SUPPLEMENTARY FIGURES

Figure S1, related to Figure 1. TBS (arrowhead) does not induce iLTD in layer II/III pyramidal cells in the presence of the PLC blocker U73122 (A) or in the presence of the mGluR5 antagonists MPEP (B). Symbols: open and filled circles in A are data obtained with 0.01% DMSO and 10μM U73122, respectively. Open and filled circles in B are data with ACSF and 10 μM MPEP, respectively. Numbers in parentheses indicate the number of rats and cells.

Figure S2, related to Figure 2. Developmental loss of iLTD is not due to the inability to detect iLTD A) High concentration of extracellular Ca^{2+} enhances transmission in cells from both 3 week (open circles) and 5 week (filled circles) old rats, but it does not rescue iLTD in 5 week-old rats. B) TBS continues to induce a decrease in PPD in 3 week-old, but not 5 week-old rats, in the presence of high extracellular Ca^{2+}.

Figure S3, related to Figure 3. Dark-rearing increases CB1R level in visual cortex.
(A) Dark-rearing 3-weeks-old mice for 2 weeks (3N2D) increased CB1R level compared to age-matched 5-weeks-old normal-reared (5N). Top left: CB1R antibody signal was absent in the visual cortex sample from CB1R KO. Top right: A representative immunoblot showing an increase in CB1R signal in 3N2D cortex. *: t-test, $p<0.005$
(B) Unlike CB1Rs, the 1-subunit of GABA-A receptor levels did not change in the visual cortex of dark-reared group compared to normal-reared (5N: 100 ± 3.5% of

average NR, 3N2D: 94 ± 5.0% of average NR, n = 5 each; t-test, p=0.38). Top: A representative immunoblot.

Figure S4, related to Figure 5. Age and endocannabinoids reduce the variability of the IPSCs evoked by prolonged irregular stimulation. The stimulus consisted of 150 pulses with Poisson distributed inter-stimulus intervals (Average frequency: 30 Hz). A) Example of IPSC's recorded in cells from a 3 week-old rat (3W: black line) and from a 5 week-old rat (5W: red line). The traces are averages of 20 consecutive normalized responses showing the first and last 0.5 sec of the train. Stimulus pulses indicated by inverted triangles. B) Same as in A, but showing the effects of WIN. The average traces were recorded before (vehicle control: black line) and 20 min after bath application of WIN (10μM, 10 min), chased with AM251 (10 μM, red line). C) Effects of age and WIN on the relative amplitude of IPSCs evoked by the 150 pulse stimulation train in cells from 3 week-old (grey circles) and 5 week-old rats (red circles), as well as before (Control: blue circles) and after WIN application (WIN: yellow circles). The bottom graph shows the differences between the curves obtained with cells from 5 and 3 weeks rats (red), and before and WIN application (yellow) In both cases the differences were larger than zero most of the times. D,E) Changes in the cumulative probability distribution of the IPSC's amplitude associated with age (D. 3 weeks: black; 5 weeks: red) and WIN (E. Before: black; after: red). F,G) Changes in the coefficient of variation (CV) associated with age (F) and WIN application (G). Open circles represent individual cells; filled circles, averages. The IPSC amplitude in C-E was determined by fitting IPSCs templates (average of 20 responses taken at 1 Hz before the trains) to the average of 15 to 20 train

responses. The CVs and the amplitude distribution in D-G were computed with IPSC's colleted during the last second of the train (shaded area in C), representing a steady state condition.

Figure S5, related to Figure 7. Endoannabinoid modulation of synaptic transmission from fast-spiking interneuron to pyramidal cell in layer II/III in rat visual cortex. A) Example of high-frequency firing without accommodation of a fast-spiking interneuron evoked by a 500 msec depolarizing current pulse. B) Average traces for ten responses recorded before (grey) and 20 min after (black) bath application of WIN (10 μM). Top: interneuron action potentials; bottom, corresponding pyramidal cell IPSCs. C) Effects of 10 μM WIN on uIPSCs recorded in cell pairs from 3 week-old rat (filled circles). Open symbols show the stability of IPSC amplitude in the absence of WIN. D) Decrease in paired pulse depression induced by exposure to WIN. E) CV analysis. For each cell the normalized changes in $1/CV^2$ induced by WIN are plotted against the corresponding normalized changes in uIPSC amplitude.

Supplementary methods

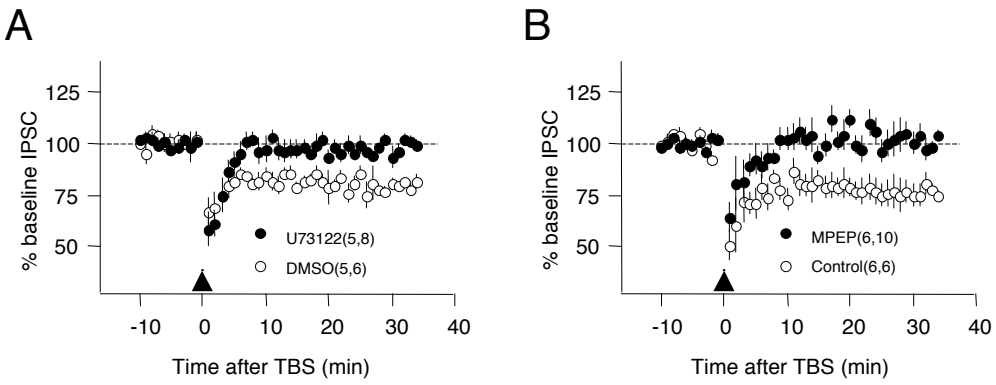
Immunoblot

Visual cortices dissected from mice were quickly frozen on dry ice. The samples were homogenized in ice-cold lysis buffer (in mM: 20 Na_3PO_4, 150 NaCl, 10 EDTA, 10 EGTA, 10 $Na_4P_2O_7$, 50 NaF, and 1 Na_3VO_4, pH 7.4; 1 µM okadaic acid; 10 U/ml aprotinin) and crude membranes prepared as previously described (Lee et al. 2000). After normalizing the protein concentration in gel sample buffer, the samples were run on 10% SDS-PAGE gels and transferred to PVDF (Millipore) membranes. The primary antibodies (anti-CB1R antibody from Dr. Mackie, 1:500; anti-GABA 1R antibody from Abcam, 1:2,000) were diluted in blocking buffer (1% bovine serum albumin and 0.1% Tween-20 in PBS), and after washes in blocking buffer, the blots were incubated in 2nd antibodiess linked to Cy3 or Cy5. After washes, blots were scanned using Typhoon Trio (GE Health), and signals were quantified using Image Quant TL software (GE Health). The fluorescence intensity value for each band was then normalized to the average value of all NR samples on the same blot to obtain the % of average NR values, which were compared across different experimental groups using unpaired Student's t-test.

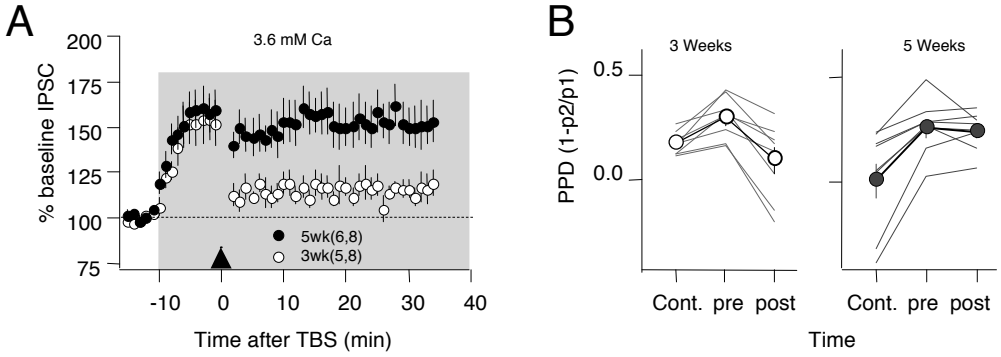
Spontaneous IPSCs

Spontaneous IPSC's (sIPSC) were recorded in ACSF with lowered divalent (1mM CaCl2, 1mM MgCl2) to reduce the event frequency and event superoposition. Control data (in 0.01% DMSO) was recorded for a period of10 minutes and WIN (10 mM) was applied for 15 min. Data was filtered at 5 KHz, digitized at 20 KHz and analyzed with Mini Analysis Program (Synaptosoft) as previously described (Morales et al 2002). For event discrimination we used a threshold of 3 times the RMS noise. To minimize biases

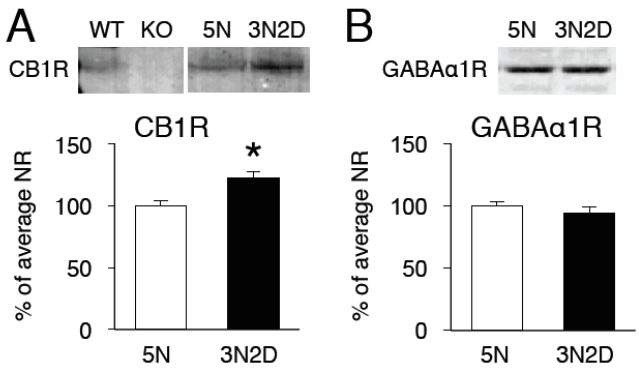
introduced by dendritic filtering, we adopted the standard criteria of analyzing only those cases in which the rise time did not show a negative correlation with the amplitude of the events. Those cases showing a drift in event frequency larger that 10% during baseline were discarded. In the computation of the amplitudes we excluded “bursts” with highly superimposed events. Comparisons were with the last 300 events recorded in control and the last 300 events recorded during WIN application. For the computation of kinetic parameters, 70 to 100 fully isolated events were averaged per cell per experimental condition (control and WIN).


A
B
125
100
75
50
% baseline IPSC
U73122(5,8)
DMSO(5,6)
MPEP(6,10)
Control(6,6)
-10
0
10
20
30
40
Time after TBS (min)

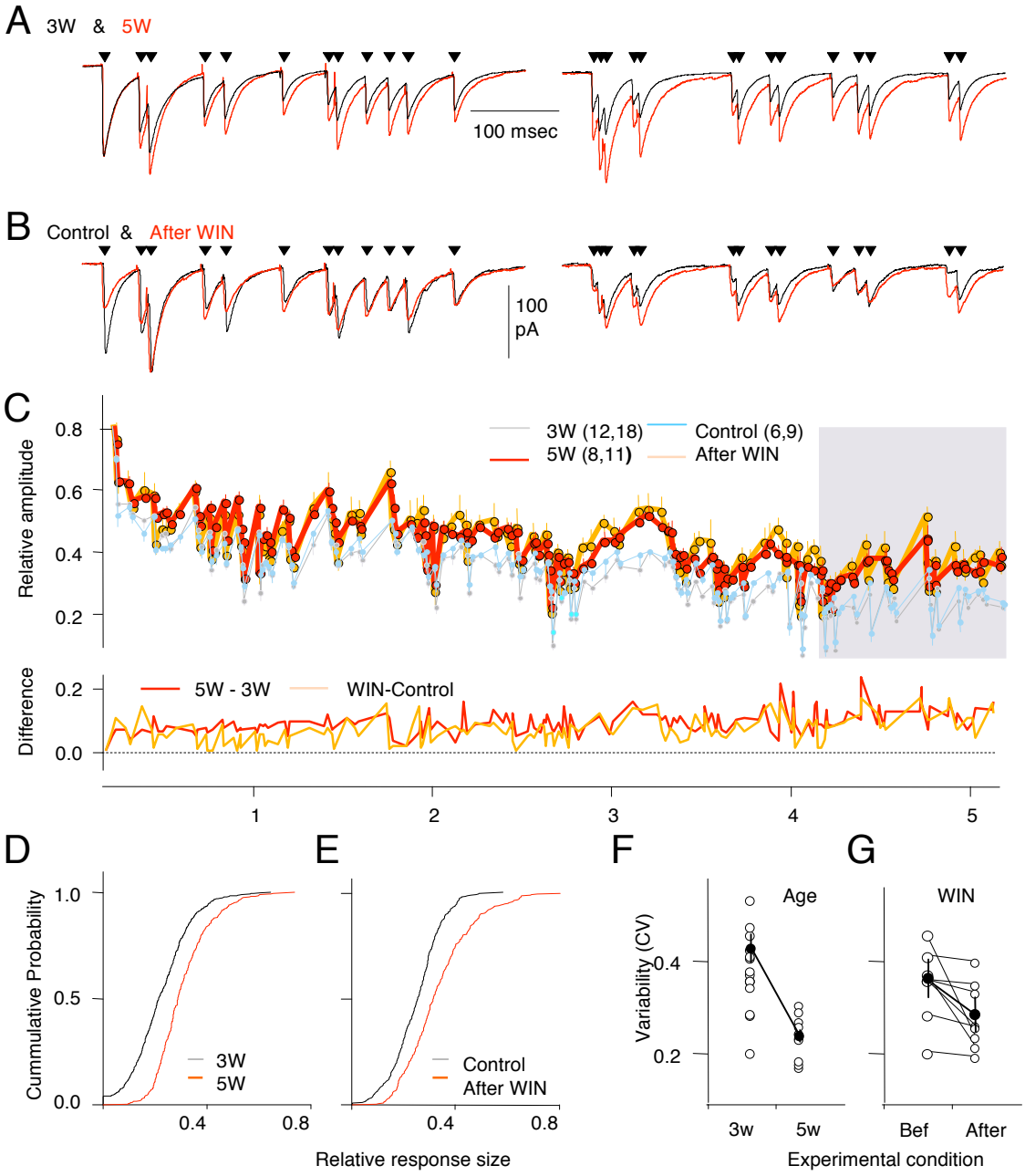
Suppl.1


A
3.6 mM Ca
% baseline IPSC
200
175
150
125
100
75
5wk(6,8)
3wk(5,8)
-10
0
10
20
30
40
Time after TBS (min)
B
3 Weeks
5 Weeks
PPD (1-p2/p1)
0.5
0.0
Cont.
pre
post
Cont.
pre
post
Time

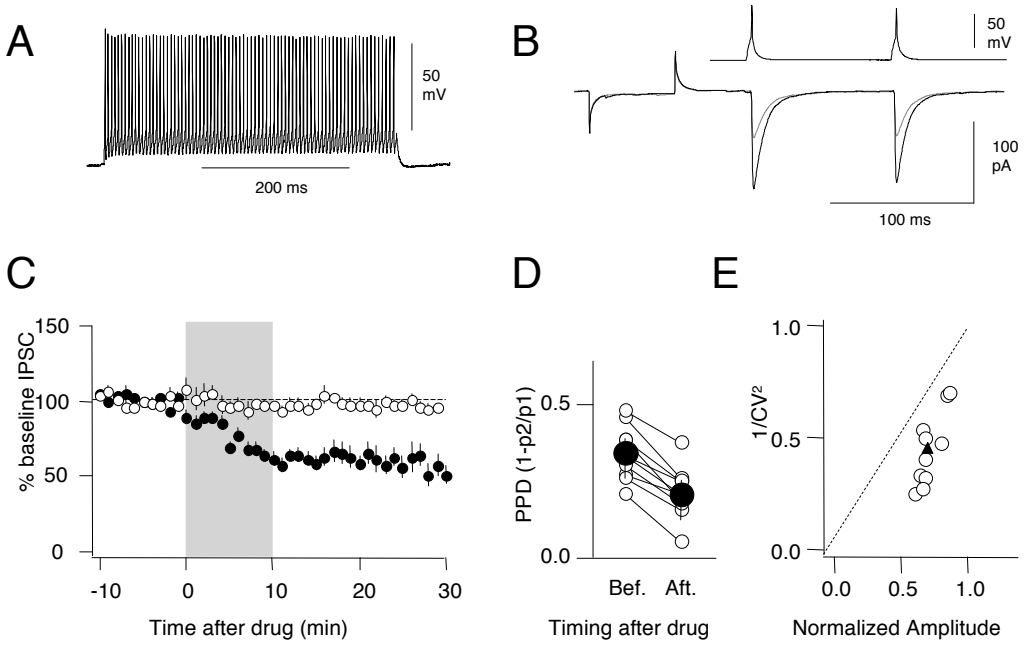
Suppl.2


A
WT KO 5N 3N2D
CB1R
CB1R
% of average NR
150
100
50
0
*
5N 3N2D
B
5N 3N2D
GABAα1R
GABAα1R
% of average NR
150
100
50
0
5N 3N2D

Suppl.3


A
3W & 5W
100 msec
B
Control & After WIN
100
pA
C
Relative amplitude
0.8
0.6
0.4
0.2
3W (12,18)
5W (8,11)
Control (6,9)
After WIN
Difference
0.2
0.0
5W - 3W
WIN-Control
1
2
3
4
5
D
E
F
G
Cummulative Probability
1.0
0.5
0.0
3W
5W
0.4
0.8
Control
After WIN
Relative response size
Variability (CV)
0.4
0.2
Age
3w
5w
WIN
Bef
After
Experimental condition

Suppl.4


A
50
mV
200 ms
B
50
mV
100
pA
100 ms
C
% baseline IPSC
150
100
50
0
-10
0
10
20
30
Time after drug (min)
D
PPD (1-p2/p1)
0.5
0.0
Bef.
Aft.
Timing after drug
E
1/CV²
1.0
0.5
0.0
0.0
0.5
1.0
Normalized Amplitude

Suppl.5