Supporting Information

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SI Materials and Methods

Solution Composition.

(*i*) Lysis buffer: 320 mM sucrose, 5 nM Hepes, 50 mM NaF, 1% SDS with Protease Inhibitor Mixture and Phosphatase Inhibitor Mixtures I and II diluted 1:100 (Sigma).

(*ii*) artificial cerebrospinal fluid (aCSF): 128 NaCl, 3 KCl, 1.25 NaH_2PO_4, 10 D-glucose, 24 $NaHCO_3$, 2 $CaCl_2$, and 2 $MgSO_4$ oxygenated with 95% O_2 and 5% CO_2 (pH 7.35, 295–305 mOsm). Sucrose aCSF was derived by fully replacing NaCl with 254 mM sucrose.

(*iii*) Pipette solution: For cell-attached, partial-access, and whole-cell recordings, patch pipettes (3–5 MΩ) were filled with an internal solution containing (in mM) 115 potassium gluconate, 20 KCl, 10 Hepes, 1.0 EGTA, 4 ATP-Mg, and 0.3 GTP (pH 7.2, 280–290 mOsm). For extracellular single-unit potential measurements, recording pipettes were filled with 2.0 NaCl.

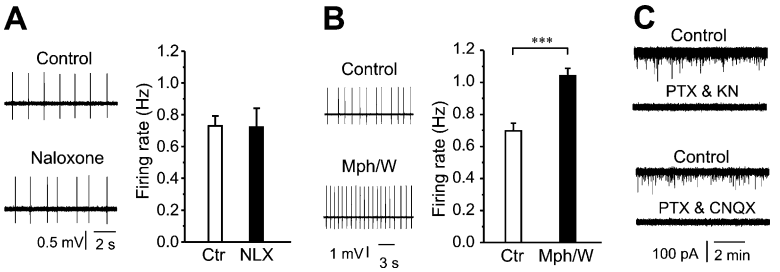

Fig. S1. (*A*) One micromolar naloxone itself had no effect on locus coeruleus (LC) firing rates in morphine-naïve slice cultures. Ctr, control; NLX, naloxone. (*B*) The 5-μM morphine-induced increase in LC firing was also observed in the absence of naloxone by washing morphine out of the slice for 90 min. Mph/W, morphine withdrawal. (*C*) Residual spontaneous synaptic activity mediated by $GABA_A$ and glutamate receptors was seen in our LC slice cultures. The residual activity was abolished completely by coapplication of the $GABA_A$ receptor antagonist picrotoxin (PTX; 100 μM) and the nonselective ionotropic glutamate receptor antagonist kynurenic acid (KN; 2 mM) or competitive AMPA/kainate receptor antagonist CNQX (10 μM) in all cells tested. Asterisks indicate statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, no significant difference).

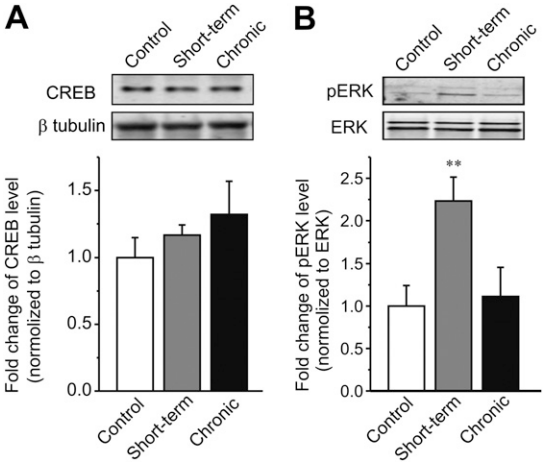

Fig. S2. (*A*) Neither short-term (30–60 min) nor chronic (2–3 d) morphine treatment of slice cultures significantly altered the total amount of cAMP response-element binding protein (CREB) protein in the LC. (*B*) Although short-term morphine treatment induced a transient increase in the level of phospho-ERK in the LC, this effect was absent when slice cultures were treated chronically with morphine. Asterisks indicate statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, no significant difference).

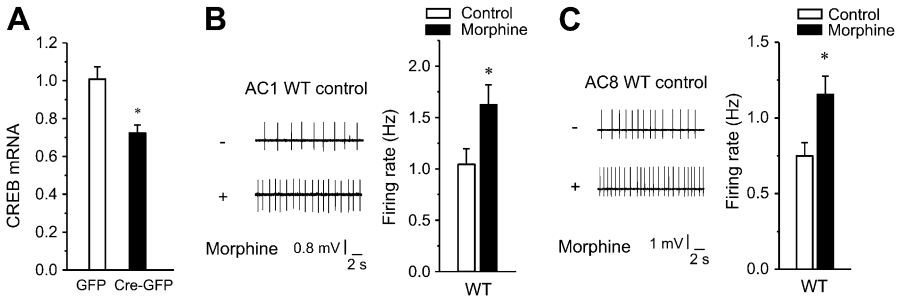

Fig. S3. (*A*) The level of CREB mRNA was decreased by overexpression of Cre-GFP in LC slice cultures obtained from floxed CREB mice. (*B*) Chronic (2–3 d) morphine treatment of slice cultures taken from the wild-type (WT) littermates of adenylyl cyclase 1 (AC1) knockout mice increased the firing rate of LC neurons. (*C*) Similar morphine-induced firing increase was seen in LC slice cultures obtained from wild-type littermates of AC8 knockout mice. Asterisks indicate statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, no significant difference).

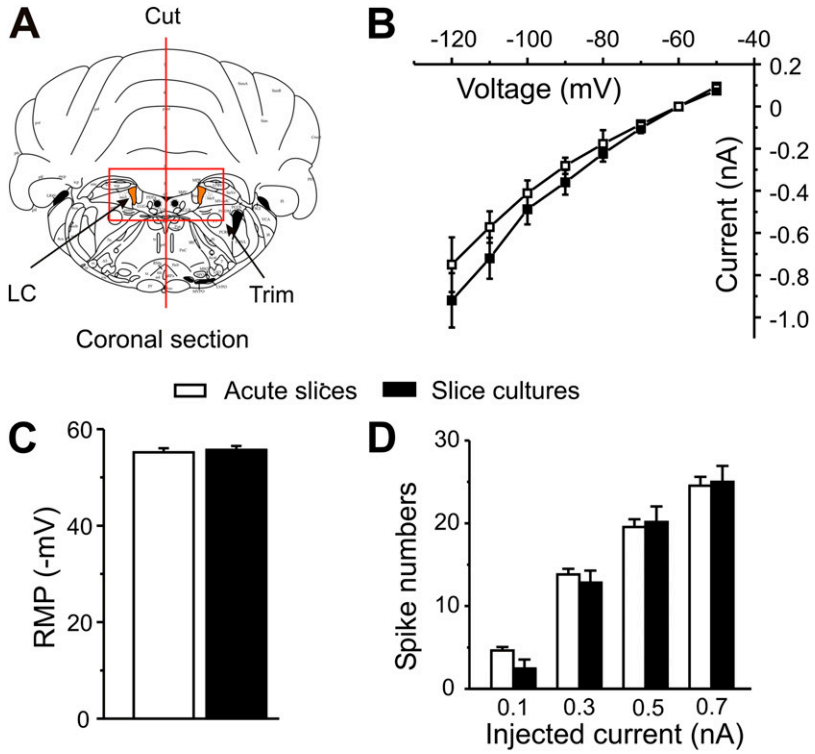

Fig. S4. (*A*) Coronal sections containing the LC were trimmed (red lines) by use of visible anatomical landmarks to obtain the more narrowly dissected sections used for the slice cultures. Such trimming improves slice health during chronic culturing. (*B–D*) The electrical properties of LC neurons in slice cultures were compared with those in acute brain slices. No significant differences were observed between these two conditions with respect to voltage-current relationship, resting membrane potential (RMP), and current injection-evoked spike numbers. Asterisks indicate statistically significant differences (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, no significant difference).