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Brain Cannabinoid CB_2 Receptors Modulate Cocaine's Actions in Mice

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Abstract

The presence and function of cannabinoid CB_2 receptors in the brain have been subject to debate. We report here that systemic, intranasal or intra-accumbens local administration of JWH133, a selective CB_2 receptor agonist, dose-dependently inhibits intravenous cocaine self-administration, cocaine-enhanced locomotion, and cocaine-enhanced accumbens dopamine (DA) in wild-type (WT) and CB_1 receptor-knockout ($CB_1^{-/-}$), but not $CB_2^{-/-}$, mice. This inhibition is mimicked by GW405833, another CB_2 receptor agonist with a different chemical structure, and is blocked by AM630, a selective CB_2 receptor antagonist. Intra-accumbens JWH133 alone dose-dependently decreases, while intra-accumbens AM630 elevates, extracellular DA and locomotion in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice. Intra-accumbens AM630 also blocks the reduction in cocaine self-administration and extracellular DA produced by systemic administration of JWH133. These findings, for the first time, suggest that brain CB_2 receptors modulate cocaine's rewarding and locomotor-stimulating effects, likely by a DA-dependent mechanism.

Keywords

Cannabinoid; CB_2 receptors; JWH133; cocaine; dopamine; self-administration

Behavioral and psychoactive effects of cannabinoids are mediated by activation of brain cannabinoid receptors[1, 2]. Two major cannabinoid receptors (CB_1 and CB_2) have been identified. Since CB_1 receptors are highly expressed in the brain[2, 3] and CB_2 receptors are

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The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

Z.-X.X. developed the original research proposal, designed and supervised all experiments, analyzed all data and wrote the manuscript. X.-Q.P., X.L. and R.S. conducted the cocaine self-administration experiments. X.L., G.-H.B and H.-Y.Z. conducted the *in vivo* microdialysis experiments. X.L., H.-J.Y, R.S. and J.L. conducted the locomotor behavioral experiments. X.-Q.P, R.S. and H.-J.Y conducted the conditioned place preference/aversion experiments. Q-R.L. contributed to the original research proposal. E.L.G. contributed to the original conceptualization of this work and was responsible for overall supervision of the research and for revisions and modifications to the manuscript.

found primarily in the periphery[4, 5], it has been heretofore generally believed that the behavioral and psychotropic effects of cannabinoids are CB_1-mediated[1, 2] and that CB_2 receptor ligands have no psychoactive effects[6]. However, the purported lack of brain CB_2 receptors has been challenged by recent reports of low densities of CB_2 receptors on microglia[7] and neuronal[8–11] cells in several brain regions - including the anterior olfactory nucleus, cerebral cortex, cerebellum, hippocampus, striatum and brainstem. Further, activation of CB_2 receptors by 2-arachidonoylglycerol, JWH015 or JWH133 inhibits locomotion[10, 11], morphine-6-glucuronide-induced emesis[11] and neuropathic pain[12, 13], while stimulating neural progenitor proliferation[14] and producing neuroprotective effects[15, 16]. More recent studies suggest that CB_2 receptor activation inhibits neuronal firing in dorsal-root ganglia and spinal cord[17, 18] and GABAergic transmission in rat cerebral cortex[19]. These data suggest that functional CB_2 receptors may be expressed on central nervous system neuronal cells, prompting us to re-examine the role of CB_2 receptors in drug reward and addiction. To this end, we here used highly selective CB_2 receptor agonists and antagonists, combined with specific CB_1 receptor-knockout ($CB_1^{-/-}$) and CB_2 receptor-knockout ($CB_2^{-/-}$) mice, to investigate possible involvement of brain CB_2 receptors in cocaine's behavioral and neurochemical effects.

RESULTS

JWH133 inhibits intravenous cocaine self-administration

To determine whether CB_2 receptor activation alters intravenous cocaine self-administration, we used JWH133, a highly selective CB_2 receptor agonist (200-fold selectivity for CB_2 *versus* CB_1)[20, 21], and AM630, a highly selective CB_2 receptor antagonist (160-fold selectivity for CB_2 *versus* CB_1)[20, 21], as pharmacological tools. We found that over 50% of wild-type (WT) (20 of 34) and $CB_2^{-/-}$ (22 of 36) mice, while only about 30% of $CB_1^{-/-}$ (10 of 36) mice acquired stable intravenous cocaine self-administration, defined as 20 or more infusions per 3-h session, with a regular pattern of self-administration achieved after 10 days of training (Supplementary Fig. 1). Strikingly, $CB_1^{-/-}$ mice displayed a significant reduction in both total number and rate (infusions per h) of cocaine infusions on days 1–5, compared to WT or $CB_2^{-/-}$ mice (Supplementary Fig. 1a, b). In addition, the majority of $CB_1^{-/-}$ mice (7 of 10) displayed a distinct "burst-like" drug-taking pattern with long inter-burst intervals, while WT and $CB_2^{-/-}$ mice displayed evenly-paced drug-taking without significant difference between the two strains (Supplementary Fig. 1c). These findings suggest that deletion of CB_1 receptors may lower cocaine's rewarding efficacy, leading to a compensatory increase in drug intake during each individual drug-taking episode. This is further supported by the finding that $CB_1^{-/-}$ mice displayed a significant reduction in break-point level for cocaine self-administration under progressive-ratio (PR) reinforcement, compared to WT mice (Supplementary Fig. 1d). Since PR break-point, defined as maximal work performed by the animal to get a cocaine infusion, is cocaine dose-dependent and positively correlated to reward strength[22], the reduction in PR break-point observed in $CB_1^{-/-}$ mice suggests a reduction in cocaine's reward strength and/or motivation for cocaine-taking behavior. This is consistent with previous findings that CB_1 receptor deletion impairs cocaine's rewarding, locomotor-stimulating, and DA-elevating effects[23, 24].

Intraperitoneal (i.p.) administration of JWH133 (10, 20 mg/kg) produced a significant and dose-dependent reduction in cocaine self-administration and cocaine intake in both WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice (Fig. 1a). This inhibition lasted for no longer than 24 hrs after 20 mg/kg JWH133 (Fig. 1b, c). Pretreatment with AM630, a selective CB_2 receptor antagonist, but not with AM251, a selective CB_1 receptor antagonist[25], significantly attenuated JWH133-induced inhibition of cocaine self-administration (Fig. 1d). This suggests that JWH133's attenuating effect is mediated by activation of CB_2, not CB_1, receptors. This conclusion is further supported by the additional finding that systemic administration of GW405833 (3, 10 mg/kg, i.p.), another highly selective but structurally distinct CB_2 receptor agonist[26], also inhibited cocaine self-administration in WT mice (Fig. 2a).

To determine whether JWH133-induced attenuation of cocaine self-administration was due to a reduction in cocaine's rewarding efficacy, we studied JWH133's effect on i.v. cocaine self-administration under PR reinforcement. We found that systemic administration of JWH133 (10, 20 mg/kg, i.p.) significantly lowered the PR break-point for cocaine self-administration in WT mice (Fig. 2b), suggesting a reduction in cocaine's reward strength and/or motivation for drug-taking behavior after JWH133 administration. We previously showed that CB_1 receptor blockade by AM251 significantly lowered the PR break-point for cocaine self-administration in rats[27]. We therefore also tested AM251 in the present study, and found that AM251 (3 mg/kg) lowered the PR break-point for cocaine self-administration in WT mice (Fig. 2b). These data suggest that the JWH133-induced reduction in cocaine self-administration resulted from a reduction in cocaine's rewarding efficacy.

JWH133 inhibits cocaine self-administration by activation of brain CB_2 receptors

To further determine whether JWH133's action was mediated by activation of brain or peripheral CB_2 receptors, we first studied the effects of intranasal microinjections of JWH133 on i.v. cocaine self-administration. Extensive studies have shown that a wide variety of compounds that cannot penetrate the blood-brain barrier can be delivered directly from nose into brain[28]. We found that intranasal microinjections of JWH133 (50, 100 μg/10 μl/side) dose-dependently inhibited i.v. cocaine self-administration (Fig. 2c). To explore the possibility that effects of intranasal JWH133 might be mediated by drug absorption into the nasal vasculature with subsequent venous delivery of drug to pharmacological site(s) of action, we observed the effects of i.v. injection of the same micro-quantities of JWH133 as used intranasally on cocaine self-administration. We found that i.v. microinjections of JWH133 (100, 200 μg) had no effect on cocaine self-administration (Fig. 2d). These data suggest that intranasal JWH133-induced pharmacological effects are mediated by activating brain rather than peripheral CB_2 receptors. To further explore this issue, we observed the effects of local administration of JWH133 into the nucleus accumbens (NAc) on cocaine self-administration. We found that intra-NAc microinjections of JWH133 (0.3, 1, 3 μg/side) significantly and dose-dependently inhibited cocaine self-administration in WT mice (Fig. 2e), but not in $CB_2^{-/-}$ mice (Fig. 2f). This inhibition was blocked by intra-NAc co-administration of AM630 (3 μg/side).

JWH133 itself has no reinforcing or aversive effects

We further examined whether JWH133 itself has cocaine-like rewarding effects. To address this issue, we first trained mice to acquire stable cocaine self-administration, and then cocaine was replaced by JWH133 (1 mg/kg/infusion) or vehicle. We found that neither JWH133 nor vehicle sustained stable self-administration in mice previously trained for cocaine self-administration (Supplementary Fig. 2a). In fact, the self-administration behavior underwent gradual extinction over the 5 days of substitution testing. This extinction pattern was essentially identical to that seen when vehicle was substituted for cocaine. However, when JWH133 or vehicle was replaced by cocaine, self-administration behavior returned to levels previously observed during stable cocaine self-administration. In addition, we also found that cocaine (10, 20 mg/kg, i.p.) produced a significant conditioned place preference, while JWH133, at the same doses, produced neither conditioned place preference nor place aversion in WT mice (Supplementary Fig. 2b). These findings suggest that JWH133 has no cocaine-like reinforcing nor aversive effects in mice.

JWH133 inhibits cocaine-enhanced locomotion

To determine whether JWH133's effect on cocaine self-administration generalizes to other cocaine actions, we investigated the effects of JWH133 on cocaine-enhanced locomotion. Systemic administration of 10 mg/kg cocaine produced a significant increase in locomotion in all 3 mouse strains (Fig. 3). Pretreatment with JWH133 (10, 20 mg/kg, 30 min prior to cocaine) dose-dependently attenuated cocaine-enhanced locomotion in WT (Fig. 3a) and $CB_1^{-/-}$ (Fig. 3b) mice, but not in $CB_2^{-/-}$ (Fig. 3c) mice. Systemic administration of the same doses of JWH133 alone also significantly inhibited locomotion in a dose-dependent manner in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice (Fig. 4a), suggesting an effect mediated by activation of CB_2 receptors. Since the same doses of JWH133 alone failed to alter locomotor performance on a fast-running rotarod device in all 3 mouse strains (Supplementary Fig. 3), we infer that JWH133's inhibition of cocaine self-administration or locomotion is not produced by nonspecific impairment of locomotor capacity.

To further determine whether such locomotor inhibition is mediated by activation of brain CB_2 receptors, we observed the effects of intra-NAc JWH133 and/or AM630 on locomotion. We found that intra-NAc microinjections of JWH133 (1, 3 μg/side) significantly inhibited locomotion in WT or $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice (Fig. 4b), in a dose-dependent manner similar to systemic administration (Fig. 4a). We note that systemic administration of AM630 failed to alter locomotion in any mouse strain tested (Fig. 4c). However, when locally administered into the NAc, AM630 (1, 3, 10 μg/side) significantly increased locomotor activity (Fig. 4d) in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice. These data suggest that CB_2 receptors may tonically modulate locomotion. A higher brain AM630 level, achieved by local rather than by systemic administration, appears to be required to block endocannabinoid action on brain CB_2 receptors.

JWH133 inhibits cocaine-enhanced extracellular DA in the nucleus accumbens

Given the crucial role of the mesolimbic DA system in cocaine self-administration and modulation of locomotion[29], we further investigated the effects of JWH133 on basal and cocaine-enhanced DA in the NAc by *in vivo* microdialysis. We did not see significant

differences in basal levels of extracellular NAc DA between WT and $CB_2^{-/-}$ mice (Supplementary Fig. 4). However, $CB_1^{-/-}$ mice displayed a significant basal reduction, compared to WT mice (Supplementary Fig. 4). Consistent with the findings in cocaine self-administration and locomotion, systemic administration of JWH133 (3, 10, 20 mg/kg, i.p.) also significantly and dose-dependently lowered extracellular NAc DA in WT (Fig. 5a) and $CB_1^{-/-}$ (Fig. 5b) mice, but not in $CB_2^{-/-}$ (Fig. 5c) mice. This reduction in NAc DA was blocked by AM630 (10 mg/kg, i.p.) in $CB_1^{-/-}$ mice (Fig. 5b), suggesting that JWH133's DA-inhibiting effect is mediated by activation of CB_2 receptors. Moreover, pretreatment with the same doses of JWH133 also significantly attenuated cocaine-enhanced NAc DA in WT (Fig. 5d), $CB_1^{-/-}$ (Fig. 5e), but not in $CB_2^{-/-}$ (Fig. 5f) mice.

To determine whether this inhibition is mediated by activation of brain or peripheral CB_2 receptors, we also observed the effects of intranasal or intra-NAc local administration of JWH133 on extracellular DA. We found that intranasal administration of JWH133 (100 μg/nostril) produced a significant reduction in extracellular NAc DA in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice (Fig. 6a). Similarly, intra-NAc local perfusion of JWH133 (1–1000 μM) significantly lowered extracellular DA in both WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice (Fig. 6b). In fact, an unexpected increase in extracellular DA was observed in $CB_2^{-/-}$ mice after local administration of JWH133. The underlying mechanisms are unclear. One possibility is that JWH133 may bind to other (non-CB_2) receptors in $CB_2^{-/-}$ mice, producing an increase in extracellular DA. Congruent with this finding, intra-NAc local perfusion of AM630 (1, 10, 100 μM) elevated extracellular DA in a concentration-dependent manner in both WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice (Fig. 6c), suggesting that endocannabinoids tonically modulate NAc DA release by activation of brain CB_2 receptors. Further, AM630-enhanced extracellular DA appears more robust in $CB_1^{-/-}$ mice than in WT mice (Fig. 6c), suggesting higher endocannabinoid tone on brain CB_2 receptors in $CB_1^{-/-}$ mice. Moreover, intra-NAc local perfusion of AM630 also blocked the reduction in extracellular NAc DA produced by systemic administration of JWH133 seen in WT and $CB_1^{-/-}$ mice (Fig. 6c, d), suggesting that JWH133-induced inhibition of DA release is mediated by activation of NAc CB_2 receptors. The locations of microdialysis probes or microinjection cannulae were within the NAc (Supplementary Fig. 5).

DISCUSSION

Here we report that systemic administration of the CB_2 receptor agonist JWH133 significantly and dose-dependently inhibits intravenous cocaine self-administration under both FR1 and PR reinforcement and inhibits cocaine-enhanced locomotion and extracellular NAc DA in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice. This effect was mimicked by GW405833 (another selective CB_2 receptor agonist), and blocked by AM630, a selective CB_2 receptor antagonist, but not by AM251, a selective CB_1 receptor antagonist, suggesting an effect mediated by activation of CB_2 receptors. Further, intranasal microinjections of JWH133, but not intravenous injections of the same micro-quantities of JWH133 as injected intranasally, also significantly and dose-dependently inhibited intravenous cocaine self-administration, suggesting an effect mediated by activation of brain, not peripheral, CB_2 receptors. This is further supported by the finding that local intra-NAc administration of JWH133 also significantly inhibited cocaine self-administration in a dose-dependent

manner, an effect that was blocked by intra-NAc co-administration of AM630. In addition, intra-NAc local administration of JWH133 dose-dependently lowered, while AM630 elevated, basal levels of locomotion and extracellular NAc DA. Intra-NAc local perfusion of AM630 blocked the reduction in cocaine self-administration and NAc DA produced by systemic administration of JWH133. These data suggest that both the behavioral and neurochemical effects of JWH133 are mediated by activation of brain CB_2 receptors.

We note that systemic administration of AM630 failed to alter, while intra-NAc local administration of AM630 elevated, extracellular DA and locomotion, suggesting that local AM630 administration is more effective than systemic administration. This may be related to AM630's relatively poor pharmacokinetic properties and/or blood-brain barrier passage. In addition, we also note that intra-NAc AM630 significantly elevated extracellular DA and locomotion, but failed to alter cocaine self-administration. This may be related to previous findings that locomotion is largely DA-dependent[30], while cocaine self-administration is dependent on both DA and non-DA mechanisms[31]. We also note that pharmacological blockade of NAc CB_2 receptors elevated, while genetic deletion of CB_2 receptors did not alter, basal extracellular DA in the NAc. The reasons are unclear. One possibility is that CB_2 receptor deletion-induced disinhibition of NAc DA release may be compromised by actions in other brain loci that modulate the mesolimbic DA system. Another possibility is that neuroadaptative processes may antagonize CB_2 receptor inactivation-induced DA neuronal disinhibition after CB_2 receptor deletion. Whatever the exact mechanism(s), the present data strongly suggest that brain CB_2 receptors functionally modulate the mesolimbic DA system and DA-related functions. Activation of brain CB_2 receptors by JWH133 inhibits both the behavioral and neurochemical effects of cocaine. Since JWH133 neither alters locomotor performance as assessed by the rotarod test, nor produces drug rewarding or aversive effects as assessed by i.v. self-administration and conditioned place preference, JWH133-induced inhibition of cocaine self-administration is most likely mediated by attenuation of cocaine's rewarding efficacy secondary to the reduction in cocaine-enhanced NAc DA rather than by nonspecific locomotor impairment or malaise.

We fully recognize that these findings challenge the currently accepted opinion that selective CB_2 receptor agonists have no CNS effects. This opinion is largely based upon previous reports that the selective CB_2 receptor agonist AM1241 neither inhibits locomotion or rotarod performance, nor produces catalepsy or hypothermia in rats or mice[32]. In addition, AM1241 also failed to alter brain functional activity as assessed by pharmacological MRI[33]. The ineffectiveness of AM1241 may be related to the relatively lower doses (30 μg-3.3 mg/kg) used in those studies, relatively poor selectivity, and species differences in CB_2 receptor response to AM1241[34–36]. For example, AM1241 is reported to act as a full or partial agonist at human CB_2 receptors[35], while acting as an inverse agonist at rodent CB_2 receptors[36]. Further, the analgesic effects produced by AM1241 are reported to be blocked by the opioid receptor antagonist naloxone[37], suggesting that AM1241 may interact with other, non-cannabinoid, receptors. However, the CB_2 receptor agonist GW405833, at high doses (30–100 mg/kg), produces significant CNS effects such as analgesia, sedation and catalepsy[26], consistent with our finding that GW405833 (3–10 mg/kg) significantly inhibits cocaine self-administration in mice.

The presence of CB_2 receptors in the CNS, in particular on neurons, has been subject to debate[10, 38]. Previous studies using *in situ* hybridization and Northern blot assays failed to detect CB_2 receptor mRNA in brain[5, 39, 40]. However, recent studies with more sensitive RT-PCR and immunolabeling techniques have claimed to find significant CB_2 receptor expression in microglia and subpopulations of neuronal cells in brain[7–11]. By using highly sensitive and specific Taqman probes, we have recently identified two CB_2 receptor isoforms (CB_{2A}, CB_{2B}) in both brain and peripheral tissues, which display significant species differences in both structure and expression between humans, rats and mice[41]. It is now well accepted that CB_2 receptors are expressed on microglia and a subset of neurons with levels increasing under certain pathological conditions such as neuroinflammation and brain injury[38]. There are two possibilities to explain the present findings. First, a low density of CB_2 receptors may be expressed on mesolimbic DA neurons. Since CB_2 receptors are $G_{i/o}$ coupled[42], activation of CB_2 receptors on DA neurons in the midbrain ventral tegmental area (VTA) may directly inhibit VTA DA neurons and decrease NAc DA release, and therefore inhibit intravenous cocaine self-administration and cocaine-enhanced locomotion as observed in the present study. Although direct evidence of CB_2 receptor expression in the mesolimbic DA neurons is lacking at present, functional CB_2 receptors are found in other neurons. For example, CB_2 receptor mRNA is expressed on striatal GABAergic neurons in non-human primates[43], and activation of CB_2 receptors inhibits GABAergic neurotransmission in the medial entorhinal cortex of the rat[19]. In addition, CB_2 receptors are also found on neurons in the dorsal-root ganglion (DRG) and spinal cord (SC)[44, 45], and activation of CB_2 receptors on DRG-SC neurons inhibits neuronal response to noxious stimuli[45, 46], thereby contributing to the antinociceptive effects of CB_2 receptor agonists[47]. The second possibility is that activation of CB_2 receptors located on microglial cells or astrocytes in the VTA and/or NAc may indirectly inhibit NAc DA release by releasing cytokines and inflammatory factors[48], thereby inhibiting cocaine self-administration and cocaine-enhanced locomotion as observed in the present study.

Whatever the mechanisms, the present findings, for the first time, suggest that activation of brain CB_2 receptors inhibits cocaine's rewarding and psychomotor-stimulating effects, which is congruent with a rapidly expanding corpus of published reports implicating brain CB_2 receptors in modulating a variety of CNS functions such as locomotion[10], pain[13, 47], emesis[11], neurogenesis[14], and neuroprotection[15]. This finding not only challenges current views that CB_2 receptors are absent from the CNS and that CB_2 receptor ligands lack CNS effects, but also suggests that brain CB_2 receptors may be a novel target for the pharmacotherapy of drug abuse and addiction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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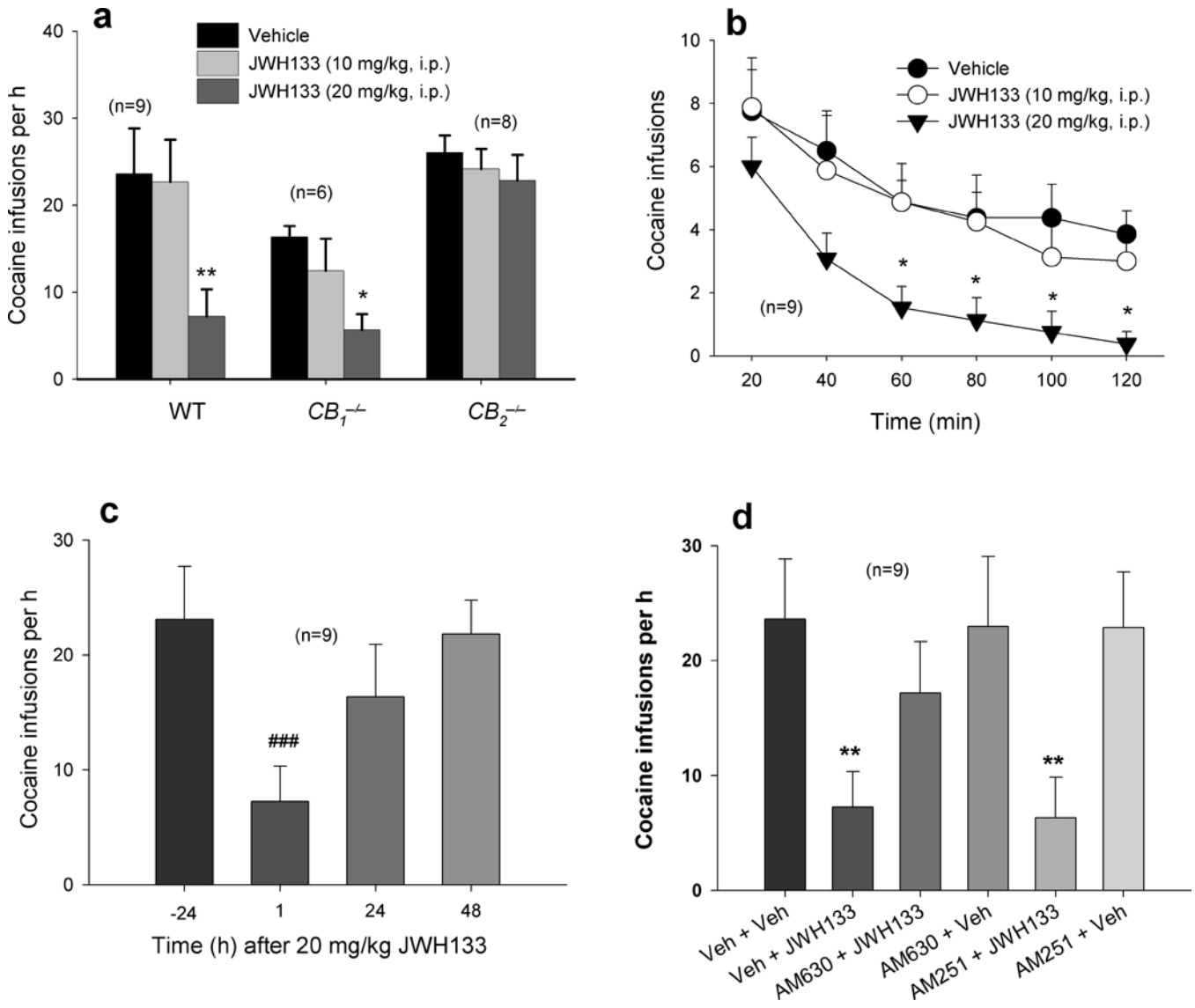

Figure 1.

Effects of JWH133 on cocaine self-administration. (**a**) Systemic administration of JWH133 (10, 20 mg/kg, i.p., 30 min prior to testing) inhibits cocaine self-administration under FR1 reinforcement in WT (one-way ANOVA, $F_{2,16} = 13.09$, $P < 0.001$) and $CB_1^{-/-}$($F_{2,10} = 5.01$, $P < 0.05$), but not $CB_2^{-/-}$ ($F_{2,14}$=0.56, $P = 0.58$), mice. (**b**) Time course of JWH133's attenuation of cocaine self-administration in WT mice on the test day. (**c**) Time course of recovery of cocaine self-administration in WT mice after JWH133 administration. (**d**) In WT mice, JWH133-induced attenuation of cocaine self-administration is prevented by pretreatment with the CB_2 receptor antagonist AM630 (10 mg/kg, i.p., 30 min prior to JWH133), but not by pretreatment with the CB_1 receptor antagonist AM251 (3 mg/kg, i.p.) ($F_{5,40} = 6.31$, $P < 0.001$). Neither AM630 nor AM251 altered cocaine self-administration in WT mice. Data are means ± s.e.m. * $P < 0.05$, ** $P < 0.01$, compared to vehicle (Veh) control groups. ### $P < 0.001$, compared to pre-JWH133 (−24 h) condition.

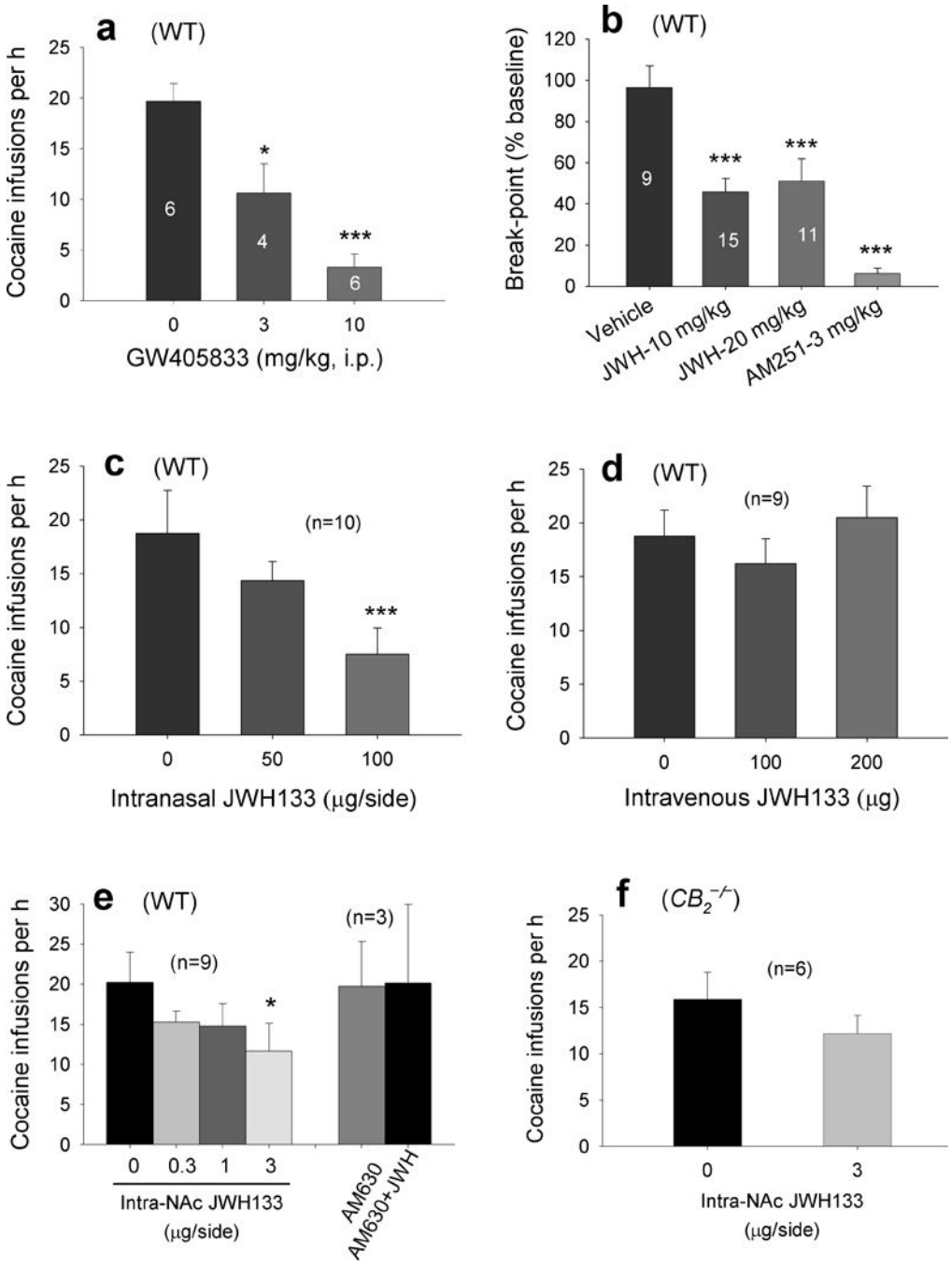

Figure 2.

Effects of GW405833 or JWH133 on cocaine self-administration. **(a)** GW405833 (3, 10 mg/kg, i.p.) dose-dependently inhibited cocaine self-administration under FR1 reinforcement in WT mice (one-way ANOVA, $F_{2,6} = 20.03$, $P < 0.01$). **(b)** JWH133 (10, 20 mg/kg) or AM251 (3 mg/kg, i.p.) significantly lowered the cocaine self-administration break-point under PR reinforcement in WT mice ($F_{3,37} = 13.83$, $P < 0.001$). **(c)** Intranasal microinjections of JWH133 (50, 100 μg/nostril) dose-dependently inhibited cocaine self-administration under FR1 reinforcement ($F_{2,18} = 14.34$, $P < 0.001$). **(d)** Intravenous

injection of the same micro-quantity (100, 200 μg) of JWH133 as used intranasally had no effect on cocaine self-administration ($F_{2,16} = 1.59$, $P = 0.23$). **(e)** Intra-NAc microinjections of JWH133 (0.3, 1, 3 μg/side) dose-dependently inhibited cocaine self-administration under FR1 reinforcement in WT mice. This inhibition was blocked by intra-NAc co-administration of AM630 (3 μg/side) ($F_{3,24} = 4.49$, $P < 0.05$). **(f)** Intra-NAc administration of JWH133 (3 μg/side) had no effect on cocaine self-administration in $CB_2^{-/-}$ mice ($F_{1,10} = 2.37$, $P = 0.15$). Data are means ± s.e.m. * $P < 0.05$, *** $P < 0.001$, compared to vehicle control group.

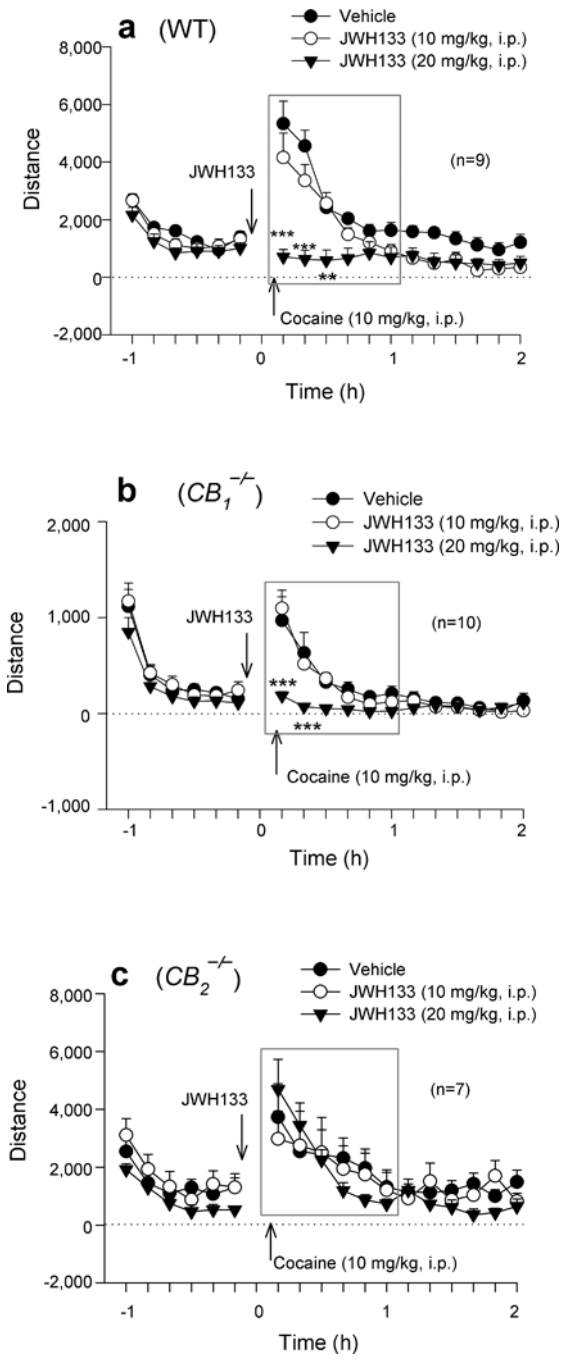

Figure 3.

Systemic administration of JWH133 (10, 20 mg/kg, i.p., 30 min prior to cocaine) dose-dependently inhibited cocaine-enhanced locomotion in WT (**a,** two-way ANOVA for repeated measures over time, $F_{2,16} = 14.45$, $P < 0.001$) and $CB_1^{-/-}$ (**b,** $F_{2,18}=12.57$, $p<0.001$), but not in $CB_2^{-/-}$ (**c,** $F_{2,12} = 0.17$, $P = 0.85$), mice. Data are means ± s.e.m. ** $P < 0.01$, *** $P < 0.001$, compared to vehicle treatment group.

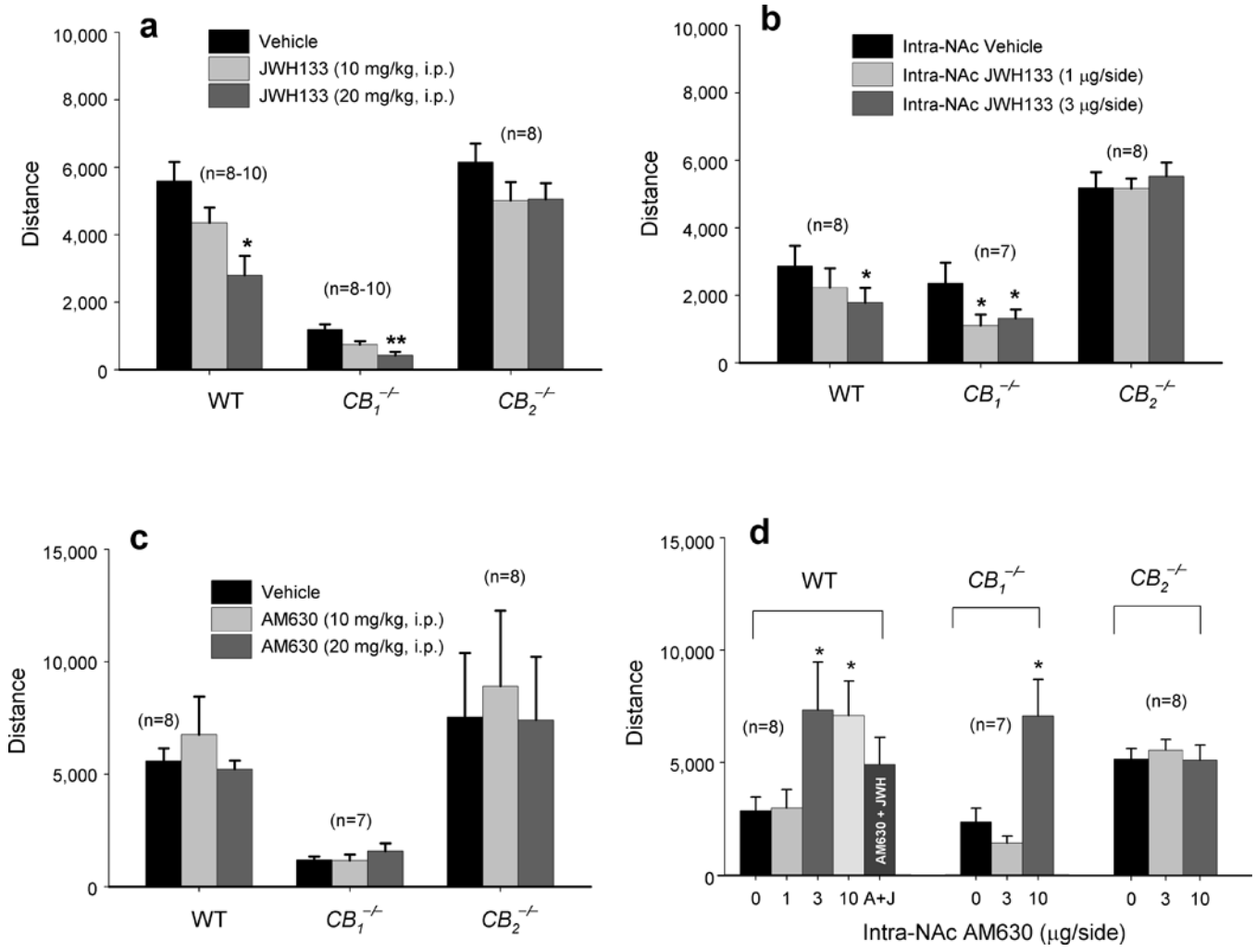

Figure 4.

Effects of systemic or local intra-NAc administration of JWH133 or AM630 on locomotor activity. **(a)** Systemic administration of JWH133 (10, 20 mg/kg, i.p.) dose-dependently inhibited locomotion in WT (one-way ANOVA, $F_{2,24} = 8.03$, $P = 0.002$) and $CB_1^{-/-}$ ($F_{2,25} = 13.44$, $P < 0.001$) mice, but not in $CB_2^{-/-}$ ($F_{2,14} = 3.36$, $P > 0.05$) mice. **(b)** Intra-NAc microinjections of JWH133 (1, 3 μg/side) significantly inhibited locomotion in WT ($F_{2,14}$=4.17, p<0.05) and $CB_1^{-/-}$ ($F_{2,12} = 4.91$, $P < 0.05$), but not in $CB_2^{-/-}$ ($F_{2,14} = 0.04$, $P > 0.05$), mice (**c**) Systemic administration of AM630 failed to alter locomotion in any strain of mice. **(d)** Intra-NAc administration of AM630 (1, 3, 10 μg/side) significantly augmented locomotion in WT ($F_{3,21} = 4.62$, $P < 0.05$) and $CB_1^{-/-}$ ($F_{2,12} = 10.57$, $P < 0.01$), but not in $CB_2^{-/-}$($F_{2,14} = 0.05$, $P > 0.05$), mice. Data are means ± s.e.m. * $P < 0.05$, ** $P < 0.01$, compared to vehicle control group.

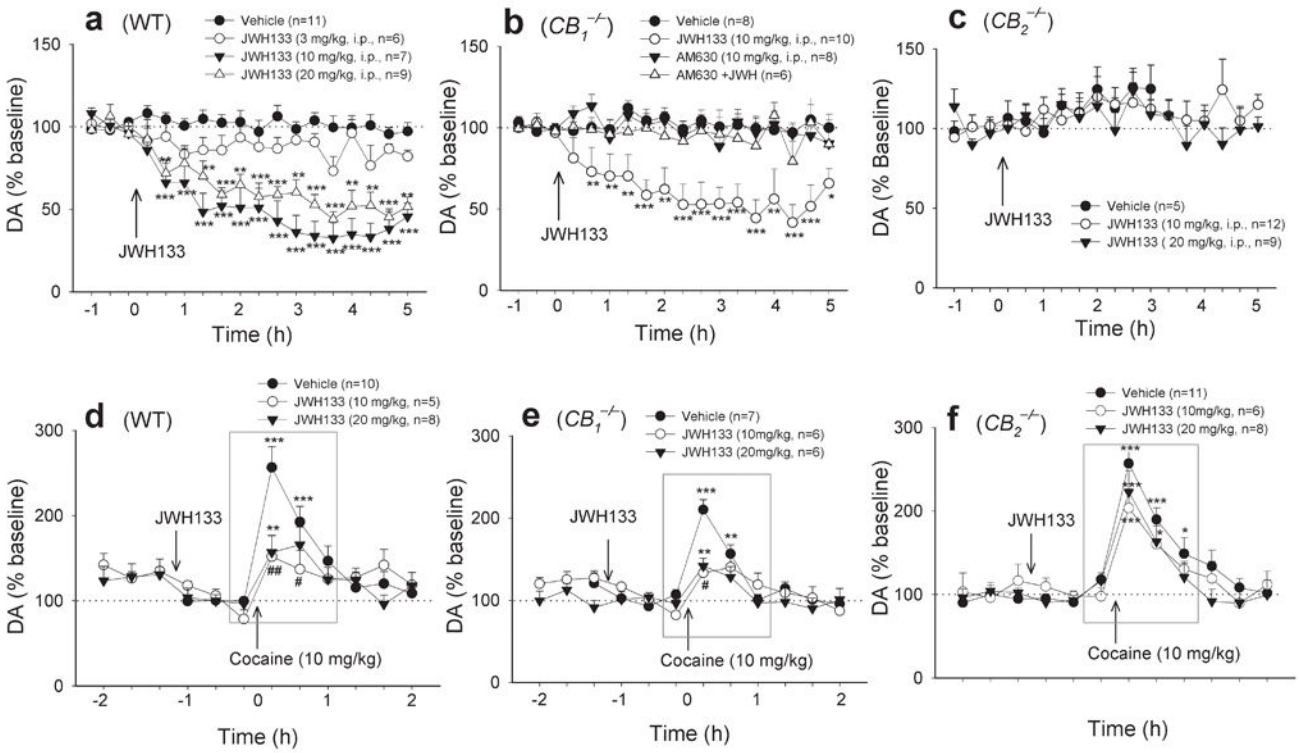

Figure 5.

Systemic administration of JWH133 (3, 10, 20 mg/kg, i.p.) dose-dependently inhibited basal (**a**, **b**, **c**) or cocaine-enhanced (**d**, **e**, **f**) extracellular NAc DA in WT (**a**, two-way ANOVA for repeated measures over time, $F_{3,29} = 25.97$, $P < 0.001$; **d**, $F_{2,19} = 4.47$, $P < 0.05$) and $CB_1^{-/-}$ (**b**, $F_{3,28} = 10.07$, $P < 0.001$; **e**, $F_{2,16} = 4.78$, $P < 0.05$) mice, but not in $CB_2^{-/-}$ (**c**, $F_{2,23} = 0.10$, $P > 0.05$; **f**, $F_{2,22} = 1.53$, $P > 0.05$) mice. AM630 alone (10 mg/kg, i.p.) failed to alter NAc DA in $CB_1^{-/-}$ mice, while AM630 pretreatment (10 mg/kg, i.p.) prevented JWH133-induced inhibition of NAc DA in $CB_1^{-/-}$ mice (**b**). Data are means ± s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to pre-drug baseline.$^{\#}$ $P < 0.05$,$^{\#\#}$ $P < 0.01$, compared to vehicle treatment group.

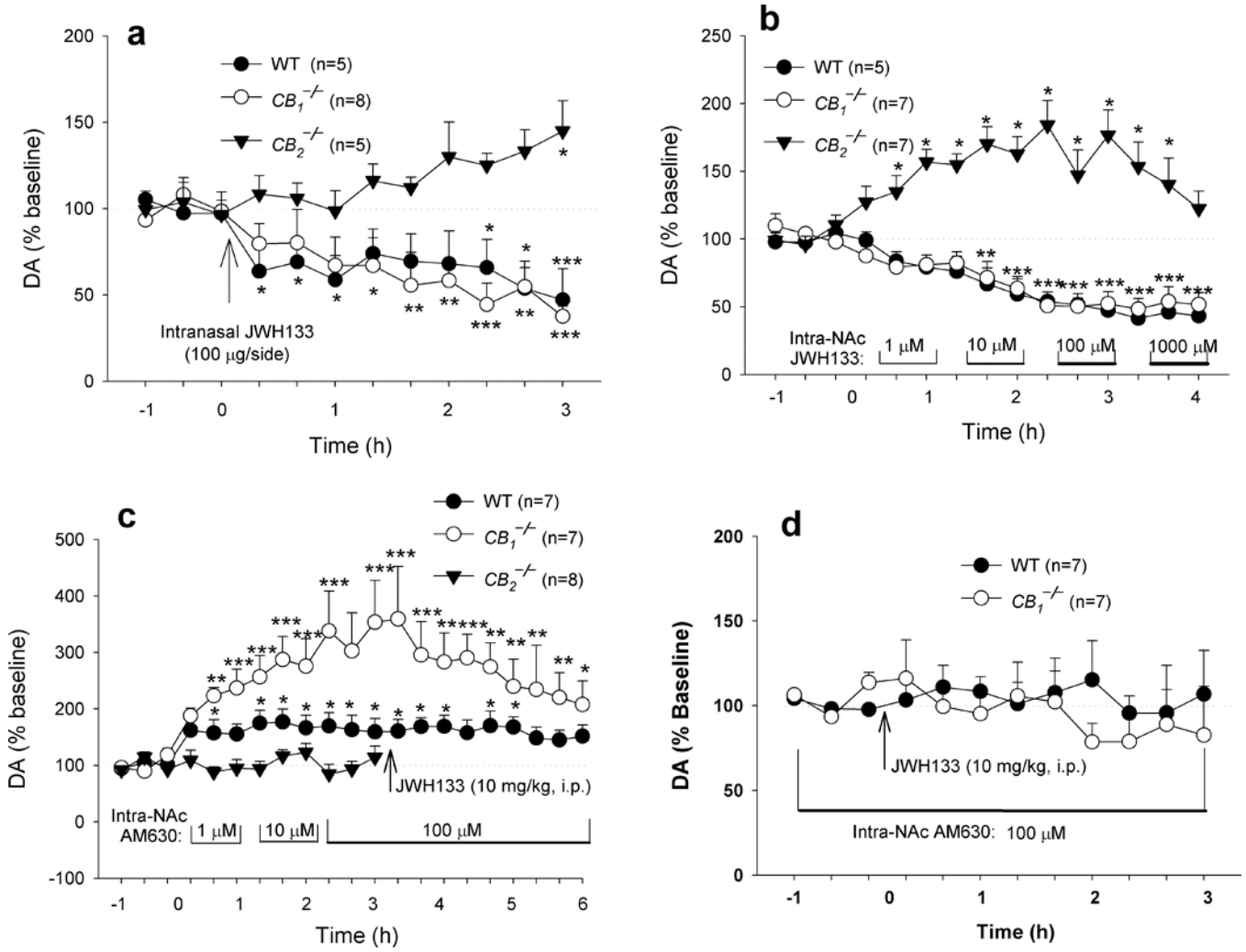

Figure 6.

Effects of intranasal or intra-NAc local perfusion of JWH133 or AM630 on extracellular NAc DA. (**a**) Intranasal administration of JWH133 (50 μg/nostril) significantly lowered extracellular DA in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice (two-way ANOVA for repeated measures over time, $F_{2,15} = 10.81$, $P = 0.001$). (**b**) Intra-NAc local perfusion of JWH133 lowered extracellular DA in WT and $CB_1^{-/-}$ mice in a dose-dependent manner, while elevating extracellular DA in $CB_2^{-/-}$ mice ($F_{2,18}$=47.00, $P < 0.001$). (**c**) Intra-NAc local perfusion of AM630 elevated extracellular DA in WT and $CB_1^{-/-}$ mice, but not in $CB_2^{-/-}$ mice ($F_{2,18} = 12.13$, $P < 0.001$). Further, AM630-enhanced extracellular DA appears more robust in $CB_1^{-/-}$ mice than in WT mice ($F_{1,12} = 7.50$, $P < 0.05$). (**d**) Renormalized data over new baselines 1 h before JWH133 administration from the data in Panel **c**, illustrating that intra-NAc local perfusion of AM630 blocked JWH133's action on extracellular DA in WT and $CB_1^{-/-}$ mice. Data are means ± s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to pre-drug baseline.