# Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus

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**The hypothalamic arcuate nucleus (ARC) integrates and responds to satiety and hunger signals and forms the origins of the central neural response to perturbations in energy balance. Here we show that rat ARC neurons containing neuropeptide Y (NPY) and agouti-related protein (AgRP), which are conditional pacemakers, are activated by orexigens and inhibited by the anorexigen leptin. We propose a neuron-specific signaling mechanism through which central and peripheral signals engage the central neural anabolic drive.**



Central and peripheral signals involved in the maintenance of energy homeostasis, including leptin[1], insulin[2], ghrelin[3] and orexin[4], have been shown to modulate electrical activity of ARC neurons. It is poorly understood, however, how ARC neurons integrate and formulate appropriate output responses to these signals. In addition, the membrane mechanisms mediating these effects and the functional differentiation between neurons targeted by these signals (as suggested by the heterogeneity of projections and diversity of chemical phenotype[5]) remain to be determined.

We made electrophysiological recordings in brain slices from adult Wistar rats (**Supplementary Methods** online) to investigate how ARC neurons integrate central and peripheral signals indicating energy status. We identified a functionally and pharmacologically distinct subpopulation of ARC neurons, henceforth referred to as ARC pacemaker neurons. ARC pacemaker neurons were located in the medial ARC proximal to the third ventricle, an area known to contain NPY- and AgRP-expressing neurons that are activated by fasting. Morphologically these neurons are characterized by small, round cell bodies (diameter 13.4 ± 1.2 μm; $n = 8$) with 1–3 primary dendrites (**Fig. 1a**). Chemical phenotype was investigated using single-cell RT-PCR, which showed that these neurons express NPY and AgRP ($n = 3$; **Fig. 1b**). ARC pacemaker neurons showed a combination of several unique membrane properties compared to other ARC neurons, including anomalous inward rectification ($I_{AN}$; voltage-dependent potassium conductances activated at potentials more negative than roughly –80 mV) and a transient outward rectifying conductance ($I_{TR}$). $I_{TR}$ manifest as a delayed return to rest of the response to negative current injection (**Fig. 1c**). Functionally, $I_{TR}$ conductances are voltage-dependent potassium conductances that regulate neuronal firing frequency. The mean resting membrane potential and input resistance of these neurons was –48.3 ± 1.4 mV and 1,286 ± 116 MΩ, respectively ($n = 33$). Notably, and in contrast to other cell types in the ARC ($n = 88$), the orexigenic signals ghrelin (100–500 nM) and orexin (50–200 nM) induced characteristic pacemaker activity ($n = 15$). Pacemaker activity comprised of regular bursts of action potentials corresponding with underlying oscillations in membrane potential (**Fig. 1d**). Orexigen-induced pacemaker activity was sustained, persisting up to 30 min after exposure to the orexigen. The frequency and amplitude of orexigen-induced membrane potential oscillations underlying burst firing were 0.041 ± 0.002 Hz and 7.2 ± 1.8 mV ($n = 10$), respectively. This is within a range reported previously for the release of neuropeptides from hypothalamic neurons[6,7]. The membrane potential oscillations underlying pacemaker activity persisted in TTX ($n = 7$; **Fig. 1e,f**) suggesting mediation through intrinsic cellular mechanisms. Orexin and ghrelin applied to the same AP neurons revealed induction of membrane potential oscillations by both neuropeptides ($n = 3$; data not shown). In contrast to orexigenic signals, the anorectic hormone leptin (50 nM, 30 min) induced a slow, progressive membrane hyperpo-

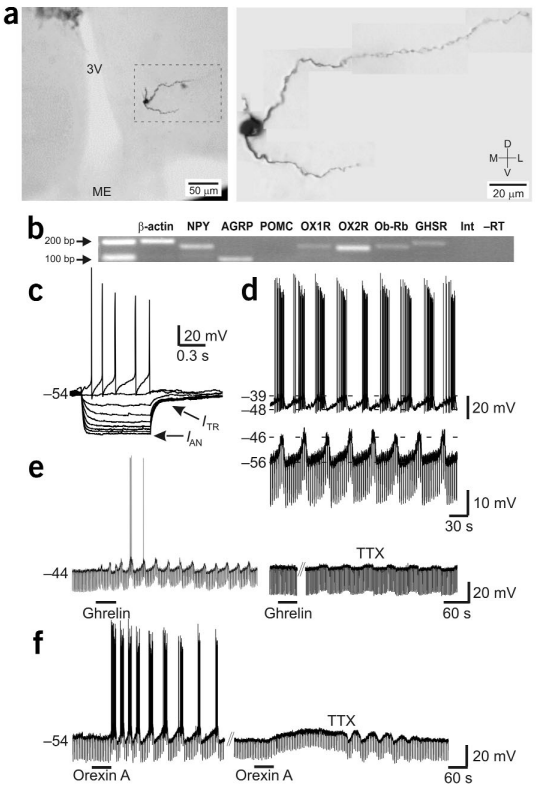


**Figure 1** Orexigen-sensitive pacemaker activity in ARC NPY/AgRP neurons. (**a**) Location (left) in the medial aspect of the arcuate nucleus, close to the third ventricle (3V) and morphology of ARC pacemaker neurons (ME, median eminence). (**b**) Using cytoplasmic contents from a single ARC pacemaker neuron, we detected mRNA encoding NPY, AgRP, orexin 1 receptor (OX1R), orexin 2 receptor (OX2R), leptin receptor (Ob-Rb), the growth hormone secretagogue receptor (GHSR) and β-actin. These neurons did not express mRNA for proopiomelanocortin (POMC) (**Supplementary Methods** and **Supplementary Table 1** online). (**c**) Characteristic electrophysiological properties of ARC pacemaker neurons included $I_{AN}$ and $I_{TR}$. (**d**) Pacemaker activity comprised bursts of action potentials (top) superimposed upon underlying oscillations in membrane potential (bottom). (**e**) Ghrelin-induced pacemaker activity in the absence and presence of TTX (1 μM). (**f**) Pacemaker activity induced by orexin in the absence and presence of TTX. All experiments were done in accordance with the guidelines of the Home Office and the University of Warwick Ethical Review Committee.

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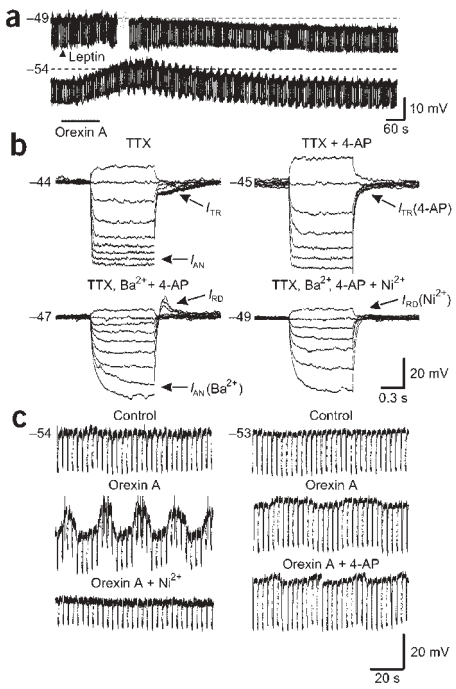


**Figure 2** Mechanisms underlying leptin-induced inhibition of and orexigen-induced activation of ARC pacemaker cells. (**a**) Continuous recording showing the slow inhibitory action of leptin on ARC pacemaker neurons and subsequent induction of pacemaker activity by orexin. (**b**) Current-voltage relations in the presence of TTX. AP neurons were characterized by $I_{AN}$, which is observed as an instantaneous fall in input resistance at negative membrane potentials, and $I_{TR}$, observed as a delayed return to resting membrane potential at the offset of the response to hyperpolarizing current injection (top left). $I_{TR}$ was blocked by 4-AP (1 mM, top right) and $I_{AN}$ was sensitive to $Ba^{2+}$ (100 μM, bottom left). The combination of these ion channel blockers revealed a rebound depolarization ($I_{RD}$) at the offset of the response to negative current injection (bottom left), which was blocked by $Ni^{2+}$ (200 μM, bottom right). (**c**) The role of these conductances in generating pacemaker activity was probed with $Ni^{2+}$ and 4-AP. Samples of a continuous recording showing that orexin-induced oscillations were abolished in the presence of $Ni^{2+}$ (left) and increased in frequency in the presence of 4-AP (right).

larization (–4.5 ± 2.2 mV) associated with a decrease in membrane resistance of 35.4 ± 6.9% and a cessation of all activity ($n$ = 3; **Fig. 2a**). This effect of leptin was similar to that previously reported for ARC neurons, through activation of ATP-sensitive potassium channels[1]. Subsequent application of orexin restored membrane potential oscillations and pacemaker activity (**Fig. 2a**). The expression of the ghrelin receptor (growth hormone secretagogue receptor (GHSR)), the long form of the leptin receptor (Ob-Rb), and orexin receptors (OX1R and OX2R) were confirmed with single-cell RT-PCR (**Fig. 1b**).

The mechanisms intrinsic to these cells, engaged by ghrelin and orexin and underlying the generation of pacemaker activity, were investigated further using potassium and calcium channel blockers. Whole-cell current-voltage ($I$-$V$) relationships obtained from ARC pacemaker neurons showed expression of a barium-sensitive (100 μM) $I_{AN}$ and a 4-aminopyridine-sensitive (4-AP; 1 mM) $I_{TR}$ ($n$ = 4; **Fig. 2b**). Pharmacological inhibition of $I_{TR}$ with 4-AP uncovered a rebound depolarization at the offset of the response to negative current injection, reminiscent of the nickel-sensitive, low-threshold, T-type calcium conductance reported in other central neurons and shown to be involved in generating pacemaker-like activity[8]. Similarly, in ARC pacemaker neurons, rebound depolarization was blocked by 100–200 μM nickel ($n$ = 3; **Fig. 2b**). The role of T-type calcium conductances and $I_{TR}$ in generating pacemaker activity was then investigated. Nickel (100–200 μM) abolished membrane potential oscillations underlying pacemaker activity in all neurons tested ($n$ = 3; **Fig. 2c**), suggesting this conductance is crucial for driving pacemaker activity. In TTX (1 μM), to eliminate indirect effects from other neurons, application of 1 mM 4-AP increased the frequency of oscillations from 34 ± 7 mHz in control to 52 ± 6 mHz ($n$ = 3; **Fig. 2c**), suggesting $I_{TR}$ regulates the frequency of bursts.

The principal orexigenic drive from the ARC originates from NPY/AgRP-expressing neurons[9], specific targets and sites of action of ghrelin and orexin[10–14] and the anorexigen leptin. Differential release of neuropeptides in the hypothalamus is crucial for the maintenance of energy homeostasis, and both ghrelin- and orexin-induced increases in food intake involve activation of NPY-dependent signaling. In the present study, we demonstrate the presence of NPY/AgRP pacemaker neurons in the ARC, in which burst firing is conditionally induced by orexigenic signals and suppressed by the anorexigen leptin. Pacemaker activity was driven by low-threshold calcium conductances, and the frequency was modulated by a transient outwardly rectifying potassium conductance. The unique response induced in these NPY/AgRP-expressing ARC pacemaker neurons to orexigenic signals and their sensitivity to the anorectic hormone leptin indicates a role for these neurons in pathways controlling energy balance. The importance of activity patterning for neuropeptide release from hypothalamic neurons is well established, with burst firing being a more effective stimulus for peptide release than fast, repetitive firing patterns of activity[15]. Taken together, these data suggest that burst firing and pacemaker activity in NPY/AgRP neurons, induced by orexigenic signals and suppressed by anorexigens, are key to the release of these peptides. Thus we propose a new neuron-specific cellular signaling pathway by which satiety and hunger signals engage the central neural anabolic drive.

*Note: Supplementary information is available on the Nature Neuroscience website.*

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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