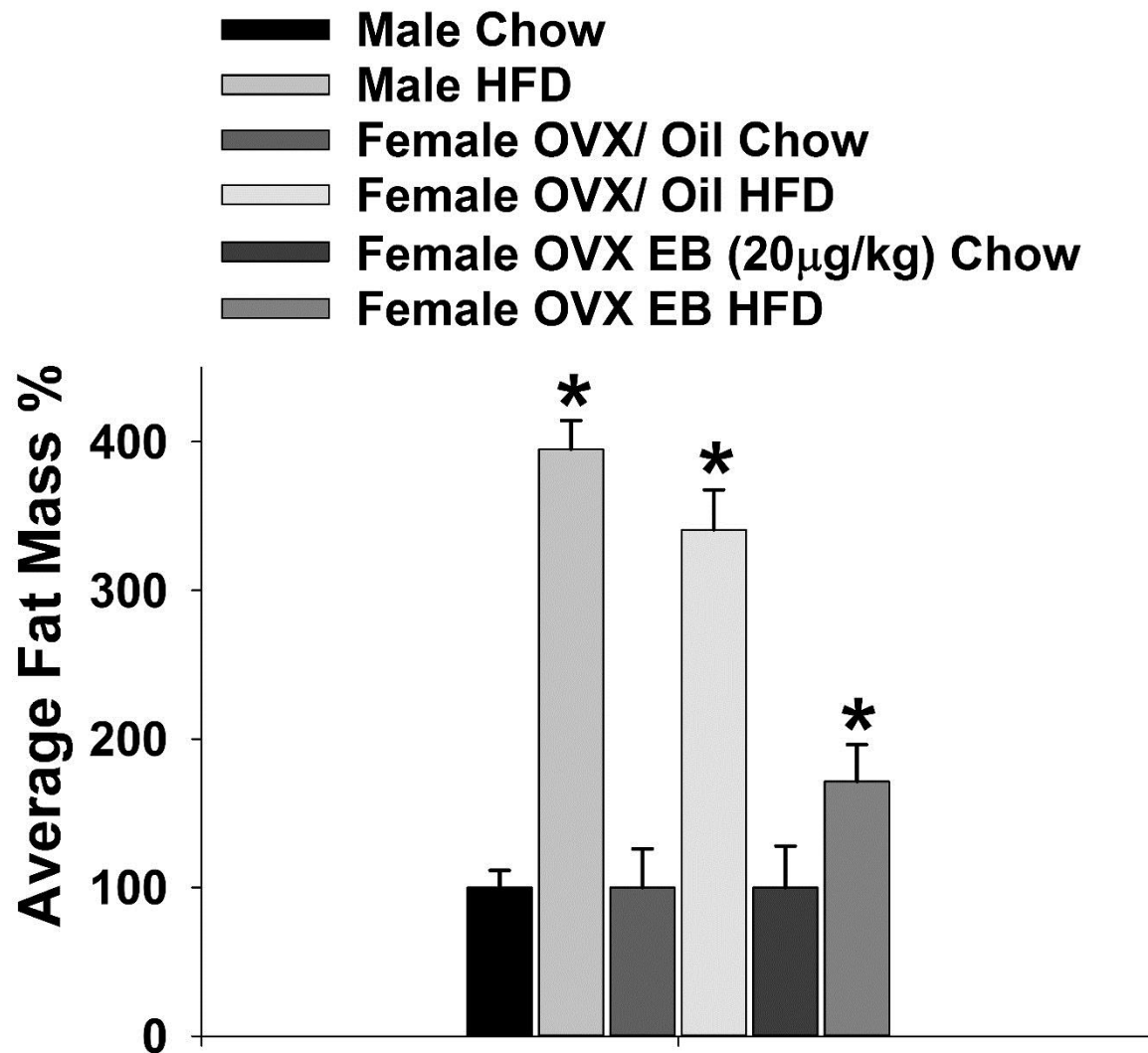
Supplementary Table 1. Proportion of POMC neurons exhibiting eEPSCs in response to electrical stimulation of the dorsomedial VMN across sex and diet. *, $p < 0.05$; Chi-Square test.

Cycle Stage	% Responsive Cells
Diestrus	16/19 (84%)
Proestrus	21/23 (91%)
Estrus	14/15 (93%)
Metestrus	4/12 (33%)*

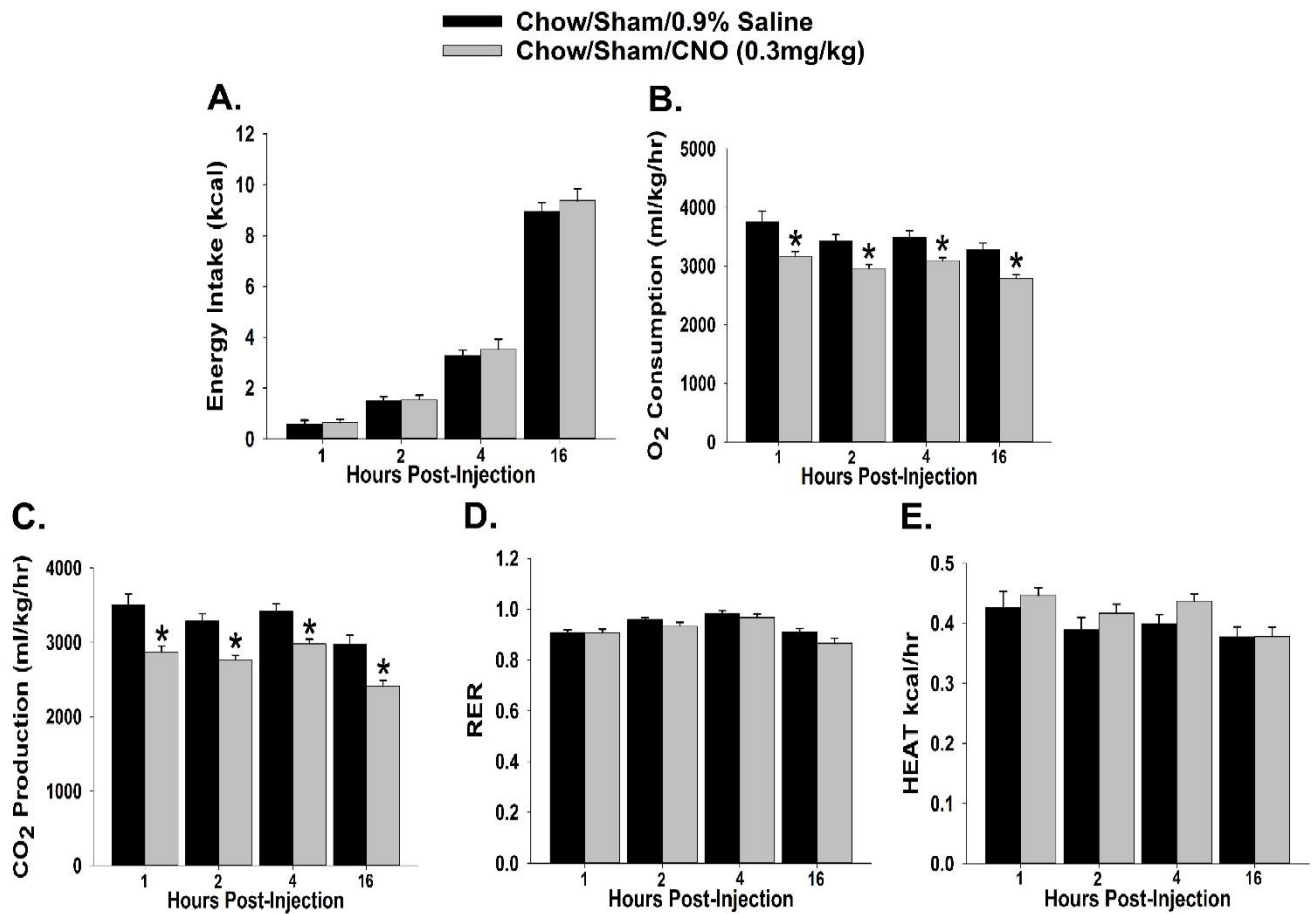
Supplementary Table 2. Proportion of POMC neurons exhibiting leEPSCs across the various stages of the cycle. *, $P < 0.05$; Chi-Square test.

Male	% Cells with eEPSCs	Female	% Cells with eEPSCs
Chow	22/30 (73%)	Chow	59/76 (78%)
HFD	18/21 (86%)	HFD	1/10 (10%)*

Supplementary Table 3. Proportion of POMC neurons exhibiting leEPSCs in response to electrical stimulation of the dorsomedial VMN across sex and diet. *, $p < 0.05$; Chi-Square test.



Supplementary Figure 2 HFD increases body weight and adiposity in NR5A1-Cre mice. Body weight was recorded on the day of harvest, and fat dissections were performed following harvest by which an incision was made through the umbilical region of the abdomen. Fat was collected from the dorsolumbar, inguinal, gluteal, epididymal, and perirenal regions of the abdomen and pelvic area. Bars represent means, and vertical lines 1 SEM. * $P < 0.05$; Student's t-test; $n=5-8$.



Supplementary Figure 3 CNO does not decrease energy intake or increase energy expenditure in sham-injected Nr5a1-cre male mice. CNO (0.3mg/kg) was without effect on cumulative energy intake (A), RER (D), or metabolic heat production (E), and actually decreased O₂ consumption (B) and CO₂ production (C). Bars represent means and lines 1 S.E.M. of the cumulative food intake, O₂ consumption, CO₂ production, RER and metabolic heat production seen in chow-fed, sham-injected NR5A1-Cre mice treated with either CNO (0.3mg/kg; n = 5), or its filtered 0.9% saline vehicle (n = 5). *, P<0.05; multifactorial ANOVA/LSD.