LETTER TO THE EDITOR

Reply by authors to letter from Ashton

Correspondence
Ken Mackie, Department of Psychological and Brain Sciences, Indiana University, 1101 E. 10th St., Bloomington, IN 47405, USA.
E-mail: kmackie@indiana.edu

We appreciate Dr Ashton's kind words about our review (Atwood and Mackie, 2010). We hoped this review would stimulate a careful reappraisal of the basis for the claims of widespread neuronal expression of CB_2 receptors and encourage a discussion of appropriate controls for these experiments. In our review, we considered CB_2 receptor knockouts to be the 'gold standard' when evaluating antibody specificity.

In his letter, Dr Ashton brings up the crucial point that, in a knockout, as the antigen (protein) is absent, not only should the staining in tissue sections be reduced, but also a qualitative difference in staining patterns should be apparent. The basis for this is that the intensity of non-specific staining in the knockout should typically be unchanged, while specific staining should be eliminated. This is particularly true for precipitation-based immunoassays, such as the peroxidase/3,3'-diaminobenzidine techniques. Ideally, sections from wild-type and knockout mice should be incubated together with the same antibody and staining solutions, and analysed by an individual unaware of the genotype.

We hope that investigators studying CB_2 receptor expression in brain will apply the guidelines outlined by Dr Ashton when analysing knockout controls.

Brady Atwood[1,2] and Ken Mackie[1]
[1]*The Gill Center and the Department of Psychological and Brain Science, Indiana University, Bloomington, IN, USA, and*
[2]*University of Washington, Program of Neurobiology and Behavior, Seattle, WA, USA*

Reference

Atwood BK, Mackie K (2010). CB_2: a cannabinoid receptor with an identity crisis. Br J Pharmacol 160: 467–479.