Supplemental Materials

Materials and Methods

Low-power images for immunofluorescence and *in situ* hybridization were taken with a dissecting microscope (SZX-12, Olympus) or a bright-field microscope (AX70, Olympus) equipped with a digital camera (DP70, Olympus).

For immunoblot, adult mouse brains were freshly removed from the skull under deep pentobarbital anesthesia (100 mg/kg of body weight, i.p.). They were homogenized using a Potter homogenizer with 15 strokes at 1000 r.p.m. in 10 volumes of ice-cold homogenizer buffer containing 0.32 M sucrose, 1 mM EDTA, 1 mM EGTA, 10 mM Tris-HCl (pH 7.2), and 0.4 mM phenylmethylsulfonyl fluoride. The protein concentration was determined by the Lowry's method. The homogenates were denatured by 50 mM (±)-dithiothreitol at 65 °C for 15 min. Proteins (20 μg/lane) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and electroblotted onto nitrocellulose membranes (BioTraceNT; PALL, Ann Arbor, MI, USA). After blocking with 5% skimmed milk for 30 min, membranes were incubated for 2 hr with the primary antibodies (1 μg/ml). Tris-buffered saline (pH 7.5) containing 0.1% Tween 20 was used for diluent and washing buffer. Immunoreaction was visualized with the ECL chemiluminescence detection system (Amersham Biosciences).

Under deep anesthesia, brains for *in situ* hybridization were freshly taken and immediately frozen in powdered dry ice. Frozen sections were prepared on a cryostat (20 μm in thickness; CM1900; Leica, Nussloch, Germany), mounted on silane-coated glass slides (Muto-Glass, Tokyo, Japan), air-dried, and stored at -80 ˚C until use for hybridization. Probes were synthesized against nucleotide residues 198-242, 631-675, 839-883 and 1081-1125 of the mouse CB_1 cDNA (Genbank, U22948), 1381-1430 of the

mouse tyrosine hydroxylase cDNA (M69200), 136-180 of the mouse serotonin transporter cDNA (AF013604). They were labeled using terminal deoxyribonucleotidyl transferase (Invitrogen, Carlsbad, CA). Sections were treated at room temperature with the following incubation media: 4% paraformaldehyde in 0.1M sodium phosphate buffer (pH 7.2) for 10 min, 2 mg/ml glycine-phosphate-buffered saline (pH 7.2) for 10 min, acetylation with 0.25% acetic anhydride in 0.1 M triethanolamine-HCl (pH 8.0) for 10 min, and prehybridization buffer containing 50% formamide, 50 mM Tris-HCl (pH 7.5), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 0.6 M NaCl, 0.25% SDS, 200 □g/ml tRNA, 1 mM EDTA, and 10% dextran sulfate for 1 hr. Hybridization was performed at 42 °C for 12 hr in the prehybridization buffer supplemented with a mixture of [^{33}P]dATP-labeled antisense oligonucleotide probes (10,000 cpm/μl for each). After confirming the same pattern of hybridization, four CB_1 probes were mixed into cocktail to increase detection sensitivity. Slides were washed twice at 55 °C for 40 min in 0.1 x SSC containing 0.1% sarcosyl. Sections were exposed to NTB-2 nuclear track emulsion (Kodak, Rochester, NY) for 4 weeks.

See procedures for immunofluorescence in the main text.

Figure Legends

Supplemental Fig. S1. Specificity of antibody against DAGLα (***A-C***), $mGluR_5$ (***D-F***), or CB_1 (***G-I***). ***A, D, G***. Immunoblot with rabbit DAGLα, rabbit $mGluR_5$, and guinea pig CB_1 antibodies using protein extracts from the adult mouse brain. The size of standard protein markers is shown to the left (kDa). Note selective detection of single protein bands at 105 kDa, 160kDa, and 47 kDa, respectively, whose size is consistent with

previous reports (Shigemoto et al., 1997; Fukudome et al., 2004; Yoshida et al., 2006). ***B, E, H***. Immunofluorescence in parasagittal forebrain sections. ***C, F, I***. Immunofluorescence in coronal forebrain sections. In the basal ganglia, DAGLα and $mGluR_5$ are high to moderate in the striatum but low in the globus pallidus and substantia nigra, whereas CB_1 is very high in the substantia nigra (SN), high in the globus pallidus (GP), and moderate in the striatum (St). Within the striatum, CB_1, and DAGLα immunoreactivities show higher distribution in the dorsolateral portion than in the remaining portions. These regional distributions are consistent with previous studies by ligand binding, *in situ* hybridization, and immunohistochemistry (Herkenham et al., 1991; Mailleux and Vanderhaeghen, 1992; Shigemoto et al., 1993; Matsuda et al., 1993; Testa et al., 1994, 1995; Pettit et al., 1998; Tsou et al., 1998; Katona et al., 1999; Egertova and Elphick, 2000; Hohmann and Herkenham, 2000; Matyas et al., 2006; Yoshida et al., 2006). Insets indicate the lack of immunofluorescent signals with use of DAGLα antibody preabsorbed with the antigen (***B***) and in gene knockout brain lacking $mGluR_5$ (***E***), or CB_1 (***H***). Cx, cortex; Hi, hippocampus; Th, thalamus. Scale bars, 1 mm.

Supplemental Fig. S2. $mGluR_1\alpha$ is low in medium spiny (MS) neurons and high in parvalbumin-positive interneurons. ***A***. Immunofluorescence for $mGluR_1\alpha$ in a parasagittal forebrain section showing that $mGluR_1\alpha$ is high in the thalamus, moderate in the neocortex, globus pallidus and hippocampus, and low in the striatum. This characteristic immunolabeling is consistent with previous reports (Martin et al., 1992, Shigemoto et al., 1992; Testa et al., 1994, 1995, 1998; Petralia et al., 1997, Berthele et al., 1998). ***B***. Triple immunofluorescence for $mGluR_1\alpha$ (red), D_1R (green), and D_2R (blue). Most perikarya of D_1R- and D_2R-positive neurons, which are very likely to be

MS neurons, are almost negative for $mGluR_1\alpha$. However, $mGluR_1\alpha$-positive puncta with low intensity are distributed around the surface of D_1R- and D_2R-positive elements in the neuropil, suggesting low $mGluR_1\alpha$ expression in MS neurons. Asterisk and arrowheads indicate soma and dendrites, respectively, of putative parvalbumin (PV)-positive interneurons, which are intense for $mGluR_1\alpha$ and express D_2R. ***C-E***. Double immunofluorescence for $mGluR_1\alpha$ (red) with PV (***C***, green), high-affinity choline transporter (***D***, CHT, green), or nitric oxide synthase (***E***, NOS, green). Note that $mGluR_1\alpha$ is intense in PV interneurons (asterisk and arrowheads), and moderate in CHT-positive cholinergic interneuron (asterisk) and NOS-positive interneuron (asterisk and arrowhead). Scale bars, ***A***, 1mm, ***B-E***, 10 μm.

Supplemental Fig. S3. Effect of WIN 55,212-2 in the presence of THL. ***A***. Representative data demonstrating suppression of IPSC by WIN 55,212-2 in the presence of THL (10 μM) following preincubation with THL (20 μM). Each point represents the average of 3 consecutive traces. Depolarizing pulses with duration of 5 s was applied before WIN 55,212-2 (1 μM) application at the time points indicated with upward arrows. *Inset*, example traces of IPSCs showing no DSI and WIN 55,212-2-induced suppression. Traces before and after treatment are superimposed. Calibration bars, 100 pA and 10 ms. ***B***. Summary bar graph for WIN-induced suppression of IPSCs. Bars represent effects of WIN 55,212-2 in control solution and in the presence of THL (10 μM) following preincubation with THL (20 μm). Effects are shown as the percentage of the control values before WIN application. Numbers of tested cells are indicated in parentheses.

Supplemental Fig. S4. Multiple immunofluorescence for CB_1 (red) with various neurochemical markers (green). ***A-G***. The lack of CB_1 staining in semithin cryosections prepared from the CB_1-knockout striatum. These images were taken as negative control experiments for Fig. 8B-H in the main text. ***H-L***. CB_1 distribution in particular striatal neurons. Weak CB_1 immunoreactivity is present in neuronal perikarya and terminals labeled for glutamic acid decarboxylase (GAD, arrows, ***H***), but absent from those labeled for high-affinity choline transporter (CHT, ***I***; Narushima et al., 2007) and calretinin (CR, ***L***). CB_1 is detected at extremely low levels in perikarya of nitric oxide synthase (NOS)-positive interneurons (arrows, ***J***), and in their axonal terminals labeled for NOS and VGAT (arrowheads, ***K***). Scale bars, ***A-G, K***, 2 μm; ***H-J, L***, 10 μm.

Supplemental Fig. S5. *In situ* hybridization for CB_1 mRNA in the striatum and neural regions projecting to the striatum. CB_1 mRNA (***A-G, I, J, L, M, O***), tyrosine hydroxylase (TH) mRNA (***H, N***), and plasmalemmal serotonin transporter (HTT) mRNA (***K***). Emulsion-dipped sections were photographed in the dark-field (***A, C, E, G, H, J, K, M, N***) or bright-field (***B, D, F, I, L, O***) microscopy. In bright-field micrographs, sections were counterstained with pyronine. Principal neurons in respective regions are labeled either positively (arrows) or negatively (arrowheads). ***A, B***. Striatum (St). Note a dorsolateral-to-ventromedial gradient in CB_1 mRNA expression. ***C, D***. Cortex (Cx). Moderate expression is seen on pyramidal neurons in the layer V. ***E, F***. Parafascicular thalamic nucleus (PF). ***G-I***. Substantia nigra pars compacta (SNc). ***J-L***. Dorsal raphe nucleus (DR). ***M-O***. Locus ceruleus (LC). In this picture, CB_1 mRNA is detected in small-to-medium neurons of the LC (arrows), but not in large neurons (arrowheads),

suggestive of CB_1 mRNA expression in interneurons. Scale bars, ***A, C, E, G, H, J, K***, 1 mm; ***M, N***, 500 μm; ***B, D, I, L, O***, 20 μm; ***F***, 10 μm.

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