Supporting Information

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SI Materials and Methods

Immunohistochemistry and Microscopy. Mice were anesthetized with sodium pentobarbital (50 mg/kg) and perfused transcardially with cold PBS (0.1 M, pH 7.4). Mice were then perfused with 150 mL of 3% (wt/vol) paraformaldehyde and 0.15% glutaraldehyde in phosphate buffer (PB; 0.1 M, pH 7.4). Brains were postfixed for 1 h at 4 °C and then sectioned coronally (50 μm) on a Vibratome (VT1200; Leica). Alternate sections were then incubated in primary antibodies for orexin A (1:250 for fluorescence, 1:30 for EM; Santa Cruz Biotechnology) or prodynorphin (1:2,500 for fluorescence, 1:30 for EM; Millipore) and subsequently AlexaFluor- (1:200; Invitrogen) or gold-conjugated secondary (1:100; Electron Microscopy Sciences) antibodies for fluorescence or EM, respectively. Immunogold-labeled tissue was silver-enhanced according to previously described procedures to distinguish antigens. Reagents were purchased from Ted Pella and Electron Microscopy Sciences. Briefly, sections were postfixed for 1 h in 1% OsO_4, dehydrated in a series of ethanol solutions (50–100%) and propylene oxide, and embedded in Epon. Blocks from the lateral hypothalamus bounded by the zona incerta and optic tract were sectioned, grid-mounted, and lightly stained with lead citrate and uranyl acetate. Both immunolabeled and unlabeled sections were then examined and photographed on a JEOL 1200EX microscope.

Electrophysiology. At the beginning of all experiments, mice were briefly anesthetized with halothane and decapitated; brains were then rapidly extracted into ice-cold sucrose solution containing 75 mM sucrose, 87 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 7 mM $MgCl_2$, 0.95 mM $CaCl_2$, and 1–1.5 mM ascorbic acid. Horizontal slices containing ventral tegmental area (VTA) were cut at 290 μm on a Vibratome (VT1200; Leica). Slices were transferred to 250 mL of artificial cerebrospinal fluid (aCSF) containing 126 mM NaCl, mM 1.6 KCl, 1.1 mM NaH_2PO_4, 1.4 mM $MgCl_2$, mM 26 $NaHCO_3$, 11 mM glucose, and 2.4 mM $CaCl_2$, and were incubated for a minimum of 60 min at 31.5 ± 1 °C before recording. All solutions were continuously saturated with 95% O_2/5% CO_2 (vol/vol). Slices were placed in the recording chamber and perfused with 34 ± 1 °C aCSF with the addition of 100 μM picrotoxin. Cells were visualized on an upright microscope using "Dodt-type" gradient contrast IR optics. Whole-cell current-clamp recordings were made using a Multiclamp 700B amplifier (Molecular Devices). Series resistance was monitored by the peak amplitude of the capacitive transient induced by a step of −5 mV applied every 4 or 6 s. Neurons in which the series resistance was ≥30 MΩ and changed by 15% during the experiment were discarded from analyses.

Fluorometric Imaging Plate Reader Assay. Cells were incubated with an equal volume of calcium-4 loading buffer (Molecular Devices) containing 2.5 mM probenecid at 37 °C for 30 min, followed by addition of N-(2-methyl-6-benzoxazolyl)-N′-1,5-naphthyridin-4-yl urea (SB334867) or nor-binaltorphimine (norBNI) (dose range: 0.1 nM–100 μM) for another 30 min. The plates were then placed into a fluorometric imaging plate reader (Molecular Devices, Inc.) to monitor fluorescence ($\lambda_{excitation}$ = 488 nm, $\lambda_{emission}$ = 540 nm) before and after the addition of EC_{90} of orexin A peptide.

The 5-Choice Serial Reaction Time Task. The front wall of each chamber had five internally illuminated apertures fitted with IR detectors for recording nose-poke responses. The rear wall held a food receptacle and dispenser, and it was also illuminated and fitted with an IR detector. During a 90-trial session (~20 min), rats are given a food pellet (45 mg; Bio-Serv) for correctly nose-poking in one of the five apertures after it was briefly illuminated (0.5 s). Rats responding before cue presentation, responding during a 5-s intertrial interval (premature response), responding incorrectly (nose-poking nonilluminated apertures), or failing to respond within the allowed 5-s response period (omitted response) were subjected to a 5-s time-out period where the apparatus house light is extinguished and no food or light cues were presented. Once rats achieved a stable criterion of >70% correct responses, with no omissions, and <5% variability in premature responses on at least 4 consecutive days, they began drug testing experiments.

I.V. Cocaine Self-Administration. Catheters consisted of a 14-cm (rat) or 6-cm (mouse) length of Silastic tubing fitted to a guide cannula (Plastics One), bent at a curved right angle, and encased in dental acrylic. The catheter tubing was passed s.c. from an animal's back to the right jugular vein, and a 1-inch (rat) or 1-cm (mouse) length of the catheter tip was inserted into the vein. In intra-VTA infusion experiments, 14-mm stylets were inserted into the cannulae and four stainless-steel skull screws and dental acrylic held the cannulae in place. Only rats with injector tips verified to be located within the VTA were included in statistical analyses. Following 7 d of surgical recovery, mice and rats were mildly food restricted to maintain 85–90% of their free-feeding body weight and trained in an operant chamber (Med Associates) to press an "active" lever for food pellets (45- mg pellets for rats, 20-mg pellets for mice; TestDiet) under a fixed ratio 5 time-out 20 s (FR5TO20) reinforcement schedule. Rats and mice were also presented with an "inactive" lever during training and testing sessions, responses on which were recorded but were without scheduled consequences. Animals responded for food until the criterion was reached (>25 pellets per daily 1-h session for ~14 d). Subsequently, they were permitted to respond for cocaine infusions on the FR5TO20 schedule during 1-h daily sessions until stable levels of cocaine intake were established (<20% variation in the number of infusions earned per session for 3 consecutive days, 7–12 sessions required). Each cocaine infusion earned resulted in the delivery of 0.5 mg/kg of cocaine per infusion (rat) or 0.3 mg/kg of cocaine per infusion (mouse) dissolved in sterile saline (0.9% wt/vol) over 3 s and initiated a 20-s time-out period signaled by a light cue located above the lever. Responding on the active lever during the postinfusion time-out period was recorded but was without scheduled consequence. Catheter patency was tested by i.v. administration of Brevital (Eli Lilly) upon completion of the cocaine self-administration experiments.

Experimental procedure: Rats. Rats self-administered cocaine during 60-min daily sessions until stable intake was achieved, defined as <20% variation in responding for 3 consecutive days. We then examined the effects of intra-VTA infusions of SB334867 on cocaine intake in rats pretreated with saline according to a crossover design. On the first test day, rats received a saline injection (i.p.) and then received an intracranial injection of SB334867 (3 μg per side) or vehicle (50:50 sterile saline solution/DMSO vehicle) directly into the VTA 30 min before their daily cocaine self-administration session. All intra-VTA infusions were administered bilaterally in a volume of 0.5 μL per side over a 60-s period. We did not observe any deleterious behavioral effects from using a 50% (vol/vol) DMSO vehicle concentration after intracerebral injection. Rats then had two daily self-administra-

tion sessions between intracranial injection test days to ensure that rates of responding for cocaine returned to preinjection baseline. Next, we examined the effects of intra-VTA infusions of SB334867 on cocaine intake in rats pretreated with norBNI, again according to a cross-over design. Because norBNI induces long-lasting inhibition of kappa-opioid receptors, we permitted animals to self-administer cocaine for an additional three daily sessions to reestablish stable responding and then assessed the effects of intra-VTA injections of SB334867 on cocaine responding. The intra-VTA injection procedure, using a cross-over design, was then repeated exactly as described above.

Experimental procedure: Mice. After training on the cocaine self-administration procedure, mice received an i.p. injection of saline (0.9% wt/vol) 30 min before the next session and responding was evaluated for 2 additional days. Next, a single i.p. injection of norBNI (10 mg/kg) was administered to mice 30 min before the daily cocaine self-administration session commenced, and cocaine intake was recorded across five daily sessions.

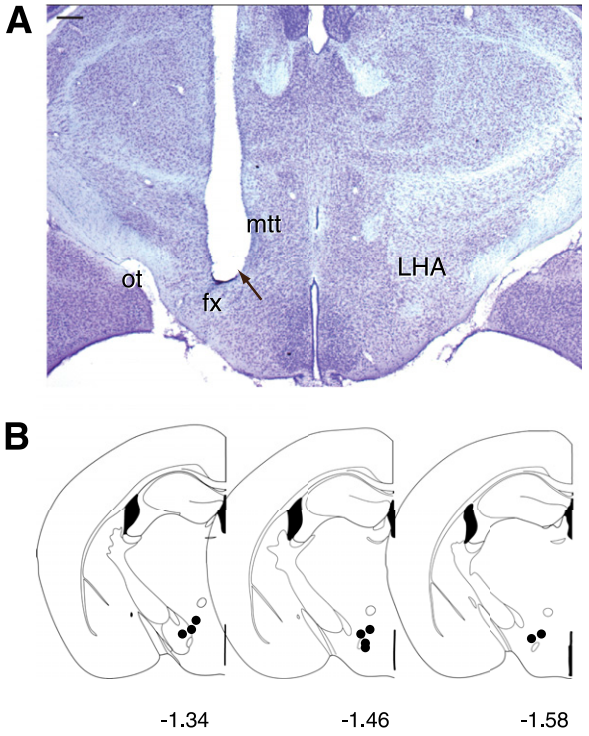

Fig. S1. (*A*) Representative micrograph demonstrates the location of the intracranial self-stimulation (ICSS) electrode in the hypothalamus (arrow). (Scale bar: 250 μm.) fx, fornix; LHA, lateral hypothalamic area; mtt, mammillothalamic tract; ot, optic tract. (*B*) Location of stimulating electrode tips in hypothalamus (●). Numbers beneath each coronal section are the distance from the bregma in millimeters.

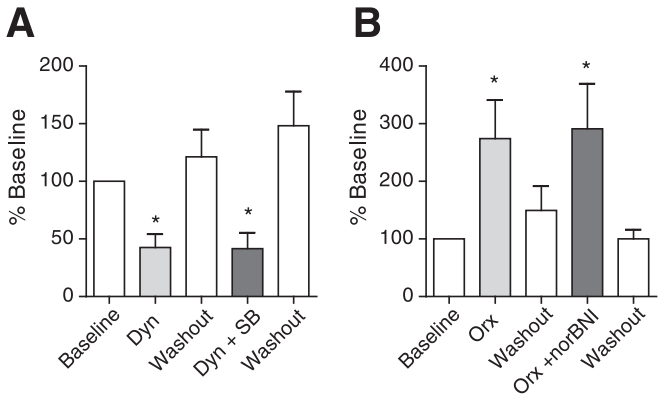

Fig. S2. No effect of coapplied peptides and antagonists. (*A*) Coapplication of dynorphin and SB334867 does not have additive effects in VTA dopamine neurons. *$P < 0.05$, difference from baseline. (*B*) Coapplication of orexin and norBNI does not have additive effects in VTA dopamine neurons. *$P < 0.05$, difference from baseline.

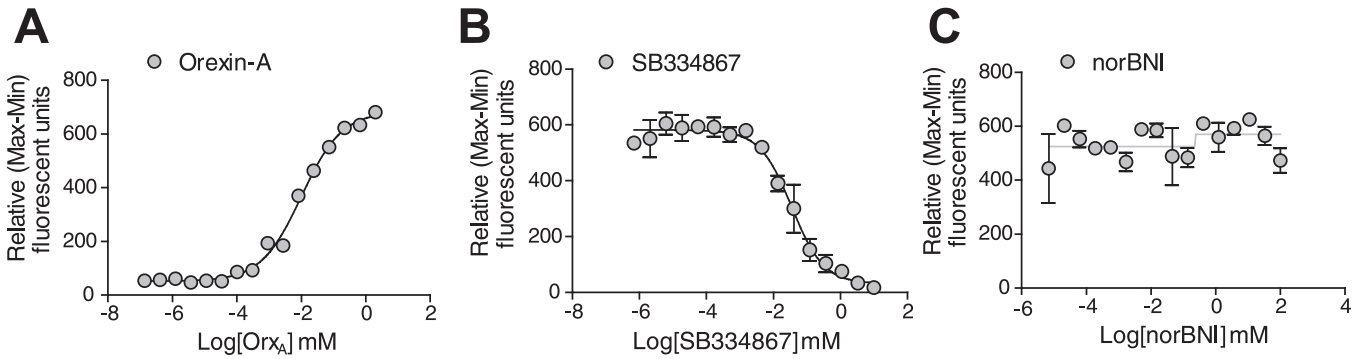

Fig. S3. norBNI shows no effect at the orexin-1 receptor (OX_1R). (*A*) Using a fluorometric imaging plate reader assay, bath-applied orexin shows the expected concentration-dependent response in intracellular Ca^{2+} release of OX_1R-expressing CHO cells. (*B*) This effect is blocked by increasing concentrations of SB334867. (*C*) Bath-applied norBNI produces no OX_1R-dependent Ca^{2+} response. Max, maximum; Min, minimum.