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# NG2 expressing cells in hippocampal cultures survive neurotoxic insult and retain the ability to divide

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Short communication

**Abstract.** It has been recently proposed that NG2 proteoglycan expressing cells may stand for a unique class of glia in adult CNS referred to as oligodendrocyte progenitor cells (OPCs). These NG2 positive cells can give rise to mature oligodendrocytes, however a large number of them persists in immature stage throughout a lifetime and responds to various types of injury. In order to investigate OPCs reactivity *in vitro*, a model of trimethyltin evoked neurodegeneration was used. The results demonstrate that NG2 expressing cells survive treatment with the neurotoxin in a concentration that injures most of neurons in the culture. Moreover, progenitors change their morphology when treated with trimethyltin, upregulate the NG2 proteoglycan expression and retain the ability to divide.

**Key words:** NG2, oligodendrocyte progenitor cells, hippocampal cultures, neurotoxic insult

NG2 proteoglycan expressing cells have been extensively studied during the last years as a unique class of glia, distinct from astrocytes, oligodendrocytes and microglia. Due to their ability to differentiate into mature oligodendrocytes (Levine et al. 1993, Nishiyama et al. 1996) they are now well accepted as oligodendrocyte progenitor cells (OPCs). However, a large pool of these cells does not pass over the immature stage and retain the ability to divide throughout a lifetime. These progenitors are evenly distributed in white and grey matter of mature CNS (Dawson et al. 2003) and comprise 5–8 % of all glial cells (Levine et al. 2001). Proliferative properties of NG2-expressing cells together with their wide occurrence in the adult brain and spinal cord raised a question about the role of these precursors in physiological and pathological conditions. Oligodendrocyte progenitor cells were shown to respond to various CNS injuries including stab wound (Hampton et al. 2004, Levine 1994), experimental allergic encephalomyelitis-EAE (Nishiyama et al. 1997), trimethyltin intoxication (Fiedorowicz et al. 2004), kainate excitotoxicity (Ong and Levine 1999) and others. Reactive oligodendrocyte progenitors highly upregulate NG2 proteoglycan expression, undergo morphological changes and tend to locate in the vicinity of degenerating neurons. However, their precise role in the pathological conditions and in the process of regeneration is still unclear.

In order to investigate the response of NG2 expressing cells to neurotoxic insult *in vitro*, a model of trimethyltin-induced neurodegeneration was used. As previously shown on mixed neuronal-glial cultures by Figiel and Fiedorowicz (2002), 1 μM of trimethyltin (TMT) evokes activation of microglia, but does not injure neurons or astrocytes, whereas 5 μM concentration of neurotoxin causes apoptosis of majority of neuronal cells and morphological changes of astrocytes.

Primary cultures were prepared from hippocampal dentate gyrus of P4 Wistar rats according to Figiel and Fiedorowicz (2002). All procedures performed on live animals were accepted by the Local Ethics Committee and were conforming to International Standards. Isolated tissue was dissociated by trypsinisation and cells were grown in Neurobasal medium (Gibco, Carlsbad, USA) supplemented with B-27 (Gibco, Carlsbad, USA), at density about 150 000/cm$^2$. After 5 days the cultures were treated with 1 μM or 5 μM TMT for 24 h and then fixed with 4% paraformaldehyde for further investigation. It was previously established by Figiel and Fiedorowicz (2002) that this *in vitro* culture consists of neurons (50%), astrocytes (40%) and microglia (10%), whereas only few mature oligodendrocytes can be identified (data not published).

The present study applied immunocytochemical staining with an antibody against NG2 proteoglycan that revealed the presence of another glia population in the culture, distinct from GFAP positive astrocytes and OX42 positive microglia (Fig.1). NG2 positive cells identified in the culture were randomly situated on the surface of astrocytes. In the untreated cultures NG2 expressing cells had round cell bodies with numerous, thin, branched processes, radially outgrowing from the cell. Studies concerning OPCs morphology *in vivo*

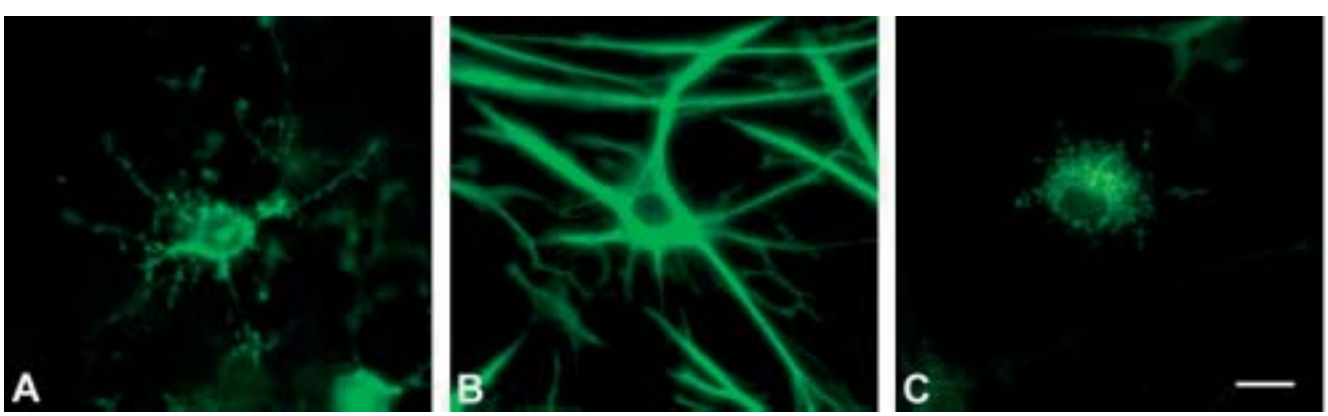


Fig. 1. Immunocytochemical staining of untreated cultures with antibodies (Chemicon, Temecula, USA) against (A) NG2; (B) GFAP; (C) OX42. Reactions developed with biotinylated antibodies (Vector, Burlingame, USA) and then visualized with avidin-FITC conjugate (Vector, Burlingame, USA). Scale bar: 10 μm.

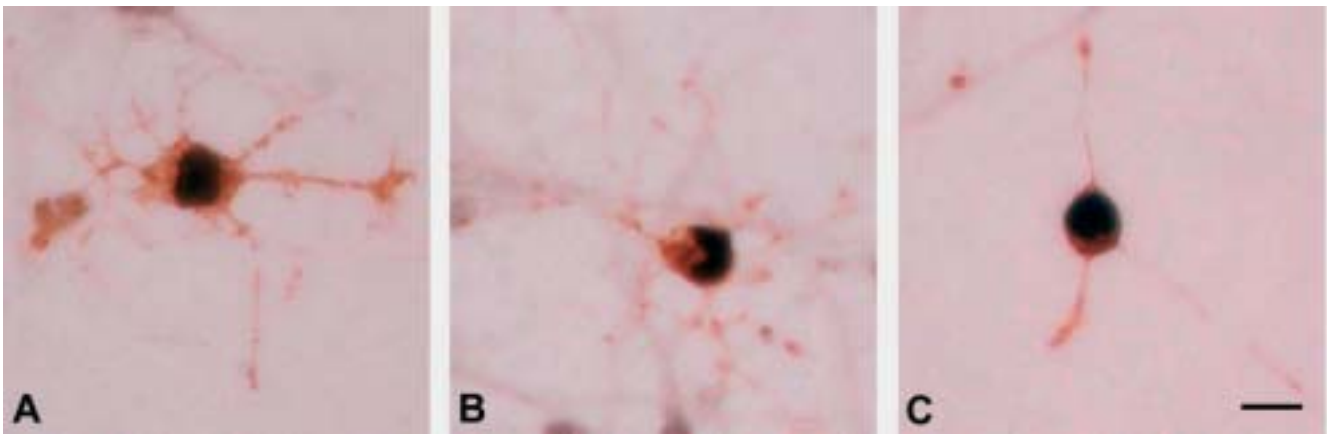


Fig. 2. Immunocytochemical staining with antibodies against NG2 (Chemicon, Temecula, USA) – brown, and PCNA (Santa Cruz Biotechnology, Santa Cruz, USA) – black. Reactions developed with biotinylated antibodies (Vector, Burlingame, USA) and then visualized with DAB (brown) or DAB + $NiCl_2$ (black). (A) Untreated culture; (B) after 24 h exposure to 1 μM of trimethyltin; (C) after 24 h exposure to 5 μM of trimethyltin. Scale bar: 10 μm.

showed that in the hippocampal gray matter processes of these cells are located around and between neuronal cell bodies (Dawson et al. 2003) and that they can interdigitate between pre- and postsynaptic terminals (Ong and Levine 1999). It was also demonstrated that oligodendroglia progenitor cells can form synaptic junctions with CA3 pyramidal neurons (Lin and Bergles 2002) suggesting their role in neuron–glia signaling in physiological and pathological conditions.

In the present study, exposure of NG2 positive cells to 1 μM TMT resulted in changing the morphology of the cells. Their processes were shorter than in control cultures, less branched and formed a number of beads at their tips (Fig. 2B). These structures can be referred to as filo- or lamellopodia which are essential for the cell migration (Levine et al. 2001) but they can also represent the sites of contact with other cells, as suggested by Dawson and coauthors (2003). These morphological changes did not affect all of the cells and were less evident than in cultures treated with 5 μM of the toxin. Exposure to 5 μM TMT led to significant retraction of the cell processes or even to complete loss of them. Furthermore there was a strong increase in NG2 proteoglycan expression illustrated by more intensive immunocytochemical staining with anti-NG2 antibody (Fig. 2C and Fig. 3A). Regular morphology of the

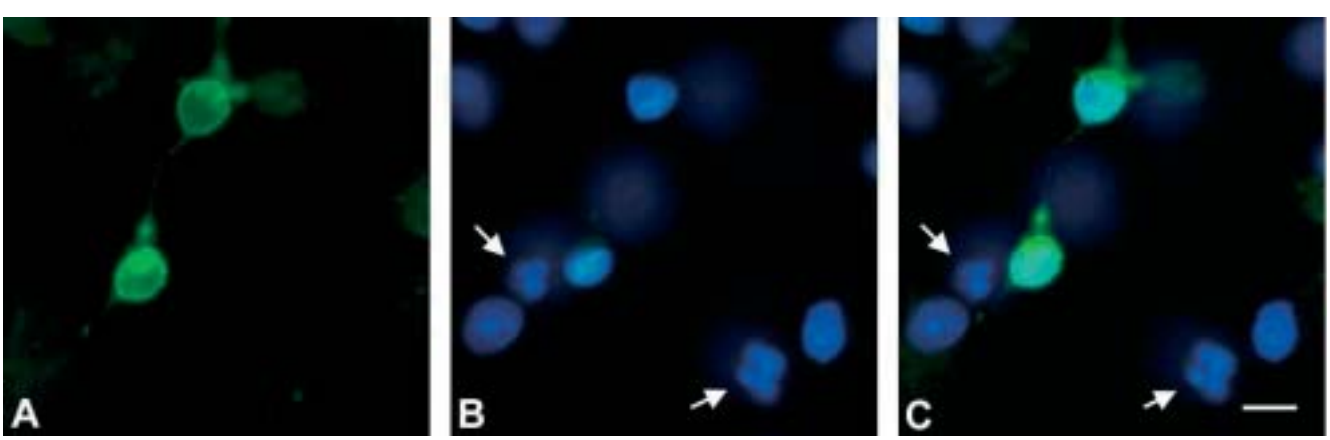


Fig. 3. (A) Immunocytochemical staining with antibody against NG2 (procedure described in Fig. 1 legend); (B) nuclei staining with Hoechst 33258 (Sigma, St. Louis, USA); (C) merge (A + B). Arrows in B and C show typical apoptotic bodies identified previously in neurons (see Figiel and Fiedorowicz 2002). Cultures treated for 24 h with 5 μM of trimethyltin. Scale bar: 10 μm.

cell nuclei demonstrated by Hoechst 33258 staining indicated that progenitors did not undergo apoptosis (Fig. 3), whereas most of the neurons were shown to possess typical chromatin condensation after 5 μM TMT (Figiel and Fiedorowicz 2002). Double immunostaining for a marker of proliferating cells – PCNA and NG2 proteoglycan revealed that a large majority of oligodendroglia progenitors continue to divide in control, as well as in TMT treated cultures (Fig. 2). There were hardly any cells found to be NG2+/PCNA-. According to *in vivo* studies on adult rat, 70% of BrdU labeled proliferating cells in hippocampus are also immunoreactive for NG2 proteoglycan (Dawson et al. 2003). This is consistent with available data that oligodendrocyte progenitor cells are the main population of cells that proliferate in mature central nervous system.

In conclusion, the results obtained in the present study indicate that OPCs retain their ability to proliferate in culture. These cells do not undergo apoptosis when treated with trimethyltin in the concentration that was shown to injure most of the neurons. However, since it was established that there are very few mature oligodendrocytes in this *in vitro* model, the fate of dividing NG2-expressing cells needs further investigation. Although recent studies suggest that NG2-positive cells can differentiate into functional hippocampal neurons (Aguire et al. 2004, Belachew et al. 2003), multipotent properties of these enigmatic cells are still not fully established.

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