

Homeostatic Regulation of Interneuron Apoptosis During Cortical Development

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ABSTRACT: The mammalian cortex consists of two main neuronal types: the principal excitatory pyramidal neurons (PNs) and the inhibitory interneurons (INs). The interplay between these two neuronal populations – which drive excitation and inhibition (E/I balance), respectively – is crucial for controlling the overall activity in the brain. A number of neurological and psychiatric disorders have been associated with changes in E/I balance. It is not surprising, therefore, that neural networks employ several different mechanisms to maintain their firing rates at a stable level, collectively referred as homeostatic forms of plasticity. Here, we share our views on how the size of IN populations may provide an early homeostatic checkpoint for controlling brain activity. In a recent paper published in *Cell Reports*, we demonstrate that the extent of IN apoptosis during a critical early postnatal period is plastic, cell type specific, and can be reduced in a cell-autonomous manner by acute increases in neuronal activity. We propose that a critical interplay between the physiological state of the network and its cellular units fine-tunes the size of IN populations with the aim of stabilizing network activity.

KEYWORDS: Cortical interneurons, cell death, neuronal activity, Lhx6 transcription factor

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A feature of neural development is an initial large-scale proliferation followed by a decrease in overall neuron numbers through apoptosis. During early postnatal stages, the overall levels of apoptosis are very high, reaching 50% in some regions of the cortex.¹ This prominent event will have a large impact in establishing the correct numbers of distinct neuronal populations in mature circuits, which is crucial for the ratio between pyramidal neurons (PNs) and inhibitory interneurons (INs), and excitation/inhibition (E/I) balance in the brain. Interestingly, the ratio of PNs to INs is tightly controlled throughout pallial development² and is conserved across mammalian species and different brain regions.³ How the output of developmental programmes that regulate the number and type of neuron matches the functional requirements of mature cortical circuits remains mostly unclear.

Early studies have postulated that the size of peripheral neuron pools is determined by the presence of limiting amounts of retrograde pro-survival signals present within the relevant target region.^{4,5} However, this neurotrophic theory has been less successful at explaining how apoptosis is controlled in the central nervous system (CNS).¹ In vivo and in vitro studies have demonstrated that the survival of cortical PNs is enhanced by network activity and that NMDA (N-methyl-D-aspartate) receptor-mediated synaptic currents modulate rates of apoptosis.^{6,7} Similarly, olfactory bulb interneuron survival can be dramatically influenced by the activity of the mature networks

they integrate into.⁸ Recently, Southwell and colleagues have demonstrated that the number of cortical INs is also determined through cell apoptosis. However, in contrast to the above studies, they suggested that INs are eliminated by a programme of apoptosis that is intrinsically determined.⁹ We sought to address the question whether IN survival is determined by a rigid cell-intrinsic programme operating at the progenitor level or whether there is developmental plasticity driven by network activity.

Cortical INs comprise a diverse group of neurons. At least, 15 functionally distinct IN subtypes are derived from three proliferating regions of the embryonic basal telencephalon: the medial and caudal ganglionic eminences (MGE–CGE) and the preoptic area (POA). Each subpallial domain is characterized by the expression of a unique combination of transcription factors that regulate the development of IN subtypes born in each domain.¹⁰ Among them, the LIM-homeodomain transcription factor Lhx6 is expressed only in the MGE lineage and regulates the development of IN subtypes that are characterized by the expression of the calcium-binding protein parvalbumin (PV) and the neuropeptide somatostatin (SST),^{11,12} which represent 70% of all cortical INs.³ We took advantage of null (Lhx6⁻) and cell lineage-specific mutants (Lhx6fl) for Lhx6, and performed lineage tracing experiments to ask whether apoptosis of developing cortical interneurons was regulated by the environment on the developing cortex.¹³



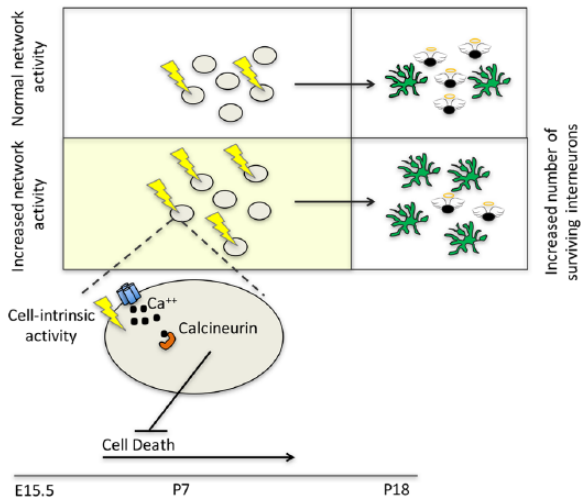


Figure 1. Proposed model for an activity-dependent control of inhibitory interneuron (IN) survival. Under normal network activity levels, a constant proportion of immature cortical INs dies due to apoptosis. When network activity is increased, the fraction of INs that undergoes apoptosis is significantly reduced. The survival of INs is dependent on a cell-autonomous induction of activity-dependent pro-survival pathways. Most probably, these pathways are dependent on the function of calcium-binding phosphatase calcineurin.

First, we introduced the Cre-dependent fluorescent reporter Rosa26-tdTomato (tdT, Ai14) into the *Lhx6^{fl/-}* genetic background (Ai14;*Lhx6^{fl/-}*), and used two Cre drivers to ablate *Lhx6* and follow the fate of all cortical INs (*VgatCRE*) or just the ones that arise from the MGE (*Nkx2.1CRE*). We found that although *Lhx6* activity is required for the survival of MGE-derived INs, surprisingly the total number of INs remained similar between control and *Lhx6* mutant mice. This observation prompts us to think that another *Lhx6*-independent lineage might compensate for the loss of MGE-derived *Lhx6* mutant INs. Indeed, when we analysed the CGE lineage by crossing the *5Htr3a* Cre deleter line to the Ai14;*Lhx6^{fl/-}* genetic background, we observed a dramatic increase in the number of CGE-derived INs in *Lhx6* mutant brains compared with control littermates. By further examining the above mutant mice at different developmental stages and in combination with TUNEL (Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling) analysis of apoptosis, we found that the increased representation of CGE-derived INs in the cortex of *Lhx6*-deficient mice results from a decrease in their rate of developmental cell death during the normal postnatal period of IN apoptosis. In parallel experiments, we performed cell transplantations of wild-type (wt) INs into the cortex of newborn *Lhx6*-deficient mice and their control littermates. We observed enhanced survival of the grafted wt INs in *Lhx6* mutant cortices compared with controls. Collectively, the above results argue against a set cell-intrinsic programme that determines the survival of developing cortical INs, and suggest an interplay between the emerging cortical environment and its cellular components that fine-tune their fate.

What is so unique therefore about the cortical environment of *Lhx6*-deficient mice? Recent and previous studies from our lab provide molecular and electrophysiological evidence for increased network activity in the cortex of *Lhx6* null mice. Importantly, we observed a dramatic upregulation in the expression of a number of activity-dependent immediate early genes in *Lhx6* mutant brains during the normal peak of interneuron cell death. Even more remarkable, these markers were upregulated not only in projection neurons but also in the CGE-derived IN lineage, which reduces its apoptosis to compensate for the loss of MGE-born INs. We therefore reasoned that a cell-autonomous increase in the activity levels of cortical INs might trigger pro-survival or block pro-death signalling. To directly test this hypothesis, we employed chemogenetics to transfect immature INs with Designer Receptors Exclusively Activated by Designer Drugs (DqDREADD),¹⁴ so that they are activated on administration of the appropriate ligand Clozapine-N-Oxide (CNO). By performing similar intracranial cell transplantations as described above, we observed that administration of CNO results in an increase in the proportion of IN expressing DqDREADD relative to transfection controls, compared with the ratios found in vehicle-administrated cortices. These results strongly suggested that IN survival is promoted by electrical activity.

Our work was further supported by recent findings from G. Fishell's lab.¹⁵ This study extended our observations by showing that reducing activity promotes cell death, revealing a bimodal regulation of apoptosis by neuronal activity. Interestingly, both studies revealed that CGE-derived VIP (Vasoactive-Intestinal polypeptide) expressing INs (an IN subtype that specifically targets other INs and has been shown to have a disinhibitory effect¹⁶) do not adjust apoptosis in response to activity changes. Collectively, both studies suggest that signals from brain microenvironment together with activity-dependent intrinsic signalling pathways determine whether a particular IN will survive or die (Figure 1). We propose that IN survival will be dependent on (1) the presence of extracellular pro-survival factors (some of which will depend on PN activity, such as tonic release of neurotransmitters or growth factors); (2) composition of transmembrane molecules (receptors and ion channels), which will be specific to different IN subtypes; and (3) intracellular cascades, such as calcineurin, implicated in pro-survival and IN maturation signalling,¹⁴ whose function depends on activity (intracellular Ca^{2+} concentration).

These findings represent a novel mechanism for regulating the relative numbers of excitatory and inhibitory neurons in the brain that contribute to stabilizing levels of network activity. This adds developmental apoptosis to an expanding list of steps in IN development that are modulated by network activity (either spontaneously generated or driven by sensory stimulation) such as migration, morphological and physiological maturation.^{17–21}

Author Contributions

MD took the lead in writing the manuscript. GN, JB and VP provided critical feedback and helped to the final version of the manuscript.

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