ONLINE METHODS:

Animals. Male wild-type (WT) and CB_1 receptor knockout ($CB_1^{-/-}$) mice with C57BL/6J genetic backgrounds were bred at the National Institute on Drug Abuse (NIDA) from three $CB_1^{+/-}$ breeding pairs[49] generously donated by Dr. Andreas Zimmer of the National Institute of Mental Health (Bethesda, MD, USA). $CB_2^{-/-}$ mice with C57BL/6J genetic backgrounds were bred at NIDA from three $CB_2^{+/-}$ breeding pairs[50] generously donated by Dr. George Kunos of the National Institute on Alcohol Abuse and Alcoholism (Rockville, MD, USA). Genotyping was performed by Charles River Laboratories before experiments were begun. About 50% of utilized mice were re-genotyped in our own laboratory for genotype confirmation after completion of experiments. All animals used in the present experiments were matched for age (8-14 weeks) and weight (25-35 grams). They were housed individually in a climate-controlled animal colony room on a reversed light-dark cycle (lights on at 7:00 PM, lights off at 7:00 AM) with free access to food and water. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Research Council, and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health.

Cocaine Self-Administration. *Surgery.* Mice were prepared for experimentation by surgical catheterization of the right external jugular vein. Catheterization was performed under an anesthetic mixture of ketamine (60 mg/kg, i.p.) and xylazine (12 mg/kg, i.p.) using aseptic surgical technique. A 6.0 cm length of MicroRenathane tubing (ID: 0.012";

OD: 0.025") (Braintree Scientific Inc., Braintree, MA, USA) was inserted 1.2 cm into the right jugular vein and anchored to a 24-gauge steel cannula (Plastics One, Roanoke, VA, USA) that was bent at a right angle and mounted to the skull with cyanoacrylate glue and dental acrylic. A 2.5 cm extension of flexible tubing was connected to the distal end of the cannula. For intracranial microinjections, two guide cannula (MAB 4.15.IC, SciPro Inc., Sanborn, NY, USA) were surgically implanted into the NAc (AP +1.4 mm, ML ±1.5 mm, DV -3.8 mm with an angle of 8º from vertical) in separate groups of mice. To keep the implanted catheters patent, they were flushed daily with a 0.05 ml saline solution containing 20 IU/ml heparin and 0.33 mg/ml gentamycin. To avoid cocaine overdose, each animal was limited to a maximum of 30 cocaine injections per 3 hr session.

Apparatus. Intravenous cocaine self-administration experiments were conducted in operant response test chambers (Model ENV-307A, Med Associates, Georgia, VT, USA). Each test chamber had 2 levers located 2.5 cm above the floor, 1 active and 1 inactive. A cue light and a speaker were located 5 cm above the active lever. A house chamber light was on during each 3 h session. Depression of the active lever activated the infusion pump; depression of the inactive lever was counted but had no consequence. Each drug infusion was paired with conditioned cue-light and a cue-sound (tone).

Procedure. After recovery from surgery, each mouse was placed into a test chamber and allowed to lever-press for i.v. cocaine (1 mg/kg/infusion) delivered in 0.015 ml over 4.2 sec, on an FR1 reinforcement schedule. During the initial 3-5 days, all animals received 5 free cocaine infusions within a 10-min time interval to prime the animal for drug-seeking and drug-taking behavior. These 5 free drug infusions were subtracted from the total number of drug infusions for data analysis. During the 4.2 sec

injection period, additional responses on the active lever were recorded but did not lead to additional infusions. Each session lasted 3 h. After 1-2 weeks of cocaine self-administration, the cocaine dose was switched from 1 mg/kg/infusion to 0.5 mg/kg/infusion for an additional 1-2 weeks of cocaine self-administration until stable day-to-day self-administration was established, which was defined as ≥ 20 cocaine infusions per session with a steady self-administration pattern for at least 3 consecutive days. Then, subjects randomly received one dose of JWH133 (10, 20 mg/kg, i.p.), GW405833 (3, 10 mg/kg, i.p.), AM630 (10 mg/kg, i.p.) or vehicle (Tocrisolve 100) 30 min prior to cocaine self-administration. After each test, animals received an additional 5-7 days of cocaine self-administration until baseline response rate was re-established prior to testing the next dose of drug.

Intranasal or intra-NAc microinjections. To determine whether the effect of JWH133 on cocaine self-administration was induced by activation of brain or peripheral CB_2 receptors, we tested the effects of intranasal (50, 100 μg/10 μl/nostril) or intra-NAc (0.3, 1, 3 μg/1 μl/side) microinjection of JWH133 on cocaine self-administration. Intranasal drug administration was performed under inhalant isoflurane anesthesia using the Fluovac System (Harvard Apparatus, Holliston, MA, USA). To determine whether the effects of intranasal JWH133 on cocaine self-administration might be mediated by absorption into the nasal vasculature with subsequent venous delivery to peripheral sites of action, the same micro-quantities of JWH133 as used intranasally (100, 200 μg) were injected intravenously in a separate experimental session via the implanted jugular catheter 30 min before cocaine self-administration.

Progressive-ratio (PR) cocaine self-administration. Additional groups of animals were initially trained under FR1 reinforcement as outlined above. After stable cocaine self-administration under FR1 reinforcement was established, animals were switched to PR reinforcement, under which the work requirement (lever presses) to receive a cocaine infusion was progressively raised within each test session[22]. The PR break-point was defined as the maximal number of lever presses completed for the last cocaine infusion prior to a 1 h period during which no infusions were obtained by the animal. Animals were allowed to continue daily sessions of cocaine self-administration under PR reinforcement conditions until day-to-day variability in break-point fell within 1-2 ratio increments for 3 consecutive days. Once a stable break-point was established, subjects randomly received one dose of JWH133 (10, 20 mg/kg, i.p.), vehicle (Tocrisolve 100), or AM251 (3 mg/kg, i.p.) 30 min prior to PR cocaine self-administration testing.

JWH133 self-administration in mice formerly self-administering cocaine. After stable cocaine self-administration under FR1 reinforcement was established for at least 3 consecutive days, the animals were divided into 2 groups and cocaine was replaced by JWH133 (1 mg/kg/infusion) or by vehicle (Tocrisolve-100) for 5 days. Since animals might take several days to acquire self-administration for a novel reinforcer, each replacement test was repeated for 5 days.

Conditioned place preference or aversion. Four groups of WT mice (n=12 each group) were used to study cocaine (10, 20 mg/kg) or JWH133 (10, 20 mg/kg)-induced conditioned place preference (CPP) or aversion (CPA). A three-chamber place preference apparatus (Med Associates, Georgia, VT, USA) was used in this study. The apparatus

consisted of two large compartments (16.8 × 12.7 × 12.7 cm^3), and one small compartment (7.2 × 12.7 × 12.7 cm^3) which separated the large compartments. The two large compartments had different visual and tactile cues. One compartment was black with a stainless steel rod floor. The other compartment was white with a stainless steel mesh floor. The small compartment was gray with a smooth polyvinyl chloride floor. The apparatus had a clear Plexiglas top with a light on it. During the preconditioning phase (days 1–2), mice were placed in the small compartment and were allowed to freely explore the three compartments for 15 min daily. The time spent in each compartment was recorded. We used an unbiased CPP procedure. Mice spending over 500 s in either compartment were excluded. The next 10 days (days 3–12) constituted the conditioning phase, with one session per day. Each mouse received an i.p. injection of the same dose of test drug (cocaine or JWH133) on days 3, 5, 7, 9, 11, and was then confined in one large compartment for 30 min. On days 4, 6, 8, 10, 12, each mouse received an i.p. injection of vehicle (saline or Tocrisolve-100) and was then confined in the other large compartment for 30 min. Test drug was paired with either white or black compartment in a counterbalanced manner. On test day (24 hrs after the last injection), mice were allowed to freely explore the three compartments for 15 min without injections, and the time spent in each compartment was recorded. All behavioral testing was performed during the light phase of the light/dark cycle.

Locomotor activity. Before drug administration, each animal was placed in a locomotor detection chamber (Accuscan Instruments, Inc., Columbus, OH, USA) for 3 days (4 h per day) for environmental habituation. On each test day, mice were placed in the chamber

for 1 h of habituation, and then removed and given either saline or one dose (10, 20 mg/kg i.p., 30 min prior to cocaine) of JWH133. Animals were then placed back into the locomotor chambers to observe the effects of JWH133 alone on locomotion for 3 h. Additional groups of animals were used to study the effects of JWH133 pretreatment on cocaine-enhanced locomotion. Cocaine (10 mg/kg, i.p.) was given 30 min after JWH133 administration. Then, animals were placed back into the locomotor chambers to monitor locomotor activity for 3 h. To determine whether intracranial microinjections of CB_2 ligands produce similar effects as systemic administration, two separate groups of mice with intracranial guide cannula implantation received intra-NAc local administration of JWH133 (1, 3 μg/side) or AM630 (1, 3, 10 μg/side), a mixture of JWH133 and AM630 (3 μg each per side), or vehicle (Tocrisolve-100). After each test, animals received an additional 3-5 days of locomotor habituation prior to testing the next dose of drug. The order of testing for the various doses of the drugs was counterbalanced. Data were collected in 10 min intervals using the VersaMax data analysis system (Accuscan Instruments, Inc., Columbus, OH, USA). Total distance was used to evaluate the effects of different treatments on locomotor behavior.

Rotarod performance. A four-station mouse rotarod device (AccuScan Instruments Inc., Columbus, OH, USA) was used to study the effects of JWH133 on operant locomotor performance. The speed of rotation of the rotarod was increased from 2.5 to 20 rpm over 2 min and the time (sec) the animal remained on the rod was determined as the mean of three trials. After 5-7 days of habituation and training on the rotarod device, animals randomly received either vehicle or one dose (10, 20 mg/kg, i.p.) of JWH133 before

rotarod testing began. After the drug injection, animals were placed on the rotarod device and their locomotor performance was assessed every 30 min for 3 h. After each test, animals then received 3-5 days of additional rotarod habituation until baseline response was re-stabilized prior to testing the next dose of drug. The various drug doses were given randomly and counterbalanced.

***In vivo* microdialysis.** All mice were surgically implanted with intracranial guide cannulae (MAB 4.15.IC, SciPro Inc., Sanborn, NY, USA) into the NAc. The surgery was performed under an anesthetic mixture of ketamine hydrochloride (80 mg/ml) and xylazine hydrochloride (12 mg/ml), using standard aseptic surgical and stereotaxic technique. The stereotaxic coordinates for the NAc were AP +1.4 mm, ML ±1.5 mm, DV -3.8 mm with an angle of 8º from vertical. The guide cannulae were fixed to the skull with dental acrylic. *In vivo* brain microdialysis was performed at least 7 days after surgery. The procedures of the microdialysis and the subsequent dialysate DA quantification were identical to those we have reported previously[24].

Drugs. Cocaine HCl (Sigma-Aldrich., Saint Louis, MO, USA) was dissolved in physiological saline. JWH133, AM251 and AM630 were obtained from Tocris Bioscience (Ellisville, MO, USA). They were dissolved in Tocrisolve-100 (vehicle).

Data analyses. All data are presented as means (± s.e.m.). One-way analysis of variance (ANOVA) was used to analyze the effects of JWH133 or other drugs on cocaine self-administration. Two-way ANOVA for repeated measures over time was used to analyze

the effects of JWH133 on locomotion or extracellular DA. Individual group comparisons were carried out using the Student-Newman-Keuls or Bonferroni methods.