# Comment on "Ccl2, Cx3cr1 and Ccl2/Cx3cr1 chemokine deficiencies are not sufficient to cause age-related retinal degeneration" by Luhmann et al. (Exp. Eye Res. 2013; 107: 80.doi: 10.1016).

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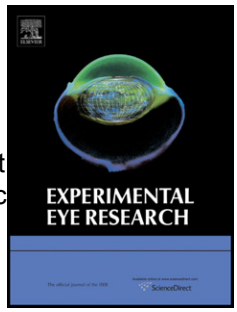


Comment on “Ccl2, Cx3cr1 and Ccl2/Cx3cr1 chemokine deficiencies are not sufficient to cause age- related retinal degeneration” by Luhmann et al.(Exp Eye Res. 2012 Dec 8. doi:pii: S0014-4835(12)00342-9.)

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Letter to the Editors

# **Comment on "Ccl2, Cx3cr1 and Ccl2/Cx3cr1 chemokine deficiencies are not sufficient to cause age- related retinal degeneration"** by Luhmann et al.(Exp Eye Res. 2012 Dec 8. doi:pii: S0014-4835(12)00342-9.)

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We are writing to you concerning a recent publication in this journal by Luhmann *et al*. (Exp Eye Res. 2013;107:80). In this manuscript the authors describe the absence of age-related subretinal macrophage/microglial cells (Mϕ/MC) and retinal degeneration in notably the $Cx3cr1^{-/-}$ mice. The authors suggest that their study contradicts Chinnery *et al*. (Neurobiol Aging. 2012; 33(8):1769) and our previous reports of age- and light-induced (Combadiere *et al.*, J Clin Invest. 2007;117(10):2920 ; Raoul *et al.*, J Neuroimmunol. 2008;198(1-2):56) subretinal Mϕ/MC accumulation and associated photoreceptor degeneration. This phenotype was described in both, pigmented C57BL/6 and BALB albino background, in $Cx3cr1^{-/-}$ knockout and $Cx3cr1^{GFP/GFP}$ knockin mice (see references cited above).
The title of the manuscript could suggest to the reader that our results are not reproducible and the authors state that their "findings are in clear contrast to several reports that describe an age-related retinal degeneration in other chemokine knockout mouse lines", including the $Cx3cr1^{-/-}$ mice. We would like to explain why we think that such conclusions cannot be drawn from the presented data.

A contamination with the *rd8* mutation of the Crb1 gene has recently been recognized to cause early onset, severe retinal degeneration in $Ccl2^{-/-}$ $Cx3cr1^{-/-}$ mice, as described in several publications, independently of their Ccl2 and Cx3cr1 deletions. Luhman *et al.* question "whether the previously reported phenotypes of different chemokine single knockout mice might also have been affected by the rd8 mutation". As communicated to the authors, the $Cx3cr1^{-/-}$ mice and $Cx3cr1^{GFP/GFP}$ mice used in our experiments are not contaminated by the *rd8* mutation.

We have previously reported that ambient light conditions are crucial for the Cx3cr1-dependent increase in subretinal Mϕ/MC accumulation, as raising $Cx3cr1^{-/-}$ BALB mice in the dark prevented (Combadiere *et al.* 2007, cited above), and a bright-light-challenge that does not induce subretinal inflammation in C57BL6/J mice, induced the phenotype in $Cx3cr1^{GFP/GFP}$ C57BL6/J mice (Raoul *et al*. 2008, cited above). We observed the age-dependent presence of Mϕ/MC in $Cx3cr1^{-/-}$ and $Cx3cr1^{GFP/GFP}$ C57BL6/J mice that were raised under 12-h light/12-h dark cycles (100–500 lux at the cage level, with no additional cover in the cage; Combadiere *et al.* 2007, cited above). Since our publication in 2007, we have observed this phenotype in over 30 Cx3cr1-deficient C57BL6/J mice with age (12 months and older; raised in the indicated light conditions) and in over 40 light-exposed Cx3cr1-deficient C57BL6/J mice, compared to a similar amount of wildtype controls. Chinnery *et al.* reproduced the increased subretinal Mϕ/MC accumulation in $Cx3cr1^{GFP/GFP}$ C57BL6/J and $Cx3cr1^{GFP/GFP}$ BALB mice (also *rd8*-free, personal

communication) at 230–275 lux in young adults. Under their conditions the accumulation further increased with age and was not statistically different anymore in $Cx3cr1^{GFP/+}$ $Cx3cr1^{GFP/GFP}$ at a massive 180 Mϕ/MCs/$mm^2$ in 20-month-old BALB mice, suggesting that a maximal Mϕ/MC cell accumulation can be reached in the subretinal space.

Ng and Streilein (Invest Ophthalmol Vis Sci. 2001;42(13):3301) showed that subretinal Mϕ/MC accumulate in albino mice and subretinal Mϕ/MCs are cleared when transferred from light to dark conditions. In $Cx3cr1^{-/-}$ mouse brains, macrophages clear less efficiently from the injection site compared to wildtype macrophages (Cardona *et al*., Nat Neurosci. 2006;9(7):917). In preliminary studies (Levy *et al*., ARVO 2011), we have shown that $Cx3cr1^{-/-}$ macrophages present a similar impaired clearance from the subretinal space, which could explain the subretinal Mϕ/MC accumulation in Cx3cr1-deficient animals we described.

The mice used in the studies described by Luhmann *et al*. were raised under a 12h/12h dark-light cycle with a mean luminescence during the light period at the bottom of the cage of 33 ± 28 lx. Furthermore, the animals had access to protection from light exposure (*e.g*., paper roll and excess of bedding) inside the cage, which allowed them to burrow. The light-conditions used in the described experiments were, therefore, significantly dimmer than the light conditions used in the previous publications.

We never suggested that $Cx3cr1^{-/-}$ or $Cx3cr1^{GFP/GFP}$ C57BL6/J mice raised under such dim-light conditions accumulate subretinal Mϕ/MCs or display age-related photoreceptor degeneration. In fact we have emphasized in all our related publications that light conditions are crucial for obtaining the phenotype.

Therefore, we believe that the only conclusion that can be drawn safely from the presented data is that none of the mouse strains used develop subretinal Mϕ/MC accumulation or retinal degeneration under very dim light conditions. The comparison of theresults described by Luhmann *et al*. to those obtained in our studies and their interpretation as a “clear contrast” to our published findings (which suggests our results were not reproducible in their laboratory) are not justified, as the conditions used in the two studies were not comparable.