Supplemental Information

Concerted action of CB1 cannabinoid receptor and Deleted in Colorectal Cancer (DCC) in axon guidance

Abbreviated title: CB1R and RGC axon guidance

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Supplementary figure legends

Figure 1. Expression of the eCB system. A, primary cortical neurons immunolabeled for DAGLα, MGL and NFL. B, Western blot analysis of CB1R expression in primary neuron cultures at several DIVs. Molecular weight markers are indicated on the right side of the panel. C, Photomicrographs of retinal cross-sections showing CB1R, eCB synthesizing (NAPE-PLD, DAGLα) and eCB degrading (FAAH, MGL) enzyme expression (magenta) during early postnatal development. Syntaxin was used to label retinal projections (green). ONL, Outer nuclear layer; OPL, outer plexiform layer; INL, Inner nuclear layer; IPL, Inner plexiform layer; GCL, Ganglion cell layer; GCFL, Ganglion cell fiber layer. D, Photomicrographs of retinal tissues from CB1R−/− and FAAH−/− mice and matched wild type animals showing CB1R and FAAH antibodies specificity.

Figure 2. CB1R activity and second-messenger cascades. CB1R agonist, inverse agonist and antagonist did not activate the PI3K, ERK1/2 or mTOR pathways following a 20 min treatment (A). Molecular weight markers are indicated on the right side of the panel. Quantification of the optical density for P-AKT (B), P-ERK1/2 (C) and P-S6 (D). CB1R stimulation following KCl induced depolarisation (E) or insulin treatment (F) failed to recruit PI3K, ERK1/2 or mTOR second messenger cascades. Molecular weight markers are indicated on the right side of the panel.

Figure 3. DCC regulates CB1R induced reorganization of the GC. A, Photomicrographs of primary neuron cultures treated with αDCCfb followed by the
addition of either a CB1R inverse agonist or antagonist (AM251 or O2050, respectively) or FSK. GC photomicrographs of \( \text{dec}^{-/-} \) (B) and \( \text{dec}^{+/+} \) (C) primary neuron cultures treated with either ACEA or AM251.

**Figure 4. Intraocular injections and mechanism by which cannabinoids modulate GC steering.** A, Schema illustrating vitreal injection and CB1R, FAAH and MGL expression analysis sites during retinal projection development. B and C, Illustrations of the methods used to quantify retinal projection branches length (B) and the number of retinal axon branches (C) in the DTN. Arrowed dotted lines indicate the distance between the lateral border of the thalamus and the tip of the farthest projections (B). D, Photomicrographs of optic nerves following vitreal injections of CTb-546 and CTb-488 in to the left and right eye, respectively. E, A model illustrating the interactions between the CB1R and DCC during axon navigation. Antagonizing the CB1R increases intracellular cAMP levels, triggering a PKA-dependent translocation of DCC to the plasma membrane and resulting in GC expansion, whereas CB1R agonists induce the opposite resulting in GC collapse.
Supplementary Figure 1

A. DAGLα, NFL, Overlay, MGL, NFL, Overlay

B. DIV 1 3 5 7 9 10 12 14 16 18 21 23

CB1R, SVP38, Glur1, NCAM, β-Actin

C. P1 Retina

CB1R, Syntaxin, Overlay, 50 µm

DAGLα, Syntaxin, Overlay

NAPe-PLD, Syntaxin, Overlay

FAAH, Syntaxin, Overlay

P3 Retina

CB1R, Syntaxin, Overlay

NAPE-PLD, Syntaxin, Overlay

FAAH, Syntaxin, Overlay

D. WT, KO

CB1R, FAAH
Supplementary Figure 3

A

Control AM251 O2050 FSK
αDCCfb AM251-αDCCfb O2050-αDCCfb FSK-αDCCfb

B
dcc

Control ACEA AM251

C
dcc

Control ACEA AM251
Supplementary Figure 4

A

Intraocular injection
in vitreous chamber without
touching or damaging the lens
or the ciliary bodies

Optic chiasm
Optic nerve
Retina
Lateral geniculate nucleus
Dorsal terminal nucleus
Superior colliculus

B

C

DTN

0 100 200µm

D

Optic Nerve

E

eCBs
Netrin-1
Inverse agonists

Out
In

AC
PKA
cAMP
GC reorganization

DCC
PKA
DCC
DCC

DCC
DCC