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Neural Activity and Survival in the Developing Nervous System

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Abstract

Recent evidence suggests that blockade of normal excitation in the immature nervous system may have profound effects on neuronal survival during the period of natural cell death. Cell loss following depression of electrical activity in the central nervous system (CNS) may explain the neuropsychiatric deficits in humans exposed to alcohol or other CNS depressants during development. Thus, understanding the role of electrical activity in the survival of young neurons is an important goal of modern basic and clinical neuroscience. Here we review the evidence from in vivo and in vitro model systems that electrical activity participates in promoting neuronal survival. We discuss the potential role of moderate elevations of intracellular calcium in promoting survival, and we address the possible ways in which activity and conventional trophic factors may interact.

Index Entries: Neurodevelopment; calcium; apoptosis; programmed cell death.

Introduction

It is well accepted that natural cell death (NCD; also called programmed cell death or physiological cell death) is an important means by which final numbers of neurons (and glia) in the nervous system are sculpted. Up to 70% of neurons, depending on the region examined, may be lost during development *(1).* Reactivation of the machinery responsible for cell death may also be important in the etiology of neurodegenerative and neuropsychiatric disorders in the mature or aging nervous system. Therefore, understanding the control and mechanisms of NCD is an important goal. According to classical mechanisms of neuronal survival during development, neurons are dependent on target-derived neurotrophic factors at or around the time of synapse forma-

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tion. It is also clear that afferent activity supports neuronal survival in a number of brain regions and model systems, although it is not certain how activity interacts with conventional trophic molecules to support survival. In vitro models of peripheral and central neurons demonstrate fairly conclusively that activity itself can be survival promoting, particularly when conventional exogenous trophic factors are absent. Drawing on recent results, we consider possible ways in which activity may interact with traditional neurotrophic factors to sculpt final numbers of neurons in the central nervous system (CNS).

The Classical View of Regulation of Developmental Neuronal Survival

Studies performed with chick embryos showed that extirpation or addition of limb muscle led to loss or gain of motorneurons, respectively, compared to normal embryos *(2).* These experiments suggested that a target-derived substance controls the degree of NCD among motor neurons. Subsequent experiments in peripheral neurons including the sympathetic nervous system culminated in the purification of nerve growth factor (NGF), a peptide that supports survival of sympathetic and many sensory neurons and that is derived from target tissues of dependent neurons. Target-derived neurotrophic factors act through receptor tyrosine kinases and downstream intracellular cascades in the presynaptic cell to support survival *(3).* The growth factors are generally believed to be present in limiting quantities, causing death of neurons that are at a competitive disadvantage for obtaining or utilizing the factor. The precise biochemical pathways by which neurotrophic factors support neuronal survival remain uncertain but may involve activation of specific kinases and the inhibition of several checkpoints in the intracellular cascade that would otherwise culminate in neuronal death *(4,5).*

The classical view of target-derived neurotrophism is supported by recent gene-deletion studies of neurotrophic molecules and receptors *(3).* CNS phenotypes in these knockout animals are not as severe as the peripheral nervous system (PNS) phenotypes, which show nearly absolute dependence on neurotrophic factors *(3,6).* There are several possible explanations for these apparent difference between PNS and CNS. One possibility is that other factors, including neuronal activity, serve as redundant survival-promoting influences in the CNS, although some recent evidence suggests that activity is actually a prerequisite for CNS neurotrophic-factor signaling *(7,8).* Even in the PNS, activity levels are likely to participate in neuronal survival through alternate or synergistic mechanisms *(9).*

Afferent-Dependent and Activity-Dependent Survival In Vivo

In the CNS, circumstantial evidence for activity-dependent survival comes from observations that afferent input is necessary for survival of post-synaptic neurons in the visual, olfactory, and auditory sensory systems, among others. In each of these systems, deletion of axons that normally excite target neurons results in widespread death of the target neurons *(10).* The required presence of afferents does not alone distinguish between activity-dependent vs activity-independent mechanisms of support. In studies designed to distinguish these mechanisms, tetrodotoxin (TTX), a sodium channel blocker, and the microtubule and axonal transport disruptor colchincine kills post-synaptic tectal neurons in the developing chick *(11).* Superficial targets, however, are preferentially susceptible to colchicine. Therefore, it seems likely that anterograde, activity-independent trophic factors support neurons in some cases. On the other hand, deep tectal neurons in the chick *(11)* and a substantial number of rat tectal neurons *(12)* are preferentially susceptible to tetrodotoxin, suggesting a role for

activity-dependent survival in some neuronal populations.

In the chick auditory system, transection or electrical block of the auditory nerve causes loss of nucleus magnocellularis neurons *(13)*, the avian equivalent of the mammalian cochlear nucleus. Glutamate promotes survival in this system by activating a metabotropic glutamate receptor, which diminishes potentially apoptosis-inducing rises in intracellular Ca^{2+} ($[Ca^{2+}_i]$) that result from high spontaneous firing levels in magnocellularis neurons *(14)*. This model system may be somewhat atypical in that glutamate plays an essentially inhibitory neuroprotective role. While toxic rises in $[Ca^{2+}]_i$ participate in many forms of excitotoxicity, NCD during development may be most often associated with toxic decreases in $[Ca^{2+}]_i$, as outlined below. Nevertheless, cell death in the nucleus magnocellularis provides a clear example of a conventional neurotransmitter playing a survival-promoting role in vivo.

Other recent evidence hints that activity-dependent survival may be a widespread feature throughout the developing CNS. Olney and colleagues recently showed that systemic administration of N-methyl-D-aspartate (NMDA) receptor antagonists, $GABA_A$ receptor potentiators, or ethanol (which has both effects) markedly increases the rate of apoptosis throughout the rat forebrain during a critical window in the early post-natal period, roughly corresponding to the last trimester of human embryogenesis *(15,16)*. It is likely that the effects of ethanol are relevant to disruption of CNS function associated with fetal alcohol syndrome in humans *(16)*. The basis for the critical window of susceptibility to over-inhibition is not clear, but could involve maturation of compensatory receptor systems or other mechanisms by which neurons become weaned of activity dependence.

Another recent study found that mice deficient in munc 18-1, a neuron-specific protein involved in vesicle trafficking, exhibit augmented neuronal loss immediately following the period of synaptogenesis. These mice have no spontaneous or action potential-evoked transmitter release in the PNS or CNS *(17)*, and show normal development up to and including synaptogenesis, the point at which NCD typically commences. After synaptogenesis, brain regions undergo massive apoptosis, resulting in the virtual deletion of brainstem, midbrain, and basal forebrain, all regions that undergo early synaptogenesis. Animals die before neuronal survival in cortex and other later-developing structures can be assessed. These results are provocative in the context of activity-dependent survival and suggest a prominent role for vesicular neurotransmission in survival of diverse types of CNS neurons.

Evidence suggests that at least some neurons may depend on activity not only during development but throughout maturity. In the adult rat, ablation of the olfactory bulb results in anterograde apoptosis of a subset of superficial post-synaptic pyramidal neurons in the piriform cortex *(18)*. Anatomically, a remarkable feature of the susceptible cells is that they possess no basal dendrites and thus receive excitation mainly from olfactory bulb but not intracortical fibers. It is tempting to speculate that these neurons are susceptible to loss of olfactory-bulb input because they receive no alternative sources of excitation to support their survival. Some evidence for this speculation comes from the observation that in lesioned neonates, intracortical fibers sprout and re-innervate deafferented neurons, apparently protecting them from apoptosis *(19,20)*.

Likewise, in the adult rat cerebellum, deafferentation of granule neurons causes granule-neuron apoptosis. To achieve robust and widespread granule-cell loss, contralateral and ipsilateral excitatory afferents must be cut *(21)*. These results are notable because primary culture preparations of cerebellar neurons (described below) are widely used as a model of activity-dependent cell loss.

The results of the above in vivo work are consistent with the idea that over-inhibition is directly toxic to many CNS neurons during development and perhaps into maturity. However, all of these studies fall short of defini-

tively determining whether the relevant survival-promoting activity is activity *per se* vs activity-dependent release of a conventional trophic peptide. For instance, brain-derived neurotrophic factor (BDNF), a conventional neurotrophic factor, is located in presynaptic terminals *(22,23),* where it could be released in an activity-dependent manner. Thus, the in vivo work does not definitively determine the relationship between activity-dependent support of neurons and conventional trophic factor support. Studies described below with peripheral and central neurons maintained in vitro may come closer to answering these questions.

Cell-Culture Models and Activity-Dependent Survival

Cell-culture models, because of their enhanced accessibility and experimental control, are useful for defining the role of activity in control of neuronal survival. Depolarization, usually achieved with elevated extracellular potassium ($[K^+]_o$), supports enhanced survival of many peripheral and central neuronal phenotypes in culture. These include sympathetic neurons, dorsal-root ganglia sensory neurons *(24,25),* cholinergic parasympathetic neurons from the ciliary ganglion *(26,27),* hypothalamic neurons *(28),* cerebellar Purkinje cells *(29),* retinal ganglion cells *(30,31),* hippocampal neurons *(32),* and neocortical neurons *(7,33).* The wide variety of cells supported by chronic deplorization suggests a fundamental and ubiquitous mechanism of support by depolarization. It seems likely that activity-dependent support involves Ca^{2+}-dependent gene regulation or Ca^{2+}-dependent control over postranslational modifications in proteins involved in the apoptotic cascade. Some specific candidates are discussed in the following sections.

Early studies with peripheral neurons in culture led to a concrete, general hypothesis of how activity and conventional neurotrophic influences interact. Sympathetic neurons deprived of NGF, upon which these cells are absolutely dependent during the period of NCD, could be protected from cell death if grown in an elevated extracellular K^+ concentration *(34).* The action of K^+ appears to be through chronic depolarization and the chronic activation of L-type Ca^{2+} channels; dihydropyridines like nifedipine and nitrendipine block the survival-promoting effects of K^+ *(34,35).* Additionally, Bay K 8644, a dihydropyridine Ca^{2+} channel agonist, promotes survival *(34,36).* Other means of raising $[Ca^{2+}]_i$, including thapsigargin to release Ca^{2+} from intracellular stores, can substitute for the activation of voltage-gated channels *(37).* NGF itself does not raise $[Ca^{2+}]_i$ *(38,39),* nor does NGF deprivation cause depression of $[Ca^{2+}]_i$ *(38).* Therefore, survival promoted by neurotrophic factors and activity may arise through different mechanisms.

Based on work with sympathetic neurons, the calcium set-point hypothesis has been proposed to explain the observed relationship between neurotrophic factor-dependent survival and activity-dependent survival *(34,36).* The calcium set-point hypothesis suggests that neurotrophic factors normally support survival during the period of NCD, at which time basal $[Ca^{2+}]_i$ levels are not conducive to survival in the absence of trophic factors. If stimuli are present to drive $[Ca^{2+}]_i$ to extremely low levels, trophic factors may not be able to protect neurons *(36),* although if the intracellular pathways activated by $[Ca^{2+}]_i$ and neurotrophic factors are separate, it is possible that neurotrophic factors can protect against extremely low $[Ca^{2+}]_i$ (Fig. 1A). Likewise, in the absence of trophic factor, modest elevations of $[Ca^{2+}]_i$ are protective. However, if $[Ca^{2+}]_i$ is driven too high for too long, excitotoxicity *(40,41)* or Ca^{2+}-dependent apoptosis *(42)* ensues and is generally insensitive to trophic factor intervention (Fig. 1).

Developmental changes in the calcium set-point may dictate the period of trophic-factor dependence *(43).* In this view (Fig. 1B), the period of trophic-factor dependence is marked by a decrease in $[Ca^{2+}]_i$, which in the absence

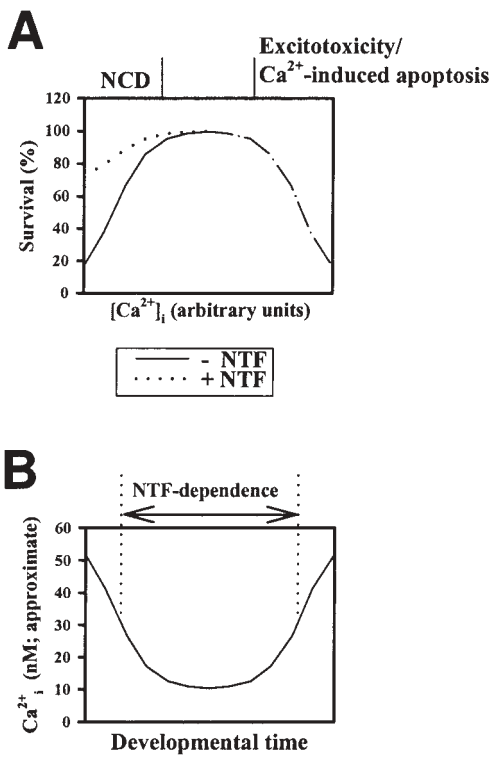

Fig. 1. Hypotheses relating $[Ca^{2+}]_i$ to neuronal survival during development. **(A)** The calcium set-point hypothesis suggests that if levels of calcium are too low or too high, survival decreases. Different mechanisms are presumably involved in the toxic vs protective effects of $[Ca^{2+}]_i$. The dotted line denotes survival in the presence of neurotrophic factor (NTF) **(B).** It has also been suggested that depressed $[Ca^{2+}]_i$ initiates the onset of neurotrophic-factor (NTF) dependence during development. Likewise, developmental programs that increase $[Ca^{2+}]_i$ may dictate the period of neurotrophic-factor weaning. Figures are adapted from refs. *(36,43).*

of neurotrophic factors initiates apoptosis. Trophic-factor support aborts the suicide program that would normally be triggered by the developmental decrease in $[Ca^{2+}]_i$. As mentioned earlier, neurotrophic factors themselves and withdrawal of neurotrophic factors do not alter basal $[Ca^{2+}]_i$. however, correlated with the loss of extrinsic trophic-factor dependence, peripheral neurons exhibit an increase in $[Ca^{2+}]_i$ *(25,44).* Thus, by maintaining $[Ca^{2+}]_i$ within a higher concentration range, cells may survive in the absence of trophic-factor support. An interesting implication of this model is that while cells may be weaned of their dependence on trophic factors, they may not be weaned of their dependence upon activity, or at least their dependence on moderately elevated $[Ca^{2+}]_i$.

It is not clear, however, that decreases in $[Ca^{2+}]_i$ alone can explain the development of neurotrophic-factor dependence, as chick nodose ganglia neurons are sensitive to depression of $[Ca^{2+}]_i$ slightly before the onset of trophic-factor dependence *(45).* On the other hand, artificially driving $[Ca^{2+}]_i$ to low levels is mature sensory neurons is able to induce NGF dependence in cells previously weaned of trophic-factor dependence *(46).*

Pros and Cons of the Calcium Set-Point Hypothesis

The calcium set-point hypothesis is a useful starting point for understanding the relationship between activity and survival. This section is devoted to reviewing recent literature in the context of the calcium set-point hypothesis. The goal is to highlight recent results that are not easily explained by the calcium set-point hypothesis and to suggest conditions under which the hypothesis might not apply. Some of these results suggest, contrary to the calcium set-point hypothesis, that activity simply potentiates neurotrophic-factor mediated signaling by increasing neurotrophic-factor synthesis, release, or responsiveness.

One alternative explanation for results in sympathetic neurons is that activity simply increases calcium-dependent release of a neurotrophic factor that substitutes for NGF and supports survival. Because activity-dependent transmitter release typically relies on activation of N or P/Q type Ca^{2+} channels, the preferential involvement of L-type Ca^{2+} channels in survival of most cell types suggests that K^+-induced support may arise directly rather than through Ca^{2+}-dependent release of a peptide

trophic factor. In addition, survival of sympathetic neurons and response to K^+ does not depend upon the seeding density of the cultures *(38)*. This may suggest that accumulation of a trophic molecule is not the basis for K^+ protection. More importantly, K^+-mediated support of NGF-deprived sympathetic neurons is independent of trkA (the NGF receptor tyrosine kinase) autophosphorylation and is unaffected by trkA blockage *(9,38)*. Nevertheless, it is difficult to exclude the possibility that L-type Ca^{2+} channels drive release of an alternate trophic factor capable of substituting for NGF in supporting survival.

Experiments with other cell types, particularly some CNS neurons, support the idea that in some situations, activity can result in accumulation of a trophic factor or increased responsivity to trophic factor, suggesting that activity may simply promote signaling via conventional neurotrophic factors. Retinal ganglion cells grown in primary culture are susceptible to cell death induced by TTX *(30)*. However, conditioned medium from cultures grown in the absence of TTX (but not medium from TTX-reared cultures) is protective *(30)*. This suggests that spontaneous activity results in the accumulation of a survival-promoting substance capable of protecting these cells. Similarly, K^+-induced depolarization of cortical neurons promotes survival by increasing BDNF *(7)*, and Ca^{2+} influx causes up-regulation of BDNF expression in cortical neurons *(47)*. Also, the survival effects of low-level glutatmate receptor activation and the toxic effects of ethanol, which depresses NMDA-receptor function, may be mediated by BDNF levels in cerebellar granule-neuron cultures *(48)*. In CNS noradrenergic cells, BDNF is found in pre-synaptic terminals *(22)*, where it could potentially be released in a Ca^{2+}-dependent manner.

Other results suggest activity could participate in survival by increasing release of neurotrophic factors from non-neuronal cells. A candidate glial-derived trophic factor (activity-dependent neurotrophic-factor peptide-9) is released in response to neuronal activity *(49,50)* and protects against apoptotic insults *(51)*. This mechanism is unlikely to explain protective effects of activity in cerebellar granule-cell cultures or sympathetic neuron cultures as non-neuronal cells typically represent <5% of the total number of cells. Nevertheless, in vivo it is possible that glia have an important role in modulating activity-dependent survival, and this issue remains relatively unexplored. In addition, glia could regulate ambient glutamate levels and the tonic level of glutamate-receptor activity *(52,53)* through glutamate transporters, thus adjusting neuronal $[Ca^{2+}]_i$ and influencing survival.

Recent studies of other CNS cells have suggested that activity may upregulate neurotrophic-factor responsiveness rather than neurotrohic-factor production and release. Retinal ganglion cells cultured in defined medium are insensitive to conventional neurotrophic factors unless K^+ or cAMP is added to the culture medium *(31)*. Neither K^+ nor cAMP alone diminished NCD. These results seem difficult to incorporate into the Ca^{2+} set-point hypothesis, although in this study $[Ca^{2+}]_i$ was not directly measured. The lack of effect of neurotrophic factors in the absence of K^+ might be explained by extremely low basal $[Ca^{2+}]_i$, a concentration at which neurotrophic support cannot rescue the cells *(36)*. However, it is difficult under the Ca^{2+} set-point hypothesis to explain the lack of effect of K^+ alone on survival unless the basal $[Ca^{2+}]_i$ achieved simply never reaches a neuroprotective level without additional neurotrophic support.

Further work with this model suggests that depolarization and increases in cAMP increase survival of retinal ganglion cells and spinal motor neurons by promoting the rapid insertion of trkB receptors into the plasma membrane *(8)*. In this study, the responsivity of sympathetic neurons cultured in the same in vitro environment was directly compared with the two CNS neuron types. The peripheral neurons were inherently responsive to neurotrophic support, while the CNS neurons required depolarization or cAMP to become responsive to neurotrophic factors. Thus, it is

possible that there is a fundamental difference in the mechanisms by which activity promotes survival in peripheral and central neurons.

Results from postnatal cerebellar granule neurons, the most popular model of NCD in a CNS neuronal-cell type, are broadly similar to work with peripheral neurons and seem, to the extent that it has been experimentally tested, to be consistent with the calcium set-point hypothesis. Granule neurons die in culture unless supported by elevated (25–30 m*M*) concentrations of K^+ *(54,55).* Although the endogenous neurotrophic factor(s) that support granule neurons in vivo are not clear, the neurotrophic factors insulin-related growth factor-1 (IGF-1) and BDNF can protect granule neurons in culture from death in low $[K^+]_o$ *(55–57).* Therefore, unlike retinal ganglion cells and spinal neurons grown in defined medium, granule neurons are responsive to neurotrophic factors even when not stimulated with depolarization or exogenous cAMP. To our knowledge, specific aspects of the calcium set-point hypothesis have not been tested in granule neurons, including whether granule neurons undergo a decrease in $[Ca^{2+}]_i$ correlated with the onset of trophic-factor dependence and whether an increase in $[Ca^{2+}]_i$ may accompany weaning from trophic-factor dependence. Answering these questions is hindered by the fact that the endogenous trophic factors responsible for supporting granule neurons are not clear and by the apparent dependence of even "mature" granule neurons (grown 7–10 d in vitro) on chronic depolarization *(58).* Interestingly, even granule neurons in the adult, intact cerebellum are apparently dependent upon afferent support *(21).*

In granule cells, neurotrophic signaling and activity-dependent signaling may converge upstream at the level of $[Ca^{2+}]_i$. As mentioned, $[Ca^{2+}]_i$ does not change in sympathetic neurons when the cells are exposed to NGF. On the other hand, it was reported that IGF-1 protection of granule cells exposed to low $[K^+]_o$ is associated with functional increases in L-type calcium-channel activity, and that dihydropyridine Ca^{2+}-channel blockers decrease the protective effects of IGF-1 *(59).* It is unclear whether these results extend to other neurotrophic factors that support granule-neuron survival, such as BDNF. Although these results differ from those obtained in sympathetic neurons, they apparently are consistent with the calcium set-point hypothesis in that the results suggest an optimal, moderately elevated $[Ca^{2+}]_i$ can directly support neuronal survival.

Other observations in cerebellar granule neurons appear difficult to reconcile with the calcium set-point hypothesis. One group found no detectable difference in basal $[Ca^{2+}]_i$ in granule cells grown in 5 m*M* K^+ vs cells grown in 30 m*M* K^+ *(60,61).* The authors propose that the neuroprotective effect and the lack of elevation of basal $[Ca^{2+}]_i$ may result from increased Ca^{2+} efflux by the Ca^{2+}/Na^+ exchanger. An alternative explanation might be that a transient $[Ca^{2+}]_i$ increase induced initially upon switch to high $[K^+]_o$ medium is responsible for the neuroprotective effect of K^+. In fact, it was recently found that a pulse (several minutes) application of IGF-1 or of K^+ was sufficient to protect granule cells from death in 5 m*M* K^+ *(59).*

$[Ca^{2+}]_i$ may not be relevant at all for K^+-induced support of some CNS neurons subjected to apoptotic stimuli. In embryonic cortical neurons, preventing K^+ efflux is sufficient to protect neurons from several apoptotic insults *(62,63).* Additionally, inducing K^+ efflux by ionophores or NMDA receptor stimulation is sufficient to induce apoptosis *(62,63).* In these experiments, it was proposed that the relevant neuroprotective effect of elevated $[K^+]_o$ was a reduction of the electrochemical driving force on K^+ efflux rather than chronic activation of Ca^{2+} channels *(62,63).*

Other questions regarding the role of $[Ca^{2+}]_i$ in neuroprotection remain unanswered. For instance, chronic K^+ and glutamate incubation, which presumably elicit chronic depolarization rather than physiological spiking, are generally used as neuroprotective treatments against NCD. How these manipulations relate to physiologically relevant patterns of activity is undetermined. Our

own recent work demonstrated that over-activation of inhibitory $GABA_A$ receptors in cultures of post-natal hippocampal neurons and astrocytes induces widespread death of neurons. Moderately low concentrations of neurosteroids, which may be endogenous potentiators of $GABA_A$ mediated neurotransmission, were sufficient to promote cell death relative to control cultures *(32).* Also, bicuculline or picrotoxin alone prevented a significant fraction of the NCD in these cultures. The fact that GABA-potentiating agents and NMDA-receptor antagonists in vivo and in vitro can augment apoptosis *(16,32)* suggests that alterations of physiological patterns of activity can render neurons susceptible to cell death.

The ammended version of the calcium set-point hypothesis (Fig. 1B) suggests that neurons beyond the period of absolute dependence on trophic factors would still be sensitive to manipulations that depress $[Ca^{2+}]_i$. This prediction may be difficult to test conclusively, however. Exploration of this question in culture can be limited by the finite lifetime of primary neuronal cultures and lack of knowledge of the relevant trophic factors involved in supporting CNS neurons. In vivo, Ikonomidou et al. *(16)* found that forebrain neurons exhibited a critical window for sensitivity to $GABA_A$ potentiators and to NMDA-receptor blockers. By several weeks of post-natal life, rat neurons are no longer sensitive to overinhibition. This result could suggest that neurons lose activity dependence as well as neurotrophic-factor dependence. However, it is possible that loss of sensitivity to manipulations of $GABA_A$ receptors or NMDA receptors are compensated by changes in other receptor or transmitter systems. For instance, maturation of AMPA-receptor mediated transmission or metabotropic glutamate receptor-mediated transmission may be able to compensate in more mature animals for inhibition produced by NMDA-receptor blockade or GABA-receptor potentiation. It is likewise possible that developmental increases in basal $[Ca^{2+}]_i$ may not be greatly affected by changes in transient Ca^{2+} fluxes induced by manipulation of action potential or synaptic signaling.

Possible Downstream Mechanisms of Ca^{2+} Interaction with Survival

In the previous section, we reviewed evidence that in some cell types and under some conditions, activity and neurotrophic signaling can converge upstream with increases in activity leading to enhanced neurotrophic-factor synthesis, release, or responsiveness. By the same token, some neurotrophic factors in some cell types can elevate $[Ca^{2+}]_i$. In these examples, activity and neurotrophic support converge early and apparently promote survival through identical or very similar downstream mechanisms. However, the idea that neurotrophic factors and activity promote survival through at least partly parallel mechanisms is popular and substantial evidence supports this idea. Accordingly, there is interest in determining the downstream checkpoints at which activity promotes survival. In this section, we review evidence for candidate points at which activity (and particularly elevated $[Ca^{2+}]_i$) and neurotrophic support may converge. In a 1992 review of the calcium set-point hypothesis, Franklin and Johnson noted that Ca^{2+} is such a ubiquitous (and important) intracellular messenger that pinpointing its role in promoting survival is likely to be difficult *(36).* Nearly a decade later, much more is known about the intracellular cascades culminating in apoptosis. Unfortunately, conclusive evidence for a specific target for neuroprotective $[Ca^{2+}]_i$ is still lacking, although several candidates have recently emerged.

We briefly review the pathways leading to apoptosis *(64–66)* as a framework for understanding the potential role of specific targets of electrical activity in modulating apoptosis. Two relatively well-characterized pathways lead to apoptosis. Both pathways culminate in the activation of effector caspase enzymes, cysteine proteases that cleave protein substrates at aspartate residues *(67,68).* One pathway is

mediated by ligand-receptor interactions, which induce death-receptor trimerization and recruitment of caspase 8 to the receptor complex, where it is autocatalytically activated. Caspase 8, in turn, activates downstream effector caspases, such as caspase 3, 6, and 7. The second pathway involves release of cytochrome c from mitochondria, which is induced by the mobilization of pro-apoptotic members of the Bcl-2 family, such as Bak, Bim, Bad, and Bax *(69).* Cytosolic cytochrome c recruits Apaf-1, a cytosolic protein that oligomerizes in response to cytochrome c binding. A caspase-recruitment domain in Apaf-1 promotes recruitment of caspase 3 to the complex, where caspase 3 is activated, perhaps autocatalytically. The effector caspase, caspase 3, is likely responsible for many of the morphological changes, such as nuclear fragmentation and internucleosomal cleavage, correlated with neuronal apoptosis.

The Bcl-2 family members represent an important regulatory step in the apoptotic cascade. Anti-apoptotic members of the Bcl-2 family, such as Bcl-2 itself and a close homolog Bclx-L, prevent cytochrome c release. It is thought that neurotrophic-factor deprivation may result in the translocation of the pro-apoptotic Bax from cytoplasmic compartments to mitochondria, where it promotes cytochrome c release *(64).* Thus, the Bcl-2 family may represent pivotal candidates for modulating cell death and survival signals.

Neurotrophic factor-mediated survival pathways have recently been reviewed *(4).* In many cases, survival signals appear mediated through activation of the lipid kinase PI-3 kinase and/or the MAP kinase pathway. Both pathways are activated by the small GTP-binding protein ras, although in many systems one pathway apparently dominates survival cues. In particular, the PI-3 kinase pathway appears important in apoptosis induced by neurotrophic-factor withdrawal, and may be an important point of convergence between activity and neurotrophic-factor support. The MAP kinase pathway may be preferentially involved in promoting survival in response to cellular insults *(4).*

In considering the role of activity in promoting survival, it is possible that there are direct links with the same intracellular pathways that mediate neurotrophic-factor support. Recent attention has focused on a pathway resulting from activation of PI-3 kinase. Activation of this pathway results in activation of the serine/threonine kinase Akt, of which there are three isoforms *(70).* Akt activation can phosphorylate and cause sequestration of Bad, a pro-apoptotic Bcl-2 family member *(71),* at least in expression studies. Other targets of Akt may also participate in promoting survival *(70).* As noted earlier, growth factors are known to activate the Akt pathway *(72,73),* and Akt is therefore is a candidate for mediating survival effects of activity, as described below.

In sympathetic neurons, membrane depolarization activates parallel pathways involving Akt and calcium/calmodulin-dependent kinase II *(9,74).* Vaillant et al. *(9)* found that low levels of NGF and K^+ synergistically increased PI-3-dependent Akt phosphorylation. Although K^+ also increased activity of the MAP kinase pathway, blocking this pathway had no effect on K^+-mediated neuroprotection. Blockade of the PI-3 kinase pathway inhibited K^+ neuroprotection. Finally, blockade of calcium/calmodulin-dependent kinase II inhibited survival promoted by K^+ alone but did not affect the synergistic survival effects at low concentrations of NGF plus low concentrations of K^+.

There is conflicting evidence for the importance of the PI-3 kinase pathway in activity-dependent survival of CNS neurons. In one study of cerebellar granule cells, K^+ protection was associated with an increase in PI-3 kinase activity, and blocking activity of PI-3 kinase blocked the protective effect of K^+ *(75).* Calcium/calmodulin-dependent kinase kinase may activate Akt directly and provide a molecular link between $[Ca^{2+}]_i$ increases and survival *(76).* On the other hand, in apparent contradiction to the idea that the PI-3 kinase/Akt pathway is involved in depolarization-induced neuroprotection, others have found that blocking PI-3 kinase in cerebellar

granule cells had no effect on K^+-induced protection. In the same studies, PI-3 kinase blockade reduced the neuroprotection afforded by IGF-1 or insulin. *(77,78).*

The aforementioned potentiation of L-type Ca^{2+} channels in cerebellar granule neurons by IGF-1 may be mediated through an Akt-dependent mechanism *(59).* Dominant-negative forms of Akt expressed in cerebellar neurons prevent potentiation of L-type channels by IGF-1, and constitutively active Akt promotes increases in channel activity *(59).* IGF-1-mediated potentiation of N-type Ca^{2+} channels is not affected by manipulation of Akt. Direct phosphorylation of the L-type channels by the serine/threonine kinase Akt is unlikely to be the cause of potentiation, as it was recently shown that direct phosphorylation of these channels by a Src-like tyrosine kinase underlies the increase in channel function mediated by IGF-1 *(79).*

Transcriptional regulation is another route through which Ca^{2+} may affect apoptotic programs. It was recently found that calcium-dependent activation of a transcription factor, MEF-2, is the relevant stimulus for granule-cell survival in the presence of depolarizing concentrations of K^+ *(80).* These studies are consistent with the observation that protein-synthesis inhibitors prevent NCD *(55).* Although p38 MAP kinase phosphorylation of MEF-2 was implicated in Ca^{2+}-dependent regulation of MEF-2 activity, the precise targets of Ca^{2+} and downstream gene products remain unclear.

The preceding discussion has focused on potential ways in which modest increases in $[Ca^{2+}]_i$ might lead to survival-promoting signals. It bears mentioning that in many systems, increased $[Ca^{2+}]_i$ can trigger apoptosis *(58,81).* Furthermore, rises in $[Ca^{2+}]_i$ may lead to calcineurin-dependent dephosphorylation of Bad and translocation to mitochondria *(82),* an event that unleashes the pro-apoptotic effect of Bad. It is possible that these mechanisms explain reported components of glutamate-induced cell death that are apparently apoptotic *(42,83).* It has also been suggested that mature neurons are more sensitive to these latter mechanisms *(58).*

Summary/Conclusions: Significance of Activity-Dependent Survival

It is clear that in many CNS and PNS regions, there is a role for activity in survival of neurons. An important implication of the classical idea of limited neurotrophic availability or access is that a secondary mechanism such as electrical activity may serve as an alternative or synergistic survival cue for neurons in widespread areas of the nervous system. Neurons that would not survive on neurotrophic factor alone may enhance their chance of survival with increased activity levels. An additional corollary is that altering activity levels in the developing nervous system may have important consequences on later development. Finally, because it is not clear whether neurons become weaned of the survival-promoting influence of activity, altering activity in the mature nervous system may also affect cell survival and, in turn, behavior.

These ideas may have important implications concerning the use of neuroactive drugs in pregnant women and young children. NCD is most prominent during the period of the brain growth spurt and synaptogenesis, which occur in late gestation and the first post-natal months in the human and the first post-natal weeks in the rat *(84).* If neuronal activity is truly an important determinant of neuronal survival and can modulate the degree of NCD, then treatment of pregnant women and young children with standard anticonvulsants and anesthetics (which typically are GABA potentiators, sodium channel blockers, or NMDA-receptor blockers) should be viewed with caution. Recent in vivo data in rats suggests that exposure to activity blockers for even several hours can have profound consequences on neuronal survival *(16).* In addition, activity-dependent neuronal survival is one of many specific reasons that substances of abuse, many of which dampen neuronal activity by

interacting with the same aforementioned receptors and channels, are dangerous to the developing fetus.

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References

1. Oppenheim R. W. (1991) Cell death during development of the nervous system. *Ann. Rev. Neurosci.* **14,** 453–501.
2. Purves D. and Lichtman J. W. (1985) *Principles of Neural Development.* Sinauer Associates, Inc., Sunderland, MA.
3. Lewin G. R. and Barde Y. A. (1996) Physiology of the neurotrophins. *Ann. Rev. Neurosci.* **19,** 289–317.
4. Kaplan D. R. and Miller F. D. (2000) Neurotrophin signal transduction in the nervous system. *Curr. Opin. Neurobiol.* **10,** 381–391.
5. Putcha G. V., Deshmukh M., and Johnson E. M., Jr. (1999) BAX translocation is a critical event in neuronal apoptosis: regulation by neuroprotectants, BCL-2, and caspases. *J. Neurosci.* **19,** 7476–7485.
6. Pettmann B. and Henderson C. E. (1998) Neuronal cell death. *Neuron* **20,** 633–647.
7. Ghosh A., Carnahan J., and Greenberg M. E. (1994) Requirement for BDNF in activity-dependent survival of cortical neurons. *Science* **263,** 1618–1623.
8. Meyer-Franke A., Wilkinson G. A., Kruttgen A., Hu M., Munro E., Hanson M. G., Jr., et al. (1998) Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. *Neuron* **21,** 681–693.
9. Vaillant A. R., Mazzoni I., Tudan C., Boudreau M., Kaplan D. R., and Miller F. D. (1999) Depolarization and neurotrophins converge on the phosphatidylinositol 3-kinase-Akt pathway to synergistically regulate neuronal survival. *J. Cell Biol.* **146,** 955–966.
10. Linden R. (1994) The survival of developing neurons: a review of afferent control. *Neuroscience* **58,** 671–682.
11. Catsicas M., Pequignot Y., and Clarke P. G. (1992) Rapid onset of neuronal death induced by blockade of either axoplasmic transport or action potentials in afferent fibers during brain development. *J. Neurosci.* **12,** 4642–4650.
12. Galli-Resta L., Ensini M., Fusco E., Gravina A., and Margheritti B. (1993) Afferent spontaneous electrical activity promotes the survival of target cells in the developing retinotectal system of the rat. *J. Neurosci.* **13,** 243–250.
13. Rubel E. W., Hyson R. L., and Durham D. (1990) Afferent regulation of neurons in the brain stem auditory system. *J. Neurobiol.* **21,** 169–196.
14. Lachica E. A., Kato B. M., Lippe W. R., and Rubel E. W. (1998) Glutamatergic and GABAergic agonists increase $Ca^{2+}{}_i$ in avian cochlear nucleus neurons. *J. Neurobiol.* **37,** 321–337.
15. Ikonomidou C., Bosch F., Miksa M., Bittigau P., Vockler J., Dikranian K., et al. (1999) Blockade of NMDA receptors and apoptotic neurodegeneration in the developing brain. *Science* **283,** 70–74.
16. Ikonomidou C., Ishimaru M. J., Wozniak D. F., Koch C., Bittigau P., Price M. T., et al. (2000) Ethanol-induced apoptotic neurodegeneration and the fetal alcohol syndrome. *Science* **287,** 1056–1060.
17. Verhage M., Maia A. S., Plomp J. J., Brussaard A. B., Heeroma J. H., Vermeer H., et al. (2000) Synaptic assembly of the brain in the absence of neurotransmitter secretion. *Science* **287,** 864–868.
18. Heimer L. and Kalil R. (1978) Rapid transneuronal degeneration and death of cortical neurons following removal of the olfactory bulb in adult rats. *J. Comp. Neurol.* **178,** 559–609.
19. Friedman B. and Price J. L. (1986) Plasticity in the olfactory cortex: age-dependent effects of deafferentation. *J. Comp. Neurol.* **246,** 1–19.
20. Friedman B. and Price J. L. (1986) Age-dependent cell death in the olfactory cortex: lack of transneuronal degeneration in neonates. *J. Comp. Neurol.* **246,** 20–31.
21. Borsello T., Di Luzio A., Ciotti M. T., Calissano P., and Galli C. (2000) Granule neuron DNA damage following deafferentation in adult rats cerebellar cortex: a lesion model. *Neuroscience* **95,** 163–171.
22. Fawcett J. P., Bamji S. X., Causing C. G., Aloyz R., Ase A. R., Reader T. A., et al. (1998) Func-

tional evidence that BDNF is an anterograde neuronal trophic factor in the CNS. *J. Neurosci.* **18,** 2808–2821.
23. Zhou X. F. and Rush R. A. (1996) Endogenous brain-derived neurotrophic factor is anterogradely transported in primary sensory neurons. *Neuroscience* **74,** 945–953.
24. Scott B. S. (1977) The effect of elevated potassium on the time course of neuron survival in cultures of dissociated dorsal root ganglia. *J. Cell. Physiol.* **91,** 305–316.
25. Eichler M. E., Dubinsky J. M., and Rich K. M. (1992) Relationship of intracellular calcium to dependence on nerve growth factor in dorsal root ganglion neurons in cell culture. *J. Neurochem.* **58,** 263–269.
26. Bennett M. R. and White W. (1979) The survival and development of cholinergic neurons in potassium-enriched media. *Brain Res.* **173,** 549–553.
27. Collins F., Schmidt M. F., Guthrie P. B., and Kater S. B. (1991) Sustained increase in intracellular calcium promotes neuronal survival. *J. Neurosci.* **11,** 2582–2587.
28. Ling D. S., Petroski R. E., and Geller H. M. (1991) Both survival and development of spontaneously active rat hypothalamic neurons in dissociated culture are dependent on membrane depolarization. *Brain Res. Dev. Brain. Res.* **59,** 99–103.
29. Cohen-Cory S., Dreyfus C. F., and Black I. B. (1991) NGF and excitatory neurotransmitters regulate survival and morphogenesis of cultured cerebellar Purkinje cells. *J. Neurosci.* **11,** 462–471.
30. Lipton S. A. (1986) Blockade of electrical activity promotes the death of mammalian retinal ganglion cells in culture. *Proc. Natl. Acad. Sci. USA* **83,** 9774–9778.
31. Meyer-Franke A., Kaplan M. R., Pfrieger F. W., and Barres B. A. (1995) Characterization of the signaling interactions that promote the survival and growth of developing retinal ganglion cells in culture. *Neuron* **15,** 805–819.
32. Xu W., Cormier R., Fu T., Covey D. F., Isenberg K. E., Zorumski C. F., and Mennerick S. J. (2000) Slow death of postnatal hippocampal neurons by $GABA_A$ receptor overactivation. *J. Neurosci.* **20,** 3147–3156.
33. Baker R. E., Ruijter J. M., and Bingmann D. (1991) Elevated potassium prevents neuronal death but inhibits network formation in neocortical cultures. *Int. J. Dev. Neurosci.* **9,** 339–345.
34. Koike T., Martin D. P., and Johnson E. M., Jr. (1989) Role of Ca^{2+} channels in the ability of membrane depolarization to prevent neuronal death induced by trophic-factor deprivation: evidence that levels of internal Ca^{2+} determine nerve growth factor dependence of sympathetic ganglion cells. *Proc. Natl. Acad. Sci. USA* **86,** 6421–6425.
35. Collins F. and Lile J. D. (1989) The role of dihydropyridine-sensitive voltage-gated calcium channels in potassium-mediated neuronal survival. *Brain Res.* **502,** 99–108.
36. Franklin J. L. and Johnson E. M., Jr. (1992) Suppression of programmed neuronal death by sustained elevation of cytoplasmic calcium. *Trends Neurosci.* **15,** 501–508.
37. Lampe P. A., Cornbrooks E. B., Juhasz A., Johnson E. M., Jr., and Franklin J. L. (1995) Suppression of programmed neuronal death by a thapsigargin-induced Ca^{2+} influx. *J. Neurobiol.* **26,** 205–212.
38. Franklin J. L., Sanz-Rodriguez C., Juhasz A., Deckwerth T. L., and Johnson E. M., Jr. (1995) Chronic depolarization prevents programmed death of sympathetic neurons in vitro but does not support growth: requirement for Ca^{2+} influx but not Trk activation. *J. Neurosci.* **15,** 643–664.
39. Tolkovsky A. M., Walker A. E., Murrell R. D., and Suidan H. S. (1990) Ca^{2+} transients are not required as signals for long-term neurite outgrowth from cultured sympathetic neurons. *J. Cell Biol.* **110,** 1295–1306.
40. Zorumski C. F. and Olney J. W. (1993) Excitotoxic neuronal damage and neuropsychiatric disorders. *Pharmacol. Ther.* **59,** 145–162.
41. Choi D. W. (1994) Glutamate receptors and the induction of excitotoxic neuronal death. *Prog. Brain Res.* **100,** 47–51.
42. Ankarcrona M. (1998) Glutamate induced cell death: apoptosis or necrosis? *Prog. Brain Res.* **116,** 265–272.
43. Burek M. J. and Oppenheim R. W. (1999) Cellular interactions that regulate programmed cell death in the developing nervous system, in *Cell Death and Diseases of the Nervous System* (Koliatsos V. E. and Ratan R. R., eds.), Humana Press, Totowa, N. J. pp. 145–179.
44. Koike T. and Tanaka S. (1991) Evidence that nerve growth factor dependence of sympathetic neurons for survival in vitro may be determined by levels of cytoplasmic free Ca^{2+}. *Proc. Natl. Acad. Sci. USA* **88,** 3892–3896.

45. Larmet Y., Dolphin A. C., and Davies A. M. (1992) Intracellular calcium regulates the survival of early sensory neurons before they become dependent on neurotrophic factors. *Neuron* **9,** 563–574.
46. Tong J. X., Eichler M. E., and Rich K. M. (1996) Intracellular calcium levels influence apoptosis in mature sensory neurons after trophic factor deprivation. *Exp. Neurol.* **138,** 45–52.
47. Tao X., Finkbeiner S., Arnold D. B., Shaywitz A. J., and Greenberg M. E. (1998) Ca^{2+} influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron* **20,** 709–726.
48. Bhave S. V., Ghoda L., and Hoffman P. L. (1999) Brain-derived neurotrophic factor mediates the anti-apoptotic effect of NMDA in cerebellar granule neurons: signal transduction cascades and site of ethanol action. *J. Neurosci.* **19,** 3277–3286.
49. Bassan M., Zamostiano R., Davidson A., Pinhasov A., Giladi E., Perl O., et al. (1999) Complete sequence of a novel protein containing a femtomolar-activity-dependent neuroprotective peptide. *J. Neurochem.* **72,** 1283–1293.
50. Gozes I., Davidson A., Gozes Y., Mascolo R., Barth R., Warren D., et al. (1997) Antiserum to activity-dependent neurotrophic factor produces neuronal cell death in CNS cultures: immunological and biological specificity. *Brain Res. Dev. Brain Res.* **99,** 167–175.
51. Glazner G. W., Camandola S., and Mattson M. P. (2000) Nuclear factor-kappaB mediates the cell survival-promoting action of activity-dependent neurotrophic factor peptide-9. *J. Neurochem.* **75,** 101–108.
52. Sah P., Hestrin S., and Nicoll R. A. (1989) Tonic activation of NMDA receptors by ambient glutamate enhances excitability of neurons. *Science* **246,** 815–818.
53. Zorumski C. F., Mennerick S., and Que J. (1996) Modulation of excitatory synaptic transmission by low concentrations of glutamate in cultured rat hippocampal neurons. *J. Physiol. (Lond.)* **494,** 465–477.
54. Gallo V., Kingsbury A., Balazs R., and Jorgensen O. S. (1987) The role of depolarization in the survival and differentiation of cerebellar granule cells in culture. *J. Neurosci.* **7,** 2203–2213.
55. D'Mello S. R., Galli C., Ciotti T., and Calissano P. (1993) Induction of apoptosis in cerebellar granule neurons by low potassium: inhibition of death by insulin-like growth factor I and cAMP. *Proc. Natl. Acad. Sci. USA* **90,** 10,989–10,993.
56. Kubo T., Nonomura T., Enokido Y., and Hatanaka H. (1995) Brain-derived neurotrophic factor (BDNF) can prevent apoptosis of rat cerebellar granule neurons in culture. *Brain Res. Dev. Brain Res.* **85,** 249–258.
57. Suzuki K. and Koike T. (1997) Brain-derived neurotrophic factor suppresses programmed death of cerebellar granule cells through a post-translational mechanism. *Mol. Chem. Neuropathol.* **30,** 101–124.
58. Toescu E. C. (1998) Apoptosis and cell death in neuronal cells: where does Ca^{2+} fit in? *Cell Calcium* **24,** 387–403.
59. Blair L. A., Bence-Hanulec K. K., Mehta S., Franke T., Kaplan D., and Marshall J. (1999) Akt-dependent potentiation of L channels by insulin-like growth factor-1 is required for neuronal survival. *J. Neurosci.* **19,** 1940–1951.
60. Kohara K., Ono T., Tominaga-Yoshino K., Shimonaga T., Kawashima S., and Ogura A. (1998) Activity-dependent survival and enhanced turnover of calcium in cultured rat cerebellar granule neurons. *Brain Res.* **809,** 231–237.
61. Ono T., Kudo Y., Kohara K., Kawashima S., and Ogura A. (1997) Activity-dependent survival of rat cerebellar granule neurons is not associated with sustained elevation of intracellular Ca^{2+}. *Neurosci. Lett.* **228,** 123–126.
62. Yu S. P., Yeh C. H., Sensi S. L., Gwag B. J., Canzoniero L. M., Farhangrazi Z. S., et al. (1997) Mediation of neuronal apoptosis by enhancement of outward potassium current. *Science* **278,** 114–117.
63. Yu S. P., Yeh C., Strasser U., Tian M., and Choi D. W. (1999) NMDA receptor-mediated K^+ efflux and neuronal apoptosis. *Science* **284,** 336–339.
64. Nijhawan D., Honarpour N., and Wang X. (2000) Apoptosis in neural development and disease. *Ann. Rev. Neurosci.* **23,** 73–87.
65. Kuan C. Y., Roth K. A., Flavell R. A., and Rakic P. (2000) Mechanisms of programmed cell death in the developing brain. *Trends Neurosci.* **23,** 291–297.
66. Wilson M. R. (1998) Apoptosis: unmasking the executioner. *Cell Death Differ.* **5,** 646–652.
67. Thornberry N. A. and Lazebnik Y. (1998) Caspases: enemies within. *Science* **281,** 1312–1316.
68. Nicholson D. W. (1999) Caspase structure, proteolytic substrates, and function during apoptotic cell death. *Cell Death Diff.* **6,** 1028–1042.

69. Sadoul R. (1998) Bcl-2 family members in the development and degenerative pathologies of the nervous system. *Cell Death Diff.* **5,** 805–815.
70. Datta S. R., Brunet A., and Greenberg M. E. (1999) Cellular survival: a play in three Akts. *Genes Dev.* **13,** 2905–2927.
71. Datta S. R., Dudek H., Tao X., Masters S., Fu H., Gotoh Y., and Greenberg M. E. (1997) Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* **91,** 231–241.
72. Crowder R. J. and Freeman R. S. (1998) Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. *J. Neurosci.* **18,** 2933–2943.
73. Hetman M., Kanning K., Cavanaugh J. E., and Xia Z. (1999) Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. *J. Biol. Chem.* **274,** 22,569–22,580.
74. Ikegami K. and Koike T. (2000) Membrane depolarization-mediated survival of sympathetic neurons occurs through both phosphatidylinositol 3-kinase- and CaM kinase II-dependent pathways. *Brain Res.* **866,** 218–226.
75. Miller T. M., Tansey M. G., Johnson E. M., Jr., and Creedon D. J. (1997) Inhibition of phosphatidylinositol 3-kinase activity blocks depolarization- and insulin-like growth factor I-mediated survival of cerebellar granule cells. *J. Biol. Chem.* **272,** 9847–9853.
76. Yano S., Tokumitsu H., and Soderling T. R. (1998) Calcium promotes cell survival through CaM-K kinase activation of the protein-kinase-B pathway. *Nature* **396,** 584–587.
77. Dudek H., Datta S. R., Franke T. F., Birnbaum M. J., Yao R., Cooper G. M., et al. (1997) Regulation of neuronal survival by the serine-threonine protein kinase Akt. *Science* **275,** 661–665.
78. D'Mello S. R., Borodezt K., and Soltoff S. P. (1997) Insulin-like growth factor and potassium depolarization maintain neuronal survival by distinct pathways: possible involvement of PI 3-kinase in IGF-1 signaling. *J. Neurosci.* **17,** 1548–1560.
79. Bence-Hanulec K. K., Marshall J., and Blair L. A. (2000) Potentiation of neuronal L calcium channels by IGF-1 requires phosphorylation of the α1 subunit on a specific tyrosine residue. *Neuron* **27,** 121–131.
80. Mao Z., Bonni A., Xia F., Nadal-Vicens M., and Greenberg M. E. (1999) Neuronal activity-dependent cell survival mediated by transcription factor MEF2. *Science* **286,** 785–790.
81. McConkey D. J. and Orrenius S. (1996) The role of calcium in the regulation of apoptosis. *J. Leukoc. Biol.* **59,** 775–783.
82. Wang H. G., Pathan N., Ethell I. M., Krajewski S., Yamaguchi Y., Shibasaki F., et al. (1999) Ca^{2+}-induced apoptosis through calcineurin dephosphorylation of BAD. *Science* **284,** 339–343.
83. Choi D. W. (1996) Ischemia-induced neuronal apoptosis. *Curr. Opin. Neurobiol.* **6,** 667–672.
84. Dobbing J. and Sands J. (1979) Comparative aspects of the brain growth spurt. *Early Hum. Dev.* **3,** 79–83.