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Bimodal control of stimulated food intake by the endocannabinoid system

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Activation of cannabinoid type-1 receptors (CB_1) is universally recognized as a powerful endogenous orexigenic signal, but the detailed underlying neuronal mechanisms are not fully understood. Using combined genetic and pharmacological approaches in mice, we found that ventral striatal CB_1 receptors exerted a hypophagic action through inhibition of GABAergic transmission. Conversely, brain CB_1 receptors modulating excitatory transmission mediated the well-known orexigenic effects of cannabinoids.

CB_1 receptors are expressed in many brain regions that control food intake, where they presynaptically regulate both excitatory and inhibitory neurotransmission[1–5]. Glutamatergic and GABAergic transmission in several brain regions are known to inhibit and promote feeding behavior, respectively[6–8]. Thus, by reducing either excitatory or inhibitory neurotransmission, activation of CB_1 receptors might have opposite effects on this function. Consistently, pharmacological treatments with CB_1 agonists typically exert biphasic effects, with low-to-moderate doses inducing hyperphagia and moderate-to-high doses causing hypophagia[4,9]. It is therefore possible that endogenous and/or exogenous cannabinoids activate distinct sets of CB_1 receptors that are expressed in different neuronal populations, thereby differentially regulating feeding behavior.

To dissect the roles of CB_1 receptors on excitatory or inhibitory transmission, we recently generated conditional mutant mice[10–12]. In these mutants, the CB_1 gene (also known as *Cnr1*) is completely absent in $CB_1^{-/-}$ mice[10], primarily absent in cortical glutamatergic neurons in the dorsal telencephalon, including neurons located in neocortex, paleocortex, archicortex, hippocampal formation and cortical portions of the amygdala, in $CB_1^{loxP/loxP;\ Nex\text{-}cre}$ mice (referred to as Glu-$CB_1^{-/-}$ mice)[11,12], and primarily absent in forebrain GABAergic neurons in $CB1^{loxP/loxP;\ Dlx5/6\text{-}cre}$ mice (referred to as GABA-$CB_1^{-/-}$ mice)[11,12].

After fasting (**Supplementary Methods**), a condition that is known to activate the endocannabinoid system (ECS)[1,4], food intake was lower in $CB_1^{-/-}$ mice as compared with wild-type littermates (**Fig. 1a**). Glu-$CB_1^{-/-}$ mice had a similar phenotype (**Fig. 1b**), whereas GABA-$CB_1^{-/-}$ mice displayed hyperphagia (**Fig. 1c**). The exposure of fed animals to palatable food led to similar responses (**Supplementary Fig. 1**). GABA-$CB_1^{-/-}$ or Glu-$CB_1^{-/-}$ mice showed no alteration in body weight when fed *ad libitum* with regular chow (**Supplementary Fig. 1**),

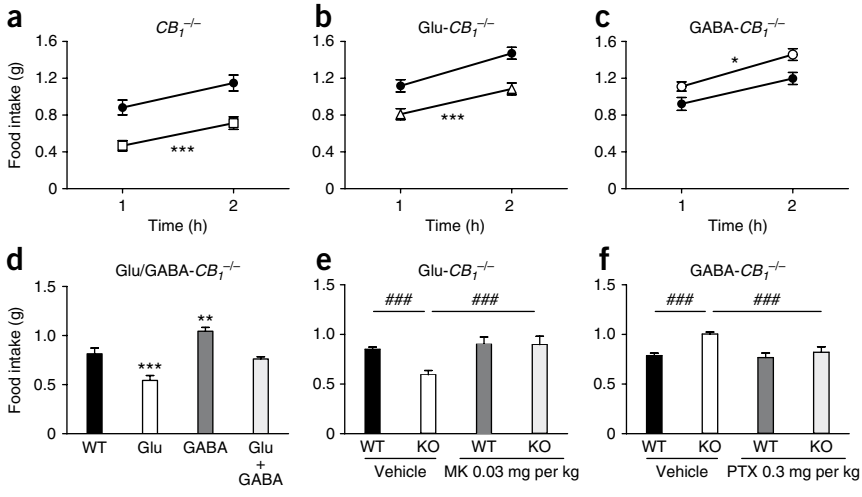

Figure 1 Deletion of CB_1 from cortical glutamatergic or GABAergic neurons has opposing effects on fasting-induced food intake. (**a**) Cumulative food intakes of $CB_1^{-/-}$ mice (open squares, $n = 12$) and wild-type littermates (black circles, $n = 11$). (**b**) Cumulative food intakes of Glu-$CB_1^{+/+}$ (black circles, $n = 15$) and Glu-$CB_1^{-/-}$ littermates (open triangles, $n = 17$). (**c**) Cumulative food intakes of GABA-$CB_1^{+/+}$ (black circles, $n = 19$) and GABA-$CB_1^{-/-}$ littermates (open circles, $n = 20$). (**d**) Food intakes of double-mutant Glu/GABA-$CB_1^{-/-}$ (light gray, $n = 15$), wild-type (black, $n = 15$), Glu-$CB_1^{-/-}$ (white, $n = 13$) and GABA-$CB_1^{-/-}$ littermates (dark gray, $n = 14$). (**e**) Effects of the NMDA receptor antagonist MK-801 (MK, 0.03 mg per kg, intraperitoneal) on wild-type (black, $n = 23$; dark gray, $n = 16$) and Glu-$CB_1^{-/-}$ littermates (KO; white, $n = 9$; light gray, $n = 6$). (**f**) Effects of the $GABA_A$ receptor antagonist picrotoxin (PTX, 0.3 mg per kg, intraperitoneal) on wild-type (black, $n = 23$; dark gray, $n = 8$) and GABA-$CB_1^{-/-}$ littermates (KO; white, $n = 22$; light gray, $n = 11$). All data are expressed as mean ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with wild-type littermate controls. $^{###}P < 0.001$. All experimental procedures were approved by the Committee on Animal Health and Care of INSERM and the French Ministry of Agriculture and Forestry (authorization number, 3306369).

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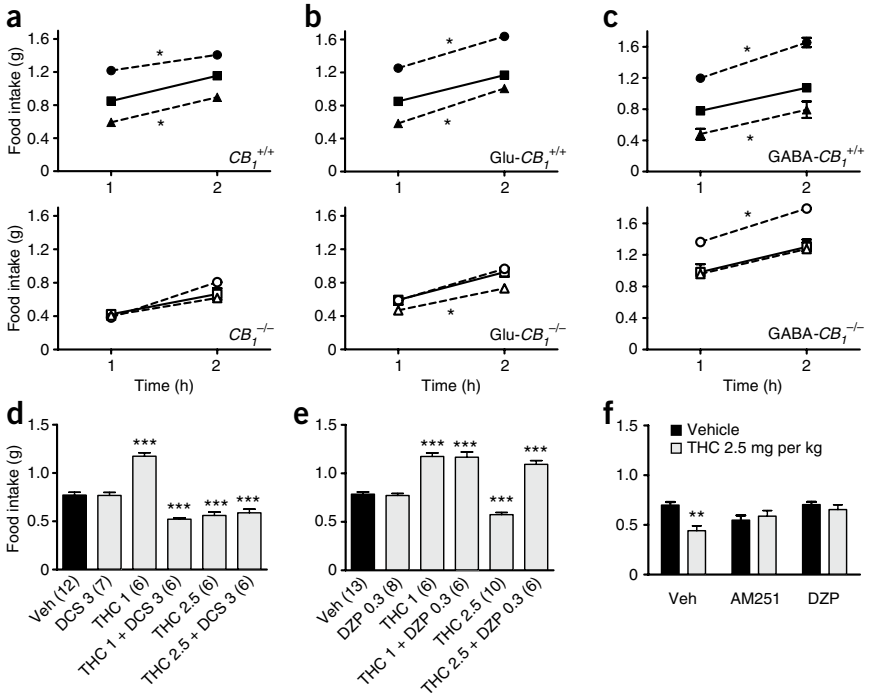

Figure 2 The hyperphagic and hypophagic effects of THC depend on CB_1-mediated modulation of glutamatergic and ventrostriatal GABAergic transmission, respectively. (**a**) Effects of intraperitoneal injections of 1 mg per kg THC (circles; $CB_1^{+/+}$, $n = 4$; $CB_1^{-/-}$, $n = 6$) and 2.5 mg per kg THC (triangles; $CB_1^{+/+}$, $n = 5$; $CB_1^{-/-}$, $n = 3$) in $CB_1^{+/+}$ mice (top) and $CB_1^{-/-}$ littermates (bottom). Squares indicate vehicle groups ($CB_1^{+/+}$, $n = 5$; $CB_1^{-/-}$, $n = 9$). (**b**) Effects of intraperitoneal injections of 1 mg per kg THC (circles; Glu-$CB_1^{+/+}$, $n = 9$; Glu-$CB_1^{-/-}$, $n = 8$) and 2.5 mg per kg THC (triangles; Glu-$CB_1^{+/+}$, $n = 8$; Glu-$CB_1^{-/-}$, $n = 5$) in Glu-$CB_1^{+/+}$ mice (top) and Glu-$CB_1^{-/-}$ littermates (bottom). Squares indicate vehicle groups (Glu-$CB_1^{+/+}$, $n = 8$; Glu-$CB_1^{-/-}$, $n = 13$). (**c**) Effects of intraperitoneal injections of 1 mg per kg THC (circles; GABA-$CB_1^{+/+}$, $n = 11$; GABA-$CB_1^{-/-}$, $n = 5$) and 2.5 mg per kg THC (triangles; GABA-$CB_1^{+/+}$, $n = 6$; GABA-$CB_1^{-/-}$, $n = 7$) in GABA-$CB_1^{+/+}$ mice (top) and GABA-$CB_1^{-/-}$ littermates (bottom). Squares indicate vehicle groups (GABA-$CB_1^{+/+}$, $n = 8$; GABA-$CB_1^{-/-}$, $n = 13$). (**d**) Effects of intraperitoneal injections of D-cyclo-serine (DCS, 3 mg per kg) in combination with 1 mg per kg and 2.5 mg per kg THC (numbers of C57BL/6NCrl mice per group are shown in parentheses). (**e**) Effects of intraperitoneal injections of DZP (0.3 mg per kg) in combination with 1 mg per kg and 2.5 mg per kg THC (numbers of C57BL/6NCrl mice per group are shown in parentheses). (**f**) Intra-ventrostriatal injections of vehicle, AM251 (1 μg per side) or diazepam (5 μg per side) in combination with intraperitoneal administration of 2.5 mg per kg THC or vehicle ($n = 4$–5 mice per group). All data are expressed as mean ± s.e.m. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with vehicle treatments.

suggesting that feeding-activating internal states are necessary to cause these phenotypes. No alterations in fasting-induced food intake were found in *Nex-cre* and *Dlx5/6-cre* transgenic mice, the appropriate genetic controls of Glu-$CB_1^{-/-}$ and GABA-$CB_1^{-/-}$ mice, respectively[11,13,14] (**Supplementary Fig. 1**). The food intake of Glu/GABA-$CB_1^{-/-}$ double-mutant mice, in which CB_1 is deleted from both cortical glutamatergic and GABAergic neurons (**Supplementary Figs. 2–5**), did not differ from wild-type littermates (**Fig. 1d**), indicating that the two genetic mutations compensate for each other in fasting-refeeding experiments. These results suggest that CB_1-dependent control of glutamatergic or GABAergic transmission exerts opposite effects on stimulated food intake.

Anatomical analyses revealed that CB_1 mRNA was not expressed in the large majority of cortical glutamatergic neurons in Glu-$CB_1^{-/-}$ mice[11], in GABAergic neurons in GABA-$CB_1^{-/-}$ mice[11] and in both neuronal populations in Glu/GABA-$CB_1^{-/-}$ double mutants (**Supplementary Figs. 2–6**). Notably, the lack of CB_1 mRNA expression resulted into an almost undetectable decrease in the levels of CB_1 protein in Glu-$CB_1^{-/-}$ mice and in a strong reduction of CB_1 protein in GABA-$CB_1^{-/-}$ mice (**Supplementary Fig. 7**), confirming that the large majority of brain CB_1 receptors are expressed in GABAergic neurons[5].

To exclude potential caveats associated with the use of genetic models, such as possible developmental alterations or undetected deletions of CB_1 receptors in additional brain areas or cell types[13,14], we carried out acute pharmacological studies in mutant mice and wild-type controls. Acute administration of an ineffective dose of the NMDA receptor antagonist MK-801 (**Supplementary Fig. 8**) abolished the phenotype of Glu-$CB_1^{-/-}$ mice (**Fig. 1e**). Similarly, an ineffective dose of the $GABA_A$ receptor antagonist picrotoxin (**Supplementary Fig. 8**) abolished the phenotype of GABA-$CB_1^{-/-}$ mice (**Fig. 1f**). These data suggest that the CB_1-dependent acute inhibition of excitatory glutamatergic transmission contributes to fasting-induced hyperphagia, whereas inhibition of inhibitory GABAergic transmission mediates a hypophagic effect of endogenous CB_1 signaling.

The CB_1 agonist Δ^9-tetrahydrocannabinol (THC) exerted a biphasic effect on food intake in fasting-refeeding experiments (**Supplementary Fig. 9**). Notably, the hyperphagic dose of THC (1 mg per kg of body weight) and the hypophagic one (2.5 mg per kg) did not alter locomotor activity during refeeding (**Supplementary Fig. 9**), indicating that altered locomotion was not involved in the effects of the drug. We then administered these doses of THC to CB_1 mutant mice and their wild-type littermates. Whatever the dose, THC was inactive in $CB_1^{-/-}$ mice (**Fig. 2a**). The hyperphagic dose of THC had no effect on Glu-$CB_1^{-/-}$ mice, whereas the higher dose decreased food intake in these mutants (**Fig. 2b**). Conversely, the lower dose of THC increased food intake in GABA-$CB_1^{-/-}$ mice, whereas the higher dose did not induce hypophagia (**Fig. 2c**). These results suggest that the orexigenic effect of a low dose of THC is mediated by CB_1-dependent inhibition of glutamate release, whereas the hypophagic effect of the higher dose occurs via CB_1-mediated inhibition of GABA release.

To further rule out possible confounding factors caused by the use of conditional mutant mice and to confirm the mechanism of the biphasic effects of THC, we carried out acute pharmacological studies in C57BL/6NCrl mice. The activation of CB_1 receptors by THC in either glutamatergic (low doses) or GABAergic neurons (high doses) should lead to a decreased release of the respective neurotransmitter, thereby reducing glutamatergic and GABAergic signaling, respectively[2,3] (**Supplementary Fig. 10**). Theoretically, these effects could be compensated for by simultaneous treatment with allosteric enhancers of the respective receptors, which would strengthen the effects of the remaining synaptic neurotransmitters (**Supplementary Fig. 10**). An ineffective dose of the allosteric NMDA receptor enhancer D-cyclo-serine (**Supplementary Fig. 11**) fully reversed the hyperphagic effect of 1 mg per kg THC, but did not alter the hypophagic

effect of the higher dose of THC (**Fig. 2d**). Conversely, an ineffective dose of the allosteric enhancer of the $GABA_A$ receptors diazepam (DZP; **Supplementary Fig. 11**) failed to change the hyperphagic effect of the low dose of THC, but fully reversed the hypophagic effect of 2.5 mg per kg THC (**Fig. 2e**).

The ventral striatum controls food intake[6,8] and contains substantial levels of CB_1-positive axons[5]. These CB_1-expressing fibers were still present in Glu-$CB_1^{-/-}$ mice, but were absent in GABA-$CB_1^{-/-}$ mice (**Supplementary Fig. 12**). Bilateral injections (**Supplementary Fig. 13**) of the CB_1 antagonist AM251 (1 μg per site) into the ventral striatum of C57BL/6NCrl mice did not alter the hyperphagic effect of 1 mg per kg THC (**Supplementary Fig. 13**), but fully blocked the effect of the hypophagic dose of the drug (**Fig. 2f**). Consistently, the local injection of an ineffective dose of DZP[8] also blocked the hypophagic effect of 2.5 mg per kg THC (**Fig. 2f**). The latter effect was also blunted in $CB_1^{loxP/loxP;\,D1\text{-}cre}$ mice (referred to as D1-$CB_1^{-/-}$ mice)[12] (**Supplementary Fig. 14**), which lack CB_1 expression in a large fraction of striatal neurons[12]. Altogether, these data indicate that CB_1-dependent inhibition of GABA release in the ventral striatum is necessary for the hypophagic effect of 2.5 mg per kg THC.

Our findings reveal two unexpected opposing brain functions of CB_1 receptors in the regulation of stimulated food intake. First, the control of glutamatergic transmission by CB_1 receptors is responsible at least in part for the well-known orexigenic role of the ECS. Second, CB_1 receptors expressed on ventrostriatal GABAergic neurons mediate, by reducing local inhibitory transmission, a previously unknown inhibitory function of the ECS on stimulated food intake. Low or high amounts of (endo)cannabinoids might differentially affect these opposing CB_1-dependent effects. The overall orexigenic role of the ECS on stimulated ingestive behavior seems to be the end product of finely regulated opposite functions. It is presently unknown how endogenous and/or exogenous CB_1 agonists could select the neuronal type in which they exert these opposing effects. The pharmacology of CB_1 receptors might vary according to the neuronal populations in which they are expressed. For example, possible cell type–specific heterodimerizations and/or conformational states of CB_1 receptors[15] might determine different cannabinoid-mediated intracellular responses. In conclusion, our findings reveal a bimodal mode of action of the ECS and exogenous cannabinoids in the control of stimulated food intake and underscore the importance of the ECS-mediated fine-tuned control of neuronal excitation and inhibition for the regulation of behavior in mammals.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

L.B. designed and performed experiments. P.L. carried out part of the food-intake experiments. A.C., N.P. and P.G. performed the anatomical experiments. D.C., F.C. and P.V.P. contributed to experimental design. P.V.P. and G.M. wrote the manuscript. All the authors edited the manuscript. G.M. conceived and supervised the project.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/natureneuroscience/.

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