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## PURPOSE

We have recently demonstrated the potential of BIO201, the di-apo-carotenoid norbixin entering clinical development, on retinal pigmented epithelium (RPE) and retina photoprotection *in vitro* and *in vivo* (Fontaine et al., 2016). We also showed that BIO201 is a dual PPAR $\alpha$ /PPAR $\beta/\delta$  ligand (Fontaine et al. 2017). Here we demonstrate that chronic BIO201 oral administration to a mouse model of dry AMD can slow down or stop the pathological course of the disease when used as preventive or curative treatment.

## METHODS

Experiments were performed using the *Abca4<sup>-/-</sup>Rdh8<sup>-/-</sup>* transgenic mouse model of dry AMD. Young mice (6-week-old males; n=10) were used to test BIO201 as a preventive treatment and older mice (12-month-old males, n=7 and females, n=10) to test it as a curative treatment. Mice were fed daily with either BIO201 supplemented (0.5 g/kg) food or regular regimen for 5 or 6 months, respectively.

Full field scotopic and photopic electroretinograms (ERG) were measured at the end of the treatment. One eye was then collected for assessing A2E accumulation, and the second was used to perform histological analyses.

Photoreceptor nuclei quantification was performed on eye cryosections. Oral bioavailability of BIO201 and A2E concentrations were measured in all mice by HPLC-MS/MS.

Statistical analyses were performed using either a *t* test or a one-way ANOVA followed by Dunnett's test.

## CONCLUSIONS

Oral administration of BIO201 (ca. 50 mg.kg<sup>-1</sup>.d<sup>-1</sup>) provides a significant protection to the retina both at histological and functional levels, provided that the retinal degradation is not too advanced when starting the treatment.

These results support the clinical development of Macuneos, a preparation based on BIO201 for dry AMD prevention and treatment.

## References

Fontaine et al. (2016) Norbixin protects retinal pigmented epithelium cells and photoreceptors against A2E-mediated phototoxicity *in vitro* and *in vivo*. PLOS ONE | DOI:10.1371/journal.pone.0167793  
Fontaine et al. (2017) Involvement of peroxisome proliferator activator receptors (PPARs) in the photoprotective activity of BIO201. Ever 2017, poster # F070

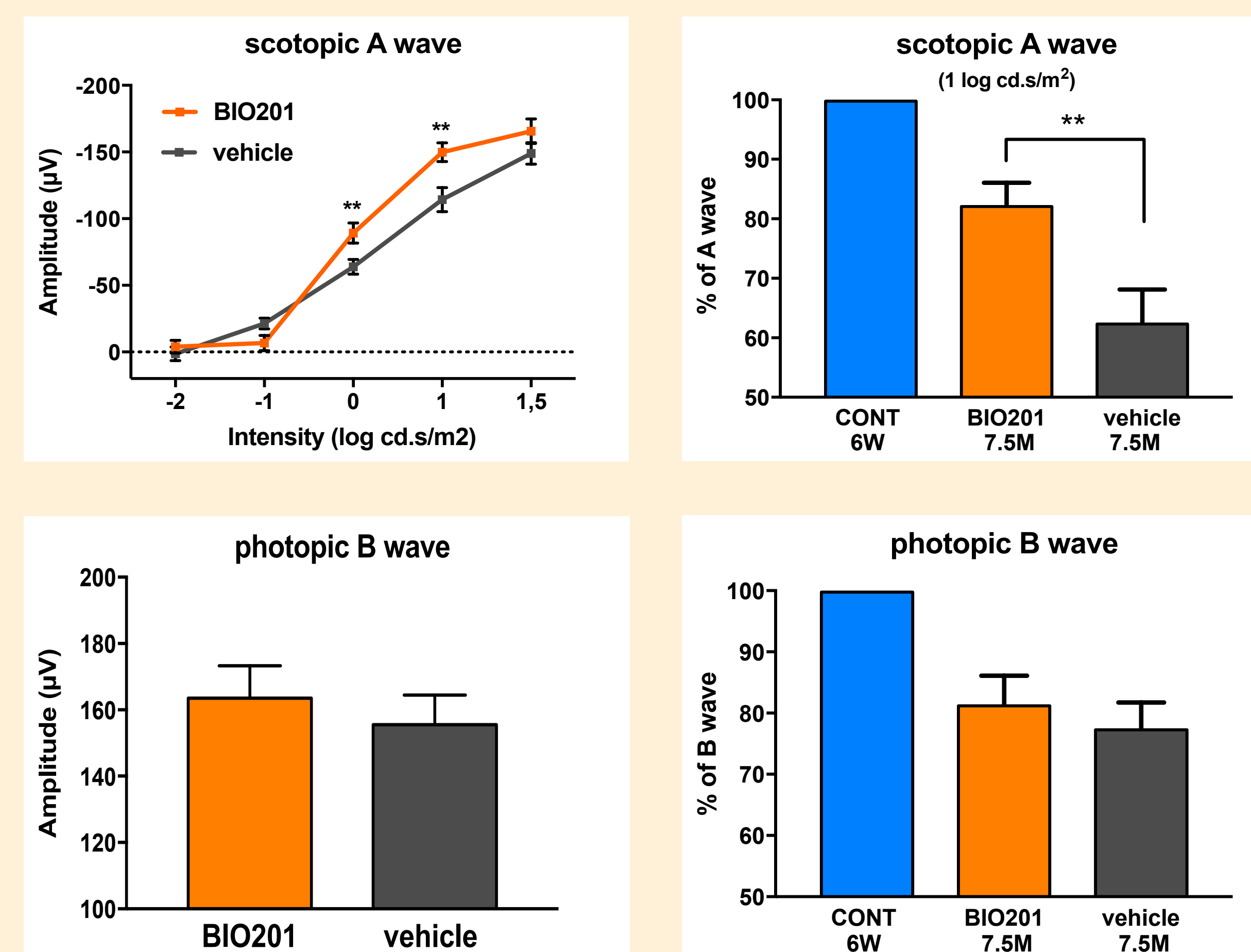
## DISCLOSURES

VF (F, P), EM (F), EB (F), MF (F), CB (E), LG (E), JAS (S, P), SV (I, E, P), PD (E), RL (I, E, P)

## RESULTS

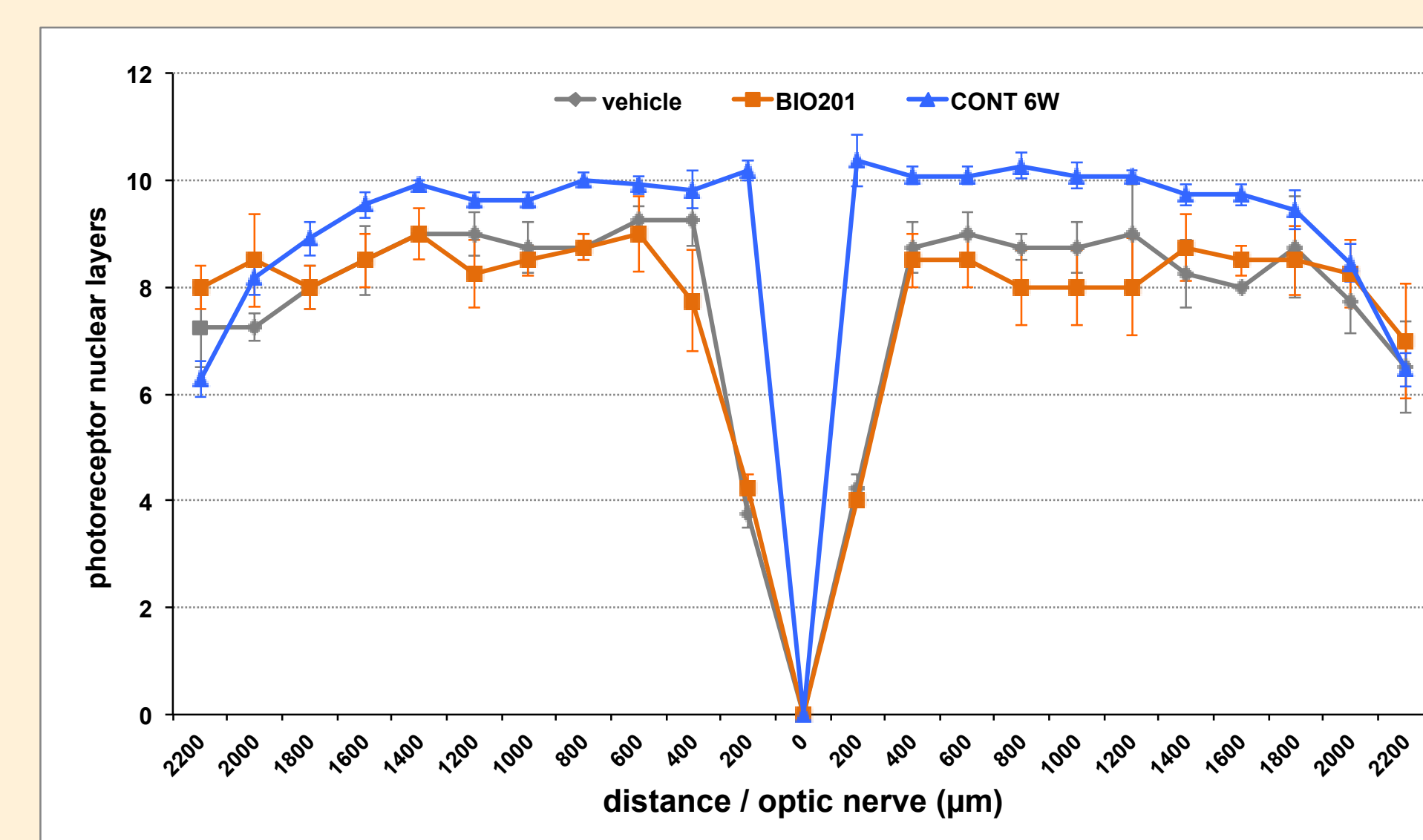
### PREVENTIVE STUDY

BIO201 protects the function of *Abca4<sup>-/-</sup>Rdh8<sup>-/-</sup>* mouse photoreceptors

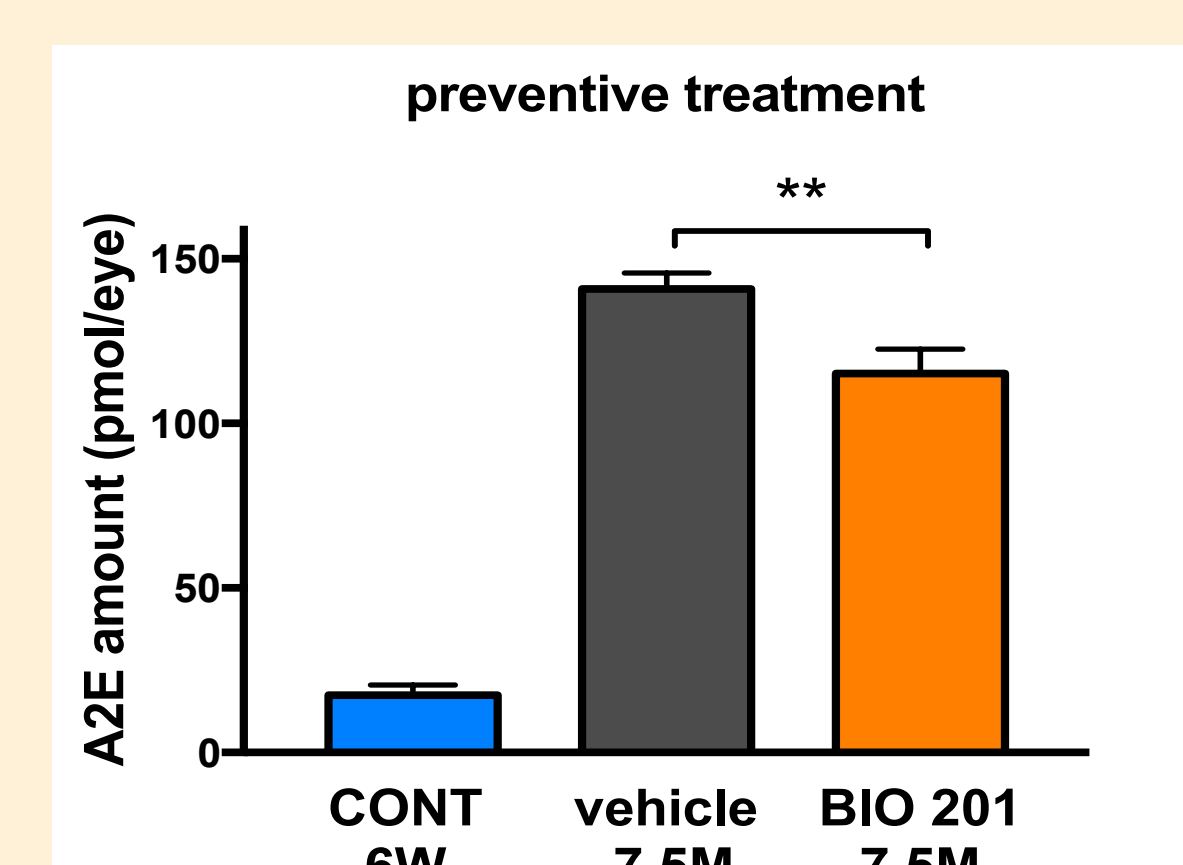


In the preventive experiment BIO201 significantly maintained the scotopic ERG A-wave at flash intensities of 1 and 10 cd.s/m<sup>2</sup>. At 10 cd.s/m<sup>2</sup> A-wave of the BIO201 treated mice was still 82% of the 6W control vs 62% for the vehicle treated mice. The photopic ERG was also better in BIO201 treated mice.

BIO201 treatment has no impact on photoreceptor degeneration in young mice



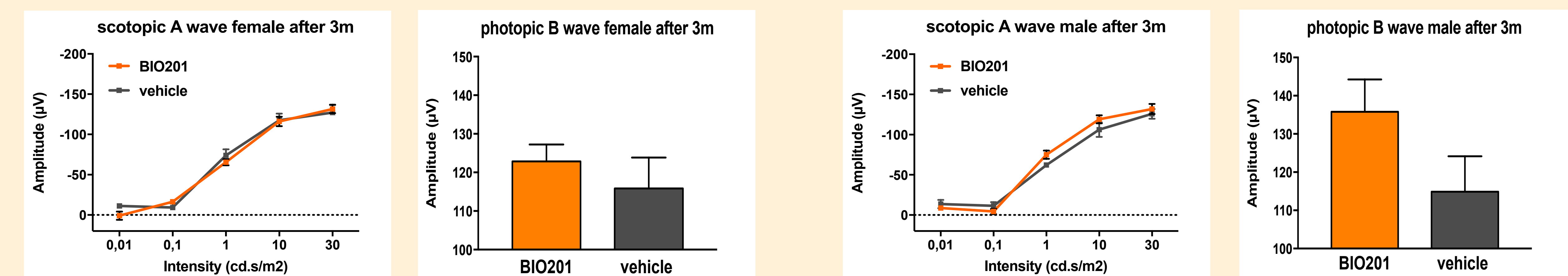
BIO201 reduces A2E accumulation



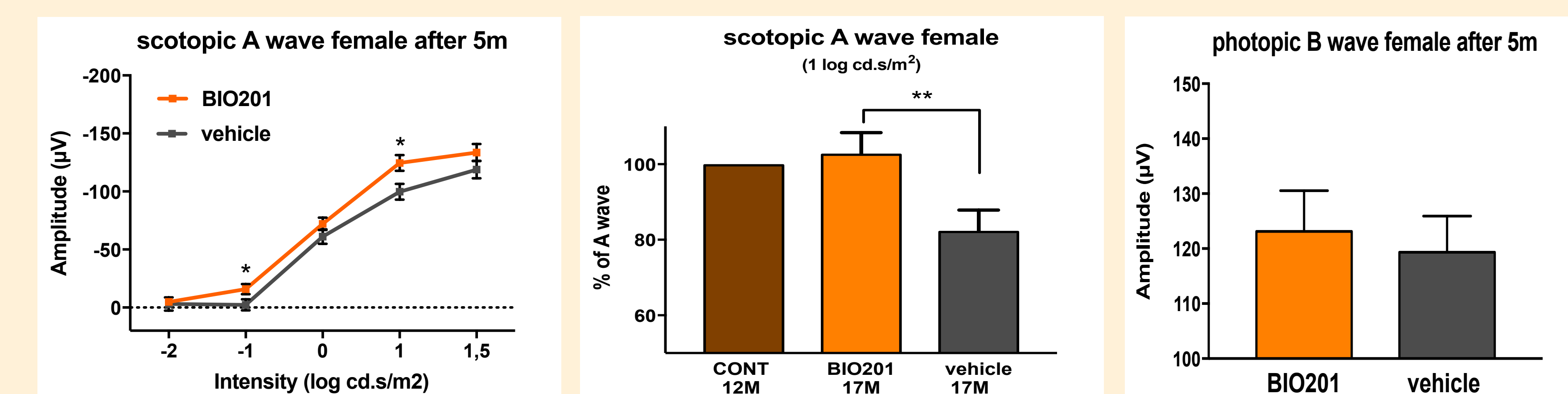
A2E accumulation was reduced by 22% (*p*<0.01) in BIO201 treated mice as compared to vehicle treated mice.

### CURATIVE STUDY

BIO201 protects the function of *Abca4<sup>-/-</sup>Rdh8<sup>-/-</sup>* mouse photoreceptors

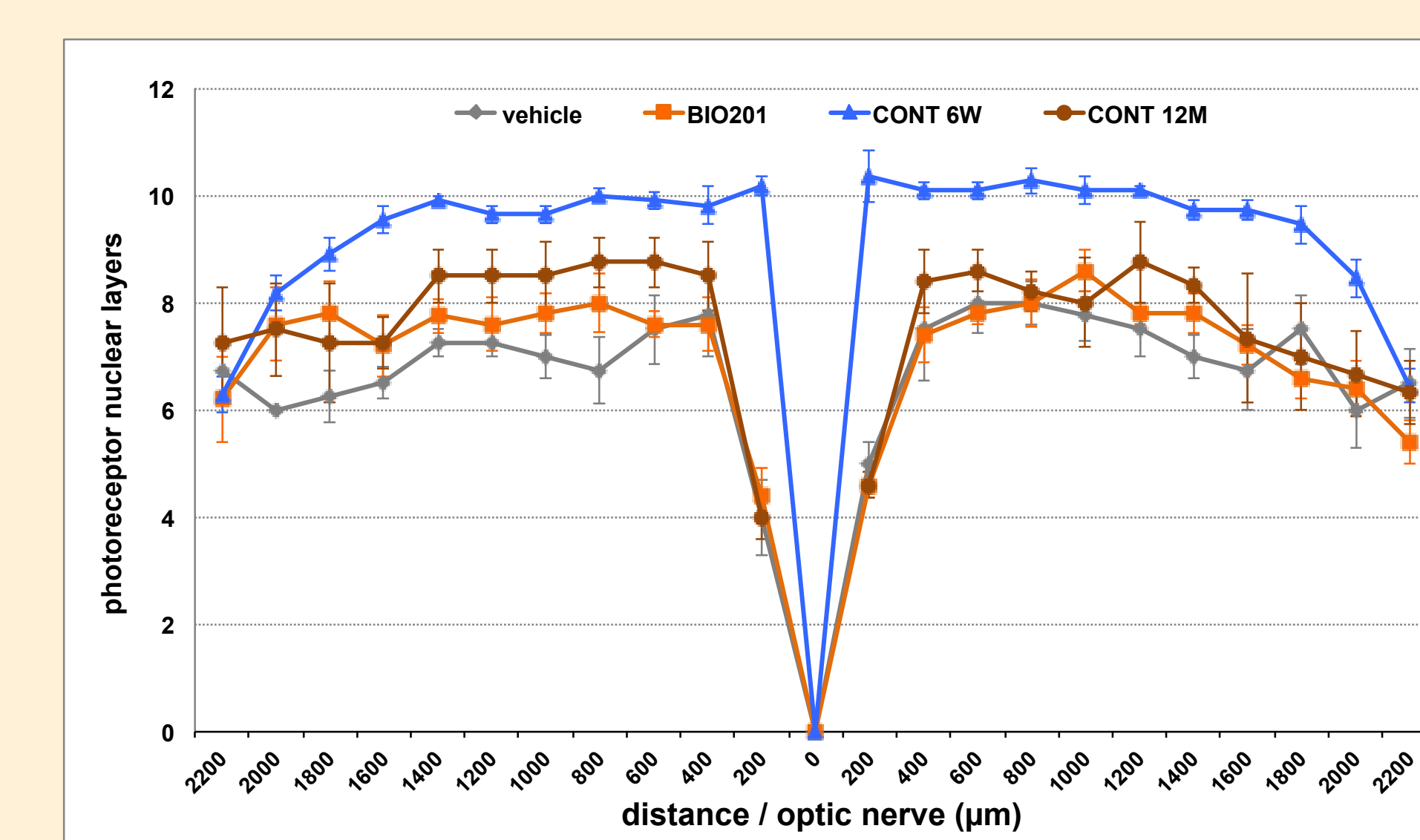


In the curative experiment 3 month-treatment of BIO201 was not highly efficient on the scotopic ERG either in females or in males but showed a slight not significant effect on photopic ERG.

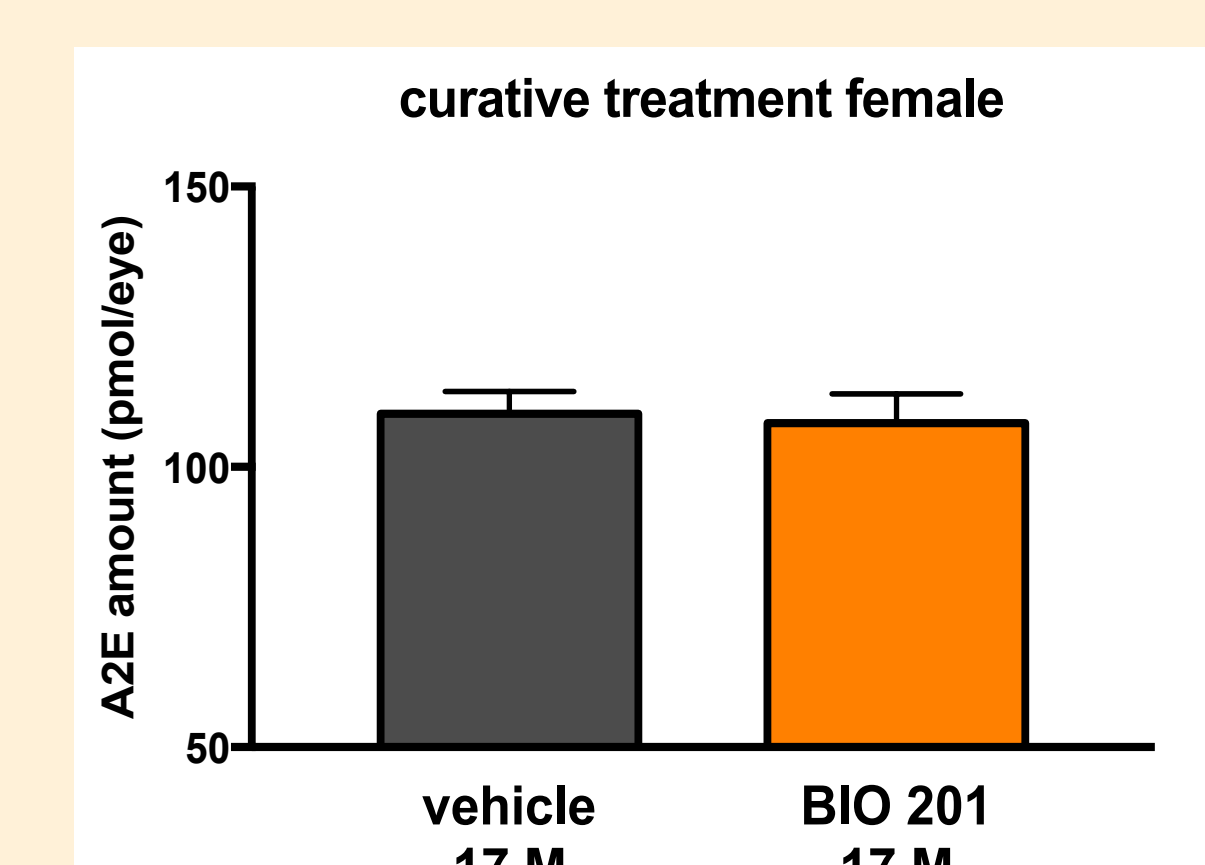


Interestingly, after 5 months of treatment BIO201 completely preserved the scotopic ERG A-wave in 17-month-old females (*p*<0,01) but not in males. The photopic ERG was slightly higher in BIO201 treated mice (not significant).

BIO201 protects residual photoreceptors in old females

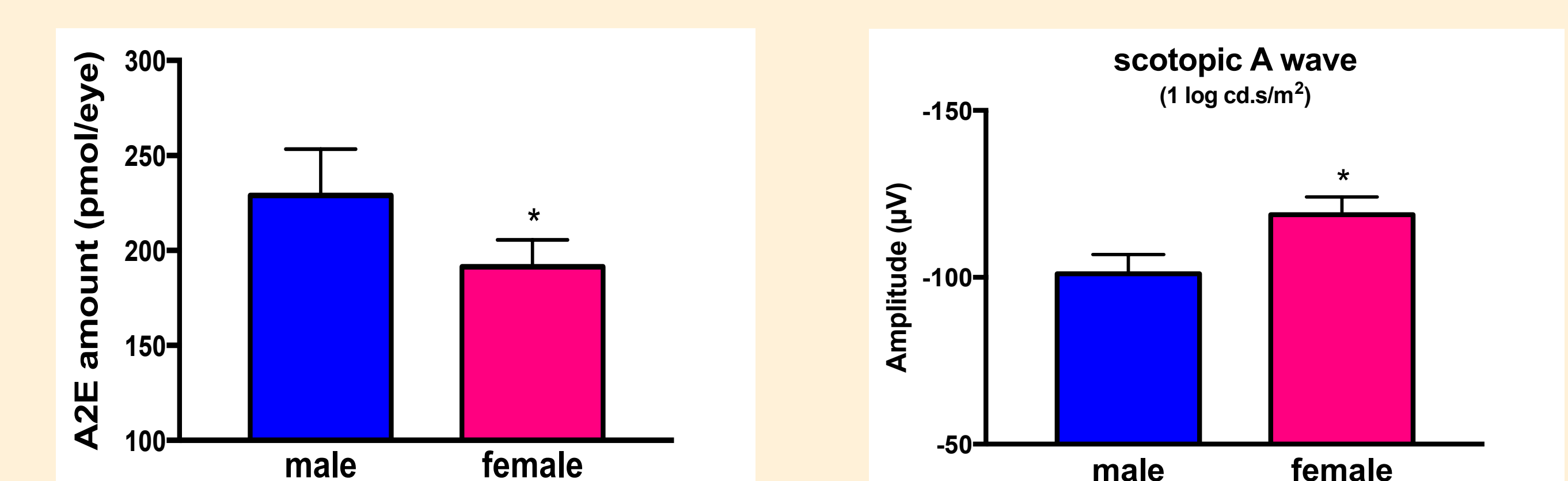


BIO201 does not reduce A2E accumulation in old females

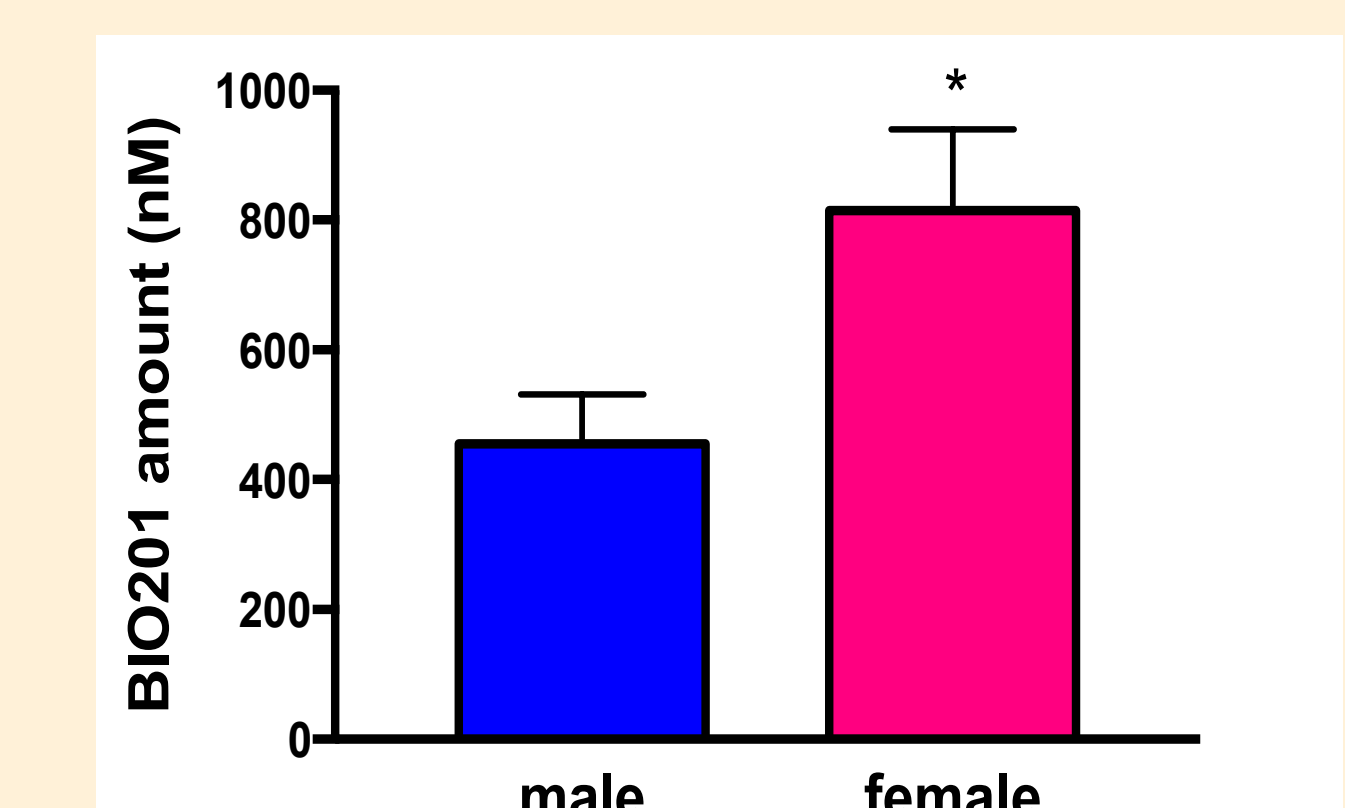


No difference in A2E accumulation was observed in 17-month-old mice.

The eyes of 12 month-old males contain more A2E and show a worsened scotopic ERG as compared to females



Males show a reduced BIO201 bioavailability as compared to females



The sex differences appear to be related to the mouse model itself rather than to the molecule. Old male mice showed an accelerated kinetics of age-related A2E accumulation in the eyes and a more deteriorated ERG at the onset of treatment. At 1 year of age, A2E level was higher in males compared to females (230.4 ± 11.5 vs 192.9 ± 6.3 pmol/eye, *p*<0.05) whereas ERG a-wave was lower (-101.8 ± 5.1 vs -119.5 ± 4.6 µV, *p*<0.05). Males also showed a reduced BIO201 bioavailability as compared to females.