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·Review·

Caspase-3 activation as a bifurcation point between plasticity and cell death

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Abstract: Death-mediating proteases such as caspases and caspase-3 in particular, have been implicated in neurodegenerative processes, aging and Alzheimer's disease. However, emerging evidence suggests that in addition to their classical role in cell death, caspases play a key role in modulating synaptic function. It is remarkable that active caspases-3, which can trigger widespread damage and degeneration, aggregates in structures as delicate as synapses and persists in neurons without causing acute cell death. Here, we evaluate this dichotomy, and discuss the hypothesis that caspase-3 may be a bifurcation point in cellular signaling, able to orient the neuronal response to stress down either pathological/apoptotic pathways or towards physiological cellular remodeling. We propose that temporal, spatial and other regulators of caspase activity are key determinants of the ultimate effect of caspase-3 activation in neurons. This concept has implications for differential roles of caspase-3 activation across the lifespan. Specifically, we propose that limited caspase-3 activation is critical for synaptic function in the healthy adult brain while chronic activation is involved in degenerative processes in the aging brain.

Keywords: caspase-3; neurodegeneration; cognition; memory; synaptic function

1 Introduction: Caspases – mediators of cell death or more?

Caspases are a class of proteases instrumental in carrying out many cellular functions including differentiation, remodeling and death. Recently there has been an increased focus on evaluating their role in non-apoptotic processes such as synaptic plasticity, spine atrophy and memory deficits, in addition to their classic role in cell death. In this review we address the question of how physiological state of a cell determines whether caspase activation triggers cell death or modulates plasticity and cognitive functions. We also discuss the different outcomes of caspase activation and their relevance for aging and Alzheimer's disease (AD).

The general mechanism of caspase-mediated cell death is highly conserved among cell types, including neurons from structures such as the cortex, hippocampus and cerebellum[1,2], and has been extensively studied and reviewed[3,4]. Caspases are synthesized in cells as inactive zymogens. Upon proteolysis, initiator caspases such as caspases-8, -9, -10, and -2 cleave the executioner caspases-3, -6, and -7. These effector or executioner proteases then degrade structural proteins, signaling molecules, and DNA repair enzymes[5-10]. Among the executioner caspases, activation of caspase-3 plays an extremely important role in neuronal apoptosis and is considered the terminal event preceding cell death. Caspase-3 is primarily activated by

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two initiator pathways driven by caspases-9 and -8, also referred to as the intrinsic (mitochondrial) and extrinsic pathways of cell death, respectively (Fig. 1).

2 Caspase-3 modulates synaptic function in the adult brain

Caspase-3 activity appears to contribute to apoptosis and connectivity in the early phases of central nervous system development, but modulates synaptic function in the adult brain. Although suppression of apoptosis in the postnatal mammalian brain coincides with decreases in caspase-3 expression[11,12], active caspase-3 has been detected in neurons and glia from hippocampal slices obtained from adult rats[13]. In healthy adult brain, caspase-3 appears to have alternative roles including modulation of synaptic plasticity and some forms of memory.

2.1 Caspases modulate synaptic plasticity Synaptic strength can be modified in response to neural activity and

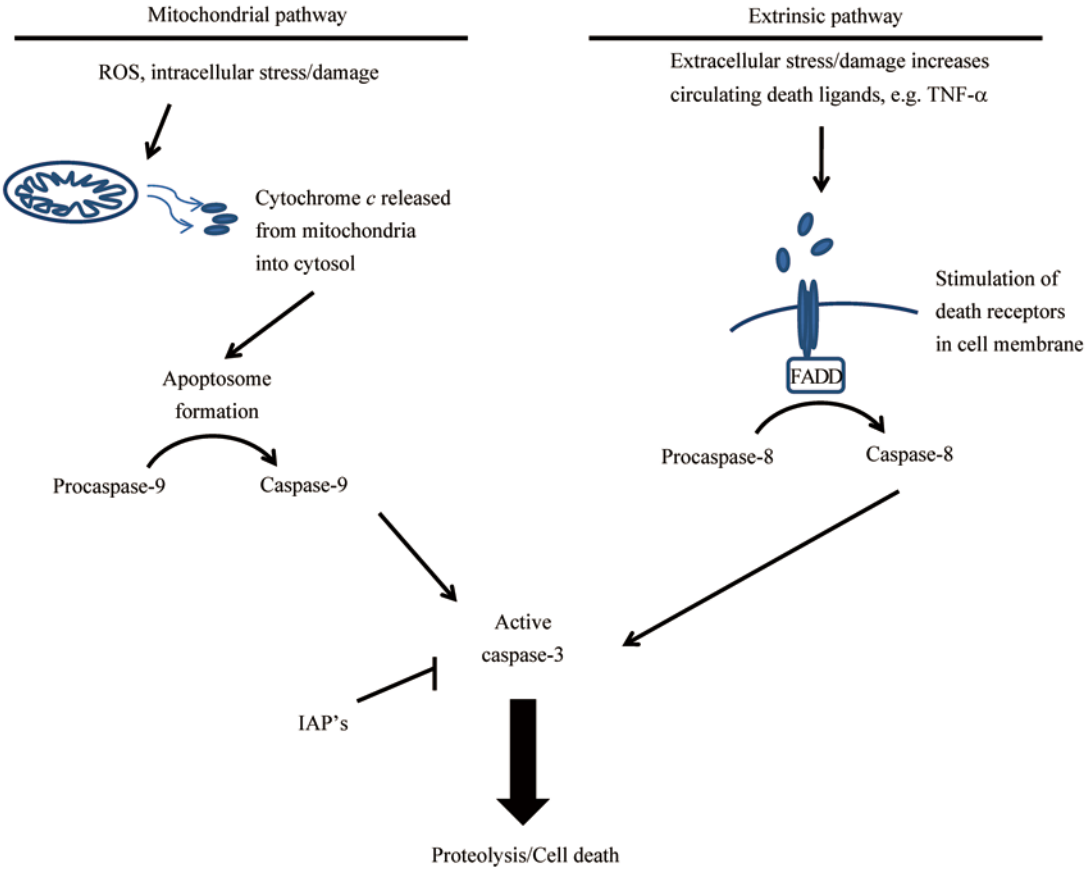

Fig. 1. Overview of the intrinsic and the extrinsic cell death pathways. The intrinsic or mitochondrial pathway is triggered by cellular stress signals such as accumulation of reactive oxygen species (ROS), DNA damage and relocalization, or activation of proapoptotic proteins. The key event in the intrinsic pathway is mitochondrial cytochrome *c* release following mitochondrial membrane permeabilization. Cytochrome *c* then binds with apoptotic protease activating factor 1 (APAF-1), facilitating formation of the apoptosome that activates procaspase-9 by proteolysis, activating downstream effector caspase-3. The extrinsic pathway is initiated by activation of cell surface receptors or death receptors belonging to the tumor necrosis factor (TNF) family. These include FAS receptor (FasR, also known as cluster of differentiation 95, CD95), TNF receptor-1 (TNFR1), DR4, and DR5. FasR is one of the best-understood death receptors and provides a model for the extrinsic pathway. Ligands bind to these receptors and induce formation of a death-inducing signaling complex (DISC) that includes the Fas-associated death domain (FADD). This complex cleaves procaspase to active caspase-8, which then leads to activation of caspase-3. Once activated, caspase-3 cleaves a wide variety of substrates in the brain resulting in loss, gain, or change of function for the target protein.

experience, and this crucial process is known as synaptic plasticity. Long-term potentiation (LTP) and long-term depression (LTD) are forms of Hebbian synaptic plasticity, characterized by rapid adjustments in the synaptic strength of individual synapses. Recent reports have suggested a non-apoptotic role for caspase-3 in synaptic plasticity and memory. In an elegant study combining pharmacological inhibition of caspase-3 and over-expression of endogenous inhibitors, Li *et al.*[14] showed that activation of caspase-3 via mitochondria is required for LTD in CA1 hippocampal neurons, which was supported by the absence of LTD in hippocampal slices from caspase-3 knockout mice. The study further demonstrated that Akt1 proteolysis by caspase-3 is necessary for the induction of LTD[14]. Interestingly, Akt1 is also a substrate of caspase-3 during apoptosis[15]. What is then the difference in the mechanisms underlying caspase-3 activation that leads to different outcomes (i.e. LTD *vs* apoptosis)? Evidence provided by the same group suggests that the intensity and duration of caspase-3 activation determines the final outcome: N-methyl-*D*-aspartate (NMDA) induces a transient activation of caspase-3, associated with LTD; whereas higher and persistent levels of active caspase-3 are induced by staurosporine[14], a widely used apoptotic stimulus[16]. Supporting the importance of the intensity and duration of caspase-3 activation in distinguishing between LTD and apoptosis, Jiao and Li[17] showed that cell death is only induced when neurons are exposed to a high concentration of NMDA (100 μmol/L) for 60 min, but not by treatment with a lower dose (30 μmol/L) or for a shorter period (10 or 30 min). Consistently, NMDA-induced LTD is associated with a moderate and transient activation of Bcl-2-associated death promoter (BAD) and BAX, the upstream factors involved in the canonical and cell death-related activation of caspase-3[17]. The spatial pattern of caspase-3 activation also appears critical in that NMDA induces LTD only by a local (dendritic) and moderate activation of the mitochondrial apoptotic pathway. This allows synaptic changes, including cleavage of Akt1, but prevents cell death[14]. In order to explain the fine-tuning of caspase-3 activation at specific synaptic sites, a model has been proposed in which caspase-3 is regulated by a mechanism for rapid release and sequestration by its endogenous inhibitor, X-linked inhibitor of apoptosis protein (XIAP)[18].

In contrast to LTD, Li *et al.*[14] found that caspase-3 inhibition by Z-DEVD-FMK does not disturb LTP in the hippocampus. This was supported by another study which reported that Z-VAD-FMK, a pan-caspase inhibitor, has no effect on LTP *per se*[19]. However, the same study also found that Z-VAD-FMK prevents the inhibition of LTP by amyloid $\beta_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) in CA1 hippocampal neurons[19]. Blockade of LTP by Aβ has been postulated as one mechanism underlying the memory deficits in AD, but the molecular details underlying Aβ actions remain unknown. The key role of caspase-3 in the inhibition of LTP by Aβ was demonstrated by experiments showing that Aβ has no effect on LTP in caspase-3 knockout mice. It was also demonstrated that cleavage of Akt1 by caspase-3 is an essential step for the inhibition of LTP by Aβ[19]. Following the canonical pathway which establishes that Akt inactivates GSK-3β by an N-terminal phosphorylation (Ser9) in neurons exposed to Aβ, the increased levels of active caspase-3 are correlated with a decrease in the levels of p-GSK-3β (Ser9). As expected, pharmacological inhibition of GSK-3β prevents the Aβ-induced inhibition of LTP, suggesting a serial mechanism in which activation of casapse-3 leads to cleavage of Akt1, which then removes a tonic inhibition of GSK-3β[19]. Although it is unknown whether this process is also associated with the role of caspase-3 in LTD[14], overall it appears that caspase-3 activation can modulate LTP and LTD differentially by modulating the Akt/GSK-3β pathway, and that caspase-3 activation is essential for normal synaptic function.

2.2 Caspases modulate learning and memory processes

In line with the increasing evidence supporting the role of caspase-3 in synaptic plasticity, caspase-3 also participates in learning and memory processes in different animal models. For instance, Huesmann and Clayton[18] reported that caspase-3 activation is required for the development of long-term habituation to a song in birds, and that inhibiting caspase-3 prevents the occurrence of a persisting memory for the song. Similarly, Stephanichev *et al.*[20] used

Z-DEVD-FMK to demonstrate that inhibition of caspase-3 levels in the adult rat brain impairs the acoustic startle response, providing strong evidence for a role of caspase-3 in normal cognitive processes in the mature brain[20]. Inhibition of caspase activity in the hippocampus has also been shown to block long-term, but not short-term, spatial memory in the water maze task[13] while Z-DEVD-FMK impairs memory in the contextual fear conditioning task in wild-type mice[21]. These reports are consistent with the emerging hypothesis that caspase-3 activation is required for learning and memory processes.

3 Role of caspase-3 in the aging brain

While caspase-3 activation is essential for some normal functions in the healthy adult brain, in the aged brain the effects of caspase-3 activation can often be detrimental. Activation of the caspase cascades and caspase-3 in particular is strongly implicated in several degenerative processes in the aging brain[22-25] and in the pathogenesis of late-onset degenerative diseases[26]. In addition to causing breakdown of structural proteins such as actin and laminins, caspase-dependent cleavage of specific proteins inactivates survival pathways, such as the phosphatidylinositol-3 kinase/Akt pathways[27,28] and mitogen-activated protein kinases (MAPKs) pathways. This modulation of various signal transduction pathways by caspases suggests a wider range of functions for the proteases.

3.1 Caspase-3 and Akt pathway Akt, also known as protein kinase B, is a serine/threonine kinase regulated by the membrane levels of phosphatidylinositol 3-phosphate. Activation of Akt requires a dual mechanism: translocation to the plasma membrane and phosphorylation at Thr308 (via PDK1) and Ser473 (via PDK2)[29,30]. Once activated, Akt shows significant anti-apoptotic signaling properties[31]. Cell survival by Akt is likely to be mediated by its ability to promote growth through mTOR[32] and to phosphorylate and inactivate several pro-apoptotic molecules including BAD[33], caspase-9[34] and Forkhead box class O[35]. Accordingly, blocking Akt signaling is associated with reduced neuronal survival[36].

In the aging rat brain, Akt p-Thr308 levels are reduced in the CA1 and increased in the CA3 regions of the hippocampus[27]. Similarly, the SAMP10 mouse model for early onset of neurodegenerative dementia shows decreased phosphorylation of Akt Ser473 in the hippocampus[28]. Furthermore, decreases in the levels of brain-derived neurotrophic factor, as found in the aging brain, may also interfere with Akt function, resulting in increased vulnerability of neurons[37]. Akt is also a direct cleavage target for caspase-3[19]. Thus, it appears likely that the protective effects of Akt signaling in the mature brain are negated with aging due to activation of caspase-3.

3.2 Caspase-3 and MAPK pathway Although many signaling cascades are affected by caspases in aging neuronal populations, very few, if any, have shown evidence of mediating a possible survival/plasticity switch. If such a switch indeed exists, it may be valuable in that it provides some insights into the role of caspases in functions other than cell death. The MAPKs are especially important in this respect. The MAPKs have three subfamilies: the extracellular signal-regulated kinases (ERKs), the c-Jun amino-terminal kinases (JNKs), and p38/HOG1 kinase, each contributing differentially to pro- and anti-apoptotic pathways[38]. While the JNK and p38 pathways are often associated with the induction of apoptosis, ERK pathway signaling is thought to protect cells from apoptosis[39-41]. ERK promotes cell survival during development and tissue homeostasis by phosphorylation and inhibition of caspase-9, which in turn leads to reduced caspase-3 activation[42]. However, an increasing number of studies have reported a role of ERK signaling in neuronal degeneration and death[22,43].

While the ERK cascade promotes cell survival in conditions of unstressed tissue homeostasis, it promotes apoptosis under elevated reactive oxygen species (ROS) or other stress conditions. In particular, non-apoptotic (caspase-independent) neuronal cell death is induced by the activation of the MEK2-ERK2 but not the MEK1-ERK1 pathway, suggesting that the ratio of ERK1/ERK2 might be a critical determinant of cell fate[44]. An upstream kinase in the ERK cascade is MEKK1. This full-length kinase plays a key role in the cell survival signal of ERK

and acts by mediating the transcription of NFκB. However, activation of caspase-3 leads to cleavage of MEKK1 to form pro-apoptotic C-terminal fragments. Cardone *et al.*[34] have also reported that these small-length fragments engage in a positive feedback loop to initiate caspase cascades. This adds to the evidence that the MEKK1-driven ERK pathway may play a significant role in controlling the survival-apoptosis switch in cells. However, this intriguing hypothesis remains to be tested.

In the aging brain, caspase-3 activation is a common convergence point for a number of toxic triggers such as oxidative damage and Aβ. Studies conducted by our group using dogs as a model of aging[25] and in human subjects[45] have shown that activated caspases are risk factors for cognitive decline and neurodegeneration. However, we also found that although caspase-3 activation occurs in the aged brain, it is not correlated with neuronal death[25]. This is in agreement with that reported in transgenic AD mouse models[46]. Our group has been using the aged dog to address whether it is possible to use lifestyle interventions to prevent age-related caspase activation in the brain. Our findings revealed that sustained changes in lifestyle, i.e. behavioral enrichment, exercise, dietary supplementation with antioxidants and mitochondrial cofactors, or a combination of these interventions, dramatically reduces the abundance of cells expressing activated caspase-3. This finding supports the concept that caspase-3 activation may be a focal point for multiple pathways that can be regulated by lifestyle interventions.

4 Caspases in AD

AD is a neurodegenerative condition characterized by progressive deposition of Aβ plaques, aggregation of paired helical filament tau in neurofibrillary tangles (NFTs), and synaptic degeneration and neuronal loss. Furthermore, caspase-3 levels are higher in AD brains than in age-matched controls[47].

There are multiple pathways by which caspase activation can exacerbate each hallmark of AD pathology and impair cognitive function. For instance, the cleavage of APP by β-secretase (BACE), an essential step in Aβ production, is up-regulated by stress and targeted for lysosomal degradation by Golgi-localized, γ-adaptin ear-containing ARF-binding protein (GGA3). Recently the Tessler-Lavigne group reported that GGA3 is a degradation target of caspase-3, suggesting that caspase activation allows accumulation of BACE and therefore increases production of Aβ[48]. The role of caspase-cleaved Akt in the effects of Aβ on the brain has also been discussed[19]. Thus, caspase activation in the AD brain appears to mediate and exacerbate the pathological effects of Aβ. Moreover, the microtubule-associated protein tau that forms NFTs is a substrate of caspase-3 and can lead to neurite degeneration by reducing the availability of full-length tau for binding to microtubules. Cleavage of tau at Asp421 also generates a positive-feedback loop for neuronal degeneration[49-51]. In a recent study by Hyman and co-workers[46], it was reported that caspase-3 activation precedes and correlates with cleaved tau and NFT formation. Calcineurin is also cleaved as a consequence of the caspase cascade, constitutively activating it. Because calcineurin is a critical mediator of LTD and spine loss, its increased activation may increase LTD and spine loss while decreasing functional connectivity.

A selective accumulation of caspase-3 in the postsynaptic density (PSD) fractions in AD patients has also been described[52]. Synapse-specific elevation of caspase-3 can lead to synapse degeneration, significant because synaptic loss is the pathology most highly correlated with the severity of cognitive impairment in AD[53,54]. In addition to its ability to induce synapse degeneration, caspase-3 activation has recently been demonstrated to decrease synaptic plasticity through internalization and dephosphorylation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors[14].

Homeostatic plasticity describes the scaling of neuronal output without changing the relative strength of individual synapses, in order to keep neurons firing within an optimal range. It is thought that homeostatic plasticity compensates for Hebbian forms of synaptic plasticity[55]. One of the mechanisms of homeostatic plasticity involves modifying miniature excitatory postsynaptic currents (mEPSCs) by scaling up AMPA receptors under

activity-deprived conditions and scaling them down during increased network excitability[56,57]. In agreement with the putative roles of caspase-3 in synaptic function, several lines of evidence suggest that caspase-3 may be involved in the mechanism controlling the availability of AMPA receptors in the synapse: first, it has been shown that caspase-3 activity is critical for the NMDA-induced internalization of AMPA receptors[14]; second, caspase-3 can alter the specific properties of AMPA receptors by cleaving subunits such as GluR1[58] and GluR4[59]; and third, anchoring and/or trafficking of the AMPA receptors can be affected by caspase cleavage of AMPA receptor-associated proteins such as PSD-95[60], CaMKII and calcineurin A[21], all of which belong to the caspase-3 substrate family. In a recent commentary[61] Hyman suggested that altering the balance between kinase cascades such as Akt and phosphatases such as calcineurin could be critical in determining the outcome of caspase activation (LTD or neurodegeneration). However, AMPA receptors are removed from their postsynaptic sites in both LTD and neurodegeneration. Evidence for deranged homeostatic synaptic scaling of AMPA receptors was also found in AD mouse models that produce humanized Aβ[62]. A similar model for the proteolytic loss-of-function of normal homeostatic processes in AD was recently described in which presenilin knock-out and presenilin mutant mice fail to activate Akt and therefore do not homeostatically scale excitatory synapses[63]. The cognitive effects of such alterations remain to be determined, but overall it appears likely that caspases play a critical role in regulation of neuronal dysfunction in neurodegenerative conditions probably due to both gain of pathological functions and loss of normal functions.

5 Control of caspase activation in brain plasticity and neuronal death

It is remarkable that a protease cascade which can trigger widespread damage and degeneration should aggregate in structures as delicate as synapses that underlie cognitive processes and memory formation. A possible explanation is that the acquisition of higher cognitive processes such as learning, memory, and problem-solving necessitates a greater amount of synaptic plasticity, which in turn, requires activation of regulatory molecules such as caspases involved in axonal sprouting and dendritic pruning. In aged cells however, which have higher levels of several misfolded proteins or other cytotoxic structures, activation of caspase-3 is more likely to cause extensive cellular damage and even eventual cell death. Thus, under conditions of low cell stress, caspase-3 activation primarily modulates plasticity, and under high-stress conditions, caspase-3 activation is likely to result in cell degeneration and death. It stands to reason then that a mechanism must exist for determining the specific role of caspase-3 in these different functions. Here we suggest that caspase-3 is actually a bifurcation point in cellular signaling, able to orient the neuronal response to stress down either pathological/apoptotic pathways or towards physiological cellular remodeling (Fig. 2).

Several regulatory mechanisms are likely determinants of caspase activity in the brain, as discussed in the following:

(1) Regulated proteolytic activation: Since caspases are produced as precursor proteins (zymogens) and must be activated by proteolytic cleavage, the intrinsic and extrinsic pathways both regulate caspase activation. Such post-translational processing may be especially relevant for aging, as accumulation of insults such as oxidative damage, stress, and decreased neurotrophic support leads to activation of the intrinsic or caspase-9-dependent pathway. Phosphorylation and inactivation of caspase-9 is considered to be a mechanism underlying the promotion of cell survival by Akt[64]. Therefore, while in the healthy brain Akt promotes cell survival by inactivating caspase-9, increased oxidative damage and accumulation of toxins like Aβ in the aging brain are likely to interfere with regulatory molecules such as Akt and may lead to a positive feedback loop to increase activation of caspase-3. It is also conceivable that activation of phosphatases such as calcineurin interferes with kinase cascades to determine the outcome of caspase activation.

(2) Transient activation: Recent evidence indicates that active caspase-3 is always present in synaptic termi-

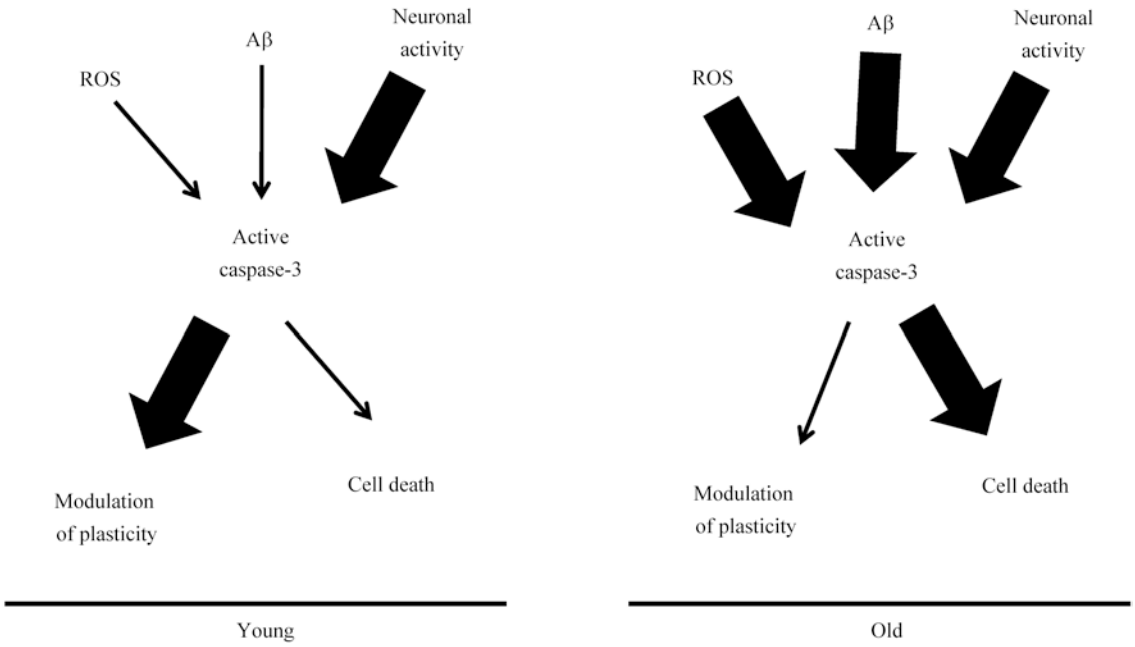

Fig. 2. Caspase-3 activation is a bifurcation point in the cellular response to stimuli, and may have different effects in a young healthy brain versus in an aged or diseased brain. The left diagram shows in a young brain, how on a background of low cell stress, caspase activation due to a specific pattern of neuronal activity could primarily modulate plasticity, and the right diagram shows under conditions of high stress such as accumulation of reactive oxygen species (ROS) or aggregated amyloid-β (Aβ) due to age, caspase-3 activation would primarily be in cell death pathways, overwhelming the normal regulation of plasticity.

nals but released only transiently to effect a synaptic process essential for memory storage. Neurons may therefore control the duration of proteolytic cleavage enacted by caspases through molecular custodians such as XIAP[18]. For instance, temporally limited activation of caspase-3 is essential for PC-12 and neurosphere differentiation[65,66]. However, under conditions of stress and injury, regulatory molecules are impaired in function and can lead to chronic activation of caspases in the brain. This has been supported by Li *et al*., who reported that transient activation of caspase-3 induced by NMDA is associated with LTD whereas a higher and persistent level of caspase-3 is induced by the pro-apoptotic reagent staurosporine[14].

(3) Spatial activation: Similarly, a study of local caspase-3 activity in both synaptic and dendritic degenerative processes showed that glutamate induces caspase-3 activation in cultured hippocampal neurons[67]. Subsequent investigations showed that limited caspase activation results primarily in proteolytic outcomes of caspases, while widespread caspase activation often results in cell death. Specifically, spatially limited caspase activation limits its destructive capacity to dendrite pruning and synapse elimination[68-71]. Furthermore, spatially or temporally limited activation of caspase-3 has been reported to down-regulate neuronal excitability through GluR1 cleavage[72]and dephosphorylation[21].

(4) Endogenous inhibitors: Caspase activation is limited by endogenous inhibitors such as the inhibitor of apoptosis proteins (IAPs), characterized by the presence of at least one baculoviral IAP repeat (BIR) domain[73]. In *Drosophila*, the caspase inhibitor drosophila/thread inhibitor of apoptosis (DIAP) binds caspases to prevent apoptosis through both caspase degradation[74] and non-degradation pathways[75]. DIAP1 in turn is modulated by the ubiquitin-proteasome system[69]. Therefore, the ratio of DIAP proteins to caspases may determine the ultimate cell fate after caspase activation.

Most studies of caspase-3 regulation have been con-

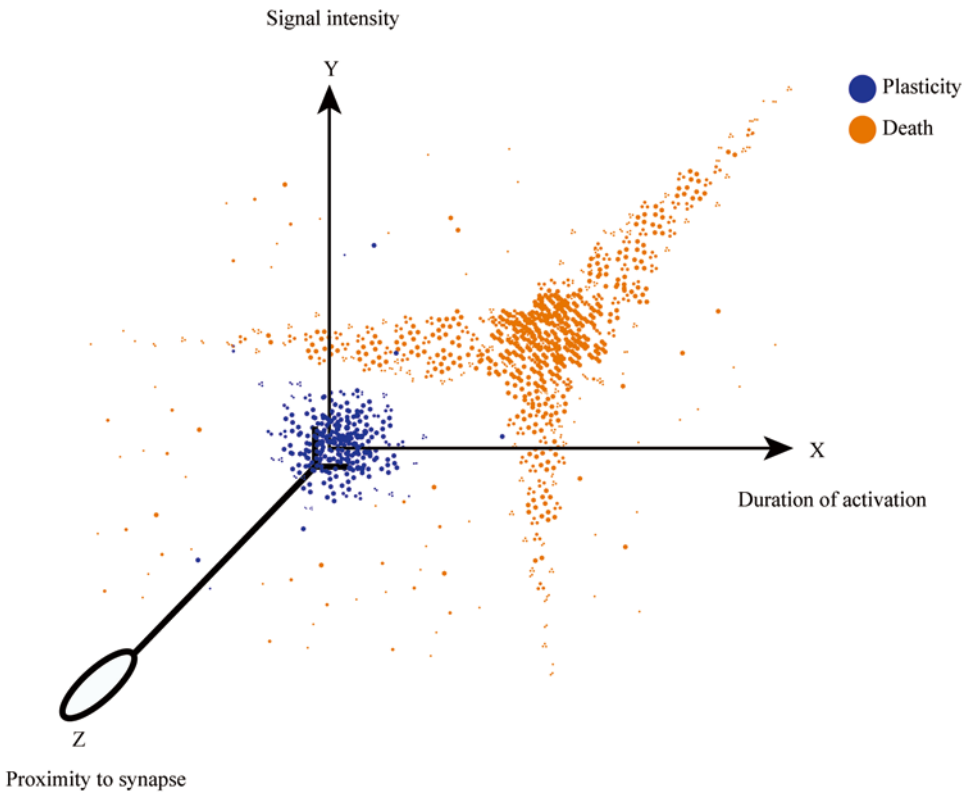

Fig. 3. Different roles of caspase-3 in the brain under different physiological states. Variables associated with the activation of caspase-3 that determine its function in neuronal circuitry are signal intensity, duration of activation and proximity to the synapse. This model suggests that the function of caspase-3 depends on the context in which it is activated. When signals detected by neurons are transduced and integrated, caspase-3 activity is modified, leading to two possible outcomes (color dots): the enrollment of caspase-3 in synaptic plasticity processes, or its participation in cell death pathways. The model predicts the existence of a threshold of caspase-3 activity that determines its role during any neuronal state; the threshold is determined by the strength and quality of all the variables involved in caspase-3 activation.

ducted with regard to cell death or structural remodeling. However, based on emerging evidence, we propose here that the site, duration and intensity of caspase activation are important determinants of whether caspase activation results in cell degeneration or synaptic plasticity (Fig. 3).

6 Conclusion

The existence of so many different regulatory mechanisms for caspase activity indicates that caspases can take on multiple roles depending on the cellular context. Indeed, while the proteolytic activity of caspases remains unchanged, the effect on different molecular targets is largely determined by temporal, spatial, and activation access provided by various regulators. It appears that a survival/plasticity switch regulates the ultimate effect of caspase-3 activation in neurons. By regarding caspase-3 as tightly-regulated cleavers of specific proteins, we may be able to integrate our understanding of its diverse roles across the lifespan. In cells with widespread caspase activation, caspases initiate apoptosis. In young cells with localized caspase-3 activation, caspases mediate branch pruning, and in healthy adults, caspase-3 activation is required for the normal maintenance of Hebbian and homeostatic plasticity. Therefore, in neurodegenerative conditions such as AD, caspase dysregulation can have a doubly detrimental effect, through both loss of normal plasticity and induction of synaptic and cellular degradation. Attempting to reduce the pathological effects of caspases through their complete

inhibition may hence result in a loss of normal physiological functions. In future, a better understanding of the complex caspase regulatory pathways may suggest the best intervention target, so that the outcome of caspase activation can be directed to a preferred outcome.

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