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Consequences of early life stress on the structure and function of the adult mouse retina

Juan S. Calanni, Ruth E. Rosenstein*

Early-life stress (ELS) refers to a period of environmental/social deprivation, physical, sexual or emotional abuse, neglect, severe and/or chronic trauma in the prenatal/early postnatal stage, which is closely related to many adverse psychiatric disorders later in life, such as depressive disorder, substance abuse, dysthymia, panic, anxiety, and suicidal behavior in adulthood (Waters and Gould, 2022). In addition to mood-related symptoms, a strong association between ELS and a wide range of adverse health outcomes in adulthood has been demonstrated, with increased risk of metabolic, musculoskeletal, neurological, respiratory, cardiovascular, and gastrointestinal symptoms (Wegman and Stetler, 2009). Therefore, ELS sequelae represent a serious public health concern. During childhood, social ties (mainly mother-infant relationship) play a key role in brain development and behavior in adulthood, and neonatal maternal separation (MS) has been shown to dramatically affect brain development, and increase the risk for several diseases (Cao et al., 2020). Since working with humans subjected to ELS is limited by the difficulty of finding a representative sample that experiences early adversities at the same stage, intensity, and duration, experimental approaches in animals are necessary to gain a deeper understanding of ELS-induced long-term consequences (Waters and Gould, 2022). In rodents, as in other mammals, the early postnatal environment is strongly determined by mother-pup interactions. In this regard, a mouse model of MS is widely recognized as a suitable model to mimic early adverse experiences, increasing depressive-like and/or anxiety-like behaviors in adulthood (Nishi, 2020), as demonstrated by behavioral tests, such as the sucrose preference test, the forced swim test, the open field test, the elevated plus-maze test, and the tail suspension tests (Nishi, 2020; Waters and Gould, 2022), some of which depend on the integrity of the visual system. In adult female Wistar rats, MS has been shown to induce a significant decrease in the thickness of the outer nuclear layer of the retina, as well as an increased retinal microglial reactivity and glial fibrillary acidic protein (a gliotic marker)-immunoreactivity (Grigoruta et al., 2020). However, despite that numerous effects of ELS on the central nervous system have been reported, its effects on the retina and visual system of adult mice remain unknown.

In this perspective, we discuss preclinical data, which for the first time indicate that ELS induces visual dysfunction and retinal structural alterations in adult mice. In combination with electrophysiological, behavioral, and neurochemical approaches, we analyze the effect of ELS on the retina of adult mice using an experimental model of maternal separation with early weaning (MSEW), which mimics early life maternal neglect. For this purpose, male and female C57BL/6 mice were subjected to progressive periods of MS, starting at postnatal day 2, and ending with weaning at postnatal day 17. At the end of each separation period, mothers were subjected to movement restriction for 10 minutes. Control pups were left undisturbed from postnatal day 0, and weaned at postnatal day 21. MSEW did not affect the eye-opening time or the corporal weight in adulthood (Calanni et al., 2022). In the adult stage, the outer and middle retinal function (assessed by the electroretinogram a- and b-wave amplitude and latency) was preserved in early stressed mice, whereas MSEW induced a significant decrease in scotopic threshold response and photopic negative response amplitude, which are the indicators to monitor retinal ganglion cell (RGC) function (Calanni et al., 2022; **Figure 1**). Moreover, MSEW induced a decrease in visual evoked potential amplitude that reflects the activity of all cells in the photoreceptors-visual cortex pathway (Calanni et al., 2022). As mentioned above, ELS affects the performance in several behavioral tests in the adult stage (Nishi et al., 2020; Waters and Gould, 2022); however, the effect of MSEW on behavioral tests specifically based on vision has not been previously described. MSEW altered the performance of adult mice in the virtual cliff test (indicative of binocular depth perception), and in the looming test, which assess the ability of mice to freeze or flee to an available refuge prior to a rapidly expanding overhead stimulus that mimics an approaching aerial predator (Yilmaz and Meister, 2013). Particularly for rodents, avoiding aerial predators (e.g., hawks and owls) is a central survival function. Assuming that these findings could be extrapolated to wild mice, it seems that MSEW could increase the risk of being detected by a predator cruising overhead, thereby reducing their survival probability (Calanni et al., 2022). MSEW, which did not affect the locomotor activity, reduced the looming stimulus-induced upregulation of the immediate-early gene c-fos (a neuronal activity proxy) in the superior colliculus, as well as the anterograde transport from the retina to the superior colliculus. In contrast to the results shown in adult female Wistar rats subjected to MS (Grigoruta et al., 2020), but consistent with our electrophysiological results, the structural analysis showed a preservation of the outer/middle retina. Moreover, decreased levels of synaptophysin (an integral membrane protein localized to synaptic vesicles) in the middle (but not outer) retina, as well as reduced RGC number were observed in adult male and female mice subjected to MSEW (**Figure 1**). MSEW induced an increased retinal Iba-1(+) area (a microglial/macrophage density index), as well as Iba-1(+) cell soma size, supporting an increased microglia/macrophages reactivity and/or infiltration, as well as changes in their phenotype, from a ramified (quiescent) to an amoeboid (activated) morphology (**Figure 1**). In addition, MSEW increased retinal microglia phagocytic activity, as evidenced by the engulfment of RGC nuclei by Iba-1(+) cells in MSEW (but not control) retinas. Interestingly, although gender/sex-dependent ELS-induced sequelae have been described in humans and rodents (Knox et al., 2021; Waters and Gould, 2022), no differences between male and female mice were observed in any of the visual/retinal parameters tested, in both control and early stressed animals. It should be noted that no functional or structural retinal alterations were observed at postnatal day 35, supporting that MSEW triggers very slow progressing retinal/visual alterations that are evident at the adult stage (i.e., at postnatal days 70–75).

Although it is well established that ELS induces a life-long impact on organismal development and physiology, it is not fully understood how ELS impacts brain regions in adulthood. In mammals, one of the major factors induced by environmental stressors is glucocorticoids. Some authors have shown that experiencing ELS, like MS, leads to persistent and life-long changes in the hypothalamic-pituitary-adrenal axis, setting it at a higher point (Amini-Khoei et al., 2019; Nishi et al., 2020). On the other hand, an increase in glucocorticoid levels during the perinatal stage (a critical period of development and growth) induces long-term behavioral, immune, and metabolic changes (reviewed by Hong, 2022). In our experimental setting, MSEW increased plasma corticosterone levels at postnatal day 10, while mifepristone (a selective blocker of glucocorticoid receptors), injected every 3 days between postnatal days 4–16, prevented MSEW-induced alterations in visual/retinal function and structure in adult mice, supporting the critical role of perinatal corticoids in the long-term consequences of ELS.

Since MSEW in mice is considered a valid preclinical model to emulate early adverse experiences that reflect behavioral deficits observed in neglected humans (Nishi et al., 2020; Waters and Gould, 2022), despite the well-known differences between mice and humans, our findings support the inclusion of a visual deficit among the threats to the quality of life caused by childhood adversities. Moreover, since depression is highly prevalent in people with visual impairment (Virgili et al., 2022), it is tempting to speculate that ELS-induced visual disturbances might contribute to psychiatric problems in people with childhood adverse experiences, and vice-versa, since anxiety/depression may affect visual functions (visual learning and memory, visual attention, and visual working memory) (Kalogerakou et al., 2015), it seems possible that the co-occurrence of visual and psychiatric disturbances might exacerbate the

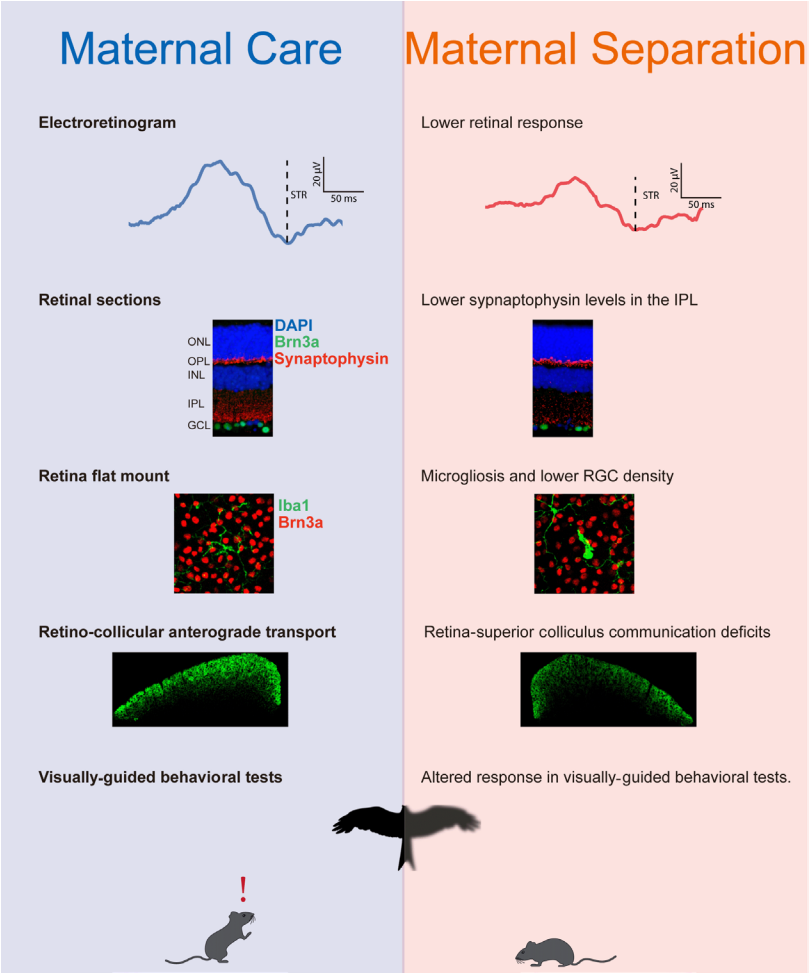

Figure 1 | **Effect of early life stress on the adult mouse visual system.**
Early life stress is defined as a period of severe and/or chronic trauma, as well as environmental/social deprivation or neglect in the pre/early postnatal stage that provokes pleiotropic adverse health outcomes in adulthood. In male and female adult mice, maternal separation with early weaning, which mimics early life maternal neglect, induces alterations in retinal ganglion cell (RGC) function (decreased scotopic threshold response (STR)), and RGC number (Brn3a-immunoreactivity), decreased synaptophysin content in the inner plexiform layer (IPL), and microgliosis, together with a retina-superior colliculus communication deficit, and decreased visual evoked potential amplitude, which translate into alterations in vision-guided behaviors (looming test). Thus, these results suggest that a visual deficit might be included among the threats to the quality of life caused by childhood adversities. Created with Adobe Illustrator 2021. GCL: Ganglion cell layer; INL: inner nuclear layer; ONL: outer nuclear layer; OPL: outer plexiform layer.

lifelong alterations induced by early life adversity. On the other hand, since the performance of mice in several behavioral tests may depend on visual capacity (Robinson et al., 2001), the present results support that the contribution of visual deficits should be taken into account when studying the behavior of ELS-subjected mice in anxiety and/or depression-like tests in adulthood. Further studies are needed to determine the precise pathway(s) involved in the long-lasting consequences of ELS on the visual system.

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Juan S. Calanni, Ruth E. Rosenstein*
Laboratory of Retinal Neurochemistry and Experimental Ophthalmology, Department of Human Biochemistry, School of Medicine/CEFyBO, University of Buenos Aires/CONICET, Buenos Aires, Argentina
***Correspondence to:** Ruth E. Rosenstein, PhD, ruthr@fmed.uba.ar.
https://orcid.org/0000-0002-8804-4395 (Ruth E. Rosenstein)

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