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Life or death: developing cortical interneurons make their own decision

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During nervous system development, programmed cell death is considered as an essential adaptive process. The mechanism by which the number of mature neurons is determined in the central nervous system is not well understood. In a recent *Nature* paper, Southwell *et al* (2012) demonstrate that cortical GABAergic interneuron cell death is intrinsically determined without the need to compete for extrinsic survival signals derived from other cell types.

Programmed cell death is evolutionarily conserved throughout the animal kingdom and affects both neurons and glia of the developing central (CNS) and peripheral nervous system (PNS) (Buss *et al*, 2006). Significant cell death also occurs during multiple stages of adult neurogenesis (Ming and Song, 2011). How neuronal population size is optimized to build mature circuits is a fundamental question in developmental neurobiology. Classic studies in the PNS led to the 'neurotrophic factor hypothesis', namely, neurons are overproduced and compete for limited amounts of survival-promoting factors from targets (Levi-Montalcini and Angeletti, 1968; Barde *et al*, 1983; Figure 1). In the developing CNS, Southwell *et al* (2012) made the interesting discovery that cortical interneurons self-regulate their number independent of survival factors released from other cell types.

To characterize developmental cell death of cortical interneurons, Southwell *et al* (2012) examined GAD67-GFP knock-in mice *in vivo* and primary interneuron precursor culture *in vitro*. Interestingly, prominent interneuron death occurs in culture during the same window when cortical interneuron number declines by ~30% *in vivo*, suggesting that interneuron death can be independent of extrinsic niche components. This developmental cell death depends on Bcl-2 associated X (Bax) and occurs uniformly across all interneuron subtypes. To corroborate their findings, they characterized interneuron death in a series of elegant transplantation experiments. First, they transplanted embryonic interneuron precursors from medial ganglionic eminence into the postnatal neocortex and showed death of transplanted interneurons occurs at a cellular age similar to that of endogenous

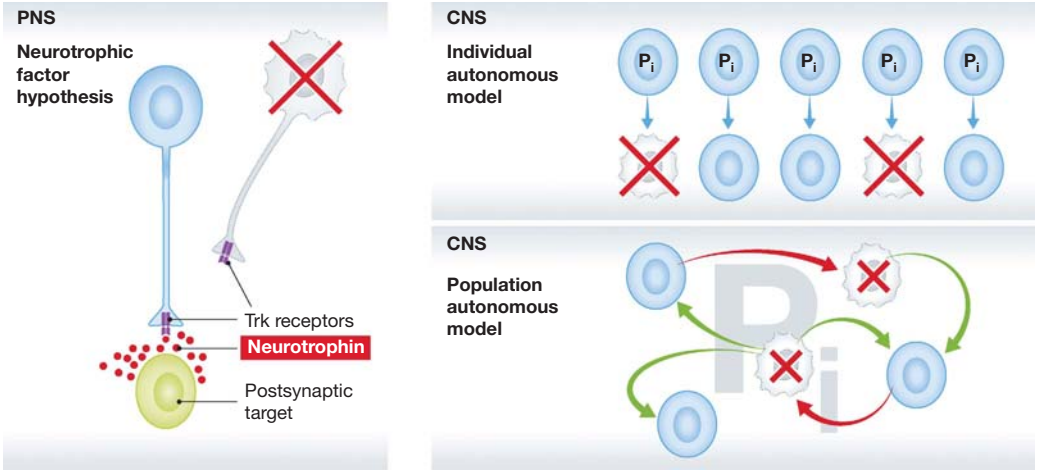

Figure 1 Neuronal number control in the developing nervous system. In the developing PNS, neurons compete for survival factors, such as neurotrophins, provided by targeted cells as a means of attaining optimal innervation. In the developing CNS, there are two proposed models for interneuron cell death. (1) 'Individual autonomous' model: interneuron cell death is intrinsically determined within each interneuron precursor in a manner independent from their interactions with other cells; each cell has a probability of Pi to survive; (2) 'Population autonomous' model: developing interneurons compete for limited survival signals produced by other interneurons born around the same time; individual neurons affect each other and the population as a whole has a probability of Pi to survive.

interneurons, suggesting a timing mechanism regulated by the intrinsic maturation state. Second, they transplanted various numbers of embryonic interneuron precursors and found that a similar fraction of transplanted interneurons survive. Third, they transplanted interneurons lacking TrkB, a neurotrophin receptor primarily expressed in the CNS, and found similar rates of cell death. These results provide strong evidence that interneuron death is not determined through competition for extrinsic survival signals. Is it then possible that transplanted interneurons compete with endogenous neurons to survive? They transplanted DsRed-expressing interneuron precursors into GAD67-GFP mice and observed equal numbers of endogenous interneurons in both recipient and control hemispheres. Together, these studies show that isochronic interneurons regulate their own numbers *in vitro*, in developing cortex *in vivo*, or after transplantation. An intrinsic regulatory mechanism is in sharp contrast to the 'neurotrophic factor hypothesis' based largely on PNS studies. There have been hints of fundamental differences in the CNS. For example, brain-derived neurotrophic factor (BDNF) is the most widely expressed neurotrophin in the CNS, but its elimination fails to affect neuronal numbers in most CNS areas (Ernfors *et al*, 1994). In a study using engineered embryonic stem cells, TrkB (although not TrkA and TrkC) does not trigger neuronal death and it was proposed that cell–cell contact and synaptic transmission control CNS neuronal survival (Nikoletopoulou *et al*, 2010).

The intriguing findings by Southwell *et al* (2012) raise more questions. First, two models have been proposed to explain cell intrinsic regulation of interneuron death in developing cortex (Figure 1). In the 'individual-autonomous' model, interneuron death is intrinsically determined within each precursor; in the 'population-autonomous' model, developing interneurons of the same age compete for limited survival signals produced by other interneurons. How to differentiate these two models experimentally? One possibility is to co-transplant a mixture of normal interneurons from GAD67-GFP mice with isochronic Bax KO interneurons. If the 'individual' model holds true, then the number of surviving GFP^+ neurons should be in proportion to total transplanted GFP^+ cells. If the 'population' model is correct, then fewer GFP^+ interneurons are expected to survive because of absence of cell death in the Bax KO population. Second, what are underlying cellular and molecular mechanisms? In the 'individual' model, is it epigenetically or transcriptionally regulated, analogous to intrinsically programmed differentiation of embryonic CNS neural stem cells (Okano and Temple, 2009)? In the 'population' model, how do interneurons achieve targeted communication with other interneurons? One possibility is via gap junctions between neocortical interneurons (Druga, 2009). A recent study showed that gap junctions allow selective communication between developing sister excitatory neurons to promote lineage-dependent microcircuit assembly in the neocortex (Yu *et al*, 2012). Interneurons also form reciprocal synaptic connections. Future genetic and pharmacological studies can test these possibilities.

Another striking finding of Southwell *et al* (2012) is that mouse neocortex has the capacity to maintain 35% more interneurons. What are the consequences of adding more neurons in the brain? Southwell *et al* (2012) performed patch-clamp recordings and found that transplanted interneurons are successfully integrated into the cortical circuitry. Interestingly, only the frequency, not the amplitude, of inhibitory events onto pyramidal neurons is increased, but not in proportion to the density of transplanted interneurons. Thus, cortical inhibition may arise through homeostatic regulation of synaptic strength and number, instead of interneuron population size. These findings have important implications for cell replacement therapy.

Given that the regulation in both number and properties of cortical interneurons under many physiological and pathological conditions, such as ageing, Alzheimer's diseases, chronic stress, schizophrenia and other severe psychiatric illnesses, and epilepsy, the findings by Southwell *et al* (2012) have significant implications for developmental neurobiology, neural plasticity and regenerative medicine.

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Conflict of interest

The authors declare that they have no conflict of interest.

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