

Feedback-mediated neuronal competition for survival cues regulates innervation of a target tissue

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Summary

Proper wiring of the nervous system requires tight control of the number of nerve terminals that innervate a target tissue. Recent work by Deppmann et al.,⁽¹⁾ now suggests that this is achieved by feedback-mediated neuronal competition for target-derived survival cues. The authors' model is inspired by the theory for pattern formation based on self-activation and lateral inhibition, proposed by Meinhardt and Gierer more than 30 years ago.⁽²⁾ *BioEssays* 30:929–933, 2008. © 2008 Wiley Periodicals, Inc.

Feedback loops in pattern formation

Animal development involves the formation of elaborate patterns that must be generated with exquisite precision, in a process that is resistant to noise. The signaling network underlying developmental patterning must therefore be precisely controlled to avoid dangerous errors. Positive and negative feedback loops have emerged as an important regulatory mechanism that ensures both precision and robustness in developmental signaling.⁽³⁾

Feedback in biological systems is an evolutionarily conserved regulatory mechanism that controls the dynamic behavior of signaling networks. By shaping the response to

external inputs in time and space, feedback—both positive and negative loops—can generate complex cellular and physiological outcomes, such as Ca^{2+} oscillations,⁽⁴⁾ circadian rhythms,⁽⁵⁾ cell polarization,^(6,7) cell fate decisions^(8,9) and patterning.^(3,10) A feedback loop can be defined as the ability of a system to adjust its output by monitoring itself. A negative feedback loop occurs when, for example, a signal induces the expression of its own inhibitor. Negative feedback brings a system back to equilibrium in response to external changes and thus generally regulates cellular homeostasis.⁽¹¹⁾ In contrast, a positive feedback loop occurs when a signal induces more of itself and drives the system away from equilibrium. Positive feedback amplifies the original perturbation and can, under the right circumstances, convert graded inputs into a switch-like, all-or-none response.⁽¹¹⁾

A ubiquitous network motif in pattern formation consists of a local positive feedback loop coupled to a long-range inhibitory signal. Since its original theoretical description in 1972 by Meinhardt and Gierer,⁽²⁾ self-activation and lateral inhibition has been proposed to underlie pattern formation in various developmental systems, including embryo segmentation,⁽¹²⁾ cell fate induction⁽¹³⁾ and single cell polarization.⁽⁷⁾ The simplest molecular representation of this network consists of a self-amplifying activator that regulates the production of its diffusible long-range antagonist. Such a mechanism can generate stable patterns in a field of cells, or at the single cell level if the spread of self-amplification is spatially restricted. This model is at first sight counterintuitive. Why doesn't the system inhibit itself, given that the source of positive feedback is also the center of inhibition? The answer to that apparent paradox came again from theoretical modeling. The rate of production of the activator must be non-linear or cooperative in nature in order to "out-compete" locally the long-range inhibitory effect.⁽¹⁰⁾ This criterion is met if two or more elements of the positive feedback circuit need to co-operate to fulfill their functions. (A classical example of cooperativity in calcium signaling is the inositol 1,4,5-trisphosphate (IP3) receptor, which requires three molecules of IP3 for activation.⁽¹⁴⁾) A central feature of this network is the ability to create stable patterns from random fluctuations, a

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Abbreviations: Akt, Protein Kinase B (PKB); Bax, Bcl-2 antagonist X; BDNF, brain-derived neurotrophic factor; IP3, inositol 1,4,5-trisphosphate; KLF 7, kruppel-like factor 7; MAPK, Mitogen-activated protein kinase; NGF, nerve growth factor; NT-4, neurotrophin-4; PNS, peripheral nervous system; TrkA, receptor tyrosine kinases A; Ras, Ras GTPase; PI3K, phosphatidylinositol 3-kinase.

property that is thought to underlie the self-organizing properties of many cells and tissues.

Feedback-mediated neuronal competition for survival cues

The combination of local positive feedback and lateral inhibition has been proposed to regulate various steps in the development of the nervous system, from the specification of neuronal cell fates⁽¹³⁾ to synapse elimination at the neuromuscular junction.⁽¹⁵⁾ In a recent issue of *Science*, Deppmann et al.,⁽¹⁾ now propose that a similar design underlies neuronal competition during target field innervation. Seminal work in the 1950s revealed that the amount of neuronal innervation that a target tissue receives depends on the amount of trophic factors that it secretes.⁽¹⁶⁾ The neurotrophin factor hypothesis⁽¹⁷⁾ further stipulates that developing neurons are overproduced, and compete for limiting quantities of target-derived survival cues. The classical target-derived growth factor NGF (Nerve Growth Factor) promotes survival, maturation and target innervation of sympathetic and sensory neurons of the peripheral nervous system (PNS).^(18–21) NGF exerts its functions by engaging its receptor tyrosine kinase TrkA on the distal axon. The NGF–TrkA complex is endocytosed and trafficked retrogradely from the periphery to the cell body where it activates an NGF-dependent transcriptional program that promotes cell survival and growth via the Ras–MAPK and PI3K–Akt signaling pathways.⁽²²⁾ Based on experimental and modeling data, the authors suggest that survival of sympathetic neurons is a highly competitive process, mediated by a local (autocrine) positive feedback loop coupled to a long-range (paracrine) apoptotic signal triggered by target-derived NGF signaling.

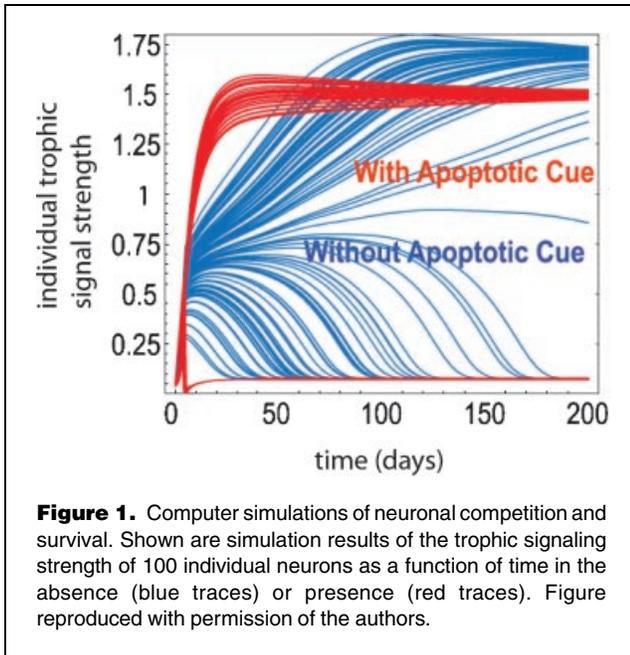
The starting point of their work is a genome-wide expression profile analysis of sympathetic neurons isolated from newly born mice with an intact or disrupted NGF locus (NGF^{-/-}). Because NGF is indispensable for neuronal survival, the authors used a NGF^{-/-} Bax^{-/-} mouse line deficient in apoptosis, a method pioneered by Snider and colleagues to maintain neuronal survival in the absence of NGF.⁽²³⁾ Interestingly, several pro-survival genes including brain-derived neurotrophic factor (BDNF), TrkA and the neurotrophin receptor p75 were downregulated in NGF^{-/-} Bax^{-/-} animals. Consistent with these *in vivo* results, TrkA expression levels and downstream signaling were significantly reduced in cultured sympathetic neurons that had been deprived of NGF for a day or two. Furthermore, the duration of pro-survival signaling, as defined by the time after NGF deprivation the pathway remains active, increased as a function of NGF exposure and neuron maturity. These observations led the authors to propose that sustained pro-survival signaling is controlled by a positive feedback loop whereby NGF induces the expression of its own receptor TrkA. As their microarray analysis suggests, multiple interconnected

feedback loops may be involved in shaping the NGF-dependent pro-survival response, as indicated by reduced expression of other neurotrophins and neurotrophin receptors (i.e. BDNF and p75) in the NGF^{-/-} Bax^{-/-} mouse. Further amplification could also result from previously described feedbacks and crosstalks in the Ras–MAPK and PI3K–Akt signaling modules.^(24–29)

To test the idea that feedback regulation in NGF signaling shapes the pro-survival response and confers competitive advantage, Deppmann et al., turned to mathematical modeling. Their model is based on two differential equations describing (1) the relative magnitude of trophic signaling (defined as the amount of NGF-bound TrkA) sensed by a single neuron and (2) the concentration of NGF available for each neuron at the target (assuming that the rate of NGF production is constant). The results of these simulations can be graphically represented as the strength of trophic signaling (i.e. concentration of NGF-bound TrkA) per unit time for each individual modeled neuron (Fig. 1). The authors considered neurons dead if their trophic signaling fell under 10% of the maximal value. To probe the role of the NGF–TrkA feedback in neuronal competition, three different scenarios were envisioned. Simulations in which both TrkA expression (i.e. signaling strength) and signaling duration were constant and independent of NGF (i.e. no feedback) led to no apparent competition. All modeled neurons survived and reached the same steady state in trophic signaling. Next, either TrkA expression or signal duration was allowed to change in response to NGF. Neurons reached a trophic signaling steady state more quickly than in the absence of feedback, but competition also failed to occur. In other words, a positive feedback loop whereby NGF-bound TrkA stimulates production of TrkA is not sufficient to elicit competition among neurons. Finally, both expression levels and signaling duration of TrkA were allowed to vary upon exposure to NGF, as the experiments suggest (the authors modeled increased signaling duration by a NGF-dependent decrease in TrkA degradation). In this paradigm, simulations led to a very interesting outcome. Some neurons reached a high trophic state and survived, while others died (Fig. 1, blue traces). The bimodal nature of these simulations indicates that neurons compete for a limiting amount of NGF. One interpretation of these simulations is that subtle neuron-to-neuron differences in the initial strength of EGF–TrkA signaling triggers the positive feedback in a subset of cells that consume the available pool of NGF by increasing both the production of TrkA (signaling strength) and duration of signaling.

Lateral inhibition by apoptotic cues

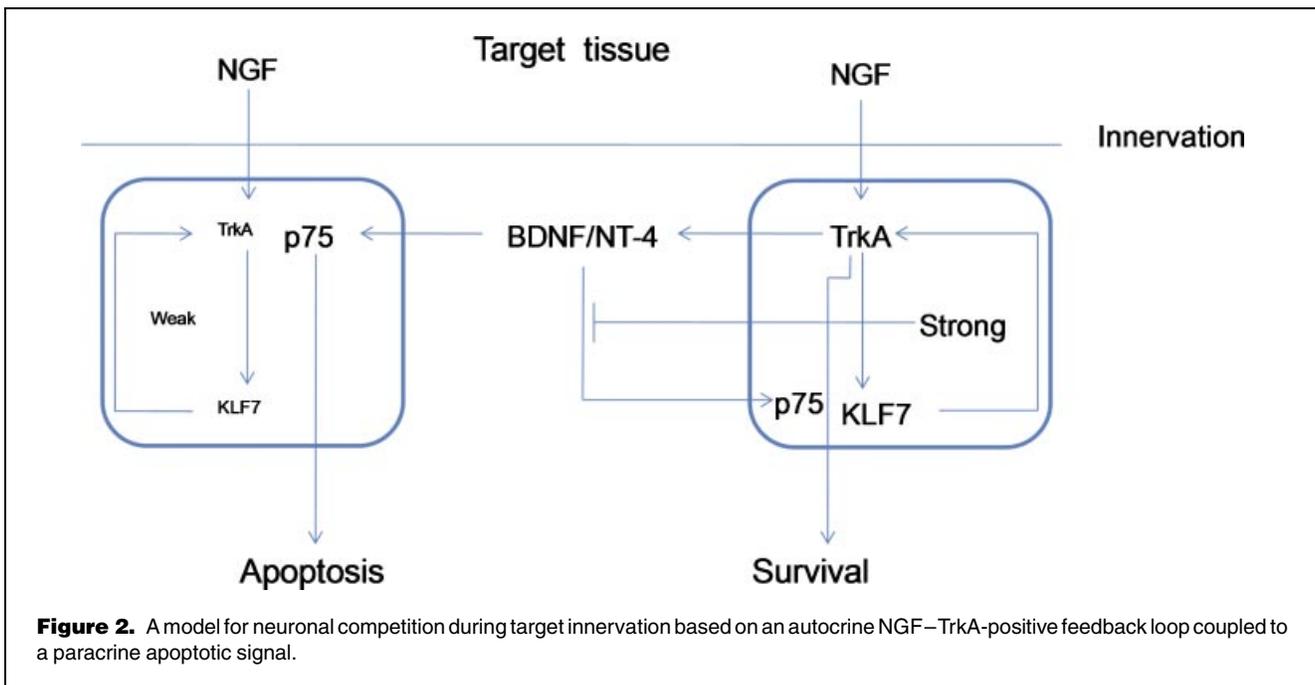
Although neuronal competition occurs in this last simulation scenario, it is relatively slow and is characterized by the presence of a number of intermediate trophic states (Fig. 1, blue traces). This prompted the authors to investigate whether



a mechanism based on lateral inhibition would promote further competition. Such a mechanism has been previously proposed to underlie synapse elimination at the neuromuscular junction during innervation of a muscle fiber by motor axons.⁽¹⁵⁾ One clue as to what this antagonistic signal may be came from the microarray data. The neurotrophin receptor p75 and the neurotrophin BDNF were two of the top three genes whose expressions were downregulated in the NGF^{-/-}

Bax^{-/-} mouse. Interestingly, p75 is a member of the tumor necrosis factor receptor family and can directly induce neuronal cell death by apoptosis,⁽³⁰⁾ which is important for the normal development of the central and peripheral nervous system.^(31,32) Furthermore, BDNF and NT-4, another member of the neurotrophin family, can promote apoptosis of sympathetic neurons through the receptor p75.^(31,33) Deppmann et al., went on to test whether high trophic signaling promotes apoptosis in neurons with low trophic signaling via a p75-mediated pathway. In agreement with the Meinhart and Gierer postulate, the putative proapoptotic cue would have to meet several criteria. It should (1) be produced by neurons with strong trophic signaling and (2) kill neurons with low trophic signaling. (3) Neurons with strong trophic signaling should be protected from the apoptotic cue. (4) Apoptotic signaling should be delayed with respect to the increase of TrkA expression upon target innervation. Previous knowledge on p75-induced apoptosis has well as an additional series of experiments performed by the authors indicate that BDNF and NT-4 satisfy these requirements. Perhaps the most-compelling arguments are that (1) BDNF and NT-4 induce apoptosis of sympathetic neurons in the presence of low concentrations of NGF, but (2) strong NGF–TrkA signaling blocks p75-mediated apoptosis of sympathetic neurons.^(1,34) (3) BDNF and NT-4 do not trigger apoptosis of p75^{-/-} neurons and (4) BDNF and NT-4 expression levels are controlled by NGF.⁽¹⁾

To evaluate the impact of apoptosis on neuronal competition, the authors turned again to computational modeling. They introduced a series of terms to their original set of equations to describe apoptotic signals (BDNF and NT-4) and their



receptors (p75), based on the premise that the apoptotic cue increases with exposure to NGF and has no effect on neurons with high NGF–TrkA signaling. The resulting simulations showed now a clear bistable behavior (Fig. 1, red traces). Neurons that did not gain a competitive advantage died ten times more quickly than in the absence of apoptotic cues, while the surviving ones reached a steady state in trophic signaling much faster. It is worth noting that the apoptotic cue speeds up competition, but does not change the final outcome of these simulations. More or less 50% of the cells end up dying with or without paracrine apoptotic signaling (Fig. 1). A testable prediction of this model is that immediately following target innervation (i.e. right after birth) the number of sympathetic neurons in p75^{-/-} mice should be higher than in control littermates whereas, in adult animals, once the competition process has been completed, the number of neurons should be identical. As predicted by the model, the number of sympathetic neurons in the superior cervical ganglia transiently increases after birth in the p75^{-/-} mouse and is back to normal in the adult animal.⁽¹⁾

Future prospects

In conclusion, the authors' model stipulates that minute differences in the amount of target-derived NGF signaling sensed by individual axons (due for example to local variations of NGF concentration or TrkA expression) are amplified through a transcription-dependent feedback loop that provides survival advantage. Competition is further strengthened by feedback-dependent expression of apoptotic cues which selectively kill neighboring neurons with low NGF–TrkA retrograde signaling (Fig. 2). This simple model allows for tight control of the number of innervating axons that synapse on the target tissue, even in a scenario in which all neurons arrive simultaneously at the target and are virtually equivalent in their initial responsiveness to NGF, assuming that there is sufficient stochastic fluctuations NGF–TrkA signaling to quick start the positive feedback.

Although Deppmann et al.,⁽¹⁾ did not show direct evidence for competitive elimination of innervating axons, their model is in agreement with recent data indicating that developmental axon pruning of sympathetic neurons projecting to the eye is mediated by BDNF–p75-dependent axon degeneration.⁽³⁵⁾ Imaging of axon terminals as they reach their target will be required in order to directly assess the contribution of the NGF–TrkA-positive feedback loop to neuronal competition. Selective disruption of this feedback circuit will necessitate the identification of the signaling components that mediate transcriptional feedback in NGF signaling. In this regard, the transcription factor Kruppel-like factor 7 (KLF 7), which regulates expression of TrkA⁽³⁶⁾ and whose expression is controlled by NGF appears to be a promising candidate. Another important unresolved issue concerns the mechanisms by which strong NGF–TrkA signaling confers resistance

to the apoptotic cues. According to the Gierer and Meinhardt postulate, positive feedback and cooperativity in NGF–TrkA signaling would be sufficient to override the apoptotic signal. Interestingly, activation of the MAPK Erk by NGF is ultrasensitive,⁽³⁷⁾ suggesting that non-linearity in the MAPK pathway in addition to the NGF–TrkA feedback loop could provide the basis for the resistance to p75-mediated cell death.

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