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No Difference Between Age-Matched Male and Female C57BL/6J Mice in Photopic and Scotopic Electroretinogram a- and b-Wave Amplitudes or in Peak Diurnal Outer Segment Phagocytosis by the Retinal Pigment Epithelium

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Abstract

Mice provide informative models of enormous utility for eye research. Sex as biological variable must be considered when conducting studies exploring mouse models. To determine if sex confounds neural retina or retinal pigment epithelium (RPE) activity in wild-type C57BL/6J mice, we compared male and female mice with respect to retinal light response and RPE phagocytosis. We tested 2-month-old mice at peak fertility and 12-month-old mice past fertility. Retinal function was assessed by quantifying a- and b-wave amplitudes of photopic and scotopic electroretinograms (ERGs). These experiments did not reveal differences between male and female mice at either age. As expected from earlier studies, 12-month-old mice showed reduced light responses compared to 2-month-old mice, but age-related decline was identical for male and female mice. RPE functionality was assessed by quantifying RPE phagosome content 1 h after light onset in mice 2 months of age, an age of maturity of the process of outer segment turnover that includes RPE phagocytosis. These experiments did not reveal differences in RPE phagocytosis between male and female mice. Altogether, male and female C57BL/6J mice do not differ in retinal light response and peak RPE phagocytic activity. Retinal activity is impaired with age to the same extent in male and female mice. Our results justify testing mixed-sex mouse cohorts in studies on outer segment renewal and RPE phagocytosis and illustrate the importance of careful consideration of cohort age.

Keywords

Sex; Biological variable; Electroretinogram; Photoreceptors; Male mice; Female mice; C57BL/6J; Phagocytosis; Outer segments; RPE; Retinal pigment epithelium

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83.1 Introduction

Sex is a biological factor that influences many biological processes; for research exploring animal models, it is therefore important to understand sex effects on the specific physiological activities that are being investigated. In recognition, consideration specifically of sex as biological variable in vertebrate animal experimentation has since recently been mandated by funding agencies such as the US DHHS National Institutes of Health. In the human eye at least of young women, estrogen receptors are expressed in the neural retina and the underlying retinal pigment epithelium (RPE) (Ogueta et al. 1999). Side-by- side comparison of visual function as tested in electroretinogram (ERG) recordings as well as gross of overall retinal morphology as tested in histology suggests that there are subtle differences in the retina of male and female albino Sprague-Dawley rats (Chaychi et al. 2015). In mouse, recent whole-transcriptome microarray analysis has shown that retinal gene expression differences between 3- and 24-month-old C57BL6/N mice are sexually divergent (Du et al. 2017). Here, we perform scotopic and photopic ERGs to directly compare retinal light responses of male and female C57BL/6J mice at ages of prime fertility and past fertility, at 2 and 12 months of age, respectively.

Sex effects on functions of mouse retinal pigment epithelium (RPE) adjacent to the neural retina have not yet been reported to our knowledge. However, mouse RPE cells may alter their activities in response to female sex hormones (Kimura et al. 2014). One of the major activities of the RPE is the diurnal phagocytosis of spent photoreceptor outer segment debris (POS), a critical part of the continuous and lifelong outer segment renewal process that is critical for vision (Strauss 2005). Here, we quantify RPE phagosome content shortly after light onset to directly test whether POS phagocytosis by RPE cells in vivo differs between male and female C57BL/6J mice at 2 months of age.

83.2 Material and Methods

83.2.1 Animals

All procedures involving animals were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th ed.) and the guidelines of the ARVO statement for “Use of Animals in Ophthalmic and Vision Research.” They were reviewed and approved by the Fordham University Institutional Animal Care and Use Committee. Wild-type C57BL/6J mice were bred and raised for experiments with food and water ad libitum and in a 12 h light: 12 h dark cycle.

83.2.2 Electroretinography

Following dark adaptation overnight, ERGs were recorded using an LKC UTAS system. Age-matched male and female mice were tested on the same day. ERGs were performed as described previously (Nandrot et al. 2004; Yu et al. 2012).

83.2.3 POS Phagosome Quantification in RPE Flatmounts

For in situ POS phagosome quantification eyes were enucleated from 2-month-old male and female mice immediately following CO_2 asphyxiation 1 h after light onset. Eyes were

dissected and RPE flatmounts processed for phagosome labeling using opsin N-terminus antibody B6–30 as primary antibody and microscopy analysis as described previously (Sethna et al. 2016). Maximal projections were generated from confocal microscopy z-stacks to visualize all POS phagosomes in RPE flatmounts regardless of depth.

83.3 Results

83.3.1 Comparing Scotopic ERGs of 2- and 12-Month-Old Male and Female C57BL/6J Mice

The ERG allows quantifying retinal responses to light with minimum discomfort to mice. Here, we used ERGs to compare cohorts of 2- and 12-month-old male and female C57BL/6J mice. We tested all mice of the same age on the same day. Quantification of resulting ERG recordings yielded a-wave and b-wave amplitudes for each mouse at each light intensity. A-wave amplitudes indicate the magnitude of light response by photoreceptors while b-wave amplitudes indicate responses of second-order retinal neurons. Average a-wave and b-wave amplitudes of dark-adapted male and female mice at 2 months of age did not differ regardless of light intensity (Fig. 83.1a, b). The same was true comparing male and female mice at 12 months of age (Fig. 83.1c, d). However, 12-month-old mice showed significantly reduced responses to light at all intensities tested compared to 2-month-old mice (Fig. 83.1, compare a to c, b to d).

83.3.2 Comparing Photopic ERGs of 2- and 12-Month-Old Male and Female C57BL/6J Mice

ERGs on dark-adapted mice will record responses by rod photoreceptors alone at light intensities and responses by both rods and cones at higher light intensities. In order to test cone responses alone, we performed photopic ERGs, which record responses to unattenuated white flash by mice that were light-adapted having been exposed to background illumination of sufficient intensity to bleach rods. Figure 83.2a, b shows that male and female mice responded identically in these photopic ERGs both at 2 months of age (Fig. 83.2a, b) and at 12 months of age (Fig. 83.2c, d). Again, 12-month-old mice responded less than 2-month-old mice (Fig. 83.2, compare a to c, b to d).

83.3.3 Comparing Peak Diurnal POS Phagosome Load of 2-Month-Old Male and Female C57BL/6J Mice

As a measure of outer segment renewal, we assessed RPE phagocytosis at its diurnal peak 1 h after light onset. RPE flatmounts were stained with opsin antibody labeling POS phagosomes which we counted. Figure 83.3 shows that POS phagosome load of the RPE is identical in male and female 2-month-old mice.

83.4 Discussion

In this study, we directly compared scotopic and photopic ERGs and POS phagosome content of the RPE between male and female C57BL/6J mice. We found that at an age of prime fertility, 2 months of age, female mice do not differ in retinal or RPE phagocytic activity from age-matched male mice. RPE phagocytosis must be in balance with POS

shedding to maintain photoreceptor integrity, and C57BL/6J mice do not exhibit signs of photoreceptor distress that would be expected to result from defective outer segment renewal. Identical levels of RPE POS phagocytosis in male and female mice are therefore indicative of robust overall outer segment turnover. Altogether, our results justify testing mixed-sex cohorts of mice in future studies on outer segment renewal and associated alterations of retinal function.

Like at peak fertility, female and male mice did not differ in retinal function at 12 months of age, past fertility. These results imply that mixed-sex cohorts of mice may be tested at different ages and in longitudinal studies without sex as confounding biological variable. Moreover, our ERG results confirm earlier reports of a decline in retinal function with age (Gresh et al. 2003).

Previous studies on rat retinal function indicated subtle differences by sex and effects of the estrus cycle (Chaychi et al. 2015). It is possible that rats differ from mice in this respect. It is important to point out that in our study, we did not synchronize our female mice by estrus cycle. However, we did not observe larger variability among female mice at fertile age (2 months of age) than among male mice or female mice past fertility. We conclude that any effect of the estrus cycle on retinal function as tested and on POS phagocytosis, if it exists at all, is very modest such that it is highly unlikely to confound testing future experimental cohorts of mice.

Acknowledgments

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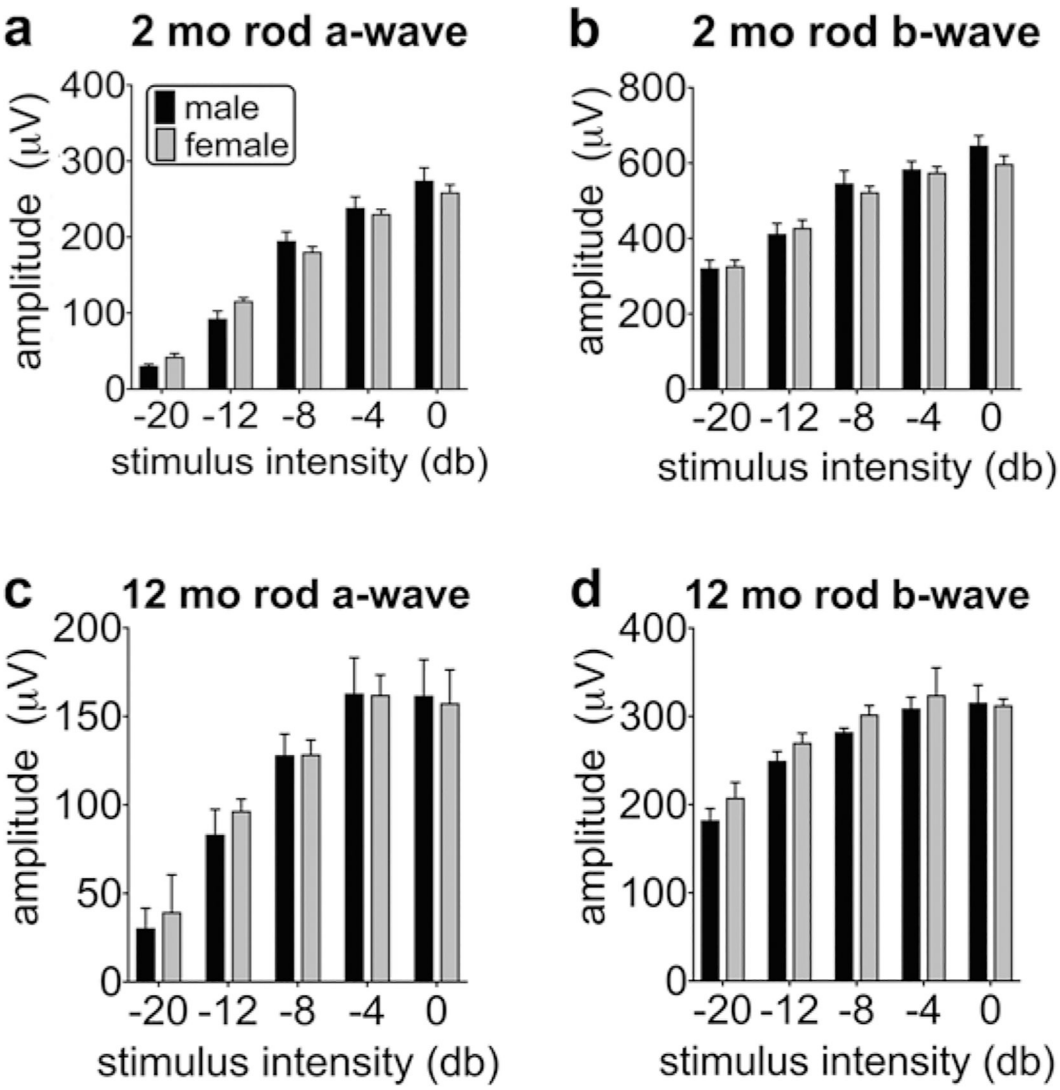

Fig. 83.1.

Scotopic ERG results comparing 2- and 12-month-old dark-adapted male and female C57BL/6J mice. (**a**, **c**) Bars show a-wave amplitudes representing photoreceptor responses to white flashes of increasing intensities at ages as indicated. (**b**, **d**) Bars show b-wave amplitudes representing second-order neuron responses at ages as indicated. All bars show mean ± s.e.m.; $n = 9$ female and 10 male mice. For all values, Student's *t*-test showed no significant difference between age-matched sexes, $P > 0.05$

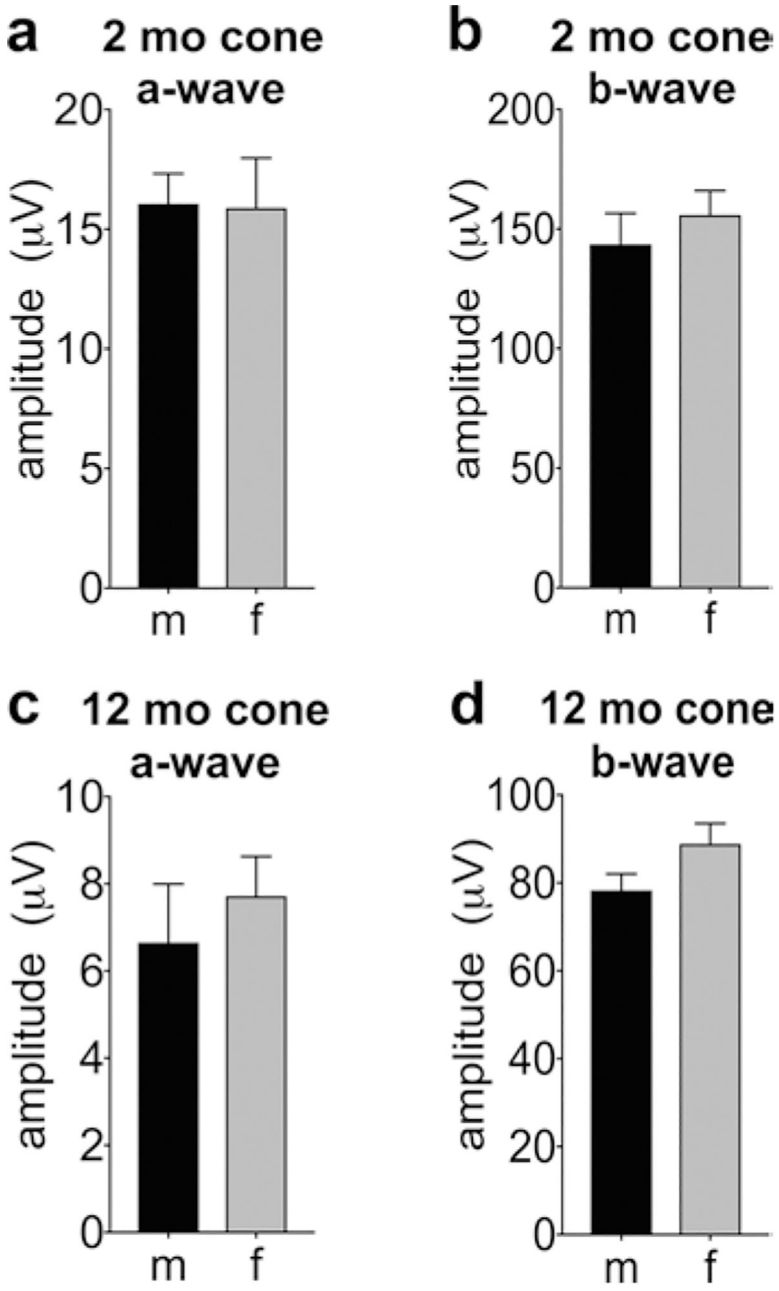

Fig. 83.2.

Photopic ERG results comparing 2- and 12-month-old male and female C57BL/6J mice pre-adapted to background illumination. (**a**, **c**) Bars show a-wave amplitudes representing cone photoreceptor responses at ages as indicated. (**b**, **d**) Bars show b-wave amplitudes representing second-order neuron responses at ages as indicated. All bars show mean ± s.e.m.; $n = 9$ female and 10 male mice. For all values, Student's *t*-test showed no significant difference between age-matched sexes, $P > 0.05$

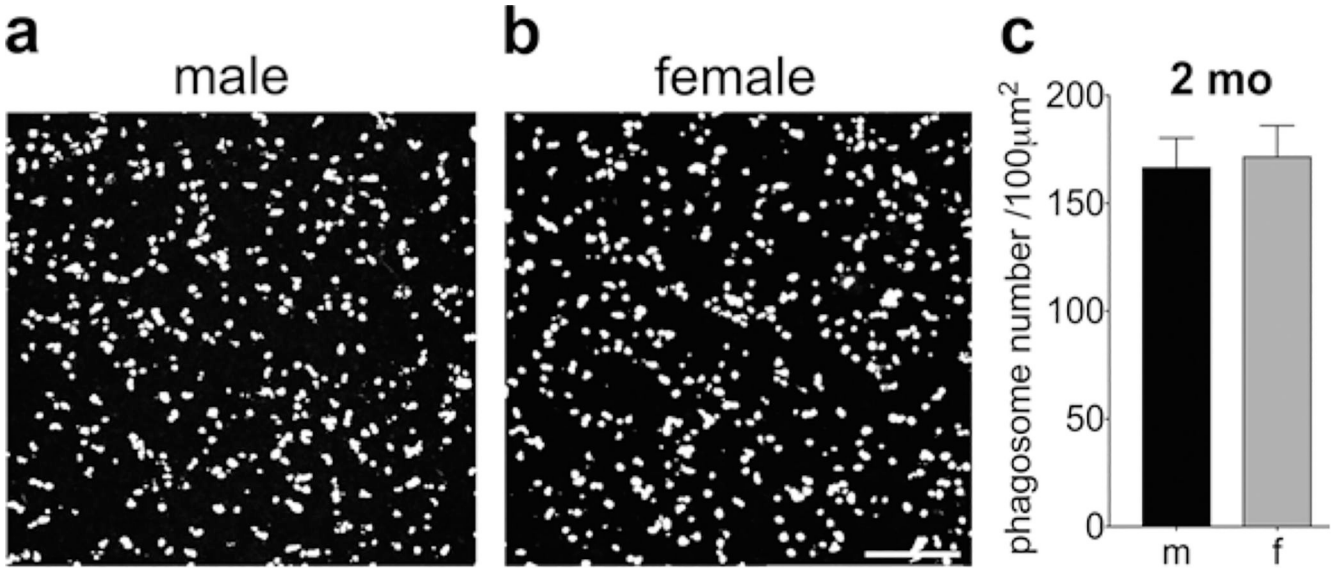

Fig. 83.3.

Comparison of RPE phagosome content early after light onset in 2-month-old male and female C57BL/6J mice. (**a**, **b**) Representative micrographs showing en face views of RPE whole mounts from male (**a**) and female mice (**b**) stained with opsin antibody labeling POS phagosomes. Mice were sacrificed 1 h after light onset. Scale bar: 25 μm. (**c**) Bars show phagosome content in RPE of males and female mice obtained from images as in **a**, **b**; mean ± s.e.m.; $n = 6$ female and 6 male mice. Student's t-test showed no significant difference between sexes, $P > 0.05$