Supporting Information

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SI Materials and Methods

GnRH-GFP, vGluT2-GFP, and GAD67-GFP Mice. Electrophysiological recordings were performed on GnRH, vGluT2, and GABAergic neurons in brain slices prepared from well-established lines of GnRH-GFP (kindly provided by Sue Moenter, University of Virginia) (1), vGluT2-GFP mice (2–5), and GAD67-GFP transgenic mice (kindly provided by K. Obata and Y. Yanagawa) (6, 7). MSDB cholinergic neurons were identified in murine brain slices in the living state, using the well-established fluorescent marker Cy3-labeled anti-NGFr (3–5 μl; 0.4 mg/ml), which was stereotaxically injected bilaterally into the lateral ventricle of anesthetized mice with a Hamilton syringe (22-gauge needle) at a rate of 0.5 μl/min (5, 8–10). Two to 5 days later, slices were prepared from fluorescently labeled mice for electrophysiological recordings. All experiments were carried out according to guidelines laid down by Yale University's animal welfare committee.

Slice Preparation and Electrophysiological Recordings. Male and female mice (2–23 weeks old) were anesthetized with chloral hydrate (400 mg/kg i.p.) and killed by decapitation. The ACSF (pH 7.35–7.38), equilibrated with 95%O_2-5% CO_2, contained the following (in mM): NaCl, 128; KCl, 3; NaH_2PO_4, 1.25; D-glucose, 10; $NaHCO_3$, 26; $CaCl_2$, 2 and $MgCl_2$, 2. After decapitation, brains were removed, placed in a Petri dish containing ACSF, and trimmed to yield a small block containing the MSDB. Coronal slices 300 μm in thickness were obtained with a Vibratome 1500 (Vibratome, St. Louis, MO) and transferred to a Plexiglas recording chamber (1.5 ml volume) on the fixed stage of an Olympus BX50WI scope for visualized whole-cell recording. The chamber was continuously perfused with normal ACSF at a rate of 2–3 ml/min and its temperature maintained at 33 ± 0.5 °C. One to 2 hours later the slice was used for recording. The effect of MCH was mostly tested in one cell per mouse with a maximum of three cells per mouse. The n values refer to the number of cells recorded; if more than one cell was recorded from a mouse in any experimental group, it is stated in Results.

Whole-cell current and voltage-clamp recordings were performed using previously described methods (11). The low-resistance (2.5–3.5 MΩ) patch pipettes were filled with a solution containing the following (in mM): K gluconate, 125; Hepes, 10; BAPTA K_4, 5; $CaCl_2$, 2.38; Mg-ATP, 4; Na phosphocreatine, 10 and Na_2-GTP, 0.3 (pH, 7.32–7.35). Data were acquired using an Axoclamp-2B and pClamp 9 (Axon Instruments, Foster City, CA). No correction was made for the calculated liquid junction potential of ≈11 mV for our internal solution.

Immunocytochemistry. Transgenic GnRH-GFP mice (n = 5) and vGluT2-GFP mice (n = 5) were given an overdose of anesthetic and were perfused transcardially with physiological saline followed by 4% paraformaldehyde. Sections 20-μm thick were cut on a cryostat in the coronal plane. After incubation in 1% normal goat serum, sections were incubated overnight in rabbit anti-MCH antibody (gift of Dr. W. Vale, Salk Institute), which is specific for MCH (12) at 1:3000. After washing, sections were incubated in a secondary antibody of goat anti-rabbit Ig conjugated to the red fluorophore, Alexa 594 at a dilution of 1:175 (Molecular Probes, Oregon). Sections were examined in an Olympus IX70 fluorescent microscope, and photomicrographs taken with a Spot camera (Diagnostic Imaging). Contrast and brightness were corrected in Adobe Photoshop; any correction was applied to the entire micrograph.

Drugs and Drug Application. MCH (Phoenix Pharmaceuticals, Burlingame, CA), kisspeptin (KiSS-1, metastin 45–54 amide, kisspeptin-10; KP-10, Sigma), and DHPG (Tocris Cookson) were diluted in ACSF from previously prepared stock solutions that were stored at −20 °C. Agonists were applied using a Y-tube (13). PMC-3881-PI, TTX (Alomone Labs, Jerusalem, Israel) and $BaCl_2$ were bath applied.

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