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Short Report

Differentiated horizontal cells seem not to be affected by apoptosis during development of the chick retina

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ABSTRACT Natural occurring cell death has been recognised as a feature of normal vertebrate development (Glucksman, 1951; Saunders, 1966). We have studied the temporal and cellular patterns of cell death in the developing chicken retina using the terminal transferase dUTP nick end-labelling (TUNEL) technique. This technique was combined with other methods to identify dying cells. Fluorescent retrograde tracing of ganglion cells or immunohistochenistry for GABA and TrkA as cell markers were combined with the TUNEL technique. The timing and distribution of dying cells suggest that each neuronal cell type in the retina dies during specific and overlapping periods: ganglion cells first, followed by amacrine cells and bipolar cells. Little cell death could be found among horizontal cells or photoreceptors. These populations seem to be protected from death. We have previously shown evidence of NGF expression among the horizontal cell population during these stages of development and also a protective role of this factor (Karlsson *et al.,* 2001) , which supports the present data.

Materials and Methods

Fertilised white Leghorn eggs were incubated in a humidified incubator and embryos were staged according to Hamburger and Hamilton (Hamburger and Hamilton, 1951). Cryostat sections parallel to the optic axis at the level of the optic nerve were processed with the TUNEL technique (Apoptosis detection system, Promega, Madison WI), according to the manufacturer. This technique was used to identify dying cells. FluoroGold (Fluorochrome, Englewood, CO) was used as a retrograde tracer for retinal ganglion cells (RGCs) and was applied to the tectum. The retinas were processed for TUNEL after retrograde tracing.

For combination of TUNEL with immunohistochemical staining, sections were processed for TUNEL and the primary antibodies were diluted in equilibration buffer and were added during the incubation with terminal tranferase. Prymary antibodies; polyclonal TrkA antibody (1 μg/ml), Anti-γ-amino butyric acid (GABA)-antibody (A-0310, SIGMA, St Louis MO) diluted 1:200. Epifluorescence using Texas Red-conjugated secondary antibodies (Vector labs, Burlingame, CA) was used to detect the antibodies.

Results

Cell death was studied in the chicken retina from embryonic day 7 (E7) to E18. The number of TUNEL positive cells increase after E7 and the majority of the first labelled cells were found in the ganglion cell layer (GCL). Peak-densities of labelled cells in the GCL were seen at E9 and E12 and in the inner nuclear layer (INL) at E11. RGCs were found labelled in both FluoroGold-tracing and TUNEL, showing which had established contact with the optic tectum, were among the cells that die (Fig. 1).

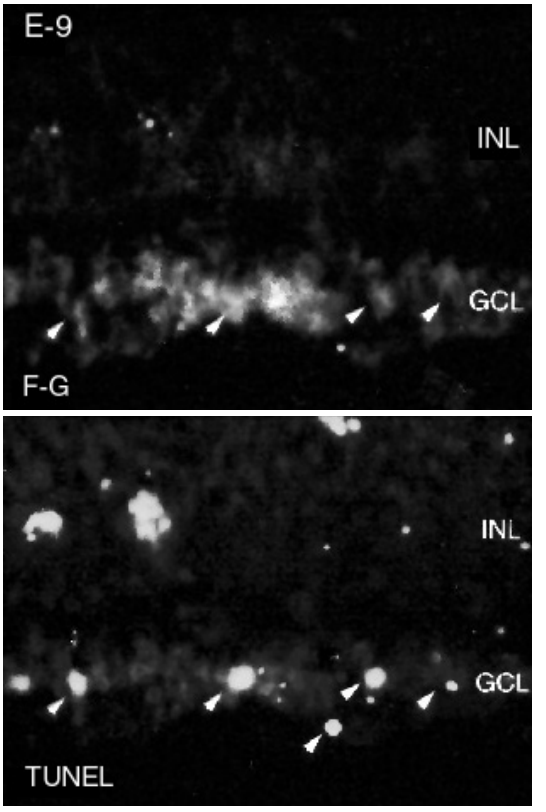

Fig. 1. Central region of a section of a retina stained for FluoroGold (F-G) and TUNEL at E9. *Arrowheads indicate the cells in the GCL that are double labelled for TUNEL and FluoroGold.*

The TUNEL cells at E12, corresponding to the minor peak, were in general smaller than those labelled at E9, suggesting that these were displaced amacrine cells. This was confirmed by combining TUNEL with immunohistochemistry for GABA, a major neurotransmiter for both displaced and non-displaced amacrine cells (Prada, 1999; Sun, 2000). Many double-labelled cells were found in the GCL as well as in the INL. GABA immuno-reaction positive cell were found close to the outer plexiform layer over

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horizontal cells, but none of those horizontal cells showed TUNEL. Over all, no or very few TUNEL-cells were detected in the external INL and outer nuclear layer (ONL) during these stages of development, which is in agreement with previous findings (Cook *et al.,*1998). TUNEL combined with immunohistochemistry for TrkA, which expressed by subpopulations of amacrine cells and horizontal cells, showed that many TrkA immuno reactive amacrine cells, but no horizontal cells were double-labelled for TUNEL. Morphologies of labelled cells and the sings of neurochemical maturation (GABA-synthesis), support, that the dying cells during these stages of development indeed are differentiating neurons. This period of cell death coincide with the establishment and maturation of functional retinal networks (Rager, 1980; Wong *et al.,* 1998).

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