


minutes. After washing, 0.3% hydrogen peroxidase was used to block endogenous peroxidase. Streptavidin HRP (1:500 in PBS) was added for 30 minutes at room temperature. DNA strand breaks were visualized by using the hydrogen peroxidase and the chromogen 3,3'-diaminobenzidine tetrahydrochloride. Positive controls were performed by incubating the slides with DNase I (1 unit/ml) for 10 minutes at room temperature; negative controls were performed by omitting TdT enzyme.

Results

Following the 50µM NMDA pulse, TUNEL-positive cells appeared dark brown (as a function of the length of incubation) as shown in Figure 1. TUNEL-positive cells were scattered throughout the inner nuclear layer (INL) and ganglion cell layer (GCL) at three hours (Panel C), and much more intense and numerous in the outer nuclear layer (ONL), INL and GCL at seven hours (Panel D). As TUNEL staining rarely labels intact nuclei (see control retinas, Panels A and B), the labeling we detected appeared to be specific for nuclear DNA strand breaks.

Summary

Evidence is presented on apoptotic cell death induced by NMDA receptors in retinal tissue as demonstrated by use of the DeadEnd™ Colorimetric Apoptosis Detection System. TUNEL-positive cells were identified in specific retinal layers predominantly affected by excitotoxic insults. 

References

1. Lipton, S.A. and Nicotera, P. (1998) *The Neuroscientist* **4**, 345.
2. Lam, T.T. et al. (1999) *Invest. Ophthalmol. Vis. Sci.* **40**, 2391.
3. Guarneri, P. et al. (1998) *Eur. J. Neurosci.* **10**, 1752.
4. *DeadEnd™ Colorimetric Apoptosis Detection System Technical Bulletin #TB199*, Promega Corporation.

Ordering Information

Product	Size	Cat.#
DeadEnd™ Colorimetric Apoptosis Detection System	40 reactions	G7130
	20 reactions	G7360

Live/Dead Assay


In situ labeling of apoptotic neurons with CaspACE™ FITC-VAD-FMK Marker

Mary L. Michaelis and Yingxue Chen

Corresponding author: mlm@ukans.edu
 Dept. of Pharmacology/Toxicology
 University of Kansas
 Lawrence, KS 66045 USA

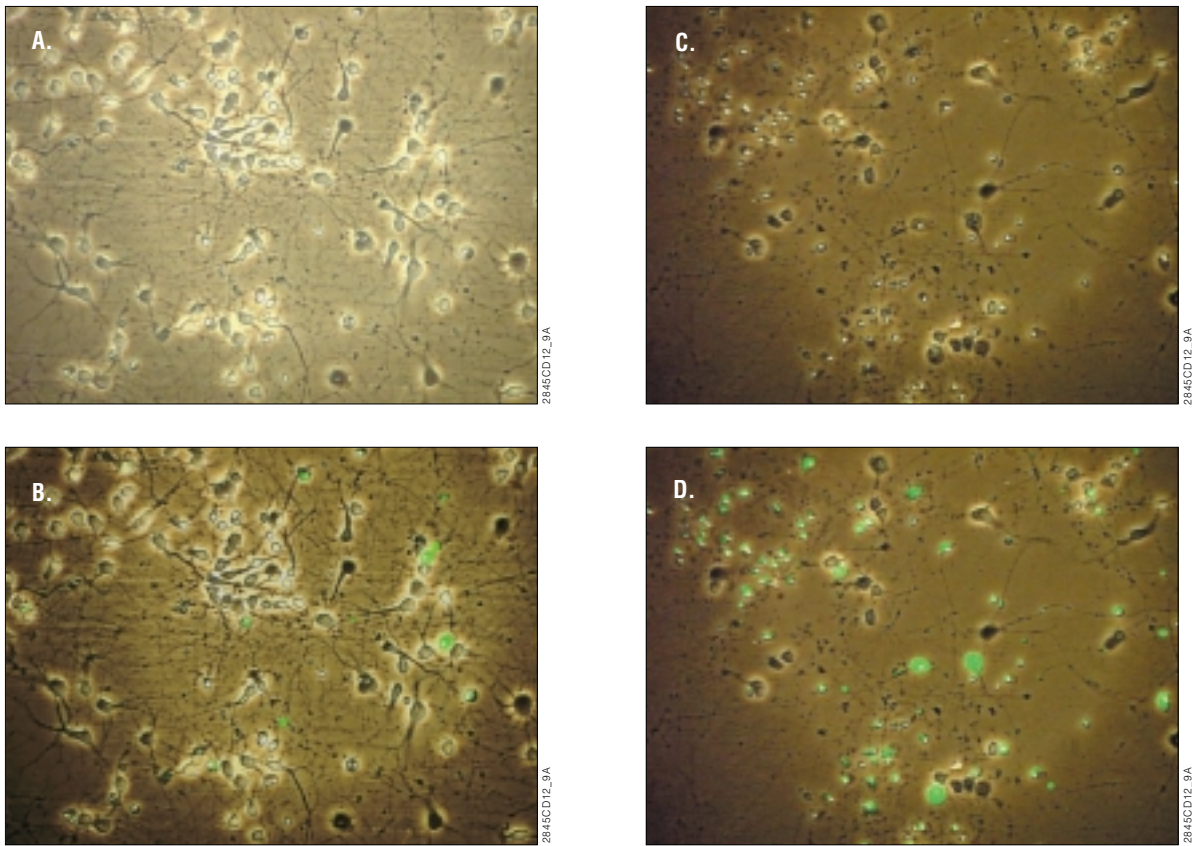
Embryonic (day 18) rat brain cortical neurons were plated at a density of 250,000 cells in 35mm glass bottom multiwell dishes and grown for 4 days in DMEM/F12 with N2 supplements. On day 4 some of the cultures were exposed to staurosporine (0.5µM) for a period of 24 hours to induce apoptosis. The cell-permeable fluorescein isothiocyanate (FITC) conjugate of the caspase inhibitor VAD-FMK (CaspACE™ FITC-VAD-FMK In Situ Marker, Cat.# G7461/G7462) was used to detect apoptotic cells. This inhibitor irreversibly binds to activated caspase, allowing for the in situ labeling of cells in which the caspase activation cascade has been initiated. Following incubation of the primary neurons with staurosporine, the CaspACE™ FITC-VAD-FMK In Situ

Marker (10µM) was added to the culture dishes and incubated at 37°C for 30 minutes. The medium was removed, and the cells were rinsed with PBS.

The dishes were placed on the stage of a Nikon® TE 200 microscope with fluorescence and DIC capabilities. The images were captured using a DAGE video camera and processed with an imaging system in Adobe® Photoshop™. Phase contrast images of the cells were obtained using DIC optics, and the fluorescence was detected at 450–490nm excitation/515nm emission. The fluorescence images were superimposed on the phase contrast images to permit both the viable and the apoptotic neurons to be observed in a single field. The total number of neurons and the percentage of apoptotic cells were easily quantified by cell counting. Primary neuronal cultures contain a small number of nonviable cells, but exposure to staurosporine markedly enhanced the number of apoptotic cells. 

Ordering Information

Product	Size	Cat.#
CaspACE™ FITC-VAD-FMK In Situ Marker	50µl	G7461
	125µl	G7462



▲ **Figure 1.**

Cortical neurons from embryonic (day 18) rat brain exposed to staurosporine to induce apoptosis. Cultures were plated at 2.5×10^5 in 35mm glass bottom microwell dishes before exposure to $0.5\mu\text{M}$ staurosporine on day 4 of culture. **Panel A:** Untreated control cultures (phase). **Panel B:** Untreated control cultures (phase and fluorescence overlay). **Panel C:** Staurosporine (phase). **Panel D:** Staurosporine (phase and fluorescence overlay). Treated cultures were incubated with staurosporine ($0.5\mu\text{M}$) for 24 hours. Cell-permeable CaspACE™ FITC-VAD-FMK In Situ Marker (Cat.# G7461, G7462) was used to detect apoptotic cells.