it was also responsible for the instability in *dw3*.

Because direct duplications are apt to undergo unequal crossing-over (*18*), could this be the mechanism by which *dw3* reverts back to *Dw3*? One way of answering this question is by identifying one or more recombinants that contain at least three copies of the duplicated region. To find such a recombinant, DNA from another 200 dwarf plants was subjected to Southern analysis (*6*). We identified a single plant that displayed a restriction pattern indicative of three copies of the duplicated region (Fig. 4E). Subsequent cloning and sequencing of this restriction fragment confirmed its triplicate nature, thereby demonstrating that *dw3* reverts back to *Dw3* by unequal crossing-over.

Interestingly, a dwarf plant with a restriction band diagnostic of wild-type revertants was also found among these 200 plants (Fig. 4E). PCR amplification and subsequent sequencing of its product indicated that unequal recombination had removed the duplicated part of the gene but introduced a number of simple nucleotide changes in the copy that was left behind (fig. S4). These changes disrupted the reading frame of DW3 and also truncated the protein by about 200 amino acids, thereby explaining the mutant nature of this new allele. Because this allele, designated *dw3-sd1*, lacks the duplication, it is expected to confer a stable mutant phenotype. This was determined by generating progeny that were homozygous for the *dw3-sd1* allele. We screened more than 2400 such plants in the field and found that none reverted back to the tall type, confirming the stable dwarf nature of this mutant derivative. To determine whether imprecise recombination at *dw3* was common enough to be practically useful, we analyzed another 500 dwarf plants by PCR (*6*). One plant was identified that yielded a product indicative of a loss of duplication. Its sequence revealed that it had undergone mutational changes similar to that of *dw3-sd1* (*19*).

Concluding remarks. These findings not only resolve a long-standing puzzle in sorghum genetics and breeding but also provide a simple strategy for effectively correcting *dw3* in the sorghum germplasm. Moreover, new mutant alleles of sorghum *dw3* or of corresponding genes in other cereals may be generated by conventional mutagenesis approaches. There is also the prospect of inciting a renewed interest in this locus for maize breeding by generating new and improved alleles of *br2*. A key advantage of the dwarfing mechanism described here is its synergistic effect on stalk quality, a trait considered to be of utmost importance for enhancing crop yields beyond those that have already been achieved (*1*).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/302/5642/81/DC1
Materials and Methods
Figs. S1 to S4
References and Notes

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CB1 Cannabinoid Receptors and On-Demand Defense Against Excitotoxicity

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Abnormally high spiking activity can damage neurons. Signaling systems to protect neurons from the consequences of abnormal discharge activity have been postulated. We generated conditional mutant mice that lack expression of the cannabinoid receptor type 1 in principal forebrain neurons but not in adjacent inhibitory interneurons. In mutant mice, the excitotoxin kainic acid (KA) induced excessive seizures in vivo. The threshold to KA-induced neuronal excitation in vitro was severely reduced in hippocampal pyramidal neurons of mutants. KA administration rapidly raised hippocampal levels of anandamide and induced protective mechanisms in wild-type principal hippocampal neurons. These protective mechanisms could not be triggered in mutant mice. The endogenous cannabinoid system thus provides on-demand protection against acute excitotoxicity in central nervous system neurons.

Mnemonic processes and normal functioning of the brain require elevated neuronal activity. However, neuronal systems need to protect themselves against the risk of excessive activity, which could lead to pathological processes known as excitotoxicity (*1*). Therefore, it is conceivable that protective signaling systems exist that are able to provide on-demand defense in case of abnormally high spiking activity. The endogenous cannabinoid system in the brain is a neuromodulatory system comprising the cannabinoid receptor type 1 (CB1), its endogenous ligands (endocannabinoids), and the machinery for their synthesis and degradation (*2*, *3*). Exogenous natural and synthetic cannabinoids have been shown to exert neuroprotective functions in several models of neurotoxicity (*4*–*7*), and neuronal depolarization increases the production of endocannabinoids (*2*–*4*, *8*). However, the involvement of the endogenous cannabinoid system in physiological protection against the consequences of excessive

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neuronal activity is still a matter of debate (*4*), and even CB1 receptor–mediated neurotoxic effects have been reported (*9*–*11*).

CB1 receptors and KA-induced seizures. To test the role of the endogenous cannabinoid system in the control of excessive neuronal activity in the brain, we first compared CB1-null mutant mice ($CB1^{-/-}$) (*12*) and their $CB1^{+/+}$ control littermates in the kainic acid (KA) model of excitotoxic epileptiform seizures (*1*, *13*). In this model, the hippocampus appears as the brain region most susceptible to KA-induced effects (*1*). Injection of KA (30 mg/kg) into $CB1^{-/-}$ mice induced clearly more severe seizures than injection into $CB1^{+/+}$ littermates (genotype: $F_{1,13} = 8.8, P < 0.05$) (*13*) (Fig. 1A), and more than 75% of $CB1^{-/-}$ mice died within 1 hour after KA injection (fig. S1A). At lower doses of KA, the death rate was still significantly higher (fig. S1A) and behavioral responses were more pronounced (fig. S1B) in $CB1^{-/-}$ than in $CB1^{+/+}$ and $CB1^{+/-}$ mice (15 mg/kg, genotype: $F_{2,15} = 4.3$, $P < 0.05$; 20 mg/kg, genotype: $F_{2,15} = 4.0$, $P < 0.05$), indicating that genetic ablation of the CB1 receptor lowers the threshold for KA-induced seizures.

If CB1 receptor activation is involved in endogenous protection against KA-induced excitotoxicity, administration of KA should induce a rapid increase in the production of endocannabinoids for CB1 receptors. We therefore measured the levels of endocannabinoids in the hippocampi of wild-type mice from the C57BL/6N line, isolated at different time points after KA treatment (30 mg/kg) (*13*). Whereas the levels of the endocannabinoid 2-arachidonoyl-glycerol and of palmitoyl-ethanolamide (an endocannabinoid-related compound) remained unaltered at any time point analyzed (*14*), the tissue concentrations of anandamide (arachidonoyl-ethanolamide) markedly increased, peaked 20 min after KA injection, and returned to basal levels within 1 hour (Fig. 1B). These findings suggest a specific involvement of the endogenous cannabinoid system in acute protection against excitotoxicity induced by KA.

To substantiate the relationship between elevated levels of anandamide and activation of CB1 receptors, we tested the acute requirement of CB1 receptor activation by treating wild-type C57BL/6N mice with the specific CB1 receptor antagonist SR141716A (3 mg/kg) 30 min before KA injection (20 mg/kg) (*13*). SR141716A-treated mice experienced more severe seizures than vehicle-treated mice (treatment: $F_{1,10} = 5.0, P < 0.05$) (Fig. 1C). This effect of the antagonist was significantly more pronounced when heterozygous CB1-null ($CB1^{+/-}$) mutants, known to possess about half the density of CB1 receptors in the hippocampus (*15*), were treated with the same dose of the antagonist (treatment in $CB1^{+/-}$ mice: $F_{1,8} = 8.5, P < 0.05$; comparison C57BL/6N mice versus $CB1^{+/-}$ mice: behavioral scores of C57BL/6N: 2.9 ± 0.5 and of $CB1^{+/-}$: 5.2 ± 1.1, $P < 0.05$) (Fig. 1C). Consistently, preadministration of the selective and potent inhibitor of endocannabinoid uptake UCM707 (*16*) (3 mg/kg) significantly protected C57BL/6N mice against KA-induced seizures (35 mg/kg; treatment: $F_{1,21} = 4.8$, $P < 0.05$) (Fig. 1D), indicating that the endogenous cannabinoid system provides on-demand protection.

Role of forebrain principal neurons. In cortical areas, the CB1 receptor is highly expressed in interneurons that contain γ-aminobutyric acid (GABAergic interneurons) (*17*, *18*), but evidence exists for its presence also in principal neurons of, for example, the hippocampus (*17*, *19*). Thus, we generated a mouse line in which the CB1 coding region is flanked by two *loxP* sites (CB1-floxed mice, $CB1^{f/f}$) (Fig. 2A). By crossing this mouse line with mice that express Cre recombinase under the control of the regulatory sequences of the *Ca^{2+}/calmodulin-dependent kinase IIα* gene ($CB1^{CaMKII\alpha Cre}$ mice) (*20*), we obtained $CB1^{f/f;CaMKII\alpha Cre}$ mice (*13*) in which the CB1 receptor is deleted in all principal neurons of the forebrain but maintains its expression in cortical GABAergic interneurons (including those in the hippocampus) (Fig. 2, B to E) and in cerebellar neurons (*14*). Injection of 30 mg/kg of KA induced clearly more severe seizures in $CB1^{f/f;CaMKII\alpha Cre}$ mice than in $CB1^{f/f}$ littermates (genotype: $F_{1,16} = 14.9, P < 0.01$) (Fig. 2F) and decreased their survival rate ($P < 0.01$) (fig. S2A). Mice expressing only the transgenic Cre protein ($CB1^{CaMKII\alpha Cre}$ mice) and their wild-type littermates did not show any differences between genotypes after injection of 30 mg/kg of KA (genotype: $F_{1,18} = 0.7, P = 0.4$), thus precluding the expression of Cre recombinase as the cause of the phenotype in $CB1^{f/f;CaMKII\alpha Cre}$ mice. A comparison of behavioral scores of $CB1^{-/-}$ and $CB1^{f/f;CaMKII\alpha Cre}$ mice, and of their respective littermate controls, revealed that the development of seizures did not differ between the CB1-null mutants and the conditional CB1 knockouts (fig. S2B). Moreover, pretreatment with 3 mg/kg of UCM707 significantly protected $CB1^{f/f}$ mice against seizures induced by 30 mg/kg of KA. However, the same treatment was ineffective in $CB1^{f/f;CaMKII\alpha Cre}$ littermates (genotype and treatment: $F_{3,28} = 14.0, P < 0.001$; comparison $CB1^{f/f}$-vehicle versus $CB1^{f/f}$-UCM707, $P < 0.05$; comparison $CB1^{f/f;CaMKII\alpha Cre}$-vehicle versus $CB1^{f/f;CaMKII\alpha Cre}$-UCM707, $P = 0.95$) (Fig. 2G), thus indicating that the effects of the drug are specifically mediated by CB1 receptors on glutamatergic neurons. In addition, the blockade of CB1 receptors by treatment with 3 mg/kg of SR141716A was without any effect on seizures induced by 20 mg/kg of KA in $CB1^{f/f;CaMKII\alpha Cre}$ mice (Fig. 2H). Thus, GABAergic interneurons endowed with CB1 receptors apparently do not confer substantial protection against KA-induced acute excitotoxicity. We therefore suggest that the endogenous cannabinoid system exerts its neuroprotective action through CB1 receptors on principal glutamatergic neurons.

Dampening of KA-induced excitation. Injection of KA activates the endogenous cannabinoid system, which, in turn, protects neurons from the excitotoxic effects of this drug through the activation of CB1 receptors. How does CB1 receptor activation reduce excitotoxicity? Exogenously applied cannabinoids most commonly decrease neuronal excitability and inhibit glutamatergic transmission (*2*–*4*). It is thus conceivable to assume that an endogenously released ligand of the CB1 receptor, such as anandamide, might prevent excitotoxicity by a CB1 receptor–mediated inhibition of glutamatergic transmission. To test this hypothesis, we gauged glutamatergic excitation of CA1 pyramidal neurons in an in vitro hippocampal slice prep-

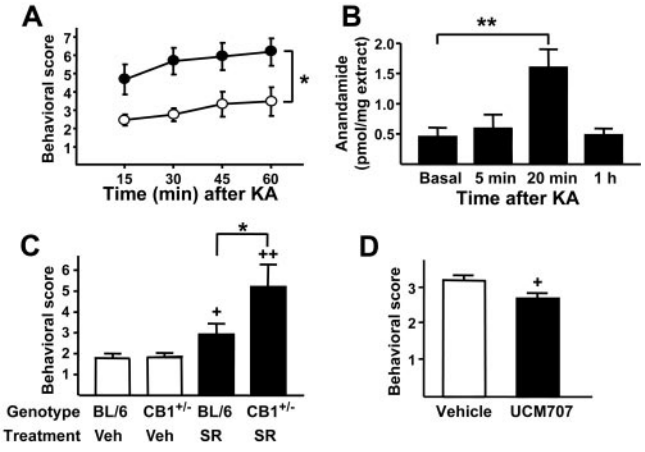

Fig. 1. The endogenous cannabinoid system is activated by KA and protects against seizures. (**A**) Seizure scoring (30 mg/kg of KA) of $CB1^{+/+}$ mice (open circles, $n = 7$) and $CB1^{-/-}$ mice (solid circles, $n = 8$). Higher scores indicate more severe seizures. (**B**) Levels of hippocampal anandamide at different time points after KA injection into C57BL/6N mice (30 mg/kg, $n = 5$ mice per group). (**C**) Effects of the CB1 receptor antagonist SR141716A (SR, solid bars) and the vehicle (Veh, open bars) on seizure scoring (20 mg/kg of KA) in C57BL/6N mice (BL/6, $n = 6$ per group) and in $CB1^{+/-}$ mice ($n = 6$ per group). (**D**) Effects of the anandamide uptake inhibitor UCM707 (solid bar) and the vehicle (open bar) on seizure scoring in C57BL/6N mice (35 mg/kg of KA, $n = 23$ to 24 per group). Means ± SEM; *, $P < 0.05$; **, $P < 0.01$; +, $P < 0.05$; ++, $P < 0.01$ versus respective vehicle-treated groups.

aration from CB1$^{f/f;CaMKII\alpha Cre}$ and CB1$^{f/f}$ littermates before (Fig. 3A) and after (Fig. 3B) bath application of 150 nM KA (*13*). At this concentration, KA did not significantly change the excitation of neurons obtained from CB1$^{f/f}$ mice. We monitored neuronal excitation as the spontaneous excitatory postsynaptic currents (EPSCs, relative excitation: 4 ± 2, $P > 0.05$, versus the baseline) (Fig. 3C). In contrast, neurons obtained from CB1$^{f/f;CaMKII\alpha Cre}$ mice showed strong excitation under these conditions (relative excitation: 17 ± 4, $P < 0.05$, versus the baseline) (Fig. 3C), which was accompanied by an increase in the frequency of EPSCs (frequency: 4.5 ± 0.5 Hz versus a baseline of 1.0 ± 0.1 Hz, $P < 0.01$).

KA-induced intracellular events. Several intracellular pathways have been implicated in the development of KA-induced excitotoxicity (*21*). In the hippocampus, injection of KA activates various kinases, including extracellular-regulated kinases (ERKs) (*21*), at different time points. Because CB1 receptor agonists stimulate the phosphorylation of ERKs (*2*), we isolated hippocampi derived from CB1$^{f/f;CaMKII\alpha Cre}$ and CB1$^{f/f}$ littermates 75 min after injection of KA (15 mg/kg) or saline, then quantified the levels of CB1 receptor–mediated activation of ERKs by Western blotting (*13*). Administration of KA induced a significant increase in phosphorylation of both p42 (phospho-p42) and p44 (phospho-p44) ERKs in CB1$^{f/f}$ mice (phospho-p42: to 173.0 ± 21.2%, $P < 0.05$; phospho-p44: to 220.1 ± 36.1%, $P < 0.01$) (Fig. 4, A and B), whereas there was no significant difference between KA- and saline-treated CB1$^{f/f;CaMKII\alpha Cre}$ mice (phospho-p42: to 101.1 ± 9.8%, $P > 0.05$; phospho-p44: to 144.0 ± 36.9%, $P > 0.05$) (Fig. 4, A and B).

KA administration rapidly induces expression of immediate early genes (IEGs) such as c-*fos* or *zif268* (*22*). This induction depends, at least in part, on the activation of ERKs (*23*). In particular, the activation of the c-*fos* gene plays a central role in protection against KA-induced excitotoxicity (*24*). Because the pharmacological stimulation of CB1 receptors induces the expression of these IEGs (*2*, *25*), we analyzed by in situ hybridization (*13*) the levels of c-*fos* and *zif268* transcripts in hippocampi from CB1$^{f/f;CaMKII\alpha Cre}$ and CB1$^{f/f}$ littermates 75 min after KA or saline injection. In saline-injected mice, the hippocampal levels of c-*fos* (Fig. 4, C, E, and O) and *zif268* transcripts (Fig. 4, G, I, and P) were similar between genotypes. However, all subregions of the hippocampi derived from KA-treated CB1$^{f/f}$ mice showed markedly increased levels of both c-*fos* (Fig. 4, D and O) and *zif268* transcripts (Fig. 4, H and P). In the hippocampi derived from KA-treated CB1$^{f/f;CaMKII\alpha Cre}$ mice, the induction of

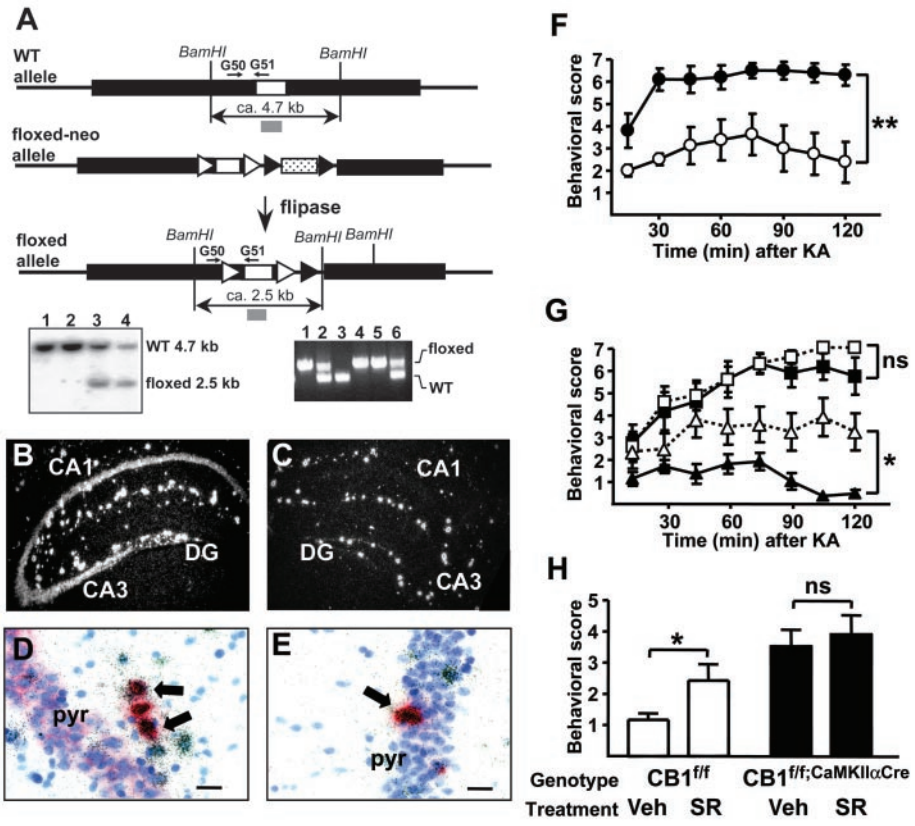

Fig. 2. Activation of CB1 receptors on principal forebrain neurons mediates protection from seizures. (**A**) Generation of the CB1$^{f/f}$ mouse line. Open box, the *CB1* open reading frame; dotted box, the phosphoglycerate kinase–neomycin phosphotransferase (*PGK-Neo*) selection cassette; open triangles, *loxP* sites; solid triangles, FLP recombinase recognition target (*FRT*) sites; gray box, the probe for Southern blot analysis; small arrows, primers for polymerase chain reaction (PCR) genotyping. Bottom left: Southern blot analysis showing CB1$^{+/+}$ mice (lanes 1 and 2) and CB1$^{f/+}$ mice (lines 3 and 4) obtained after FLP recombinase–mediated excision of *PGK-Neo* cassette. Bottom right: PCR analysis of CB1$^{f/f}$ (lanes 1, 4, and 5), CB1$^{f/+}$ (lanes 2 and 6), and CB1$^{+/+}$ (lane 3) mice. WT, wild-type; BamHI, endonuclease recognition site; G50 and G51, PCR primers (*12*, *13*); flipase, FLP recombinase. (**B** and **C**) Expression of *CB1* mRNA (dark-field) in hippocampi from (B) CB1$^{f/f}$ and (C) CB1$^{f/f;CaMKII\alpha Cre}$ mice. The CA1, CA3, and DG regions of the hippocampus are marked. (**D** and **E**) Expression of *CB1* mRNA (red staining), in combination with the GABAergic-specific marker *GAD65* (silver grains) in the CA3 region of the hippocampus in (D) CB1$^{f/f}$ and (E) CB1$^{f/f;CaMKII\alpha Cre}$ mice. *CB1* mRNA is present in pyramidal neurons in CB1$^{f/f}$ but not in CB1$^{f/f;CaMKII\alpha Cre}$ mice. Pyr, the CA3 pyramidal layer; arrows, interneurons co-expressing *CB1* and *GAD65*; blue stain, toluidine-blue counterstaining. Scale bars, 20 μm. (**F**) Seizure scoring (30 mg/kg of KA) of CB1$^{f/f}$ mice (open circles, $n = 8$) and CB1$^{f/f;CaMKII\alpha Cre}$ mice (solid circles, $n = 10$). (**G**) Effects of the anandamide uptake inhibitor UCM707 (3 mg/kg, solid symbols) and the vehicle (open symbols) on seizure scoring (30 mg/kg of KA) of CB1$^{f/f}$ mice (triangles, $n = 9$ per group) and CB1$^{f/f;CaMKII\alpha Cre}$ mice (squares, $n = 7$ per group). (**H**) Effects of the CB1 receptor antagonist SR141716A (3 mg/kg) on seizure scoring (20 mg/kg of KA) of CB1$^{f/f}$ mice (open bars, $n = 12$ to 14 per group) and of CB1$^{f/f;CaMKII\alpha Cre}$ mice (solid bars, $n = 11$ per group). Means ± SEM; *, $P < 0.05$; **, $P < 0.01$; ns, not significant.

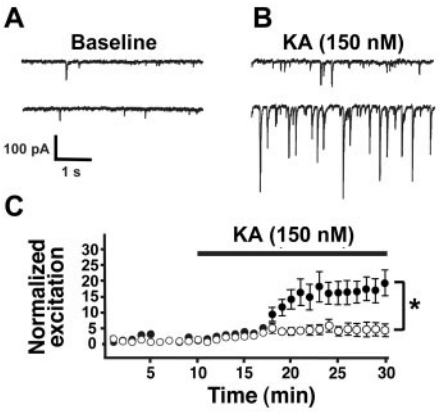

Fig. 3. On-demand activation of the endogenous cannabinoid system dampens KA-induced excitation of CA1 hippocampal pyramidal neurons. (**A**) Representative traces of CB1$^{f/f}$ (upper) and CB1$^{f/f;CaMKII\alpha Cre}$ (lower) neurons, before KA application. (**B**) Representative traces of the same neurons 20 min after KA application. (**C**) Normalized excitation values over the course of the experiments. Open circles, CB1$^{f/f}$ (7 cells from 2 mice); solid circles, CB1$^{f/f;CaMKII\alpha Cre}$ (6 cells from 2 mice). Bar represents duration of bath application of KA. Means ± SEM; *, $P < 0.05$.

c-*fos* (Fig. 4, F and O) and *zif268* expression (Fig. 4, J and P) was abolished.

The brain-derived neurotrophic factor (BDNF) exerts neuroprotective functions (*26*, *27*) and participates in c-*fos*–dependent neuronal protection against KA-induced excitotoxicity (*24*). We measured *BDNF* messenger RNA (mRNA) levels by in situ hybridization in the hippocampi of the same mice used for the analysis of c-*fos* and *zif268* expression (*13*). In saline-treated mice, *BDNF* mRNA was expressed at moderate levels in all subregions of the hippocampus (Fig. 4, K, M, and Q). Slightly but significantly lower levels of *BDNF* were observed in the CA3 region of $CB1^{f/f;CaMKII\alpha Cre}$ mice, possibly indicating a role of CB1 receptors in the basal control of *BDNF* expression (Fig. 4Q). In KA-treated $CB1^{f/f}$ mice, *BDNF* expression was strongly enhanced compared to that of saline-treated littermates in all hippocampal subregions (Fig. 4, L and Q). However, as with c-*fos* and *zif268*, no increase of *BDNF* expression was observed in KA-treated $CB1^{f/f;CaMKII\alpha Cre}$ mice as compared to saline-treated controls (Fig. 4, N and Q).

Long-term effects. Excitotoxic stimuli lead to neuronal cell death through the activation of several molecular pathways (*28*). To test the involvement of the endogenous cannabinoid system in protection against the long-term effects of KA, surviving $CB1^{f/f}$ and $CB1^{f/f;CaMKII\alpha Cre}$ mice were killed 4 days after the injection of 20 mg/kg of KA. The degree of neuronal damage in their hippocampi was evaluated by staining with terminal deoxynucleotide transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) (*13*). KA-treated $CB1^{f/f;CaMKII\alpha Cre}$ mice showed significantly higher levels of TUNEL staining in the CA1 and CA3 regions of the hippocampus ($P < 0.05$) (fig. S3, A to C), indicating higher levels of neuronal damage. Immunostaining for glial fibrillary acidic protein (*13*) in the same hippocampi revealed increased levels of gliosis in KA-treated mutants ($P < 0.05$) (fig. S3, D to F).

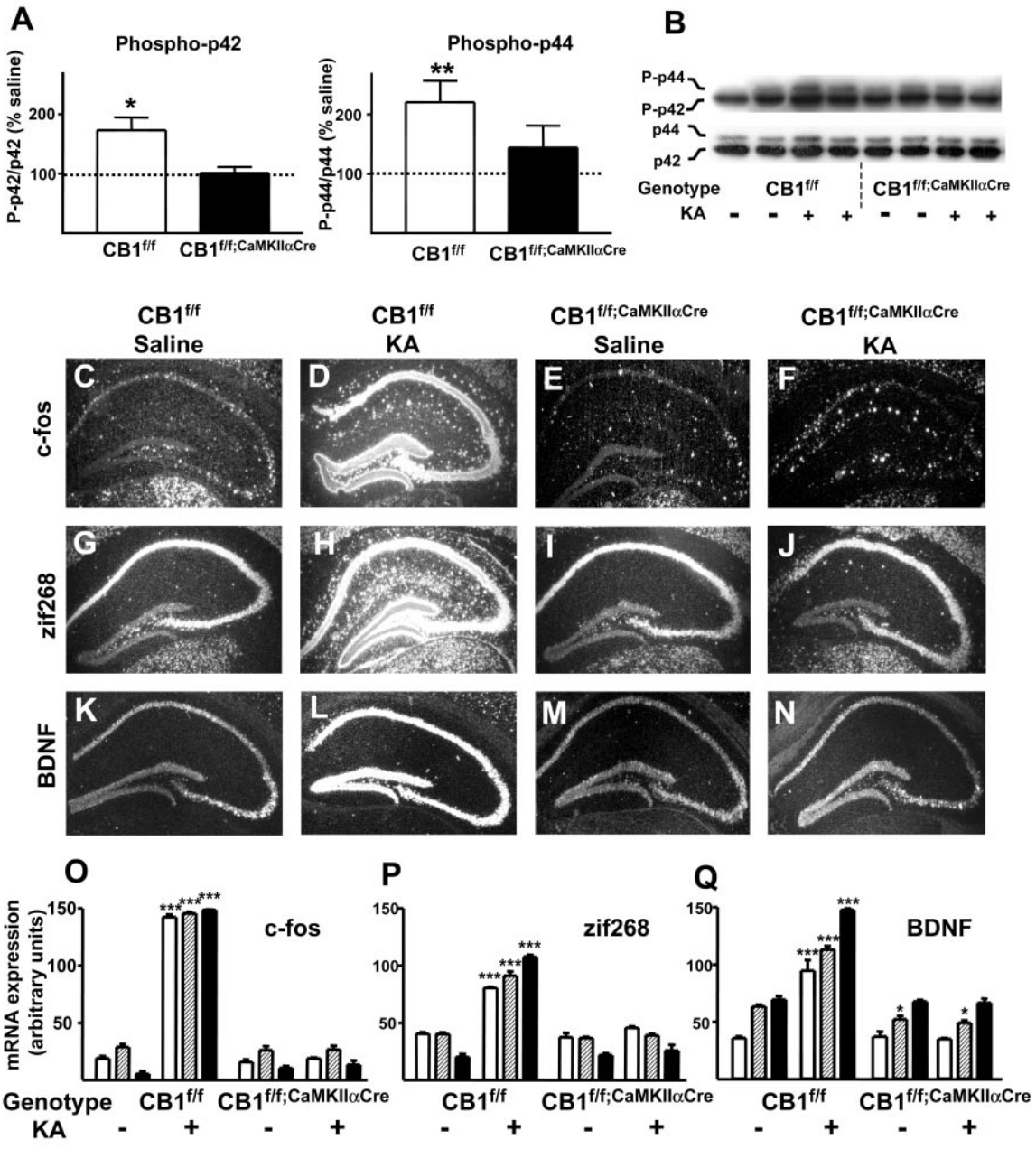

Fig. 4. On-demand activation of the endogenous cannabinoid system in principal hippocampal neurons is required to induce protective molecular cascades. (**A**) Densitometric quantification of KA-induced ERK phosphorylation in $CB1^{f/f}$ (open bars) and $CB1^{f/f;CaMKII\alpha Cre}$ (solid bars) mice, relative to saline-treated littermates (100%, dotted lines); *, $P < 0.05$; **, $P < 0.01$ versus respective controls; $n = 5$ to 6 mice per group. P-p42 and P-p44, phospho-p42 and phospho-p44. (**B**) Representative Western blots of phosphorylated ERKs (P-p42 and P-p44) and total ERKs (p42 and p44). (**C** to **N**) Representative dark-field micrographs showing expression of [(C) to (F)] c-*fos*, [(G) to (J)] *zif268*, and [(K) to (N)] *BDNF* mRNA in $CB1^{f/f}$ and $CB1^{f/f;CaMKII\alpha Cre}$ mice, 75 min after injection of KA (15 mg/kg) or saline. The dark halos in (D) and (H) are artifacts due to the excessive presence of silver grains. (**O** to **Q**) Densitometric quantification from autoradiographic films for mRNA expression of (O) c-*fos*, (P) *zif268*, and (Q) *BDNF* in the CA1 (open bars), CA3 (hatched bars), and DG (solid bars) regions of the hippocampus ($n = 5$ to 6 mice per group). Means ± SEM; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ versus saline-treated $CB1^{f/f}$.

Discussion. Taken together, these results show that endogenous activation of CB1 receptors in principal forebrain neurons promotes neuronal survival during excitotoxicity. Activation of CB1 receptors on principal forebrain neurons mediates the prominent protective role, whereas CB1 receptors on GABAergic interneurons exert only a negligible function. Considering that in other behavioral paradigms, CB1 receptors on GABAergic interneurons have been proposed to play a crucial role (*2*, *12*, *18*), our data further underline the diverse functions of the endogenous cannabinoid system in different neuronal processes.

Anandamide levels rapidly increase after KA administration and protect against excitotoxicity. The mechanisms inducing this rise in anandamide levels in the adult mouse brain are still to be determined, but they are more likely to rely on enhanced production and/or decreased degradation of this endocannabinoid than on enhanced synthesis of its biosynthetic precursors (*29*).

Cell-type specificity and dynamic regulation appear to be fundamental features of this highly efficient physiological protection system. It has been reported that pharmacological treatment of mice with CB1 receptor agonists and genetic enhancement of endocannabinoid tissue concentrations can increase susceptibility to KA-induced seizures (*10*). Some of these findings may be attributed to the lack of spatial and temporal specificity of CB1 receptor activation (i.e., CB1 receptors on both GABAergic and glutamatergic neurons are probably activated simultaneously by pharmacological application of agonists or by genetic enhancement of anandamide levels). We were able to observe significant protection induced by the anandamide uptake inhibitor UCM707 in wild-type animals but not in $CB1^{f/f;CaMKII\alpha Cre}$ mice, indicating that an enhancement of anandamide concentration at sites of synthesis is pivotal for physiological protection. The increased ability of KA to induce neuronal excitation mediated by spontaneous EPSCs in $CB1^{f/f;CaMKII\alpha Cre}$ hippocampal slices indicates a presumable CB1 receptor–mediated control of the presynaptic release of L-glutamate. CB1 receptor activation is known to induce hyperpolarization of neuronal membranes, mainly by increasing K^+ and decreasing Ca^{2+} conductance (*2*). Such a hyperpolarization, caused by an autocrine or paracrine activation of CB1 receptors by endocannabinoids (presumably anandamide), would also decrease the L-glutamate release evoked during excitotoxicity, as indicated by the higher frequency of EPSCs in $CB1^{f/f;CaMKII\alpha Cre}$ hippocampal principal neurons. Previous immunohistochemical experiments in rodent hippocampus could not detect CB1 protein associated with glutamatergic synapses (*18*). Thus, it remains to be clarified in which compartment of the projecting neurons the endogenous cannabinoid system acts. An additional postsynaptic site of action of the endocannabinoid system cannot be excluded.

CB1 receptors mediate protection against excitotoxicity not only by dampening the neuronal excitability of pyramidal neurons but also by inducing intracellular cascades, including ERK phosphorylation and the expression of IEGs that code for transcription factors (c-*fos* and *zif268*) and neurotrophins (such as BDNF). The two separate mechanisms may act in concert to provide protection against the consequences of excessive neuronal activity. Whereas lowering neuronal excitability by hyperpolarization provides rapidly available protection, the activation of the intracellular cascades might contribute to long-term adaptive cellular changes in response to the excitotoxic insult in neuronal circuits (*24*). Nevertheless, rapid effects of ERK activation or IEG expression after KA application might also contribute to the early adaptive reactions.

There is evidence from different neuropathological models that the endogenous cannabinoid system can be differentially activated in a species- and age-dependent manner (*30*–*35*) or even through non-CB1 receptor–mediated mechanisms (*36*). For instance, brain trauma induced an increase of 2-arachidonoyl-glycerol levels in adult mice (*31*), whereas in a similar experimental model in neonatal rats, the tissue concentrations of anandamide but not of 2-arachidonoyl-glycerol were increased (*37*). In neonatal rats, blocking of CB1 receptors with SR141716A induced a "paradoxical" protection against *N*-methyl-D-aspartate–induced neurotoxicity (*11*), whereas exogenous anandamide was protective in a model of ouabain-induced neurotoxicity in the same species at the same age (*7*, *34*). The reasons for these apparent discrepancies are not clear. Different processing of endocannabinoids in different species and at different developmental stages (*29*), different experimental conditions (such as the method of inducing neurotoxicity and the parameters monitored), or differences in neuronal circuitries at different ages (*38*) may be responsible for some of these divergent findings.

Our results establish the CB1 receptor–dependent activation of the endogenous cannabinoid system as a rapidly activated early step in a protective cascade against excitotoxicity in the adult mouse brain. The endogenous cannabinoid system might become a promising therapeutic target for the treatment of neurodegenerative diseases with excitotoxic events as their hallmarks (*1*, *39*–*41*).

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Supporting Online Material

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