Chapter 2
To Survive or to Die: How Neurons Deal with it

Yubin Wang, Xiaoning Bi, and Michel Baudry

Abstract Unlike the majority of cells in the organism, neurons have only two options during their entire existence, to survive or to die. As a result, they have evolved elaborate mechanisms to determine which path they will follow in response to a multitude of internal and external signals, and to the wear-and-tear associated with the aging process. Until recently, activation of the calcium-dependent protease, calpain, had been traditionally associated with neurodegeneration. This chapter will review recent findings that indicate that two of the major calpain isoforms present in the brain, calpain-1 and calpain-2, play opposite functions in neuronal survival/death. Thus, calpain-1 activation, downstream of synaptic NMDA receptors, is part of a neuronal survival pathway through the truncation of PHLPP1 and the stimulation of the Akt pathway. In contrast, calpain-2 activation is downstream of extrasynaptic NMDA receptors and is neurodegenerative through the truncation of the phosphatase, STEP, and the activation of the p38 protein kinase. These findings have major significance for our understanding of neurological conditions associated with neurodegeneration and for the development of new therapeutic approaches to prevent neuronal death in these disorders.

Keywords Calpain-1 · Calpain-2 · Neuronal death · Neuronal survival · NMDA receptors · Akt · STEP

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2.1 Introduction

Neurons have to perform several basic functions, including growing (from the time of differentiation), migrating, responding and adapting to external and internal stimuli, and surviving or dying, as a result of continuous challenges and the deleterious effects of the aging process. Numerous reviews have discussed the role of calpain in neurodegeneration in general (Vosler et al. 2008; Yildiz-Unal et al. 2015), and in stroke (Anagli et al. 2009; Koumura et al. 2008) and in traumatic brain injury (TBI) (Kobeissy et al. 2015; Liu et al. 2014). Likewise, numerous studies have attempted to use calpain inhibitors to reduce neurodegeneration in both stroke and TBI (Anagli et al. 2009; Bartus et al. 1994a, b; Cagmat et al. 2015; Hong et al. 1994; Li et al. 1998; Markgraf et al. 1998; Siklos et al. 2015; Tsubokawa et al. 2006). While some studies have reported some positive effects of calpain inhibitors in TBI (Thompson et al. 2010), other studies have not confirmed these results. In particular, overexpression of the endogenous calpain inhibitor, calpastatin, was reported to reduce the formation of the Spectrin Breakdown Product (SBDP), resulting from calpain-mediated truncation of spectrin, a widely used biomarker of calpain activation and potentially neurodegeneration (Yan and Jeromin 2012), but had no effect on neurodegeneration (Schoch et al. 2012). Another recent study concluded that even a blood-brain barrier- and cell-permeable calpain inhibitor, SNJ-1945, did not have a sufficient efficacy and a practical therapeutic window in a model of controlled cortical impact (Bains et al. 2013).

Several reasons could account for the failure to develop clinical applications of such inhibitors, including their lack of specificity/potency/selectivity (Donkor 2011), and the incomplete knowledge regarding the functions of the major calpain isoforms in the brain, calpain-1 and calpain-2 (aka μ- and m-calpain). Work from our laboratory over the last 5 years has revealed new features of these two enzymes, which significantly changed our understanding of their functions in the brain. Specifically, we found that calpain-1 and calpain-2 play opposite functions in both synaptic plasticity and neuroprotection/neurodegeneration (Baudry and Bi 2016). Thus, calpain-1 activation is required for theta burst stimulation-induced long-term potentiation (LTP) and for certain types of learning and memory, and is neuroprotective (Wang et al. 2013, 2014). Calpain-1 is neuroprotective due to the degradation of the PH domain and Leucine rich repeat Protein Phosphatase 1 (PHLPP1β) and the resulting activation of the Akt survival pathway. On the other hand, calpain-2 activation limits the magnitude of LTP and restricts learning, and is neurodegenerative due to the cleavage of STEP and the stimulation of death pathways (Wang et al. 2013, 2014). In addition, we found that ischemia-induced damage to retinal ganglion cells was exacerbated in calpain-1 knock-out mice, indicating that calpain-1 inhibition is likely to counteract the potential beneficial effects of calpain-2 inhibition if non-selective calpain inhibitors are used (Wang et al. 2016b). These findings could account for the failure of the previous studies to convincingly demonstrate the role of calpain in neurodegeneration, and for the lack of clear efficacy of the previously tested calpain inhibitors, which did not discriminate between calpain-1 and

calpain-2. It is also important to stress that calpain activation has also been implicated in diffuse axonal injury (Wang et al. 2012a), which has been proposed to represent an important component of the pathophysiology of TBI (Xiong et al. 2013), although at this point, there is no information regarding which calpain isoform is involved.

In this chapter, we will first discuss how calpain-1 and calpain-2 activation appear to be closely related to the stimulation of synaptic and extra-synaptic NMDA receptors, respectively. We will then review the mechanisms underlying the neuroprotective effects of calpain-1 activation, which will be followed by a discussion of the mechanisms involved in calpain-2-mediated neurodegeneration. These two aspects will be illustrated by studies using intra-ocular NMDA injection to produce acute neurodegeneration of retinal ganglion cells. Finally, we will discuss the potential clinical implications of these findings and our current efforts to develop selective calpain-2 inhibitors as a new approach for neuroprotection in conditions associated with acute neurodegeneration.

2.2 Calpains and NMDA Receptors

NMDARs play critical roles in both physiological and pathological conditions, and several studies have shown that NMDA receptor localization is responsible for opposite consequences of NMDA receptor stimulation for neuronal survival or death; thus, synaptic NMDAR activation provides neuroprotection, while extrasynaptic NMDARs are linked to pro-death pathways (Hardingham and Bading 2010). The Akt and MAP kinase/extracellular signal-regulated kinase (ERK1/2) pathways are two key pro-survival pathways downstream of synaptic NMDARs (Hardingham et al. 2001; Papadia et al. 2005; Wang et al. 2012b). Akt phosphorylates and inhibits various pro-apoptotic substrates, such as glycogen synthase kinase-3 (GSK3), forkhead box O (FOXO) (Soriano et al. 2006), apoptosis signal-regulating kinase 1 (ASK1) (Kim et al. 2001), p53 (Yamaguchi et al. 2001), and Bcl2-associated death promoter (BAD) (Downward 1999). On the other hand, ERK1/2 activates the survival nuclear transcription factor, cyclic-AMP response element binding protein (CREB) (Hardingham et al. 2001). Although some protein kinases linking NMDARs to Akt and ERK have been found (Krapivinsky et al. 2003; Perkinton et al. 2002), how Akt and ERK1/2 were activated by synaptic but not extrasynaptic NMDARs was not clearly understood until recently.

PH domain and Leucine rich repeat Protein Phosphatase 1 (PHLPP1) exhibits two splice variants, PHLPP1α and PHLPP1β, which share amino acid sequence similarity but have different sizes (140 kDa and 190 kDa, respectively). PHLPP1α dephosphorylates Akt at Ser473 in cancer cells (Gao et al. 2005) and neurons (Jackson et al. 2010), and its down-regulation is related to cell survival in CNS (Chen et al. 2013; Liu et al. 2009; Saavedra et al. 2010). PHLPP1β inhibits ERK1/2

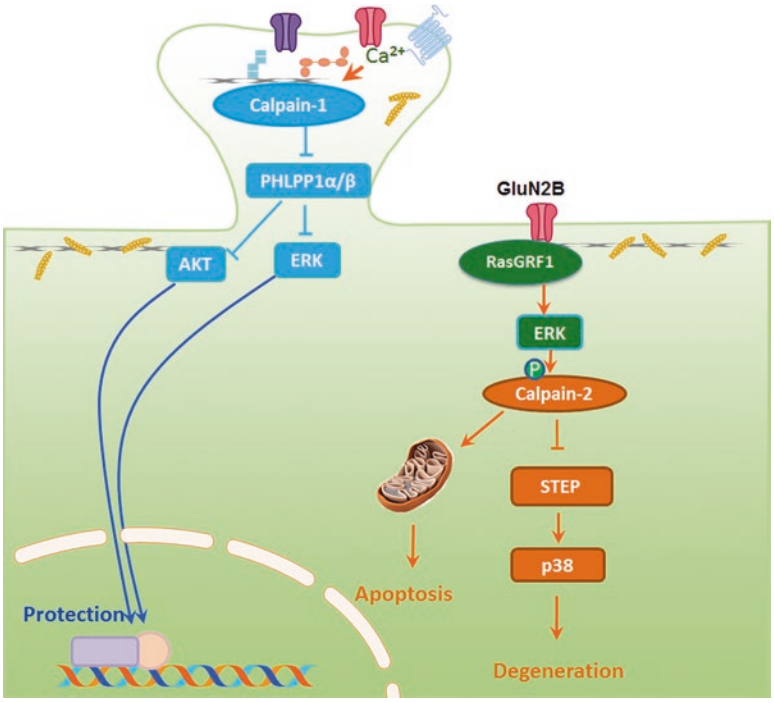

Fig. 2.1 Schematic representation of the links between synaptic and extrasynaptic NMDARs and calpain-1 and calpain-2. Calpain-1 is rapidly stimulated by the calcium influx generated by synaptic NMDA receptor activation, resulting in PHLPP1α/β degradation. This produces the activation of Akt and ERK, which triggers the stimulation of neuroprotective cascades. On the other hand, extrasynaptic NMDA receptors containing NR2B subunits trigger ERK activation, calpain-2 phosphorylation/activation and the activation of STEP and p38, leading to neurodegeneration. Moreover, calpain-2 activation has been linked to apoptosis through the truncation of anti-apoptotic factors

by binding and trapping its activator Ras in the inactive form (Shimizu et al. 2003). PHLPP1β was previously shown to be degraded by calpain in hippocampus, and its degradation contributes to novel object recognition memory (Shimizu et al. 2007). Thus, PHLPP1 was a good candidate to link NMDA receptor stimulation to Akt and ERK regulation.

Using primary neuronal cultures, we showed that calpain-1 and calpain-2 are activated by different NMDAR populations (synaptic vs. extrasynaptic NMDARs) and regulate different substrates (PHLPP1 and STEP) to produce opposite effects on neuronal fate (neuroprotection and neurodegeneration) (Fig. 2.1). Interestingly, calpain-induced cleavage of PHLPP1β and the resulting ERK activation were previously shown to regulate synaptic plasticity (Shimizu et al. 2007). We showed that calpain-1-mediated PHLPP1β degradation was specifically triggered by synaptic but not extra-synaptic NMDAR activation and contributed to the neuroprotective effects of synaptic NMDAR activation. In addition, PHLPP1α, which

dephosphorylates and inhibits Akt, was also cleaved by calpain-1 following synaptic NMDAR activation. Calpain cleavage of PHLPP1 1α and β was necessary and sufficient for synaptic NMDAR-induced activation of the Akt and ERK pathways, since calpain inhibition blocked, while PHLPP1 knockdown mimicked, the effects of synaptic NMDAR activation on Akt and ERK pathways. PHLPP1 suppressed Akt and ERK pathways under basal conditions; following synaptic NMDAR activation, calpain cleaves PHLPP1α and β, thus releasing the inhibition of these two major pro-survival signaling cascades in neurons. Consistently, calpain-1-mediated cleavage of PHLPP1 was required for the neuroprotective effects of synaptic NMDARs, as calpain inhibition blocked the neuroprotection elicited by synaptic NMDAR activation. We further confirmed these results using PHLPP1 knockdown, as down-regulation of PHLPP1 not only suppressed the blockade of neuroprotection caused by calpain inhibition but also induced neuroprotection without synaptic NMDAR activation. Consistent with our results, a recent study reported that PHLPP1 knockout mice are more resistant to ischemic brain injury (Chen et al. 2013). Thus, PHLPP1 should be considered as a novel potential target for the treatment of neurodegenerative diseases.

As previously reported (Xu et al. 2009), we found that calpain activated by extrasynaptic NMDAR stimulation cleaved STEP and caused neuronal death (Wang et al. 2013). It had previously been proposed that prolonged or excessive activation of calpain was responsible for calpain-mediated neurotoxicity, whereas brief and limited calpain activation could be involved in the regulation of synaptic plasticity. However, prolonged activation of synaptic NMDARs (by Bic and 4-AP treatment) for as long as 3 days did not result in STEP cleavage, nor in neuronal damage, but produced neuroprotection against starvation and oxidative stress. On the other hand, activation of extrasynaptic NMDARs did not affect PHLPP1 or its downstream pathways, strongly suggesting that there are two separate pools of calpain downstream of synaptic and extrasynaptic NMDARs, which regulate different substrates and therefore exert separate functions.

The possibility that calpain-1 and calpain-2 could exert different roles in CNS had not been extensively discussed. However, the discovery that calpain-2 could be activated by phosphorylation (Zadran et al. 2010), coupled with the identification of PTEN as a specific calpain-2 substrate (Briz et al. 2013), raised the possibility that calpain-1 and calpain-2 could play distinct functions. Interestingly, synaptic NMDAR activation did not result in the degradation of PTEN, a specific calpain-2 substrate, further supporting the idea that synaptic NMDAR activation does not activate calpain-2. The use of calpain-1 and calpain-2 specific inhibitors also confirmed this idea, as a calpain-2 specific inhibitor did not affect synaptic NMDAR-dependent PHLPP1 cleavage and neuroprotection but blocked extrasynaptic NMDAR-dependent STEP cleavage and neurotoxicity. In contrast, a calpain-1 specific inhibitor blocked synaptic NMDAR-mediated effects but not extrasynaptic NMDAR-mediated neurotoxicity. Down-regulation of calpain-1 and calpain-2 by specific siRNAs in cultured neurons also indicated that only calpain-1 knockdown blocked synaptic NMDAR-mediated neuroprotective pathways. In addition, knockdown of calpain-2 but not calpain-1, by AAV-shRNA transfection increased survival

of primary hippocampal neurons following NMDA treatment (Bevers et al. 2009). Results obtained in cultured neurons were further confirmed using a model of NMDA-induced neurotoxicity in acute hippocampal slices from young mice, which had previously indicated that NMDA treatment of acute hippocampal slices caused neurotoxicity in young but not adult rats (Zhou and Baudry 2006), probably because young rats have more NR2B-containing NMDARs, which are preferentially localized extrasynaptically (Tovar and Westbrook 1999). In hippocampal slices prepared from young calpain-1 knock-out mice, NMDA induced the degradation of STEP but not PHLPP1, and exacerbated neurotoxicity, as compared to slices prepared from wild-type mice. On the other hand, calpain-2 specific inhibition by applying either a selective calpain-2 inhibitor in slices from wild-type mice or a non-selective calpain inhibitor in slices from calpain-1 knock-out mice blocked NMDA-induced degradation of STEP and suppressed neurotoxicity (Wang et al. 2013).

Together, these results demonstrate that calpain-1 is preferentially activated by synaptic NMDAR stimulation, whereas calpain-2 is preferentially activated by extrasynaptic NMDAR stimulation. Calpain-1 was shown to be localized in synaptic compartments (Perlmutter et al. 1988), where it could regulate synaptic function through its action on synaptic elements such as cytoskeletal and scaffolding proteins, as well as glutamate receptors (Liu et al. 2008). Little is known regarding the ultrastructural localization of calpain-2 in neurons. One of the newly discovered physiological roles of calpain-2 is to regulate activity-dependent local protein synthesis (Briz et al. 2013; Wang and Huang 2012), which takes place not in synapses but in nearby extrasynaptic areas (Frey and Morris 1998; Steward and Wallace 1995). In addition, calpain-2 has been reported to control synaptogenesis in dendritic shafts through constitutive proteolysis of the cytoskeletal protein, cortactin (Mingorance-Le Meur and O'Connor 2009). These findings would suggest that calpain-2 is localized, at least in part, in extrasynaptic domains (Fig. 2.1).

The existence of separate signaling pathways for calpain-1 and calpain-2 suggested that these two calpain isoforms belong to different protein scaffolds, which could segregate them in different neuronal compartments. PHLPP1 could be cleaved by both purified calpain-1 and calpain-2 in membrane fractions, yet it was cleaved only by calpain-1 following synaptic NMDAR activation in hippocampal slices, suggesting that substrate specificity for calpains depends not only on amino acid sequences within substrates, but also on localization and scaffolding of both substrates and calpains in neurons. Co-immunoprecipitation experiments confirmed that NR2A-containing NMDARs, PSD95, calpain-1 and PHLPP1, form a complex in neurons. Furthermore, synaptic NMDAR activity recruited calpain-1 to this NMDAR multi-protein complex; such recruitment could facilitate the proteolysis of PHLPP1 and possibly other calpain-1 substrates in the complex. In contrast, calpain-2 was not present in this complex under basal conditions nor was it recruited by activity, consistent with the absence of calpain-2 activation following synaptic NMDAR activation. It is likely that a calpain-2-containing multi-protein complex is associated with extrasynaptic NMDARs. How could activation of extrasynaptic NMDARs results in calpain-2 activation? It has been repeatedly shown that NR2B subunits are enriched in extrasynaptic NMDARs (Papouin and Oliet 2014), and that

their activation is critical for excitotoxicity (Chazot 2004). Interestingly, NR2B directly binds RasGRF1, which provides a link between NMDAR activation and ERK activation (Krapivinsky et al. 2003). As we have shown that ERK activation directly phosphorylates and activates calpain-2 (Zadran et al. 2010), this pathway is likely responsible for the prolonged activation of calpain-2 following stimulation of extrasynaptic NMDA receptors (Fig. 2.1). In addition, we discussed elsewhere the existence of different PDZ binding domains in the C-terminal of calpain-1 and calpain-2, which could account for their differential subcellular distribution (Baudry and Bi 2016).

2.3 Calpain-1 Activation and Neuroprotection

As discussed above, calpain-1 is downstream of synaptic NMDARs and as such, we postulated that it has a neuroprotective function. This notion was supported by results obtained in cultured neurons, where we demonstrated that calpain-1 activation following stimulation of synaptic NMDARs was neuroprotective against starvation- and oxidative stress-mediated neurotoxicity (Wang et al. 2013). Previous studies have shown that normal stimulation of synaptic NMDA receptors is required to limit the extent of apoptotic neuronal death during the postnatal period, as blockade of these receptors during this period increases the extent of apoptotic neuronal death (Monti and Contestabile 2000). Calpain activity is higher in cerebellum than in cortex or hippocampus across different mammalian species (Baudry et al. 1986). An immunohistochemical study revealed that the major calpain isoform expressed in cerebellar neurons is calpain-1 (Hamakubo et al. 1986). Calpain-1 activity in cerebellum during prenatal and early postnatal period is high, as compared to that in adulthood (Simonson et al. 1985), suggesting a potential role for calpain-1 in cerebellar development. Interestingly, a *CAPN1* missense mutation in the Parson Russell Terrier dog breed has been associated with spinocerebellar ataxia (Forman et al. 2013).

Loss of cerebellar granule cells (CGCs) induced by different mechanisms results in ataxia (Hashimoto et al. 1999; Kim et al. 2009; Pennacchio et al. 1998; Shmerling et al. 1998). NMDAR activity is essential for CGC survival during the critical stage of cerebellar development (Monti and Contestabile 2000; Balazs et al. 1988; Monti et al. 2002; Moran and Patel 1989), although the underlying mechanism has remained elusive. NMDAR-induced activation of the nuclear factor CREB is required (Monti et al. 2002), and CREB is a target of the pro-survival kinase Akt (Du and Montminy 1998).

As discussed above, synaptic NMDAR-mediated calpain-1 activation results in the degradation of PHLPP1. PHLPP1 dephosphorylates and inhibits Akt, and is involved in tumorigenesis (Chen et al. 2011), circadian clock (Masubuchi et al. 2010), learning and memory process (Wang et al. 2014; Shimizu et al. 2007), and autophagy (Arias et al. 2015). Calpain-1-mediated degradation of PHLPP1 activates Akt and promotes neuronal survival (Wang et al. 2013), and we postulated that

calpain-1 mediated regulation of PHLPP1 and Akt could be involved in NMDAR-dependent CGC survival during postnatal development.

We analyzed apoptosis in the brain during the postnatal period in wild-type and calpain-1 KO mice (Wang et al. 2016a). Calpain-1 KO mice exhibited abnormal cerebellar development, including enhanced apoptosis of CGCs during the early postnatal period, and reduced granule cell density and impaired synaptic transmission from parallel fiber to Purkinje cells in adulthood, resulting in an ataxia phenotype. All these defects are due to deficits in the calpain-1/PHLPP1/Akt pro-survival pathway in developing granule cells, since treatment with an Akt activator during the postnatal period or crossing calpain-1 KO mice with PHLPP1 KO mice restored most of the observed alterations in cerebellar structure and function in calpain-1 KO mice (Wang et al. 2016a). To reverse reduced pAkt levels in cerebellum of calpain-1 KO mice during the early postnatal period, we treated them from PND1 to PND7 with a PTEN inhibitor, bisperoxovanadium (bpV) (0.5 mg/kg, i.p., twice daily), which has been shown to activate Akt (Boda et al. 2014; Li et al. 2009; Mao et al. 2013). BpV injection significantly increased pAkt levels in cerebellum of developing KO mice, and completely prevented the enhanced apoptosis in cerebellum and cerebrum of calpain-1 KO mice at PND7 (Fig. 2.2).

Thus the NMDAR/calpain-1/PHLLP1/Akt pro-survival pathway is active in developing CGCs, where it limits the extent of CGC apoptosis. Increased PHLPP1 and decreased pAkt levels were found in cerebellar homogenates of calpain-1 KO mice, indicating that calpain-1 activity normally reduces PHLPP1 levels and maintains Akt activated during the postnatal period in cerebellum. The density of pAkt-positive puncta was reduced in cerebellar granular layer but not in Purkinje or molecular layer of calpain-1 KO mice, suggesting that calpain-1-dependent regulation of Akt only takes place in CGCs but not in other cerebellar cell types. Down-regulation of PHLPP1 restored normal levels of pAkt in developing cerebellum of calpain-1 KO mice, indicating that PHLPP1 is downstream of calpain-1 and that its level is important for Akt regulation. Finally, reduced Akt activity was associated with enhanced CGC apoptosis in calpain-1 KO mice, while increased Akt activity was associated with reduced CGC apoptosis in bpV-injected WT and in mice lacking both calpain-1 and PHLPP1.

NMDAR- and calpain-1-mediated neuronal survival during brain development was not limited to CGCs, as enhanced apoptosis was present in other brain regions such as cortex, striatum and hippocampus in developing calpain-1 KO mice (Fig. 2.2). Importantly, calpain-1-mediated neuroprotection is also present in human brain, as calpain-1 mutations resulting in lack of function are associated with cerebellar ataxia (Wang et al. 2016a; Gan-Or et al. 2016). Furthermore, the important roles of calpain-1 in hippocampal neuronal survival during development and in synaptic plasticity in the adult (Wang et al. 2014; Zhu et al. 2015) may contribute to the cognitive decline found in ataxia patients with *CAPN1* mutations.

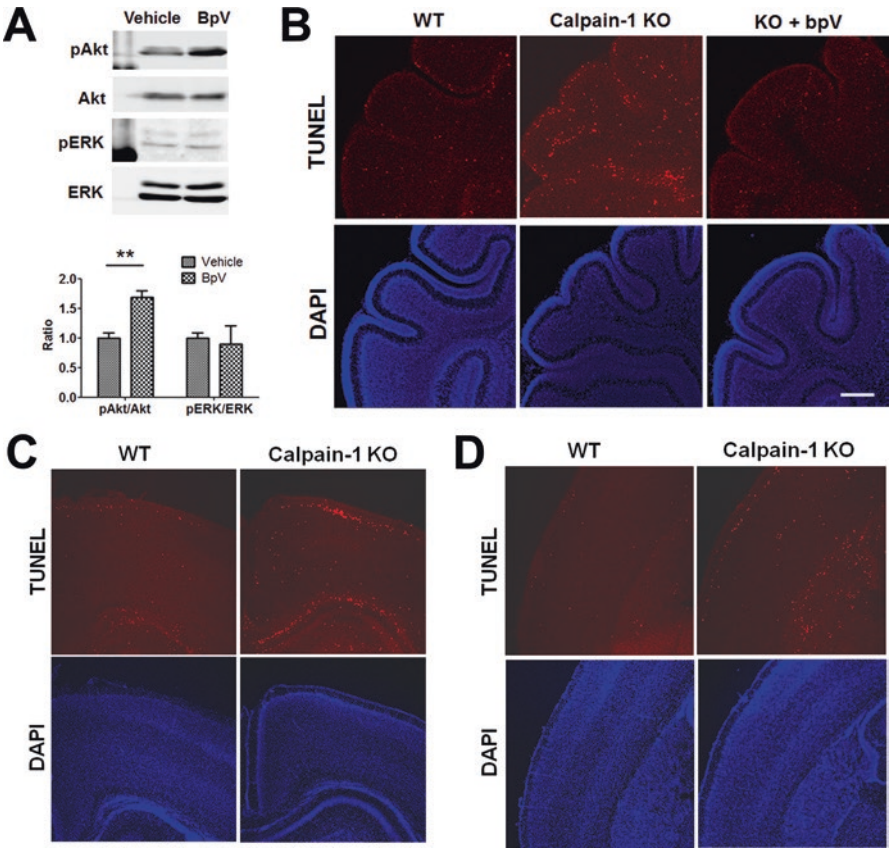

Fig. 2.2 Effects of bpV on apoptosis and Akt in telencephalon of calpain-1 (CAPN1) KO mice during the postnatal period. (**a**–**c**) TUNEL and DAPI staining of coronal sections at various anterior-posterior levels of PND7 calpain-1 KO mice injected from PND1 to PND7 with vehicle or a PTEN inhibitor, bisperoxovanadium (bpV) (0.5 mg/kg, i.p., twice daily). Note the clear decrease in TUNEL staining in bpv-injected calapin-1 KO mice. (**d**, **e**) Levels of Akt and p-Akt and ERK and p-ERK in cortex of PND7 calpain-1 KO mice injected from PND1 to PND7 with vehicle or bpv (0.5 mg/kg, i.p., twice daily). Results are expressed as means ± SEM of four experiments. **$p < 0.05$, Student's t-test

2.4 Calpain-2 and Neurodegeneration

As mentioned above, there is abundant literature linking calpain activation with neurodegeneration. However, very few studies have explored the specific contributions of calpain-1 and calpain-2 in neurodegeneration. Our *in vitro* studies clearly indicated that calpain-2 activation, but not calpain-1 activation was responsible for NMDA-induced excitotoxicity through the activation of STEP. A similar study indicated that down-regulation of calpain-2 but not calpain-1 also increased neuronal survival following NMDA treatment of cultured hippocampal neurons (Bevers et al.

2009). In order to further analyze the role of calpain-2 in neurodegeneration in vivo, we used a model consisting of direct intraocular NMDA injection in mice. Calpain activation had been previously involved in retinal cell death induced by NMDAR activation (Chiu et al. 2005; Shimazawa et al. 2010). To test the specific roles of calpain-1 and calpain-2 in this process, wild-type (WT) mice were injected systemically with a calpain-2 selective inhibitor (C2I), Z-Leu-Abu-CONH-CH_2-C_6H_3 (3, 5-$(OMe)_2$) (Wang et al. 2013, 2014), 30 min before NMDA intravitreal injection. Levels of SBDP and of PHLPP1, were determined in retinal extracts 6 h after NMDA injection (Fig. 2.3a–c). Akt levels were also measured as a loading control. Levels of SBDP were significantly increased and those of PHLPP1 decreased after NMDA injection, as compared to control (PBS intravitreal injection), suggesting that calpain was activated after NMDA injection. Systemic (intraperitoneal; i.p.) injection of C2I significantly suppressed NMDA-induced changes in SBDP but not in PHLPP1, suggesting that C2I systemic injection selectively inhibited calpain-2 but not calpain-1 activation in retina after intravitreal NMDA injection.

Six days after intravitreal injection of NMDA or PBS to WT mice, frozen retinal sections were prepared and H&E staining was performed to evaluate cell numbers in the ganglion cell layer (GCL) and the thickness of the Inner Plexiform Layer (IPL), which contains RGC dendrites. NMDA injection (NMDA plus Vehicle) significantly reduced cell numbers in the GCL and IPL thickness, while PBS injection (PBS plus Vehicle) had no effect on these parameters (Fig. 2.3d–f). Systemic injection of C2I 30 min before and 6 h after NMDA injection significantly suppressed the reduction in GCL cell numbers and IPL thickness (Fig. 2.3d–f), suggesting that calpain-2 activation contributes to NMDA-induced cell death in GCL.

In calpain-1 KO mice, GCL cell number and IPL thickness were not affected by vehicle injection. However, the effects of NMDA injection on GCL cell number and IPL thickness were larger than in WT mice (Fig. 2.3g–i). GCL cell death in calpain-1 KO mice after NMDA injection was significantly more severe than that in WT mice (Fig. 2.3j), suggesting that calpain-1 supports cell survival in GCL after NMDA injection. Systemic injection of C2I to calpain-1 KO mice partially but significantly reversed NMDA-induced decrease in GCL cell number and IPL thickness

Fig. 2.3 (continued) (2 μl of 2.5 mM). Mice were injected i.p. with vehicle (10% DMSO) or C2I (0.3 mg/kg) 30 min before intravitreal injection. Quantification of the ratios of SBDP/Akt (**b**) and PHLPP1/Akt (**c**). n = 4. *p < 0.05, ***p < 0.001. One-way ANOVA followed by Bonferroni test. (**d**) H&E staining of naive, PBS- (control) or NMDA- (2 μl of 2.5 mM) treated retina from WT mice injected i.p. with vehicle (10% DMSO) or C2I (0.3 mg/kg) 30 min before and 6 h after NMDA injection. H&E staining was performed 7 days after injection. Scale bar = 30 μm. Quantification of cell numbers in GCL (**e**) and thickness of IPL (**f**). Six sections in each eye were analyzed. n = 4–8 (eyes). *p < 0.05, **p < 0.01, One-way ANOVA followed by Bonferroni test. (**g**) H&E staining of PBS- (control) and NMDA- (2 μl of 2.5 mM) treated retina from calpain-1 KO mice injected i.p. with vehicle or C2I (0.3 mg/kg) 30 min before and 6 h after NMDA injection. H&E stain was done 7 days after injection. Scale bar = 30 μm. Quantification of cell number in GCL (**h**) and thickness of IPL (**i**). n = 6. *p < 0.05, **p < 0.01, ***p < 0.001, One-way ANOVA followed by Bonferroni test. (**j**) GCL cell numbers in NMDA-treated WT and KO mice without and with C2I treatment. n = 6. **p < 0.01. Two-tailed t-test

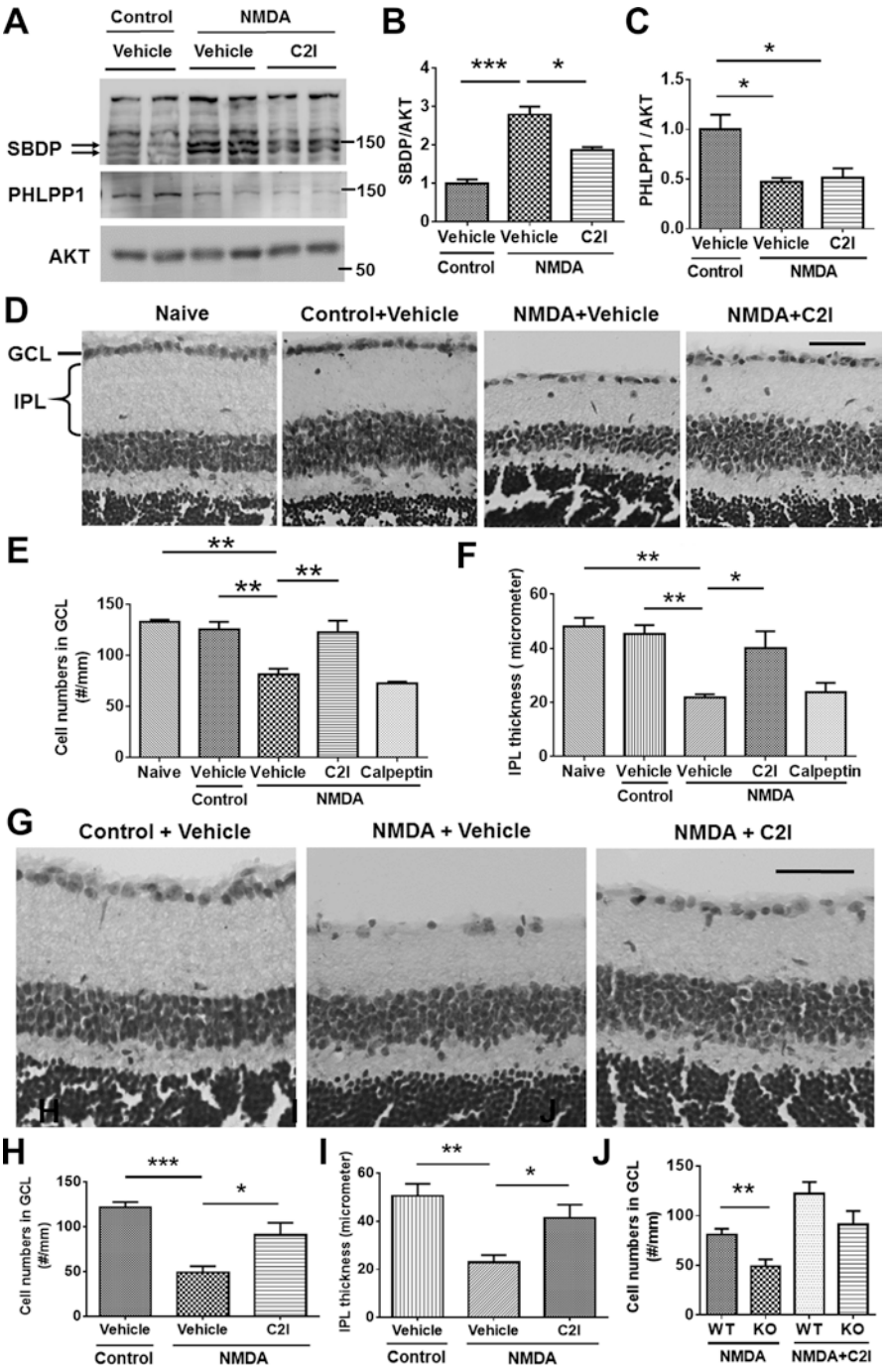

Fig. 2.3 Calpain-2 inhibition reduces, while calpain-1 knockout exacerbates cell death in ganglion cell layer induced by NMDA intravitreal injection. (**a**) Representative immunoblot of indicated proteins in mouse retinal extracts 6 h after intravitreal injection of PBS (control) or NMDA

(Fig. 2.3g–i). A very similar pattern of results was obtained in a different model of acute glaucoma, consisting in a brief period of increased intraocular pressure (Wang et al. 2016b). Furthermore, recent studies in a mouse model of TBI also support the notion that calpain-2 activation is prolonged and responsible for neuronal death, while calpain-1 activation is neuroprotective (Wang et al 2017).

2.5 Clinical Implications of Specific Calpain-2 Inhibition and Calpain-1 Activation

Our results clearly demonstrate that calpain-1 and calpain-2 have opposite functions in both synaptic plasticity and neuronal survival/death after acute insults. Thus, calpain-1 activation is required for LTP induction and for hippocampus-dependent learning and is neuroprotective both during the postnatal developmental period and in adulthood following acute insults. On the other hand, calpain-2 activation limits the extent of hippocampus-dependent learning and is neurodegenerative following acute insults, and in particular excitotoxicity. Our results have important implications for the development of new approaches for treating diseases associated with excitotoxicity, such as epilepsy, stroke, Alzheimer's and Parkinson's disease, Huntington disease and ischemia. In all these cases, it has been suggested that extrasynaptic NMDAR activation and STEP degradation are involved in neurodegeneration. Our results would, therefore, suggest that specific inhibition of calpain-2 but not calpain-1 would have neuroprotective effects under these conditions. Conversely, overexpression or activation of calpain-1, by cleaving PHLPP1 and stimulating pro-survival cascades, could also have beneficial effects. In addition, calpain-2 activation is involved in regulating the magnitude of long-term potentiation (LTP) in hippocampus, due to the existence of a molecular brake consisting in calpain-2-mediated PTEN degradation and stimulation of m-TOR dependent PHLPP1β synthesis (Wang et al. 2014). We also showed that low doses of a selective calpain-2 inhibitor facilitate learning in normal mice, while higher doses, which inhibit calpain-1, impair learning. Thus, a selective calpain-2 inhibitor could be extremely beneficial for preventing neurodegeneration, while facilitating certain forms of learning and memory. As discussed above, a selective calpain-2 inhibitor prevented death of retinal ganglion cells and maintained vision in a mouse model of acute glaucoma (Wang et al. 2016b). Calpain inhibitors have previously been proposed to represent potential treatments for a variety of eye disorders, including glaucoma and macular degeneration (Azuma and Shearer 2008; Paquet-Durand et al. 2007), and further studies are needed to assess the potential use of selective calpain-2 inhibitors for these disorders. Calpain inhibition has been proposed to represent a therapeutic approach for stroke and TBI, although this notion has not been supported by a variety of experiments. We postulate that the use of selective calpain-2 inhibitors might overcome the problems associated with that of non-selective calpain inhibitors. Our results in a mouse model of TBI supports this notion, as we have found that

post-treatment with a selective calpain-2 inhibitor provides a highly significant degree of neuroprotection and facilitates behavioral recovery (Wang et al 2017). The potential use of selective calpain-2 inhibitors for chronic neurodegenerative disorders needs to be further evaluated. It is important to note that calpain has been proposed to participate in neurodegeneration associated with Parkinson's disease as well as Alzheimer's disease, and it is tempting to speculate that selective calpain-2 inhibitors might also be beneficial in these disorders.

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References

Anagli J, Han Y, Stewart L, Yang D, Movsisyan A, Abounit K, Seyfried D (2009) A novel calpastatin-based inhibitor improves postischemic neurological recovery. Biochem Biophys Res Commun 385(1):94–99

Arias E, Koga H, Diaz A, Mocholi E, Patel B, Cuervo AM (2015) Lysosomal mTORC2/PHLPP1/Akt regulate chaperone-mediated autophagy. Mol Cell 59(2):270–284. https://doi.org/10.1016/j.molcel.2015.05.030

Azuma M, Shearer T (2008) The role of calcium-activated protease calpain in experimental retinal pathology. Surv Ophthalmol 53(2):150–163

Bains M, Cebak JE, Gilmer LK, Barnes CC, Thompson SN, Geddes JW, Hall ED (2013) Pharmacological analysis of the cortical neuronal cytoskeletal protective efficacy of the calpain inhibitor SNJ-1945 in a mouse traumatic brain injury model. J Neurochem 125(1):125–132

Balazs R, Jorgensen OS, Hack N (1988) N-methyl-D-aspartate promotes the survival of cerebellar granule cells in culture. Neuroscience 27(2):437–451

Bartus RT, Baker KL, Heiser AD, Sawyer SD, Dean RL, Elliott PJ, Straub JA (1994a) Postischemic administration of AK275, a calpain inhibitor, provides substantial protection against focal ischemic brain damage. J Cereb Blood Flow Metab 14(4):537–544. https://doi.org/10.1038/jcbfm.1994.67

Bartus RT, Hayward NJ, Elliott PJ, Sawyer SD, Baker KL, Dean RL, Akiyama A, Straub JA, Harbeson SL, Li Z et al (1994b) Calpain inhibitor AK295 protects neurons from focal brain ischemia. Effects of postocclusion intra-arterial administration. Stroke 25(11):2265–2270

Baudry M, Bi X (2016) Calpain-1 and Calpain-2: the Yin and Yang of synaptic plasticity and neurodegeneration. Trends Neurosci 39(4):235–245. https://doi.org/10.1016/j.tins.2016.01.007

Baudry M, Simonson L, Dubrin R, Lynch G (1986) A comparative study of soluble calcium-dependent proteolytic activity in brain. J Neurobiol 17(1):15–28. https://doi.org/10.1002/neu.480170103

Bevers MB, Lawrence E, Maronski M, Starr N, Amesquita M, Neumar RW (2009) Knockdown of m-calpain increases survival of primary hippocampal neurons following NMDA excitotoxicity. J Neurochem 108(5):1237–1250

Boda B, Mendez P, Boury-Jamot B, Magara F, Muller D (2014) Reversal of activity-mediated spine dynamics and learning impairment in a mouse model of Fragile X syndrome. Eur J Neurosci 39(7):1130–1137. https://doi.org/10.1111/ejn.12488

Briz V, Hsu Y-T, Li Y, Lee E, Bi X, Baudry M (2013) Calpain-2-mediated PTEN degradation contributes to BDNF-induced stimulation of dendritic protein synthesis. J Neurosci 33(10):4317–4328

Cagmat EB, Guingab-Cagmat JD, Vakulenko AV, Hayes RL, Anagli J (2015) Potential use of calpain inhibitors as brain injury therapy. In: Kobeissy FH (ed) Brain neurotrauma: molecular, neuropsychological, and rehabilitation aspects. CRC Press/Taylor & Francis, Boca Raton, FL. Chapter 40. Frontiers in Neuroengineering

Chazot PL (2004) The NMDA receptor NR2B subunit: a valid therapeutic target for multiple CNS pathologies. Curr Med Chem 11(3):389–396

Chen M, Pratt CP, Zeeman ME, Schultz N, Taylor BS, O'Neill A, Castillo-Martin M, Nowak DG, Naguib A, Grace DM, Murn J, Navin N, Atwal GS, Sander C, Gerald WL, Cordon-Cardo C, Newton AC, Carver BS, Trotman LC (2011) Identification of PHLPP1 as a tumor suppressor reveals the role of feedback activation in PTEN-mutant prostate cancer progression. Cancer Cell 20(2):173–186. https://doi.org/10.1016/j.ccr.2011.07.013

Chen B, Van Winkle JA, Lyden PD, Brown JH, Purcell NH (2013) PHLPP1 gene deletion protects the brain from ischemic injury. J Cereb Blood Flow Metab 33(2):196–204

Chiu K, Lam TT, Ying Li WW, Caprioli J, Kwong Kwong JM (2005) Calpain and N-methyl-d-aspartate (NMDA)-induced excitotoxicity in rat retinas. Brain Res 1046(1–2):207–215. https://doi.org/10.1016/j.brainres.2005.04.016

Donkor IO (2011) Calpain inhibitors: a survey of compounds reported in the patent and scientific literature. Expert Opin Ther Pat 21(5):601–636

Downward J (1999) How BAD phosphorylation is good for survival. Nat Cell Biol 1(2):E33–E35

Du K, Montminy M (1998) CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem 273(49):32377–32379

Forman OP, De Risio L, Mellersh CS (2013) Missense mutation in CAPN1 is associated with spinocerebellar ataxia in the Parson Russell Terrier dog breed. PLoS One 8(5):e64627. https://doi.org/10.1371/journal.pone.0064627

Frey U, Morris RG (1998) Synaptic tagging: implications for late maintenance of hippocampal long-term potentiation. Trends Neurosci 21(5):181–188

Gan-Or Z, Bouslam N, Birouk N, Lissouba A, Chambers DB, Veriepe J, Androschuck A, Laurent SB, Rochefort D, Spiegelman D, Dionne-Laporte A, Szuto A, Liao M, Figlewicz DA, Bouhouche A, Benomar A, Yahyaoui M, Ouazzani R, Yoon G, Dupre N, Suchowersky O, Bolduc FV, Parker JA, Dion PA, Drapeau P, Rouleau GA, Bencheikh BO (2016) Mutations in CAPN1 cause autosomal-recessive hereditary spastic paraplegia. Am J Hum Genet 98(5):1038–1046. https://doi.org/10.1016/j.ajhg.2016.04.002

Gao T, Furnari F, Newton AC (2005) PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol Cell 18(1):13–24

Hamakubo T, Kannagi R, Murachi T, Matus A (1986) Distribution of calpains I and II in rat brain. J Neurosci 6(11):3103–3111

Hardingham GE, Bading H (2010) Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. Nat Rev Neurosci 11(10):682–696

Hardingham GE, Arnold FJ, Bading H (2001) Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. Nat Neurosci 4(3):261–267

Hashimoto K, Fukaya M, Qiao X, Sakimura K, Watanabe M, Kano M (1999) Impairment of AMPA receptor function in cerebellar granule cells of ataxic mutant mouse stargazer. J Neurosci 19(14):6027–6036

Hong SC, Goto Y, Lanzino G, Soleau S, Kassell NF, Lee KS (1994) Neuroprotection with a calpain inhibitor in a model of focal cerebral ischemia. Stroke 25(3):663–669

Jackson TC, Verrier JD, Semple-Rowland S, Kumar A, Foster TC (2010) PHLPP1 splice variants differentially regulate AKT and PKCα signaling in hippocampal neurons: characterization of PHLPP proteins in the adult hippocampus. J Neurochem 115(4):941–955

Kim AH, Khursigara G, Sun X, Franke TF, Chao MV (2001) Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. Mol Cell Biol 21(3):893–901

Kim JC, Cook MN, Carey MR, Shen C, Regehr WG, Dymecki SM (2009) Linking genetically defined neurons to behavior through a broadly applicable silencing allele. Neuron 63(3):305–315. https://doi.org/10.1016/j.neuron.2009.07.010

Kobeissy FH, Liu MC, Yang Z, Zhang Z, Zheng W, Glushakova O, Mondello S, Anagli J, Hayes RL, Wang KK (2015) Degradation of βII-Spectrin protein by Calpain-2 and Caspase-3 under neurotoxic and traumatic brain injury conditions. Mol Neurobiol 52(1):696–709

Koumura A, Nonaka Y, Hyakkoku K, Oka T, Shimazawa M, Hozumi I, Inuzuka T, Hara H (2008) A novel calpain inhibitor,((1S)-1 ((((1S)-1-benzyl-3-cyclopropylamino-2, 3-di-oxopropyl) amino) carbonyl)-3-methylbutyl) carbamic acid 5-methoxy-3-oxapentyl ester, protects neuronal cells from cerebral ischemia-induced damage in mice. Neuroscience 157(2):309–318

Krapivinsky G, Krapivinsky L, Manasian Y, Ivanov A, Tyzio R, Pellegrino C, Ben-Ari Y, Clapham DE, Medina I (2003) The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1. Neuron 40(4):775–784

Li PA, Howlett W, He QP, Miyashita H, Siddiqui M, Shuaib A (1998) Postischemic treatment with calpain inhibitor MDL 28170 ameliorates brain damage in a gerbil model of global ischemia. Neurosci Lett 247(1):17–20

Li D, Qu Y, Mao M, Zhang X, Li J, Ferriero D, Mu D (2009) Involvement of the PTEN-AKT-FOXO3a pathway in neuronal apoptosis in developing rat brain after hypoxia-ischemia. J Cereb Blood Flow Metab 29(12):1903–1913. https://doi.org/10.1038/jcbfm.2009.102

Liu J, Liu MC, Wang K (2008) Calpain in the CNS: from synaptic function to neurotoxicity. Sci Signal 1(14):re1

Liu J, Weiss HL, Rychahou P, Jackson LN, Evers BM, Gao T (2009) Loss of PHLPP expression in colon cancer: role in proliferation and tumorigenesis. Oncogene 28(7):994–1004

Liu S, Yin F, Zhang J, Qian Y (2014) The role of calpains in traumatic brain injury. Brain Inj 28(2):133–137

Mao L, Jia J, Zhou X, Xiao Y, Wang Y, Mao X, Zhen X, Guan Y, Alkayed NJ, Cheng J (2013) Delayed administration of a PTEN inhibitor BPV improves functional recovery after experimental stroke. Neuroscience 231:272–281. https://doi.org/10.1016/j.neuroscience.2012.11.050

Markgraf CG, Velayo NL, Johnson MP, McCarty DR, Medhi S, Koehl JR, Chmielewski PA, Linnik MD (1998) Six-hour window of opportunity for calpain inhibition in focal cerebral ischemia in rats. Stroke 29(1):152–158

Masubuchi S, Gao T, O'Neill A, Eckel-Mahan K, Newton AC, Sassone-Corsi P (2010) Protein phosphatase PHLPP1 controls the light-induced resetting of the circadian clock. Proc Natl Acad Sci U S A 107(4):1642–1647. https://doi.org/10.1073/pnas.0910292107

Mingorance-Le Meur A, O'Connor TP (2009) Neurite consolidation is an active process requiring constant repression of protrusive activity. EMBO J 28(3):248–260

Monti B, Contestabile A (2000) Blockade of the NMDA receptor increases developmental apoptotic elimination of granule neurons and activates caspases in the rat cerebellum. Eur J Neurosci 12(9):3117–3123

Monti B, Marri L, Contestabile A (2002) NMDA receptor-dependent CREB activation in survival of cerebellar granule cells during in vivo and in vitro development. Eur J Neurosci 16(8):1490–1498

Moran J, Patel AJ (1989) Stimulation of the N-methyl-D-aspartate receptor promotes the biochemical differentiation of cerebellar granule neurons and not astrocytes. Brain Res 486(1):15–25

Papadia S, Stevenson P, Hardingham NR, Bading H, Hardingham GE (2005) Nuclear Ca2+ and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. J Neurosci 25(17):4279–4287

Papouin T, Oliet SH (2014) Organization, control and function of extrasynaptic NMDA receptors. Philos Trans R Soc B 369(1654):20130601

Paquet-Durand F, Johnson L, Ekström P (2007) Calpain activity in retinal degeneration. J Neurosci Res 85(4):693–702

Pennacchio LA, Bouley DM, Higgins KM, Scott MP, Noebels JL, Myers RM (1998) Progressive ataxia, myoclonic epilepsy and cerebellar apoptosis in cystatin B-deficient mice. Nat Genet 20(3):251–258. https://doi.org/10.1038/3059

Perkinton MS, Ip J, Wood GL, Crossthwaite AJ, Williams RJ (2002) Phosphatidylinositol 3-kinase is a central mediator of NMDA receptor signalling to MAP kinase (Erk1/2), Akt/PKB and CREB in striatal neurones. J Neurochem 80(2):239–254

Perlmutter LS, Siman R, Gall C, Seubert P, Baudry M, Lynch G (1988) The ultrastructural localization of calcium-activated protease "calpain" in rat brain. Synapse 2(1):79–88

Saavedra A, Garcia-Martinez J, Xifro X, Giralt A, Torres-Peraza J, Canals J, Diaz-Hernandez M, Lucas J, Alberch J, Perez-Navarro E (2010) PH domain leucine-rich repeat protein phosphatase 1 contributes to maintain the activation of the PI3K/Akt pro-survival pathway in Huntington's disease striatum. Cell Death Differ 17(2):324–335

Schoch KM, Evans HN, Brelsfoard JM, Madathil SK, Takano J, Saido TC, Saatman KE (2012) Calpastatin overexpression limits calpain-mediated proteolysis and behavioral deficits following traumatic brain injury. Exp Neurol 236(2):371–382

Shimazawa M, Suemori S, Inokuchi Y, Matsunaga N, Nakajima Y, Oka T, Yamamoto T, Hara H (2010) A novel calpain inhibitor, ((1S)-1-((((1S)-1-Benzyl-3-cyclopropylamino-2,3-dioxopropyl)amino)carbonyl)-3-me thylbutyl)carbamic acid 5-methoxy-3-oxapentyl ester (SNJ-1945), reduces murine retinal cell death in vitro and in vivo. J Pharmacol Exp Ther 332(2):380–387. https://doi.org/10.1124/jpet.109.156612

Shimizu K, Okada M, Nagai K, Fukada Y (2003) Suprachiasmatic nucleus circadian oscillatory protein, a novel binding partner of K-Ras in the membrane rafts, negatively regulates MAPK pathway. J Biol Chem 278(17):14920–14925

Shimizu K, Phan T, Mansuy IM, Storm DR (2007) Proteolytic degradation of SCOP in the hippocampus contributes to activation of MAP kinase and memory. Cell 128(6):1219–1229

Shmerling D, Hegyi I, Fischer M, Blattler T, Brandner S, Gotz J, Rulicke T, Flechsig E, Cozzio A, von Mering C, Hangartner C, Aguzzi A, Weissmann C (1998) Expression of amino-terminally truncated PrP in the mouse leading to ataxia and specific cerebellar lesions. Cell 93(2):203–214

Siklos M, BenAissa M, Thatcher GR (2015) Cysteine proteases as therapeutic targets: does selectivity matter? A systematic review of calpain and cathepsin inhibitors. Acta Pharm Sin B 5(6):506–519

Simonson L, Baudry M, Siman R, Lynch G (1985) Regional distribution of soluble calcium activated proteinase activity in neonatal and adult rat brain. Brain Res 327(1–2):153–159

Soriano FX, Papadia S, Hofmann F, Hardingham NR, Bading H, Hardingham GE (2006) Preconditioning doses of NMDA promote neuroprotection by enhancing neuronal excitability. J Neurosci 26(17):4509–4518

Steward O, Wallace CS (1995) mRNA distribution within dendrites: relationship to afferent innervation. J Neurobiol 26(3):447–459

Thompson SN, Carrico KM, Mustafa AG, Bains M, Hall ED (2010) A pharmacological analysis of the neuroprotective efficacy of the brain-and cell-permeable calpain inhibitor MDL-28170 in the mouse controlled cortical impact traumatic brain injury model. J Neurotrauma 27(12):2233–2243

Tovar KR, Westbrook GL (1999) The incorporation of NMDA receptors with a distinct subunit composition at nascent hippocampal synapses in vitro. J Neurosci 19(10):4180–4188

Tsubokawa T, Solaroglu I, Yatsushige H, Cahill J, Yata K, Zhang JH (2006) Cathepsin and calpain inhibitor E64d attenuates matrix metalloproteinase-9 activity after focal cerebral ischemia in rats. Stroke 37(7):1888–1894. https://doi.org/10.1161/01.STR.0000227259.15506.24

Vosler P, Brennan C, Chen J (2008) Calpain-mediated signaling mechanisms in neuronal injury and neurodegeneration. Mol Neurobiol 38(1):78–100

Wang C-F, Huang Y-S (2012) Calpain 2 activated through N-methyl-D-aspartic acid receptor signaling cleaves CPEB3 and abrogates CPEB3-repressed translation in neurons. Mol Cell Biol 32(16):3321–3332

Wang JT, Medress ZA, Barres BA (2012a) Axon degeneration: molecular mechanisms of a self-destruction pathway. J Cell Biol 196(1):7–18

Wang Y-B, Wang J-J, Wang S-H, Liu S-S, Cao J-Y, Li X-M, Qiu S, Luo J-H (2012b) Adaptor protein APPL1 couples synaptic NMDA receptor with neuronal prosurvival phosphatidylinositol 3-kinase/Akt pathway. J Neurosci 32(35):11919–11929

Wang Y, Briz V, Chishti A, Bi X, Baudry M (2013) Distinct roles for mu-calpain and m-calpain in synaptic NMDAR-mediated neuroprotection and extrasynaptic NMDAR-mediated neurodegeneration. J Neurosci 33(48):18880–18892. https://doi.org/10.1523/JNEUROSCI.3293-13.2013

Wang Y, Zhu G, Briz V, Hsu YT, Bi X, Baudry M (2014) A molecular brake controls the magnitude of long-term potentiation. Nat Commun 5:3051. https://doi.org/10.1038/ncomms4051

Wang Y, Hersheson J, Lopez D, Hammer M, Liu Y, Lee KH, Pinto V, Seinfeld J, Wiethoff S, Sun J, Amouri R, Hentati F, Baudry N, Tran J, Singleton AB, Coutelier M, Brice A, Stevanin G, Durr A, Bi X, Houlden H, Baudry M (2016a) Defects in the CAPN1 gene result in alterations in cerebellar development and cerebellar ataxia in mice and humans. Cell Rep 16(1):79–91. https://doi.org/10.1016/j.celrep.2016.05.044

Wang Y, Lopez D, Davey PG, Cameron DJ, Nguyen K, Tran J, Marquez E, Liu Y, Bi X, Baudry M (2016b) Calpain-1 and calpain-2 play opposite roles in retinal ganglion cell degeneration induced by retinal ischemia/reperfusion injury. Neurobiol Dis 93:121–128. https://doi.org/10.1016/j.nbd.2016.05.007

Wang, Y, Liu, Y, Lopez, D, Lee, M, Dayal, S, Hirtado, A, Bi, X and Baudry, M (2017) Protection against TBI-induced neuronal death with post-treatment with a selective calpain-2 inhibitor in mice. J Neurotrauma 34:1–13

Xiong Y, Mahmood A, Chopp M (2013) Animal models of traumatic brain injury. Nat Rev Neurosci 14(2):128–142

Xu J, Kurup P, Zhang Y, Goebel-Goody SM, Wu PH, Hawasli AH, Baum ML, Bibb JA, Lombroso PJ (2009) Extrasynaptic NMDA receptors couple preferentially to excitotoxicity via calpain-mediated cleavage of STEP. J Neurosci 29(29):9330–9343. https://doi.org/10.1523/JNEUROSCI.2212-09.2009

Yamaguchi A, Tamatani M, Matsuzaki H, Namikawa K, Kiyama H, Vitek MP, Mitsuda N, Tohyama M (2001) Akt activation protects hippocampal neurons from apoptosis by inhibiting transcriptional activity of p53. J Biol Chem 276(7):5256–5264

Yan X-X, Jeromin A (2012) Spectrin breakdown products (SBDPs) as potential biomarkers for neurodegenerative diseases. Curr Trans Geriatr Exp Gerontol Rep 1(2):85–93

Yildiz-Unal A, Korulu S, Karabay A (2015) Neuroprotective strategies against calpain-mediated neurodegeneration. Neuropsychiatr Dis Treat 11:297

Zadran S, Jourdi H, Rostamiani K, Qin Q, Bi X, Baudry M (2010) Brain-derived neurotrophic factor and epidermal growth factor activate neuronal m-calpain via mitogen-activated protein kinase-dependent phosphorylation. J Neurosci 30(3):1086–1095

Zhou M, Baudry M (2006) Developmental changes in NMDA neurotoxicity reflect developmental changes in subunit composition of NMDA receptors. J Neurosci 26(11):2956–2963

Zhu G, Liu Y, Wang Y, Bi X, Baudry M (2015) Different patterns of electrical activity lead to long-term potentiation by activating different intracellular pathways. J Neurosci 35(2):621–633. https://doi.org/10.1523/JNEUROSCI.2193-14.2015