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THE p75 NEUROTROPHIN RECEPTOR IS ESSENTIAL FOR NEURONAL CELL SURVIVAL AND IMPROVEMENT OF FUNCTIONAL RECOVERY AFTER SPINAL CORD INJURY

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Abstract—The mechanisms initiating post–spinal cord injury (SCI) apoptotic cell death remain incompletely understood. The p75 neurotrophin receptor (p75^{NTR}) has been shown to exert both pro-survival and pro-apoptotic effects on neural cells *in vitro*. While a previous study had shown that there is decreased oligodendrocyte apoptosis distal to a clean partial transection injury of the cord in mice with nonfunctional p75^{NTR}, most human spinal cord injuries do not involve partial transections but are rather due to compression/contusion injuries with significant perilesional ischemia. Therefore, we sought to examine the role of the p75^{NTR} in a clinically relevant clip compression model of SCI in p75 null mice with an exon III mutation. Mice with a functional p75^{NTR} had increased caspase-9 activation at 3 days after SCI in comparison to the functionally deficient p75^{NTR} mice. However, at 7 days following SCI there was no difference in the activation of the effector caspases (caspase-3 and caspase-6) at the spinal cord lesion. Moreover, at 7 days after injury, there was increased terminal deoxynucleotidyl transferase-mediated dUTP nick-end (TUNEL) positive cell death at the injury site in the functionally deficient p75^{NTR} mice. Using double labeling with TUNEL and cell specific markers we showed that the absence of p75^{NTR} function increased the extent of neuronal but not oligodendroglial cell death at the injury site. This selective loss of neuronal cells after SCI was confirmed with a decrease in levels of microtubule-associated protein 2 in the p75 null mice. Furthermore, the wild-type animals had dramatically improved survival and enhanced locomotor recovery at 8 weeks after SCI when compared with the p75^{NTR} null mice. Also at 8 weeks, there were significantly more neurons present at the injury site of wild-type mice when compared with p75 null mice. We conclude that the p75^{NTR} receptor is integral to neuronal cell survival and endogenous reparative mechanisms after compressive/contusive SCI. © 2007 Published by Elsevier Ltd on behalf of IBRO.

Key words: spinal cord injury, p75 neurotrophin receptor, caspase-3, caspase-9, caspase-8, caspase-6.

There is a critical need for more effective treatments for acute spinal cord injury (SCI) (Chu and Fehlings, 2002; Sekhon and Fehlings, 2001), a condition which leaves thousands paralyzed each year. Over the last two decades, great strides have been made to understand the CNS's response to traumatic injury. Apoptosis has been shown to be involved after SCI (Beattie et al., 2002b; Crowe et al., 1997; Fehlings and Skaf, 1998; Keane et al., 2001; Liu et al., 1997) and is considered one of the components of the 'secondary injury' cascade that occurs after neurotrauma. The mechanisms underlying posttraumatic apoptosis after SCI remains incompletely understood. However, it has been hypothesized that the apoptosis occurs after activation of death receptors such as Fas and p75 (Beattie et al., 2002a; Casha et al., 2005; Demjen et al., 2004) similar to what is noted *in vitro* with these receptors (Casaccia-Bonnefil et al., 1996; Harrington et al., 2002; Longthorne and Williams, 1997).

The p75 neurotrophin receptor (p75^{NTR}) was the first neurotrophin receptor discovered and can essentially bind to all neurotrophins with equal affinity in most cells (Roux and Barker, 2002) but it does bind to the proform of NGF with a higher affinity than the mature form (Lee et al., 2001). The main function of p75^{NTR} however, remains elusive. It has been shown to promote cell survival either in association with Trk receptors or by itself (Barker, 1998). Paradoxically, its activation has also been shown to promote apoptotic cell death (Barker, 1998; Roux and Barker, 2002). Unlike other apoptotic death receptors (Fas receptor, tumor necrosis factor receptor), p75^{NTR}-induced cell death follows the intrinsic apoptotic death pathway with release of cytochrome c from mitochondria and caspase-9 activation rather than the extrinsic pathway with caspase-8 activation (Bhakar et al., 2003). The p75^{NTR} has also been shown to be a co-receptor to the Nogo receptor thereby possibly playing a role in inhibition of axonal regeneration after SCI though *in vivo* studies have not borne out this fact (Song et al., 2004).

The p75^{NTR} has been shown to initiate the apoptotic cascade in *in vitro* studies of oligodendroglia and neurons (Bamji et al., 1998; Casaccia-Bonnefil et al., 1996). It has been shown that mice with genetic inactivation of the p75^{NTR} have decreased oligodendroglial cell death from a partial transection of the spinal cord secondary to increased pro-NGF expression at the injury site (Beattie et al., 2002a). However, the partial transection model of SCI is critically different from the most common type of SCI in humans which is a compression/contusion injury with significant associated peri-lesional ischemia (Koyanagi et al., 1993; Sekhon and Fehlings, 2001; Tator and Fehlings, 1991; Tator and Koyanagi, 1997). In the present paper, we show for the first time, using a more clinically relevant compression model of SCI, that the presence of a func-

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Abbreviations: BBB, Basso, Beattie, Bresnahan; H&E, hematoxylin and eosin; LFB, Luxul Fast Blue; Map2, microtubule-associated protein 2; p75^{NTR}, p75 neurotrophin receptor; SCI, spinal cord injury; TdT, terminal deoxynucleotidyl transferase; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end.

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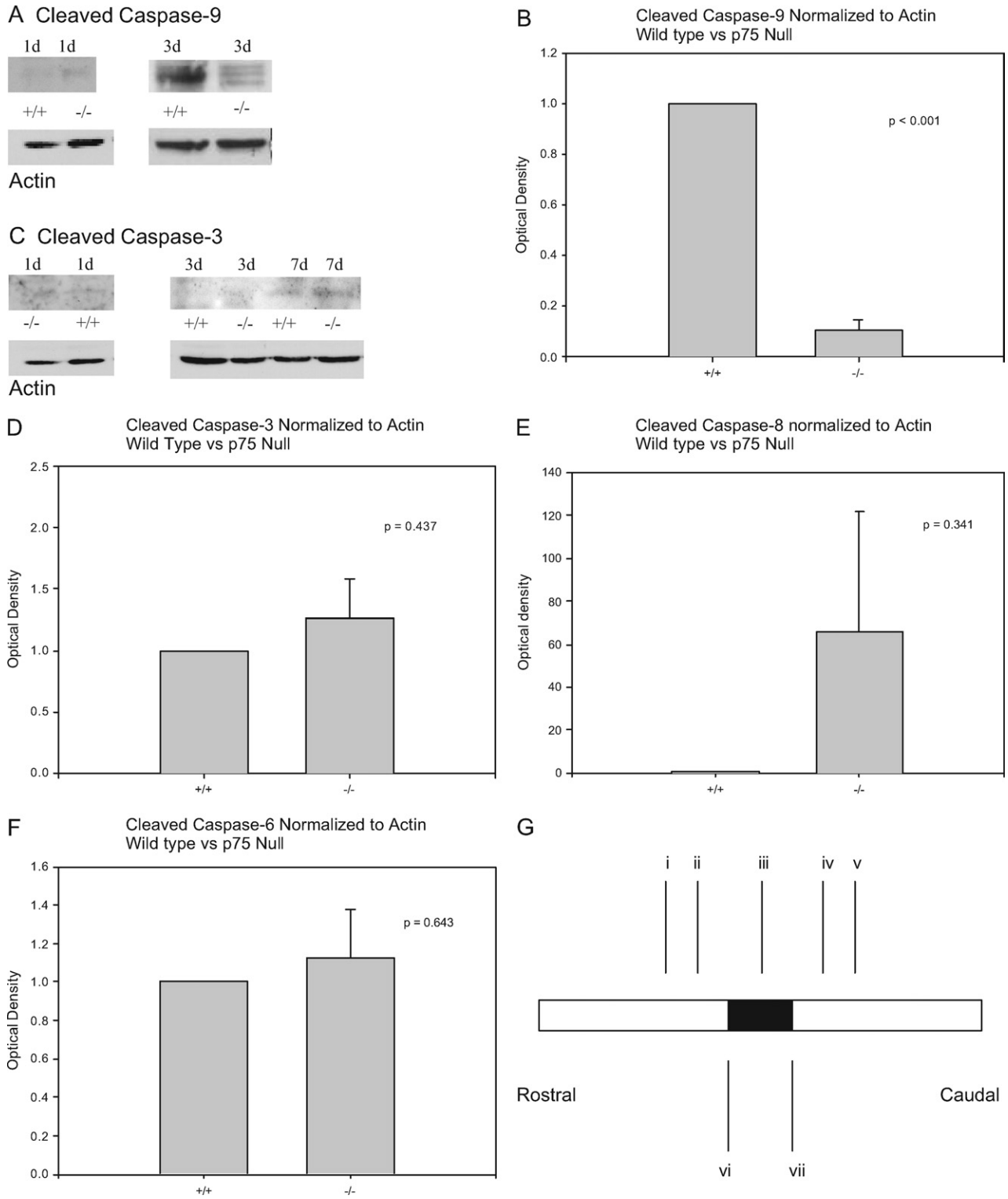

Fig. 1. p75 Null mice have decreased activated caspase-9 levels when compared with wild-type mice but not for activated caspase-3. (A) Western blot showing cleaved caspase-9 levels at the injury site from the injured mice at 1 and 3 days postinjury. The p75 null (−/−) animals have decreased levels compared with the wild type (+/+) at 3 days. At 1 day after injury, there are minimal levels of cleaved caspase-9. The actin band is shown to verify equal loading. For each group, $n=3$. (B) Bar graph demonstrating the levels of cleaved caspase-9 at the injury site, 3 days post-SCI, normalized to actin between p75 null mice and wild types. There is a significant difference between the groups at $P<0.001$. Error bars represent S.E.M. (C) Western blot showing cleaved caspase-3 levels at the injury site from the injured mice at 1, 3 days, and 7 days postinjury. At 1 day and 3 days after injury, there are minimal levels of cleaved caspase-3. At 7 days after injury, the levels are increased in both groups but there is no difference between the p75 null mice and wild types. The actin band is shown to verify equal loading. For each group, $n=4$. (D) Bar graph demonstrating the levels of cleaved caspase-3 at the injury site, 7 days post-SCI, normalized to actin between p75 null mice and wild types. There is no significant difference, with