Supplementary Methods

Contextual fear conditioning

Immediately after the 6-week feeding period, contextual fear conditioning test and extinction session were conducted as previously described (Yamada *et al*, 2009). Briefly, mice were acclimated to the test room for 2 h before fear conditioning, placed in the conditioning chamber (20 × 20 cm floor, 35 cm high; Muromachi Kikai, Tokyo, Japan), and conditioned with a single electrical footshock (0.8 mA, 2 s duration). After 1 h or 24 h, the mice were re-exposed to the chamber for 3 min without footshock to measure the freezing response. In the extinction experiment, the exposure session lasted for 10 min for both extinction training (24 h after conditioning) and test (48 h after conditioning). The freezing response is expressed as the percentage of time that the mouse spent freezing during a 180 s time window.

Analysis of fatty acids

Lipids were extracted by homogenizing tissue samples in a 4:1 (v/v) methanol/hexane solution containing 50 μg/ml butylated hydroxytoluene as an antioxidant. Methyl docosatrienoate (22:3n-3) was used as an internal standard. Fatty acids were transmethylated with 2 ml of a lipid homogenate containing acetyl chloride (200 μl) at –80°C for 1 h under nitrogen. Gas chromatographic analysis of fatty acid methyl esters was performed on an Agilent 6890N network gas chromatography system (Agilent Technologies Ltd., Tokyo, Japan) equipped with a split injector, flame ionizing detector and a fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 mm film thickness; Agilent Technologies). Fatty acid methyl esters were identified by co-chromatography with a purified standard mixture (462, Nu-Chek Prep, Inc., Elysian,

MN, USA). Fatty acid data are expressed as the percentage peak area corresponding to the weights of individual fatty acids. Analysis of the fatty acid composition of diets was performed by Japan Diet Research Laboratories (Tokyo, Japan).

Quantitative real-time PCR of CB_1 receptor and diacylglycerol-α (DGLα) in the mouse brain

After mice were anesthetized with halothane and blood was collected, the animals were then perfused with phosphate-buffered saline (PBS) for several minutes. The forebrain was quickly dissected, the basal ganglia and hippocampus were removed from the cerebrum, and the remaining tissue was stored at –80°C until use. Total RNA was isolated from the tissue using the RNeasy lipid tissue mini kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. cDNA synthesis was carried out from 1 μg of total RNA using a PrimeScript II first-strand cDNA synthesis kit (Takara Bio, Inc., Otsu, Japan). Real-time quantitative PCR was done using SYBR premix Ex Taq II (Takara Bio, Inc.) with an ABI7500 real-time PCR system (Applied Biosystems). β-actin was used as an internal standard. The sequences of PCR primers for each of the genes analyzed are described below. CB_1 receptor (GenBank Accession No. NM_007726): forward primer (F), 5'-AAGTCGATCTTAGACGGCCTT-3', reverse primer (R), 5'-TCCTAATTTGGATGCCATGTCTC-3'; DGLα (GenBank Accession No. NM_198114): F; 5'-TTCGCCGAGTTCATTGACAG-3', R; 5'-TCTCAGGCACCATCATGCA-3', β-actin (GenBank Accession No. NM_007393): F, 5'-GTGACGTTGACATCCGTAAAGA-3', R, 5'-GCCGGACTCATCGTACTCC-3'.

Slice preparation and Patch-clamp recordings

Mice were anesthetized with halothane and the brain was removed quickly. When recordings were performed from the BLA, coronal brain slices (300 μm thick) were prepared using a linear slicer Pro 7 (Dosaka EM Co., Ltd., Kyoto, Japan) in aCSF (in mM): 125 NaCl, 4.4 KCl, 1.5 $MgSO_4$, 1.0 NaH_2PO_4, 26 $NaHCO_3$, 10 glucose, 2.5 $CaCl_2$, pH7.4, 290–300 mOsm/L. The slices were maintained at 37°C for at least 30 min before recordings in aCSF continuously bubbled with 95% O_2/5% CO_2. A slice was transferred to the recording chamber and was perfused (3 ml/min) with aCSF maintained at 28–32°C. Patch electrodes (resistance 4–7 MΩ) were filled with a solution containing the following (in mM): 132 K-gluconate, 3 KCl, 10 HEPES, 0.5 EGTA, 1 $MgCl_2$, 12 sodium phosphocreatine, 3 ATP magnesium salt, 0.5 GTP, pH 7.4, with KOH, 285–290 mOsm/L. The electrophysiological signal was amplified and filtered at 5 kHz using a MultiClamp 700B patch-clamp amplifier (Axon Instruments, Union City, CA, USA). Data were digitized at 50 kHz and acquired using Clampex software (version 9.2, Axon Instruments). The access resistance, which was frequently checked during recording, was between 7 and 24 MΩ, and cells with a large drift (±20%) in resistance were excluded from the analysis. Because the reversal potential of $GABA_A$ receptor mediated synaptic currents was estimated between –60 and –70 mV when this pipette solution was used, we used a holding potential of –60 mV in voltage-clamp mode to minimize

the contribution of $GABA_A$ currents in the synaptic response. Synaptic responses were elicited by electrical stimulation through an aCSF-filled double-barrel glass pipette (5–10 μm tip diameter), which was placed within 100 μm of the soma of a recording BLA pyramidal neuron. Stimulation intensity was set at 30–50% of the maximal synaptic response amplitude. eEPSC was recorded (at 0.067 Hz) for 5 min before and 10 min after the bath application of the CB_1 agonist WIN, to check the activity of the CB_1 receptor. Before recording, some slices were incubated for 30 min in the presence of 5 mM MCD to expel cholesterol from the plasma membranes (Bari *et al*, 2005a; Maccarone *et al*, 2009). After MCD treatment, slices were gently washed in aCSF and used for recordings. The DSE test consisted of 10 stimuli (50–100 μs duration at 0.5 Hz) before and 10 stimuli after postsynaptic depolarization from –60 to 0 mV for 10 s (Ohno-Shosaku *et al*, 2002; Puente *et al*, 2011). The degree of DSE was calculated as the amplitude of one evoked response just after depolarization (post) divided by the average of 10 evoked responses just prior to depolarization (baseline). Peak amplitudes of eEPSCs were measured as the peak inward current of the response. Some slices were pre-incubated with the CB_1 antagonist RIM (5 μM) at least 30 min before the DSE test.