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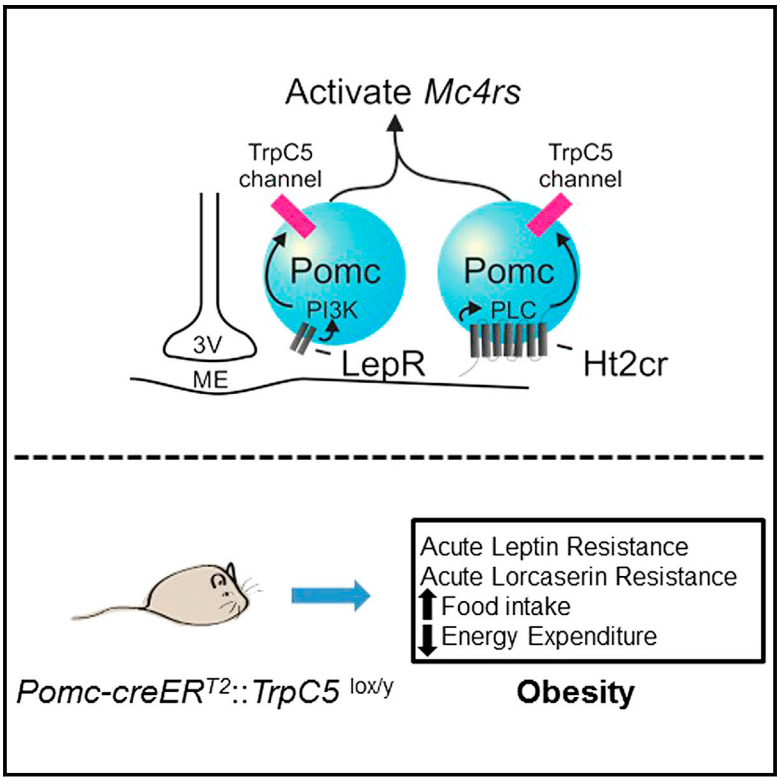
Report

# *TrpC5* Mediates Acute Leptin and Serotonin Effects via *Pomc* Neurons

## Graphical Abstract




## Highlights

- Leptin- and serotonin-induced activation of arcuate Pomc neurons requires TrpC5 subunits
- Acute effects of leptin and serotonin on food intake require TrpC5 subunits
- TrpC5 subunits are a physiological mechanism in the CNS to regulate metabolism

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## In Brief

Gao et al. show that TrpC5 subunits are essential for the negative energy balance associated with Pomc neuronal activation. TrpC5 subunits not only link the acute activities of leptin and serotonin receptors in Pomc neurons, but also modify direct effects on basal metabolism.TrpC5 subunits may provide an endogenous target to manipulate the activity of key neurons involved in the regulation of energy balance and glucose metabolism.

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# *TrpC5* Mediates Acute Leptin and Serotonin Effects via *Pomc* Neurons

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## SUMMARY

**The molecular mechanisms underlying acute leptin and serotonin 2C receptor-induced hypophagia remain unclear. Here, we show that neuronal and pro-opiomelanocortin (*Pomc*)-specific loss of transient receptor potential cation 5 (*TrpC5*) subunits is sufficient to decrease energy expenditure and increase food intake resulting in elevated body weight. Deficiency of *Trpc5* subunits in *Pomc* neurons is also sufficient to block the anorexigenic effects of leptin and serotonin 2C receptor (*Ht2Cr*) agonists. The loss of acute anorexigenic effects of these receptors is concomitant with a blunted electrophysiological response to both leptin and *Ht2Cr* agonists in arcuate *Pomc* neurons. We also demonstrate that the *Ht2Cr* agonist lorcaserin-induced improvements in glucose and insulin tolerance are blocked by *TrpC5* deficiency in *Pomc* neurons. Together, our results link *TrpC*5 subunits in the brain with leptin- and serotonin 2C receptor-dependent changes in neuronal activity, as well as energy balance, feeding behavior, and glucose metabolism.**

## INTRODUCTION

Leptin receptors (*LepR*s) in the brain produce robust anti-obesity and anti-diabetic effects (Bjørbaek and Kahn, 2004; Coll et al., 2007; Elmquist et al., 1999; Flier, 2006; Friedman, 2004; Spiegelman and Flier, 2001). Similarly, the neurotransmitter serotonin (5-hydroxytryptamine [5-HT]) contributes to the regulation of feeding behavior, energy expenditure, and glucose homeostasis by acting via serotonin 2C receptors (*Ht2Cr*s) within the CNS (Giorgetti and Tecott, 2004; Heisler et al., 2003). The hypothalamic arcuate pro-opiomelanocortin (*Pomc*) neurons are vital in mediating both leptin's and serotonin's beneficial effects on metabolism (Balthasar et al., 2004; Berglund et al., 2012; Sohn et al., 2011). Very recently, lorcaserin, an *Ht2Cr*-specific agonist, became the first US Food and Drug Administration (FDA)-approved weight-loss drug in the last 15 years independent of adverse cardiopulmonary effects. In order to better understand the effectiveness and safety of newly approved anti-obesity drugs, it is imperative to delineate cellular mechanisms underlying *Ht2Cr* and *LepR* activity in the brain.

There is an emerging role for *TrpC* channels in the regulation of energy homeostasis by leptin (Qiu et al., 2010). Similarly, activation of *Pomc* neurons by *Ht2Cr*s is mediated by a phospholipase C (*PLC*)-dependent activation of putative *TrpC* channels (Sohn et al., 2011). Single-cell RT-PCR of arcuate *Pomc* neurons revealed that *TrpC* 1, 4, and 5 subunits predominate in murine *Pomc* neurons (Qiu et al., 2010). However, *TrpC*1 alone may not be sufficient to form a functional ion channel (Strübing et al., 2001), suggesting that *TrpC4 or TrpC5* may be a common target in *Pomc* neurons of these two potent anorexigenic signals, leptin and serotonin. In the current study, we studied the requirement of *TrpC*5 in the acute effects of leptin and serotonin to depolarize hypothalamic *Pomc* neurons. We also examined the requirement of *TrpC*5 subunits in the acute effects of leptin and serotonin on energy expenditure as well as glucose homeostasis.

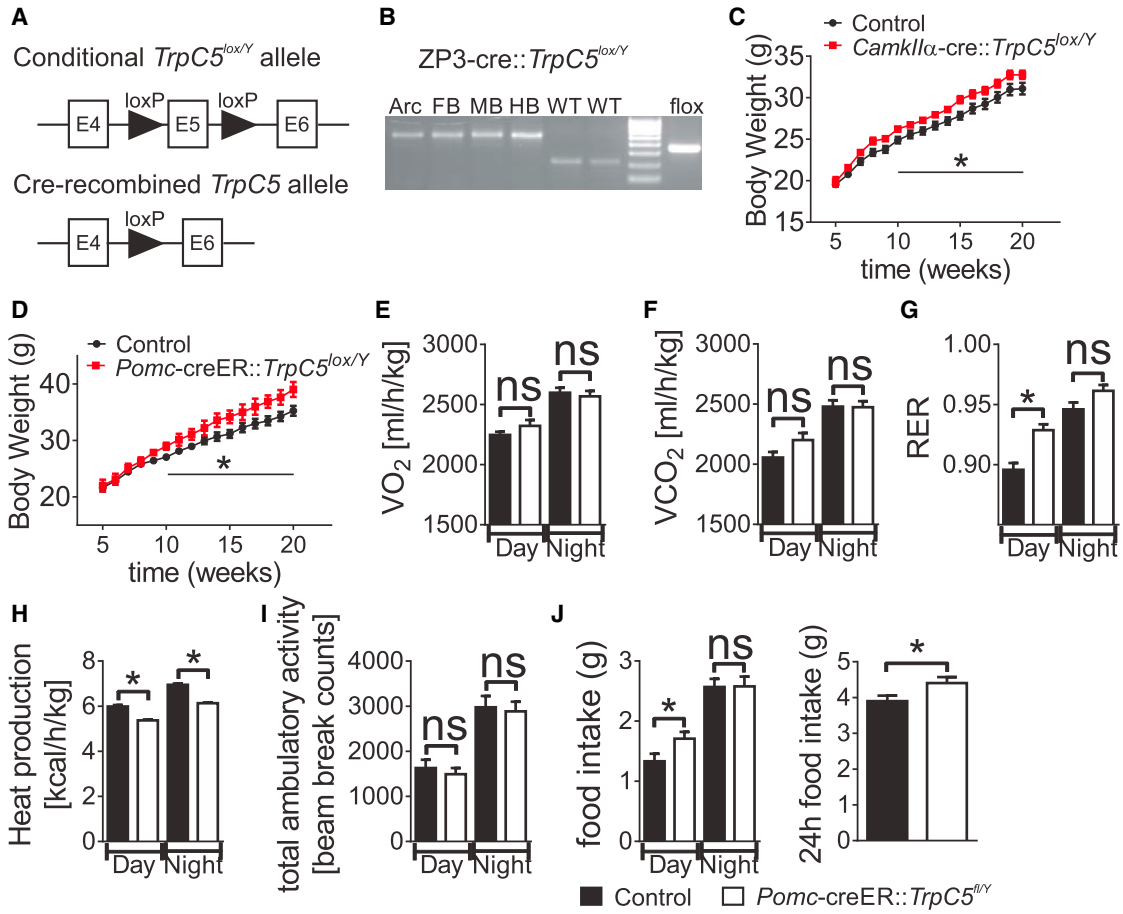


**Figure 1. Body Weight and Metabolic Assessment of Male WT and *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ Mice on Chow Diet**

(A) Schematic of genomic DNA region around exon 5 of *TrpC5* in mice carrying a targeted *TrpC5* allele (*TrpC5*$^{lox/Y}$). After Cre-mediated excision of exon 5, a PCR amplification product identifying the deleted *TrpC5* allele (*TrpC5*$^{lox/Y}$) becomes detectable.

(B) PCR amplification products from genomic DNA using primers detect wild-type, floxed, and deleted *TrpC5*. Tissues were dissected from a ZP3-cre::*TrpC5*$^{lox/Y}$ mouse. From left to right: lane 1, arcuate nucleus (positive for recombination); lane 2, forebrain (positive for recombination); lane 3, midbrain (positive for recombination); lane 4, hindbrain (positive for recombination); lanes 5 and 6, wild-type hypothalamus (negative for recombination); lane 6, DNA ladder; and lane 7, hypothalamus from floxed mouse negative for ZP3-cre.

(C and D) Body weight curve of (C) male *CamkIIα*-cre::*TrpC5*$^{lox/Y}$ and (D) male *Pomc*-cre::*TrpC5*$^{lox/Y}$ mice (*$p < 0.05$).

(E–I) Depictions of (E) unchanged oxygen consumption ($VO_2$), (F) unchanged carbon dioxide production ($VCO_2$), (G) increased respiratory exchange ratio (RER), (H) decreased heat production, and (I) unchanged ambulatory activity in male *Pomc*-cre::*TrpC5*$^{lox/Y}$ mice.

(J) Male *Pomc*-cre::*TrpC5*$^{lox/Y}$ mice exhibited increased food intake in the light cycle that resulted in hyperphagia over 24 hr.

(A–I) n = 8–15 per group; *$p < 0.05$. Error bars indicate SEM. Mice used in (E)–(I) were age-matched male littermates (8 weeks of age) and had comparable body weight and lean mass.

## RESULTS

### Neuronal *TrpC5* Subunits Are Required for Proper Energy Balance

Disturbance of acute leptin or *Ht2Cr* signaling pathways in *Pomc* neurons is correlated with blunted acute anorexia by leptin and serotonin 2C receptor-agonists, as well as deficits in energy and glucose homeostasis (Hill et al., 2008; Xu et al., 2008, 2010b). We hypothesized that leptin and *Ht2Cr*s may acutely activate *TrpC5* subunits within *Pomc* neurons, thus being required in vivo for the feeding effects of these receptors as well as regulating energy balance and glucose homeostasis. To determine the metabolic effects of *TrpC5* subunits within the CNS during postnatal development, we generated a conditional mouse model (*TrpC5*$^{lox/Y}$) in which we could selectively reduce the expression of *TrpC5* subunits in key brain sites. This was accomplished by utilizing *TrpC5*-targeted ESCs from the European Union Conditional Mouse Mutagenesis (EUCOMM) program in order to generate conditional *TrpC5*$^{lox/y}$ mice via the insertion of *loxP* sites surrounding exon 5 of the *TrpC5* gene (Figure 1A). Subsequent breeding to ZP3-cre (Figure 1B), *CamkIIα-cre*, or *Pomc*-creER$^{T2}$ mice generated mice deficient for *TrpC5* in a cell- and tissue-type-dependent manner.

When fed a chow diet, neuron- (*CamkIIα*-cre, in which Cre recombinase is expressed widely in the forebrain and hindbrain;

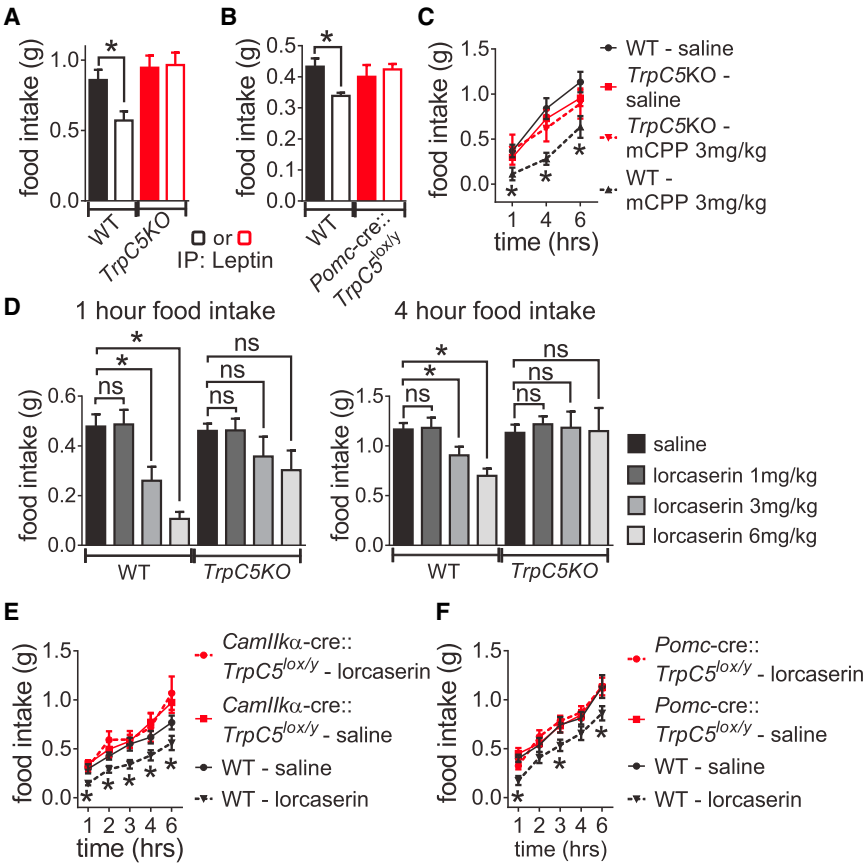


**Figure 2. Leptin-, mCPP-, and Lorcaserin-Induced Hypophagia Is Blunted in Mice Deficient for *TrpC5* Subunits**

(A and B) Food consumption was measured 1 hr after leptin administration (5 mg/kg, i.p.) and compared with food consumption in each animal following saline administration. WT versus *TrpC5KO* (A); WT versus *Pomc*-creER::*TrpC5*$^{lox/Y}$ (B).

(C and D) Cumulative food intake measured at 1, 4, and/or 6 hr after administration of *Ht2Cr* agonists mCPP (C) (3 mg/kg) or lorcaserin (D) (1, 3, or 6 mg/kg).

(E and F) Food intake measured in response to lorcaserin (3 mg/kg, i.p.). WT versus *CamIIkα*-cre::*TrpC5*$^{lox/Y}$ (E); WT versus *Pomc*-creER::*TrpC5*$^{lox/Y}$ (F).

n = 9–14 per group; *p < 0.05.

Casanova et al., 2001) or Pomc-specific (*Pomc*-creER$^{T2}$-cre allows temporal control of *cre* recombinase activity in *Pomc* neurons; Berglund et al., 2013) deficiency of *TrpC5* subunits resulted in an age-dependent increase in body weight (Figures 1C and 1D). The increased body weight was more pronounced in mice deficient for *TrpC5* subunits in *Pomc* neurons alone (Figure 1D). Age- and weight-matched *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ males were hypometabolic, as demonstrated by significant decreases in energy expenditure (Figures 1E–1H) independent of altered activity levels (Figure 1I). Components of total energy expenditure include energy required for physical activities and basal metabolism. In particular, *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ mice exhibited decreased heat production (Figure 1H) suggestive of decreased metabolic rate. *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ mice also showed increased ad libitum food intake (Figure 1J).

## Neuron- and *Pomc*-Specific *TrpC5* Deficiency Blunts the Acute Anorexia by *LepR* and *Ht2Cr* Activation

Wild-type (WT), transient receptor potential cation channel 5 knockout (*TrpC5*KO) (Riccio et al., 2009), and *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ mice were fasted overnight and intraperitoneally (i.p.) injected with either saline or leptin (5 mg/kg). Consistent with previous reports (Hill et al., 2008), i.p. injections of leptin reduced food intake in wild-type mice (at 1 hr) compared with the saline-injected group (Figures 2A and 2B). However, i.p. injections of leptin failed to decrease food intake in *TrpC5*KO and *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ mice (at 1 hr) versus the saline-injected group (Figures 2A and 2B).

We performed a similar series of experiments with both the non-selective *Ht2Cr* agonist (meta-chlorophenylpiperazine [mCPP]) (3 mg/kg) and lorcaserin (1, 3, and 6 mg/kg). As reported previously, the *Ht2Cr* agonists significantly reduced food intake in wild-type mice versus saline-injected groups (at 1, 4, and/or 6 hr; Figures 2C–2F). However, i.p. injections of mCPP or lorcaserin failed to suppress food intake (at 1, 4, and/or 6 hr) in *TrpC5*KO, *CamkIIα*::*TrpC5*$^{lox/Y}$, and/or *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ mice compared with saline injections. Of note, 3 mg/kg lorcaserin appears to be a threshold dose for the acute biological activity of food intake. Taken together, *TrpC5* subunits are required in *Pomc* neurons for the acute anorexia induced by leptin receptor and *Ht2Cr* activation.

## *TrpC5* Subunits Are Required for the Acute Activation of *Pomc* Neurons by Leptin

Whole-cell patch-clamp recordings were made in 58 arcuate *Pomc* neurons from *Pomc*-hrGFP (humanized Renilla green fluorescent protein) mice (Parton et al., 2007; Ramadori et al., 2010; Sohn et al., 2011). Twenty-six *Pomc* neurons were from wild-type and 32 were in *Pomc* neurons deficient for *TrpC5* subunits (Riccio et al., 2009). *Pomc* neurons from wild-type mice (n = 26) had a resting membrane potential of −41.5 ± 1.3 mV, a mean input resistance of 1,260 ± 80 MΩ, and overshooting action potentials. When compared with values obtained from wild-type *Pomc* neurons, *Pomc* neurons deficient for *TrpC5* exhibited a hyperpolarized mean resting membrane potential (−46.3 ± 0.9 mV; 1,085 ± 53 MΩ; n = 32; p < 0.05; Figure S1A). Analogous results were obtained in *Pomc* neurons from *Pomc*-creER$^{T2}$ versus *Pomc*-creER$^{T2}$::*TrpC5*$^{lox/Y}$ mice (resting membrane potential [RMP] = −44.3 ± 1.0 mV from wild-type Pomc neurons, n = 24; RMP = −48.0 ± 1.4 mV in *Pomc* neurons from *Pomc*-creER$^{T2}$::*Trpc5*$^{lox/Y}$ mice, n = 22; p < 0.05; Figure S1A).

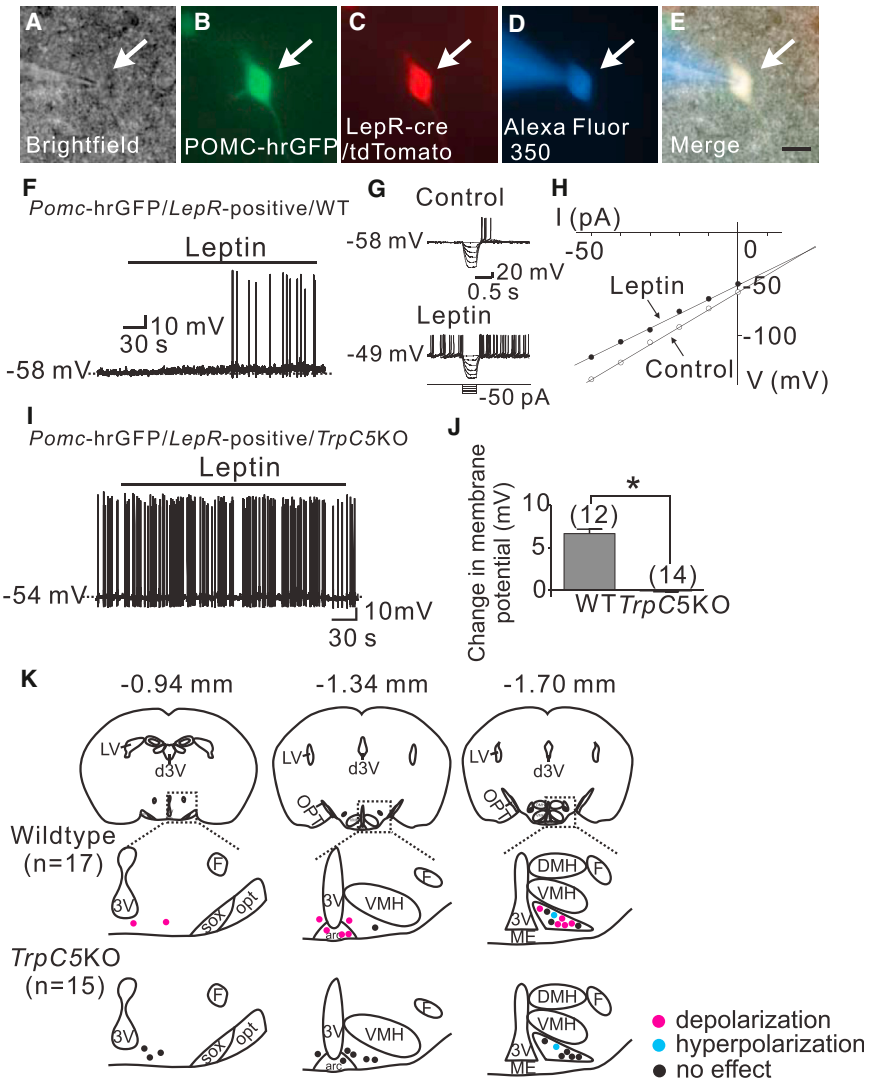


**Figure 3. *Trpc5* Subunits Are Required for the Acute Leptin-Induced Depolarization of Arcuate *Pomc* Neurons**

(A) Brightfield illumination of *Pomc*-hrGFP::*Lepr*-cre::*tdtomato* neuron from *PLT* mice.

(B and C) The same neuron under fluorescein isothiocyanate (FITC; hrGFP; B) and Alexa Fluor 594 (*tdtomato*; C) illumination.

(D) Complete dialysis of Alexa Fluor 350 from the intracellular pipette.

(E) Merge image illustrates colocalization of hrGFP, *tdtomato*, and Alexa Fluor 350 indicative of a *Pomc* neuron that expresses *Lepr*s.

(F) Electrophysiological study demonstrates a *Pomc*-hrGFP::*Lepr*-cre::*tdtomato* (green/red) neuron that is depolarized in response to leptin (100 nM).

(G) Traces showing decreased voltage deflection and increased action potential frequency after leptin application.

(H) Current versus voltage (I-V) plot from same WT neuron illustrating a characteristic decrease in input resistance subsequent to leptin application. Shown are responses before (control) and during leptin application.

(I) Demonstrates a current-clamp recording of a *Pomc*-hrGFP::*Lepr*-cre::*tdtomato*::*TrpC5KO* (green/red) neuron in which leptin fails to induce a depolarization.

(J) Histogram summarizing the acute effect of leptin on the membrane potential of *Pomc* neurons that express leptin receptors as well as express or do not express Trpc5 subunits (n = 12–14 per group).

(K) Rostro-caudal and medio-lateral distribution of electrophysiological responses to leptin from *Pomc* neurons that express leptin receptors as well as express or do not express Trpc5 subunits.

Similar to previous reports (Hill et al., 2008; Sohn et al., 2011), *Pomc* neurons that express *LepRs* were targeted from *Pomc*-hrGFP::*LepR*-cre-tdtomato (PLT) mice (Sohn et al., 2011; Sun et al., 2016; Williams et al., 2014) to test the acute cellular effects of leptin (Figures 3A–3E). Leptin (100 nM) depolarized 70.6% (12 out of 17) of wild-type *Pomc* neurons that express *LepRs* by 6.7 ± 0.5 mV (n = 12; Figures 3F and 3J; Table S1). We found one cell (5.9%) that was hyperpolarized by −10 mV, whereas the remaining four cells (23.5%) were not responsive to leptin (0.3 ± 0.3 mV, n = 4). Analysis of current-voltage relationships revealed that leptin decreased input resistance by 21.4% ± 3.0% (n = 12, from 1.2 ± 0.1 GΩ in control artificial cerebrospinal fluid (CSF) (ACSF) to 0.9 ± 0.1 GΩ in leptin) with a reversal potential of −26.0 ± 2.2 mV (n = 12) (Figures 3G and 3H). These results confirmed that leptin activates a non-selective cation conductance to depolarize *Pomc* neurons.

In contrast, leptin failed to depolarize any of 15 *Pomc* neurons that express *LepRs* from *TrpC5* knockout (PLT5KO) mice (Figures 3I and 3J; Table S2): 14 cells (93.3%) remained unresponsive to leptin (−0.1 ± 0.5 mV), whereas 1 cell (6.7%) was hyperpolarized by −9 mV. Analogous results were obtained in recordings from *Pomc* neurons selectively deficient for *TrpC5* subunits (from *Pomc*-creER$^{T2}$::*TrpC5* mice; Figure S1B). These data suggest that *TrpC5* subunits underlie the leptin-induced activation of a non-selective cation conductance resulting in the depolarization of *Pomc* neurons. Notably, the hyperpolarizing effects of leptin remained unchanged in *Pomc* neurons lacking *TrpC5* subunits, and these effects were accompanied by decreased input resistance with a reversal potential close to equilibrium potential for potassium ($E_K$). Similar results were obtained in ventral premammillary nucleus (PMv) neurons that express leptin receptors (Supplemental Information; Figure S2). Thus, the null *TrpC5* gene does not affect the functional expression of leptin receptors or other channels in our model.

After recordings, the sections were fixed in formalin to determine the location of recorded cells within the arcuate nucleus, as described previously (Sohn et al., 2011). Because acute leptin responses show a distinct distribution pattern (Williams et al., 2010), we targeted cells of similar region within the

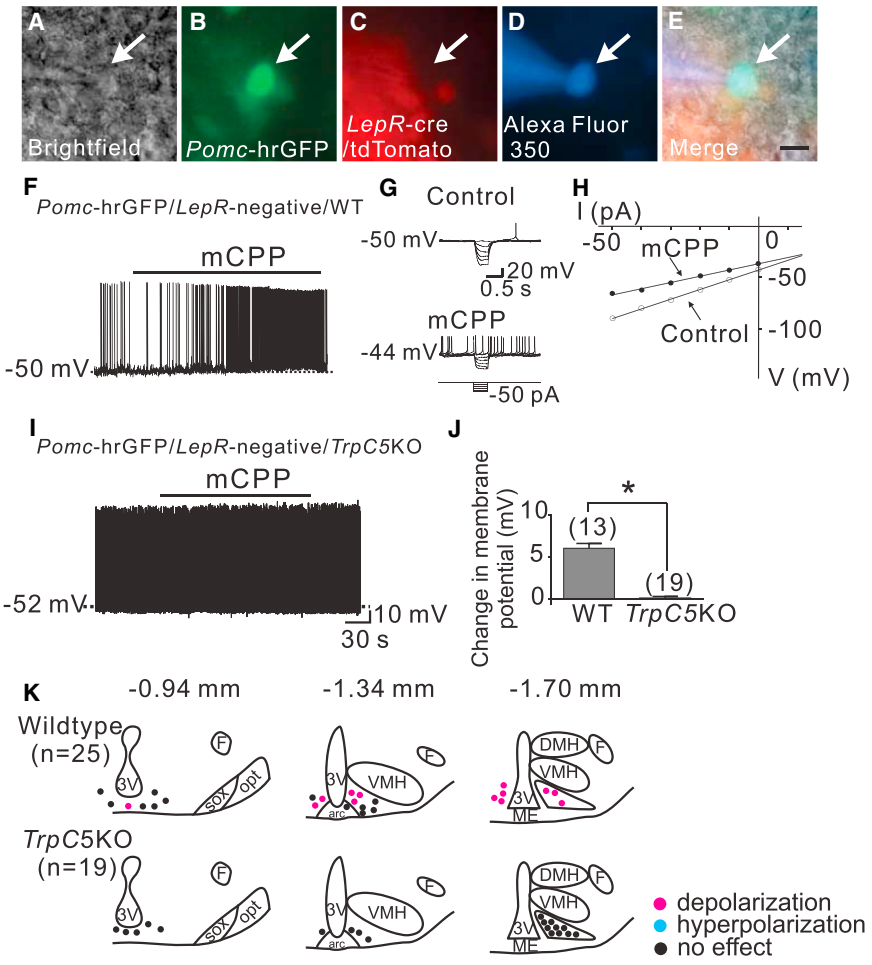


**Figure 4. *Trpc5* Subunits Are Required for the Acute mCPP-Induced Depolarization of Arcuate Pomc Neurons**

(A) Brightfield illumination of *Pomc*-hrGFP neuron from *PLT* mice.

(B and C) The same neuron under (B) fluorescein isothiocyanate (FITC) (hrGFP) and (C) Alexa Fluor 594 (*tdtomato*) illumination.

(D) Complete dialysis of Alexa Fluor 350 from the intracellular pipette.

(E) Merge image illustrates colocalization of hrGFP and Alexa Fluor 350 indicative of a *Pomc* neuron that does not express *Lepr*s.

(F) Electrophysiological study demonstrates a *Pomc*-hrGFP (green) neuron from *PLT* mice that depolarized in response to mCPP (4 μM). Traces showing decreased voltage deflection and increased action potential frequency after mCPP application.

(G) Traces showing decreased voltage deflection and increased action potential frequency after mCPP application.

(H) Current versus voltage (I-V) plot from same WT neuron illustrating a characteristic decrease in input resistance subsequent to mCPP application. Shown are responses before (control) and during mCPP application.

(I) Demonstrates a current-clamp recording of a *Pomc*-hrGFP::*Trcp5*$^{-/Y}$ (green) neuron in which mCPP fails to induce a depolarization.

(J) Histogram summarizing the acute effect of mCPP on the membrane potential of *Pomc* neurons that do not express leptin receptors as well as express or do not express Trpc5 subunits (n = 13–19 per group). *p < 0.05.

(K) Rostro-caudal and medio-lateral distribution of electrophysiological responses to mCPP from *Pomc* neurons that do not express leptin receptors as well as express or do not express Trpc5 subunits.

arcuate nucleus in both wild-type and *TrpC*5 knockout mice (Figure 3K).

## The Acute mCPP-Induced Activation of *Pomc* Neurons Requires *TrpC5* Subunits

To test the acute cellular effects of *Ht2Cr*s, we targeted *Pomc* neurons that do not express *LepR*s from PLT mice (Figures 4A–4E), as previously described (Sohn et al., 2011). We confirmed that mCPP (4 μM) depolarized 55% (5 out of 9) of wild-type *Pomc* neurons that do not express *LepR*s by 6 ± 0.6 mV (n = 5; Figures 4F and 4J; Table S1). The remaining four cells (45%) were not responsive to mCPP (0.4 ± 0.2 mV; n = 4). Application of current steps revealed that mCPP decreases input resistance by 14.8% ± 4.8% (n = 5, from 1.5 ± 0.1 GΩ in control ACSF to 1.3 ± 0.1 GΩ in mCPP) with a reversal potential of −14 ± 4.8 mV (n = 5) (Figures 4G and 4H). In contrast, mCPP failed to depolarize all *Pomc* neurons tested from *Trpc*5 knockout (PLT5KO) mice (0.1 ± 0.2 mV; n = 19; Figures 4I and 4J; Table S2). Similar to the acute leptin effects in the current study, we targeted cells in a similar region, and responses were mapped within the arcuate nucleus (Figure 4K).

## The Lorcaserin-Induced Depolarization of *Pomc* Neurons Is Dependent upon *TrpC5*

We previously confirmed that the acute effects of mCPP on *Pomc* neurons were mediated by *Ht2Cr*s (Berglund et al., 2013; Xu et al., 2010a). Thus, the observed cellular effects of mCPP in the present study should depend upon *Ht2Cr* activation, and the lack of mCPP effects on *Pomc* neurons from the *TrpC*5 knockout mice indicate that *Trpc*5 is required for the acute activation of *Pomc* neurons by *Ht2Cr*s.

However, to further examine this activity, we used lorcaserin (a specific *Ht2Cr* agonist recently approved for chronic weight management). Similar to results obtained with mCPP, lorcaserin (4 μM) depolarized 50% (6 out of 12) of wild-type *Pomc* neurons that do not express *LepR*s by 7.0 ± 0.7 mV (n = 6; Figures 5A and 5E; Table S1). The remaining six cells (50%) were not responsive to lorcaserin (0.3 ± 0.2 mV; n = 6). Application of current steps confirmed that lorcaserin decreased the input resistance by 25.6% ± 6.5% (n = 6; from 1.6 ± 0.1 GΩ in control ACSF to 1.2 ± 0.2 GΩ in lorcaserin) with a reversal potential of −13.1 ± 3.2 mV (n = 6) (Figures 5B and 5C). Similar results were observed in *Pomc* neurons that express *Ht2Cr*s (from *Pomc*:: *Ht2Cr*-cre::tdtomato

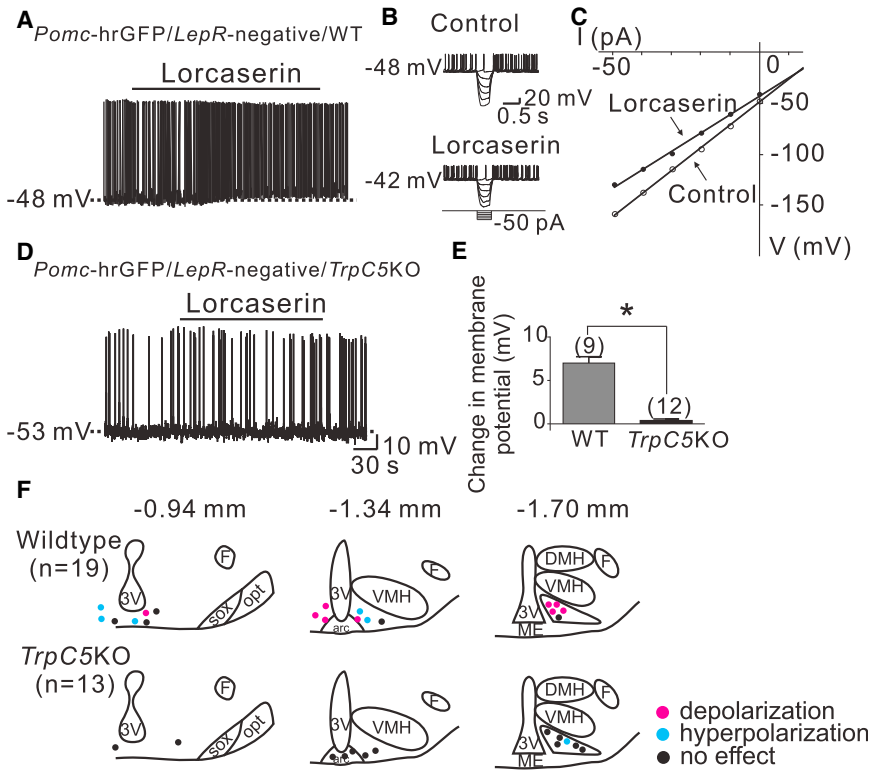


**Figure 5. *Trpc5* Subunits Are Required for the Acute Lorcaserin-Induced Depolarization of Arcuate Pomc Neurons**

(A) Electrophysiological study demonstrates a *Pomc*-hrGFP (green) neuron from *PLT* mice that depolarized in response to lorcaserin (4 μM).

(B) Traces showing decreased voltage deflection and increased action potential frequency after lorcaserin application.

(C) Current versus voltage (I-V) plot from same WT neuron illustrating a characteristic decrease in input resistance subsequent to lorcaserin application. Shown are responses before (control) and during lorcaserin application.

(D) Demonstrates a current-clamp recording of a *Pomc*-hrGFP::*Trcp5*$^{lox/Y}$ (green) neuron from *PLT* mice in which lorcaserin fails to induce a depolarization.

(E) Histogram summarizing the acute effect of lorcaserin on the membrane potential of *Pomc* neurons that do not express leptin receptors as well as express or do not express *TrpC5* subunits (n = 9–12 per group). *p < 0.05.

(F) Rostro-caudal and medio-lateral distribution of electrophysiological responses to lorcaserin from *Pomc* neurons that do not express leptin receptors as well as express or do not express *TrpC5* subunits.

[P2CT] mice; Supplemental Information; Figures S3 and S4; Table S3).

As expected, lorcaserin failed to depolarize any of 12 *Pomc* neurons from *TrpC5* knockout (PLT5KO) mice (Figures 5D and 5E; Table S2). Eleven cells (91.7%) remained unresponsive to lorcaserin (0.4 ± 0.2 mV), whereas one cell (8.3%) was hyperpolarized by −8 mV. A summary of the location of targeted cells was mapped throughout the rostro-caudal extent of the arcuate nucleus (Figure 5F). Similar results were obtained in recordings from *Pomc* neurons selectively deficient for *TrpC5* subunits (from *Pomc*-creER$^{T2}$::*TrpC5* mice; Figure S1C; Table S4). These data confirm that *TrpC5* subunits underlie the *Ht2Cr*-mediated activation of non-selective cation conductance and the depolarization of *Pomc* neuron membrane potential.

## DISCUSSION

Neuronal or *Pomc*-specific *TrpC5* deficiency resulted in a positive energy balance that contributed to excess weight gain. In addition to these physiological aberrations, global deficiency of *TrpC5* as well as neuron- or *Pomc*-specific deficiency of *TrpC5* alone abrogated the acute anorexigenic effects of both leptin and serotonin 2C receptor agonists. *TrpC5* subunits were also required for the acute effects of lorcaserin to improve both glucose and insulin tolerance (Figures S5 and S6). On a cellular level, deficiency of *TrpC5* subunits abrogates the acute effects of both leptin and serotonin 2C receptors to activate arcuate *Pomc* neurons. Together, these data demonstrate that *TrpC5* subunits in arcuate *Pomc* neurons are important regulators of the pharmacological effects of both leptin and serotonin, and link critical molecular mechanisms with recently developed anti-obesity pharmacotherapeutics, such as lorcaserin.

Multiple signaling cascades are activated in response to leptin receptor activation. In particular, phosphoinositol 3-kinase (*PI3K*) has been suggested to be required for the acute effects of leptin to stimulate *Pomc* neuronal activity via a putative activation of *TrpC* channels (Al-Qassab et al., 2009; Hill et al., 2008; Qiu et al., 2010). Pharmacological inhibition of *PI3K* or targeted disruption of *PI3K* regulatory subunits in *Pomc* neurons alone blunted the suppression of feeding elicited by central leptin administration (Hill et al., 2008; Niswender et al., 2001). Moreover, mice deficient for *PI3K* catalytic activity exhibited central leptin resistance, increased adiposity, diet-induced obesity, and impaired glucose regulation (Al-Qassab et al., 2009; Hill et al., 2009). Importantly, these data are analogous to the observations detailed in the current study, supporting a role for the *PI3K*-*TrpC5* pathway in the acute and chronic regulation of energy balance and glucose homeostasis.

It should be noted that a previous report demonstrated that global deficiency of *TrpC5* subunits (*Trpc5*KO) fails to alter body weight (Riccio et al., 2009). However, numerous reports have demonstrated that global deficiency of various genes may result in compensatory adaptation (Luquet et al., 2007; Zeltser et al., 2012). In the current study, global *TrpC5* deficiency resulted in improved glucose tolerance, whereas neuronal- or *Pomc*-specific loss of *TrpC5* failed to alter basal glucose tolerance (Figures S5 and S6). Despite the improved glucose tolerance, global *TrpC5* deficiency did not improve insulin tolerance,

suggesting that *Trpc5* subunits might ameliorate only specific components of the glucose-stimulated insulin response. Together, these data may suggest that inhibition of *TrpC5* subunits in the periphery (possibly including *TrpC5* subunits in adipose tissue) may improve glucose homeostasis; however, loss of *TrpC5* subunits in *Pomc* neurons might impair energy balance and blunt targeted improvements in glucose metabolism. Although these data support possible mechanisms for improved energy balance and glucose metabolism via *TrpC5* subunits, they also highlight the utility of cell-type targeted strategies to better identify roles for cellular signaling in energy balance and glucose metabolism.

Another salient finding is that *TrpC*5 subunits in *Pomc* neurons alter basal metabolism and feeding behavior. Several reports using chemo- and opto-genetic strategies have highlighted the role of acutely modulating melanocortin cellular activity and resulting changes in feeding behavior and body weight (Aponte et al., 2011; Zhan et al., 2013). However, identification of humoral signals linked to endogenous signaling cascades and channels that may acutely alter metabolism has remained undefined. Moreover, leptin's and serotonin's acute beneficial effects on metabolism are due at least in part to the acute activity of melanocortin neurons. In the current study we propose that activation of *TrpC5* in arcuate *Pomc* neurons, as occurs in response to *LepRs* or *Ht2Crs*, represents a physiological cellular correlate to the light- and/or chemical-induced activation of channelrhodopsins/designer receptors exclusively activated by designer drugs (DREADDs) in *Pomc* neurons previously demonstrated to stimulate a negative energy balance (Aponte et al., 2011; Zhan et al., 2013). Moreover, in addition to the pharmacological activity of leptin and *Ht2Cr* agonists, *Trpc*5 subunits contribute to a basal activity of melanocortin neurons that alters metabolic rate and feeding behavior.

*Ht2Crs* are known to regulate energy homeostasis and body weight. This regulatory impact is perhaps most notable due to the anti-obesity drug d-fenfluramine (d-Fen), which was identified to act via *Ht2Cr* (Vickers et al., 1999; Xu et al., 2008). However, the cellular mechanism required for *Ht2Cr*-expressing neurons to mediate effects on energy homeostasis remains unclear. This is topical due to the recent approval by the FDA of the anti-obesity drug, lorcaserin, which targets *Ht2Cr* for chronic weight management (Harlan et al., 2011; Martin et al., 2011). Additionally, other *Ht2Cr* agonists are also being tested in humans to treat obesity, further reinforcing a need for mechanistic insights on how these compounds exert their effects (Rodgers et al., 2012). Importantly, lorcaserin has also been associated with improvements of blood glucose levels in patients with type 2 diabetes (O'Neil et al., 2012). Notably, in the current study, lorcaserin-induced acute improvements in glucose and insulin tolerance were observed and required *TrpC*5 subunits in *Pomc* neurons. This observation suggests potential weight-independent effects of lorcaserin and *TrpC* channels on blood glucose levels, which should be studied further.

It should be noted that, in addition to classic neuropeptides (α-MSH [melanocyte-stimulating hormone], β-endorphin, neuropeptide Y [NPY], agouti related peptide [AgRP], etc.), there is an emerging role for the fast-acting neurotransmitters (glutamate and gamma-Aminobutyric acid [GABA]) in regulating energy balance and glucose metabolism(Krashes et al., 2014; Liu et al., 2012; Pinto et al., 2004; Tong et al., 2008; Yang et al., 2011). Arcuate *Pomc* neurons co-express glutamate (Collin et al., 2003; Kiss et al., 2005), independent of NPY/AgRP and to a variable extent GABA (Hentges et al., 2004, 2009; Horvath et al., 1997; Ovesjö et al., 2001; Wittmann et al., 2013; Yee et al., 2009). It is currently unclear how *TrpC5*-dependent activation of arcuate *Pomc* neurons might differentiate between the release of either neurotransmitters or neuropeptides. Similarly, recent work suggests a sensory component of Pomc or NPY/AgRP neuronal activity that is likely independent of endogenous peptidergic signals (Betley et al., 2015; Chen et al., 2015). Although currently unclear, involvement of pharmacological levels of leptin and serotonin 2C receptor agonists might act on these sensory cues influencing feeding behavior. Thus, the role of either neurotransmitters or neuropeptides in the *Pomc*-specific *TrpC5*-dependent regulation of metabolism warrants further investigation.

In summary, activation of arcuate *Pomc* neurons results in a negative energy balance (Aponte et al., 2011; Zhan et al., 2013). Numerous peptides and neurotransmitters have been demonstrated to acutely stimulate the activity of arcuate *Pomc* neurons, including leptin and serotonin. Although several signaling mechanisms have been identified, the channel required for these activities has remained largely undefined. In the current study, we demonstrate that *TrpC*5 subunits are essential for the negative energy balance associated with *Pomc* neuronal activation. *TrpC*5 subunits not only link the acute activities of leptin and serotonin receptors in Pomc neurons, but also modify direct effects on basal metabolism. Similar to opto- and chemo-genetic strategies, *TrpC*5 subunits may provide an endogenous target to manipulate the activity of key neurons involved in the regulation of energy balance and glucose metabolism.

## EXPERIMENTAL PROCEDURES

### Animals

Male (4- to 16-week-old) pathogen-free *CamkIIα*-cre, *Pomc*-creER$^{T2}$, *Pomc*-hrGFP::*LepR*-cre::tdtomato (PLT), *Pomc*-hrGFP::*Ht2Cr*-cre::tdtomato (P2CT), *TrpC5KO*, and *TrpC5$^{fl/y}$* (described below) mice (Berglund et al., 2013; Parton et al., 2007; Ramadori et al., 2010; Riccio et al., 2009; Sohn et al., 2011; Sun et al., 2016) were used for all experiments. All mice were housed under standard laboratory conditions (12 hr on/off; lights on at 7:00 a.m.) and temperature-controlled environment with food and water available ad libitum. All experiments were performed in accordance with the guidelines established by the NIH *Guide for the Care and Use of Laboratory Animals* and approved by the University of Texas Institutional Animal Care and Use Committee.

### Generation of a cre-Conditional TrpC5 Allele (*TrpC5$^{fl/y}$*)

The *TrpC5*-targeted clone obtained from EUCOMM was injected into blastocysts to obtain highly chimeric mice. These mice were then bred to obtain germline transmission of the targeted *TrpC5* allele. The *TrpC5$^{lox/Y}$* mice were generated following flp-mediated removal of the neo cassette, leaving two *lox*P sequences flanking exons 5 of the *TrpC5* gene. Deletion of exon 5 creates a frameshift mutation.

### Tamoxifen Treatment to Induce Adult-Onset Ablation of *TrpC5* in *Pomc* Neurons

Tamoxifen (0.15 mg/g; Sigma-Aldrich) dissolved in corn oil (Sigma-Aldrich) was administered i.p. for 5 consecutive days to 6-week-old male *TrpC5$^{fl/y}$*

mice (controls) and *TrpC5*$^{fl/y}$ × *Pomc*-cre*:ERT2* littermate mice. Corn oil was used as a vehicle control.

### Electrophysiology

Whole-cell patch-clamp recordings from *Pomc*-hrGFP neurons with or without leptin receptors were maintained in hypothalamic slice preparations, and data analysis was performed as previously described (Hill et al., 2008). Briefly, 4- to 16-week-old male mice were anesthetized and transcardially perfused with a modified ice-cold artificial CSF (ACSF) (described below), in which an equiosmolar amount of sucrose was substituted for NaCl. The mice were then decapitated, and the entire brain was removed and immediately submerged in ice-cold, carbogen-saturated (95% O2 and 5% CO2) ACSF (126 mM NaCl, 2.8 mM KCl, 1.2 mM MgCl2, 2.5 mM CaCl2, 1.25 mM NaH2PO4, 26 mM NaHCO3, and 5 mM glucose). Coronal sections (250 μm) were cut with a Leica VT1000S Vibratome and then incubated in oxygenated ACSF at room temperature for at least 1 hr before recording. Slices were transferred to the recording chamber and allowed to equilibrate for 10–20 min before recording. The slices were bathed in oxygenated ACSF (32°C–34°C) at a flow rate of ~2 mL/min.

The pipette solution for whole-cell recording was modified to include an intracellular dye (Alexa Fluor 594 or Alexa Fluor 350) for whole-cell recording: 120 mM K-gluconate, 10 mM KCl, 10 mM HEPES, 5 mM EGTA, 1 mM CaCl2, 1 mM MgCl2, 2 mM MgATP, and 0.03 mM Alexa Fluor 594 or Alexa Fluor 350 hydrazide dye (pH 7.3). Epifluorescence was briefly used to target fluorescent cells, at which time the light source was switched to infrared differential interference contrast imaging to obtain the whole-cell recording (Zeiss Axioskop FS2 Plus or Nikon FN1 equipped with a fixed stage and a QuantEM:512SC electron-multiplying charge-coupled device camera). Electrophysiological signals were recorded using an Axopatch 700B amplifier (Molecular Devices), low-pass-filtered at 2–5 kHz, and analyzed offline on a PC with pCLAMP programs (Molecular Devices). Recording electrodes had resistances of 2.5–5 MΩ when filled with the K-gluconate internal solution. Input resistance was assessed by measuring voltage deflection at the end of the response to a hyperpolarizing rectangular current pulse steps (500 ms of −10 to −50 pA).

Leptin (100 nM; provided by A.F. Parlow, through the National Hormone and Peptide Program), mCPP (4 μM; Sigma-Aldrich), and lorcaserin (4 μM; provided by Kathryn Cunningham) were added to the ACSF for specific experiments. Solutions containing leptin, mCPP, or lorcaserin were typically perfused for 2–4 min. A drug effect was required to be associated temporally with peptide application, and the response had to be stable within a few minutes. A neuron was considered depolarized or hyperpolarized if a change in membrane potential was at least 2 mV in amplitude.

### Analyses of Leptin-, mCPP-, and Lorcaserin-Induced Hypophagia

Leptin (5 mg/kg; provided by A.F. Parlow, through the National Hormone and Peptide Program), mCPP (3 mg/kg; Sigma-Aldrich), lorcaserin (1, 3, and 6 mg/kg; provided by Kathryn Cunningham), and vehicle (sterile saline) were administered i.p. in a counterbalanced manner to chow-fed 18 hr overnight-fasted mice as previously described (Berglund et al., 2013; Williams et al., 2014; Xu et al., 2010b). Food intake was measured hourly for 6 hr and then a single measurement at 24 hr.

### Analyses of Lorcaserin-Induced Alterations in Glucose Tolerance Test and Insulin Tolerance Test

For glucose tolerance tests (GTTs), mice were intraperitoneally injected with 1.5 g/kg body weight of dextrose after an overnight fast. Mice were injected with lorcaserin (1.5 mg/kg) or vehicle 45 min prior to glucose injection. We drew blood samples at 0, 15, 30, 60, 90, and 120 min time points and measured glucose levels with a Bayer contour glucometer.

For insulin tolerance tests (ITTs), mice were fasted for 3 hr with water ad libitum. Mice were injected with lorcaserin (1.5 mg/kg) or vehicle 45 min prior to insulin injection. After measurement of basal levels of glucose, insulin (1.2 U/kg; Eli Lilly and Company) was administrated i.p. Blood glucose levels were monitored at given time points after insulin injection.

### Statistics

Statistical analysis was carried out using GraphPad 5 (GraphPad) software. All data were evaluated using a two-tailed Student's t test or ANOVA where appropriate, with $p < 0.05$ being considered significant. In all instances, data are presented as mean ± SEM. Degrees of freedom (df) for *t* statistics are marked as $t_{(df)}$.

## SUPPLEMENTAL INFORMATION

Supplemental Information includes six figures and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.12.072.

## AUTHOR CONTRIBUTIONS

K.W.W. conceived and designed the study. Y.G., T.Y., Z.D., and J.-W.S. are co-first authors. Y.G. and J.-W.S. performed electrophysiological experiments, analyzed data, and wrote the manuscript. T.Y. and Z.D. designed and performed all experiments except electrophysiological experiments, analyzed the data, and wrote the manuscript. J.S. and Y.H. assisted performing experiments. X.K., K.Y., R.W., H.C., H.G., and J.Y. designed experiments and edited the manuscript. K.A.C. provided lorcaserin and edited the manuscript. Y.C., T.L., and K.W.W. are corresponding authors who supervised development of the mouse models, designed experiments, and edited the manuscript. K.W.W. is the lead contact of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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## REFERENCES

Al-Qassab, H., Smith, M.A., Irvine, E.E., Guillermet-Guibert, J., Claret, M., Choudhury, A.I., Selman, C., Piipari, K., Clements, M., Lingard, S., et al. (2009). Dominant role of the p110beta isoform of PI3K over p110alpha in energy homeostasis regulation by POMC and AgRP neurons. Cell Metab. *10*, 343–354.

Aponte, Y., Atasoy, D., and Sternson, S.M. (2011). AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. Nat. Neurosci. *14*, 351–355.

Balthasar, N., Coppari, R., McMinn, J., Liu, S.M., Lee, C.E., Tang, V., Kenny, C.D., McGovern, R.A., Chua, S.C., Jr., Elmquist, J.K., and Lowell, B.B. (2004). Leptin receptor signaling in POMC neurons is required for normal body weight homeostasis. Neuron *42*, 983–991.

Berglund, E.D., Vianna, C.R., Donato, J., Jr., Kim, M.H., Chuang, J.C., Lee, C.E., Lauzon, D.A., Lin, P., Brule, L.J., Scott, M.M., et al. (2012). Direct leptin action on POMC neurons regulates glucose homeostasis and hepatic insulin sensitivity in mice. J. Clin. Invest. *122*, 1000–1009.

Berglund, E.D., Liu, C., Sohn, J.W., Liu, T., Kim, M.H., Lee, C.E., Vianna, C.R., Williams, K.W., Xu, Y., and Elmquist, J.K. (2013). Serotonin 2C receptors in

pro-opiomelanocortin neurons regulate energy and glucose homeostasis. J. Clin. Invest. *123*, 5061–5070.

Betley, J.N., Xu, S., Cao, Z.F., Gong, R., Magnus, C.J., Yu, Y., and Sternson, S.M. (2015). Neurons for hunger and thirst transmit a negative-valence teaching signal. Nature *521*, 180–185.

Bjørbaek, C., and Kahn, B.B. (2004). Leptin signaling in the central nervous system and the periphery. Recent Prog. Horm. Res. *59*, 305–331.

Casanova, E., Fehsenfeld, S., Mantamadiotis, T., Lemberger, T., Greiner, E., Stewart, A.F., and Schütz, G. (2001). A CamKIIalpha iCre BAC allows brain-specific gene inactivation. Genesis *31*, 37–42.

Chen, Y., Lin, Y.C., Kuo, T.W., and Knight, Z.A. (2015). Sensory detection of food rapidly modulates arcuate feeding circuits. Cell *160*, 829–841.

Coll, A.P., Farooqi, I.S., and O'Rahilly, S. (2007). The hormonal control of food intake. Cell *129*, 251–262.

Collin, M., Bäckberg, M., Ovesjö, M.L., Fisone, G., Edwards, R.H., Fujiyama, F., and Meister, B. (2003). Plasma membrane and vesicular glutamate transporter mRNAs/proteins in hypothalamic neurons that regulate body weight. Eur. J. Neurosci. *18*, 1265–1278.

Elmquist, J.K., Elias, C.F., and Saper, C.B. (1999). From lesions to leptin: hypothalamic control of food intake and body weight. Neuron *22*, 221–232.

Flier, J.S. (2006). Neuroscience. Regulating energy balance: the substrate strikes back. Science *312*, 861–864.

Friedman, J.M. (2004). Modern science versus the stigma of obesity. Nat. Med. *10*, 563–569.

Giorgetti, M., and Tecott, L.H. (2004). Contributions of 5-HT(2C) receptors to multiple actions of central serotonin systems. Eur. J. Pharmacol. *488*, 1–9.

Harlan, S.M., Morgan, D.A., Agassandian, K., Guo, D.F., Cassell, M.D., Sigmund, C.D., Mark, A.L., and Rahmouni, K. (2011). Ablation of the leptin receptor in the hypothalamic arcuate nucleus abrogates leptin-induced sympathetic activation. Circ. Res. *108*, 808–812.

Heisler, L.K., Cowley, M.A., Kishi, T., Tecott, L.H., Fan, W., Low, M.J., Smart, J.L., Rubinstein, M., Tatro, J., Zigman, J.M., et al. (2003). Central serotonin and melanocortin pathways regulating energy homeostasis. Ann. N Y Acad. Sci. *994*, 169–174.

Hentges, S.T., Nishiyama, M., Overstreet, L.S., Stenzel-Poore, M., Williams, J.T., and Low, M.J. (2004). GABA release from proopiomelanocortin neurons. J. Neurosci. *24*, 1578–1583.

Hentges, S.T., Otero-Corchon, V., Pennock, R.L., King, C.M., and Low, M.J. (2009). Proopiomelanocortin expression in both GABA and glutamate neurons. J. Neurosci. *29*, 13684–13690.

Hill, J.W., Williams, K.W., Ye, C., Luo, J., Balthasar, N., Coppari, R., Cowley, M.A., Cantley, L.C., Lowell, B.B., and Elmquist, J.K. (2008). Acute effects of leptin require PI3K signaling in hypothalamic proopiomelanocortin neurons in mice. J. Clin. Invest. *118*, 1796–1805.

Hill, J.W., Xu, Y., Preitner, F., Fukuda, M., Cho, Y.R., Luo, J., Balthasar, N., Coppari, R., Cantley, L.C., Kahn, B.B., et al. (2009). Phosphatidyl inositol 3-kinase signaling in hypothalamic proopiomelanocortin neurons contributes to the regulation of glucose homeostasis. Endocrinology *150*, 4874–4882.

Horvath, T.L., Bechmann, I., Naftolin, F., Kalra, S.P., and Leranth, C. (1997). Heterogeneity in the neuropeptide Y-containing neurons of the rat arcuate nucleus: GABAergic and non-GABAergic subpopulations. Brain Res. *756*, 283–286.

Kiss, J., Csaba, Z., Csáki, A., and Halász, B. (2005). Glutamatergic innervation of neuropeptide Y and pro-opiomelanocortin-containing neurons in the hypothalamic arcuate nucleus of the rat. Eur. J. Neurosci. *21*, 2111–2119.

Krashes, M.J., Shah, B.P., Madara, J.C., Olson, D.P., Strochlic, D.E., Garfield, A.S., Vong, L., Pei, H., Watabe-Uchida, M., Uchida, N., et al. (2014). An excitatory paraventricular nucleus to AgRP neuron circuit that drives hunger. Nature *507*, 238–242.

Liu, T., Kong, D., Shah, B.P., Ye, C., Koda, S., Saunders, A., Ding, J.B., Yang, Z., Sabatini, B.L., and Lowell, B.B. (2012). Fasting activation of AgRP neurons requires NMDA receptors and involves spinogenesis and increased excitatory tone. Neuron *73*, 511–522.

Luquet, S., Phillips, C.T., and Palmiter, R.D. (2007). NPY/AgRP neurons are not essential for feeding responses to glucoprivation. Peptides *28*, 214–225.

Martin, C.K., Redman, L.M., Zhang, J., Sanchez, M., Anderson, C.M., Smith, S.R., and Ravussin, E. (2011). Lorcaserin, a 5-HT(2C) receptor agonist, reduces body weight by decreasing energy intake without influencing energy expenditure. J. Clin. Endocrinol. Metab. *96*, 837–845.

Niswender, K.D., Morton, G.J., Stearns, W.H., Rhodes, C.J., Myers, M.G., Jr., and Schwartz, M.W. (2001). Intracellular signalling. Key enzyme in leptin-induced anorexia. Nature *413*, 794–795.

O'Neil, P.M., Smith, S.R., Weissman, N.J., Fidler, M.C., Sanchez, M., Zhang, J., Raether, B., Anderson, C.M., and Shanahan, W.R. (2012). Randomized placebo-controlled clinical trial of lorcaserin for weight loss in type 2 diabetes mellitus: the BLOOM-DM study. Obesity (Silver Spring) *20*, 1426–1436.

Ovesjö, M.L., Gamstedt, M., Collin, M., and Meister, B. (2001). GABAergic nature of hypothalamic leptin target neurones in the ventromedial arcuate nucleus. J. Neuroendocrinol. *13*, 505–516.

Parton, L.E., Ye, C.P., Coppari, R., Enriori, P.J., Choi, B., Zhang, C.Y., Xu, C., Vianna, C.R., Balthasar, N., Lee, C.E., et al. (2007). Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. Nature *449*, 228–232.

Pinto, S., Roseberry, A.G., Liu, H., Diano, S., Shanabrough, M., Cai, X., Friedman, J.M., and Horvath, T.L. (2004). Rapid rewiring of arcuate nucleus feeding circuits by leptin. Science *304*, 110–115.

Qiu, J., Fang, Y., Rønnekleiv, O.K., and Kelly, M.J. (2010). Leptin excites proopiomelanocortin neurons via activation of TRPC channels. J. Neurosci. *30*, 1560–1565.

Ramadori, G., Fujikawa, T., Fukuda, M., Anderson, J., Morgan, D.A., Mostoslavsky, R., Stuart, R.C., Perello, M., Vianna, C.R., Nillni, E.A., et al. (2010). SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity. Cell Metab. *12*, 78–87.

Riccio, A., Li, Y., Moon, J., Kim, K.S., Smith, K.S., Rudolph, U., Gapon, S., Yao, G.L., Tsvetkov, E., Rodig, S.J., et al. (2009). Essential role for TRPC5 in amygdala function and fear-related behavior. Cell *137*, 761–772.

Rodgers, R.J., Tschöp, M.H., and Wilding, J.P. (2012). Anti-obesity drugs: past, present and future. Dis. Model. Mech. *5*, 621–626.

Sohn, J.W., Xu, Y., Jones, J.E., Wickman, K., Williams, K.W., and Elmquist, J.K. (2011). Serotonin 2C receptor activates a distinct population of arcuate pro-opiomelanocortin neurons via TRPC channels. Neuron *71*, 488–497.

Spiegelman, B.M., and Flier, J.S. (2001). Obesity and the regulation of energy balance. Cell *104*, 531–543.

Strübing, C., Krapivinsky, G., Krapivinsky, L., and Clapham, D.E. (2001). TRPC1 and TRPC5 form a novel cation channel in mammalian brain. Neuron *29*, 645–655.

Sun, J., Gao, Y., Yao, T., Huang, Y., He, Z., Kong, X., Yu, K.-J., Wang, R.-T., Guo, H., Yan, J., et al. (2016). Adiponectin potentiates the acute effects of leptin in arcuate Pomc neurons. Mol. Metab. *5*, 882–891.

Tong, Q., Ye, C.P., Jones, J.E., Elmquist, J.K., and Lowell, B.B. (2008). Synaptic release of GABA by AgRP neurons is required for normal regulation of energy balance. Nat. Neurosci. *11*, 998–1000.

Vickers, S.P., Clifton, P.G., Dourish, C.T., and Tecott, L.H. (1999). Reduced satiating effect of d-fenfluramine in serotonin 5-HT(2C) receptor mutant mice. Psychopharmacology (Berl.) *143*, 309–314.

Williams, K.W., Margatho, L.O., Lee, C.E., Choi, M., Lee, S., Scott, M.M., Elias, C.F., and Elmquist, J.K. (2010). Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. J. Neurosci. *30*, 2472–2479.

Williams, K.W., Liu, T., Kong, X., Fukuda, M., Deng, Y., Berglund, E.D., Deng, Z., Gao, Y., Liu, T., Sohn, J.W., et al. (2014). Xbp1s in Pomc neurons connects ER stress with energy balance and glucose homeostasis. Cell Metab. *20*, 471–482.

Wittmann, G., Hrabovszky, E., and Lechan, R.M. (2013). Distinct glutamatergic and GABAergic subsets of hypothalamic pro-opiomelanocortin neurons revealed by in situ hybridization in male rats and mice. J. Comp. Neurol. *521*, 3287–3302.

Xu, Y., Jones, J.E., Kohno, D., Williams, K.W., Lee, C.E., Choi, M.J., Anderson, J.G., Heisler, L.K., Zigman, J.M., Lowell, B.B., and Elmquist, J.K. (2008). 5-HT2CRs expressed by pro-opiomelanocortin neurons regulate energy homeostasis. Neuron *60*, 582–589.

Xu, Y., Berglund, E.D., Sohn, J.W., Holland, W.L., Chuang, J.C., Fukuda, M., Rossi, J., Williams, K.W., Jones, J.E., Zigman, J.M., et al. (2010a). 5-HT2CRs expressed by pro-opiomelanocortin neurons regulate insulin sensitivity in liver. Nat. Neurosci. *13*, 1457–1459.

Xu, Y., Jones, J.E., Lauzon, D.A., Anderson, J.G., Balthasar, N., Heisler, L.K., Zinn, A.R., Lowell, B.B., and Elmquist, J.K. (2010b). A serotonin and melanocortin circuit mediates D-fenfluramine anorexia. J. Neurosci. *30*, 14630–14634.

Yang, Y., Atasoy, D., Su, H.H., and Sternson, S.M. (2011). Hunger states switch a flip-flop memory circuit via a synaptic AMPK-dependent positive feedback loop. Cell *146*, 992–1003.

Yee, C.L., Wang, Y., Anderson, S., Ekker, M., and Rubenstein, J.L. (2009). Arcuate nucleus expression of NKX2.1 and DLX and lineages expressing these transcription factors in neuropeptide Y(+), proopiomelanocortin(+), and tyrosine hydroxylase(+) neurons in neonatal and adult mice. J. Comp. Neurol. *517*, 37–50.

Zeltser, L.M., Seeley, R.J., and Tschöp, M.H. (2012). Synaptic plasticity in neuronal circuits regulating energy balance. Nat. Neurosci. *15*, 1336–1342.

Zhan, C., Zhou, J., Feng, Q., Zhang, J.E., Lin, S., Bao, J., Wu, P., and Luo, M. (2013). Acute and long-term suppression of feeding behavior by POMC neurons in the brainstem and hypothalamus, respectively. J. Neurosci. *33*, 3624–3632.