Supplementary Information

Estrogen-Sensitive Medial Preoptic Area Neurons Coordinate Torpor in Mice

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Source_Data.xlsx



Supplementary Figure 1. Activating ER α + MPA neurons alters temperature and heat loss. a, ER α immunoreactivity (magenta) in the MPA of adult male mice. Image representative of n = 4 mice. Scale bar: 200 µm. 3v, third ventricle; ac, anterior commissure; MnPO, median preoptic nucleus; MPA, medial preoptic area; MPN, medial preoptic nucleus; oc, optic chiasm; VLPO, ventrolateral preoptic nucleus; VMPO, ventromedial preoptic nucleus. b, Quantification of mean ER α intensity in rostral MPA and MPN; c, mCherry reporter expression in the MPA of *Esr1Cre* males following stereotaxic delivery of AAV-flex-hM3Dq-mCherry to the MPA. Images representative of n=9 mice. Scale bar: 200 µm. d, Core body temperature measured every 5 minutes before and after injection (dotted line at x=0) of saline (black) or CNO (blue) in n=9 *Esr1*Cre male mice. e, Per-animal averages of core temperature before (-120 min to 0 min) and after (120 min to 180 min) saline or CNO injection for animals shown in d. f, Core body temperature before and after injection of saline (black) or CNO (pink) in wide-type females (n=6). g, Core body temperature before and after injection of saline (black), CNO (pink) or C21

(purple) in *Esr1Cre* females (n=6). **h**, Schematic showing the strategy of measuring core (T_{core}), brown adipose tissue (T_{BAT}) and tail (T_{tail}) temperature. **i**, qPCR analysis of thermogenic gene expression in BAT 90 min after saline or CNO injection in *Esr1Cre* females expressing hM3Dq in the MPA (n=6 per group). **j**, heat loss index (HLI) calculated by attached tail thermo-logger before and after injection (dotted line at x = 0) of saline (black, n=10 mice) or CNO (pink, n=6 mice) in *Esr1Cre* females expressing hM3Dq in the MPA. **k**, Changes in HLI from baseline (-120 min to 0 min) to after (25 min to 35 min) saline or CNO injection. Statistical significance denoted by NS, not significant; **, p<0.05; ***, p<0.01; ****, p<0.0001 for Sidak's multiple comparison tests following a significant effect of treatment or the interaction of treatment with region (**b**) time (**d-g**, **i**) or gene (**h**) in a two-way ANOVA (**b**, **i**) or RM ANOVA (**d-g**, **j**). ***, p<0.001 for (**k**) paired, two-tailed student's t-test. All error bars show SEM.



Supplementary Figure 2. Chemogenetic activation of ERα+ MPA neurons induces a torpor-like state. a, Frequency distribution showing duration of hypothermia bouts (Tcore $< 31^{\circ}$ C) after CNO injection (n=31 mice), **b**. Linear regressions for body weight vs. age, body weight vs. minimum core temperature (Tmin), and BW vs. bout duration after CNO injection (n=42 female Esr1Cre mice). c, Core temperature after CNO injection in mice (n=4 Esr1Cre females) during exposure to an ambient temperature (Ta) of 30°C (red bar) then 22°C (green bar). d, e, Energy expenditure (EE or heat generation kcal/h/kg) (d) and respiratory exchange ratio (RER) (e) before (-40 min to 0) and after (120 min to 180 min) saline or CNO injection calculated from indirect calorimetry data and normalized to lean body mass (n = 6 Esr1Cre females). f, Respiratory rate (breaths/min) quantified from >1 min videos of Esr1Cre mice 3 hours after saline (n = 3 females) or CNO (n = 5 females, 1 male) injection. g, Representative ECG in mice 4 h after saline or CNO injection. Red arrowheads indicate skipped heart beats. h, Relative delta power from 4-day consecutive EEG recordings with saline NREM vs. CNO and saline wake vs. CNO. White and grey bars represent light and dark periods. ZT, zeitgeber time. i-j, Eight-day consecutive recordings of core temperature (i) and movement (j) after two single injections of CNO (dotted line) in a cohort of Esr1Cre female mice showing long torpor bouts (n = 4 Esr1Cre females). Lines show group means, error bars or shaded area (c, h-j) show SEM, faded lines (d, e) show individual animals. ****, p<0.0001 for Sidak's multiple comparison tests comparing saline and CNO following a significant effect of treatment (d and i) in a two-way RM ANOVA or the interaction between treatment and time in a linear mixed effects model (h). **, p<0.01 for unpaired, 2-tailed student's t-test (f).



Supplementary Figure 3. Neuronal activity of ER α + MPA neurons during temperature challenge. a, Fasting induced hypothermia as model for torpor (n=6, females). Shaded areas show SEM. b, Representative trace recorded during gradual warm (40 °C) and cold (15 °C) temperature exposure. c-e, Individual mouse responses during each temperature exposure period (5 min), including variance (c), area under the curve, AUC (d) and total peak area (e). f, Distribution of neurons by firing rate changes in response to temperature change. Neurons showing <=41.4% change in firing rate were classified as non-responsive (NR, grey) and neurons showing >41.4% change in firing rate were classified as temperature responsive (TR, red). n = 179 cells from 4 female mice. g, Pie charts showing percentages of TR (red) and NR (grey) neurons in the medial preoptic nucleus, (MPN), rostral MPA, ventrolateral preoptic nucleus (VLPO), and ventromedial preoptic nucleus (VMPO). h. Heat map comparing transcriptomes of NR (n = 3 samples, two neurons per sample) or TR (n = 4 samples, 2 neurons per sample) neurons when exposed to 25 °C and 30 °C. i, Volcano plots comparing gene expression in TR versus NR ERa neurons in the MPN. All genes shown as grey dots, those with significant differential expression (adjusted p<0.05 using the Benjamini-Hochberg procedure) denoted by dark grey dots, and transcripts of interest denoted by pink dots. Transcripts of interest include markers of GABAergic neurons, glutamatergic neurons, distinct Esr1expressing clusters in the MPA (from Ref. 14), and markers of neurons activated by torpor (from Ref. 39). Gene symbols are provided for genes that are significantly enriched (right) or depleted (left) in TR ERα neurons. Genes that were detected in TR and NR but not differentially expressed in TR neurons include Gabra2, Gabbr1, Gad2. Slc32a1 for GABAergic markers, Slc17a6, Gls, Glul for glutamatergic markers, Trhr, Nts, Calcr, Tac2, Etv1, Mc4r for Esr1 cluster markers, and Sntg2, Nts, Pde1c, Map3k15 for torpor neuron markers.



Supplementary Figure 4. Sex differences after ablating ER α + MPA cells in baseline activity and fastinginduced torpor. **a** and **c**, Physical activity over 24 h, measured every 5 min for 3 days. Group averages shown for control (black, n=8) and ablated (pink, n=8 female; blue, n=7 male) mice. Shading along the curve denotes the SEM. **b** and **d**, Sum of activity from mice shown in panel **a** and **c** highlighting per animal in light (7:00 to 19:00), dark (19:00 to 7:00), and total 24 h periods. **e**, Duration of the longest bout of Tcore \leq 31 °C, lowest Tcore and body weight during the fasting period for control females (n=7 mice), ablated females (n=7 mice), control males (n=6 mice), and ablated males (n=8 mice). Error bars show SEM. Statistical significance denoted by NS, not significant effect of treatment in a two-way RM ANOVA (**b** and **d**), *, p<0.05 and **, p<0.01, ****, p<0.0001 for twoway ANOVA (**e**).



Supplementary Figure 5. Projections of ERa+ neurons from the MPA. a, Schematic strategy for visualizing descending target structures from the MPA using Cre-dependent virus expressing GFP. **b**, GFP expression in ERa+ cell bodies at the injection site (green dashed outline in Bregma: 0.0 mm) and in fibers from the major projection targets. Projection profile is shown 2 weeks after GFP injection. Images representative of n=3 *Esr1Cre* female mice. 3v, third ventricle; ac, anterior commissure; ArcMP or ArcLP, arcuate nucleus, medial posterior part (ArcMP) or lateroposterior part (ArcLP); BNST, bed nucleus of stria terminalis; f, fornix; D3V, dorsal third ventricle; DMC (DMD/DMV), dorsal medial hypothalamus, compact part (or dorsal/ventral parts); MPA, medial preoptic area; MS, medial septum nucleus; PAG, periaqueductal gray; PVH, paraventricular hypothalamus; RML, retromammillary nucleus, lateral part; SNCD, substantia nigra, compact part, dorsal tier; VMH (vI), ventromedial hypothalamus (ventral lateral part); VTA (R), ventral tegmental area (rostral part). Scale bars, 250µm. **c**, Schematic summary of the major projection brain areas from ERa+ MPA neurons.

Supplementary Table 1. qPCR primer sequences

Gene name	Symbol	Forward	Reverse
Uncoupling protein 1	Ucp1	CACGGGGACCTACAATGCTT	TAGGGGTCGTCCCTTTCCAA
Deiodinase, iodothyronine, type II	Dio2	CCTCAGAAGGGCTGCGCTGTG	TCAGCGGTCTTCTCCGAGGC
PR domain containing 16	Prdm16	GAAGTCACAGGAGGACACGG	TCATTGCATATGCCTCCGGG
Peroxisome proliferator- activated receptor gamma, coactivator 1 alpha	Pgc1a	CAGTACAGCCCCGATGACTC	GAAAGCTCGTCCACGTCAGAC
Adrenergic receptor, beta 3	Adrb3	GGAAGCTTGCTTGATCCCCA	GCCGTTGCTTGTCTTTCTGG