

AUTOPHAGY GOOD AND BAD: A GENUINE TARGET FOR RUBBISH REMOVAL IN NEUROPATHOLOGIES?

Philip M. Beart,

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Phillip Nagley



Gavin Higgins



Yea Seul Shin

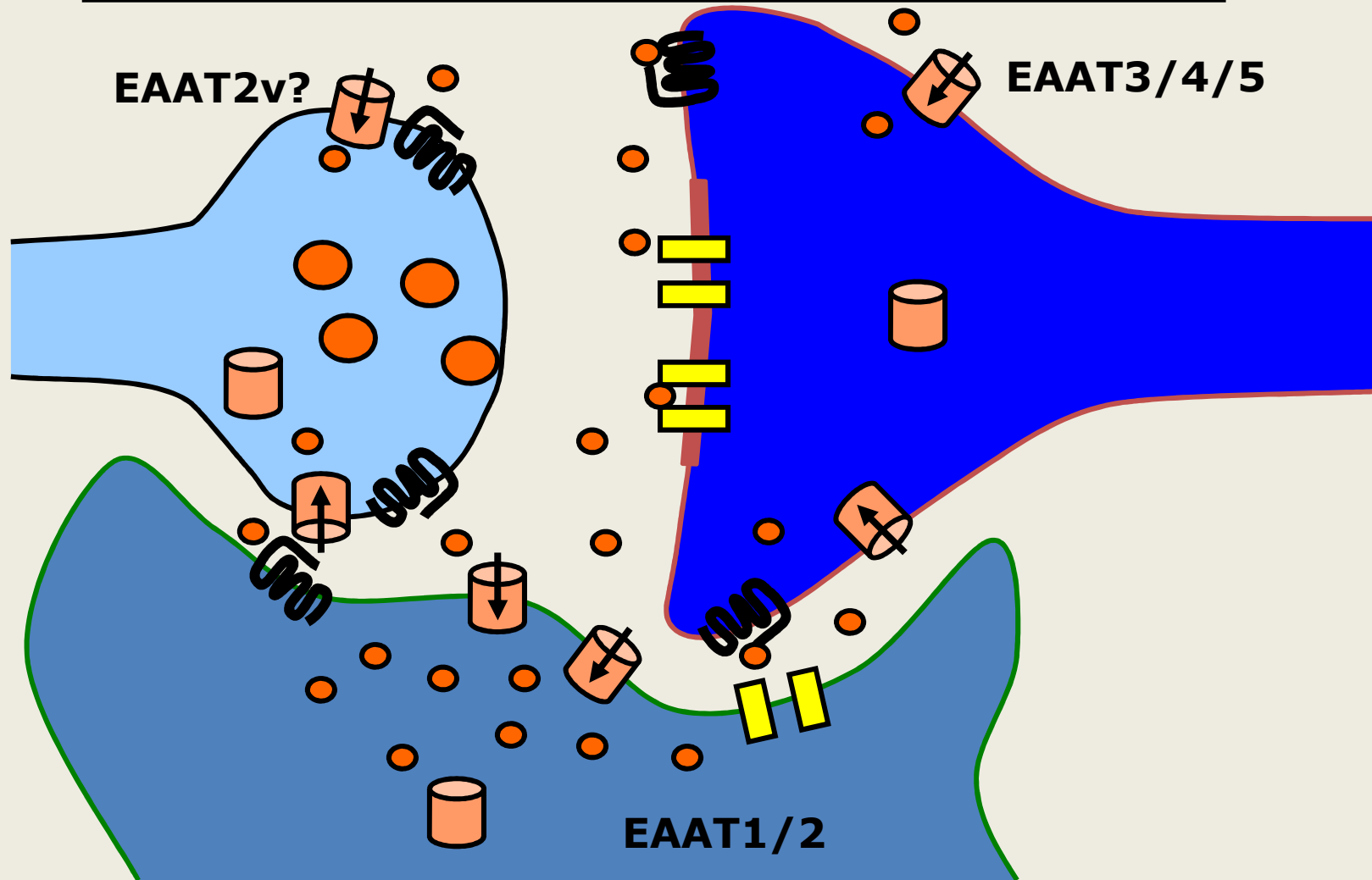


Linda Mercer



Rod Devenish

Excitatory Amino Acid Transmission



Blockade by polyamine NMDA antagonists related to ifenprodil of NMDA-induced synthesis of cyclic GMP, increases in calcium and cytotoxicity in cultured neurones

Philip M. Beart, *Arne Schousboe & *Aase Frandsen

▼
Patent with Merck (Europe)

BAY36-7620: A Potent Non-Competitive mGlu1 Receptor Antagonist with Inverse Agonist Activity.

FIONA Y. CARROLL, ANDREAS STOLLE, **PHILIP M. BEART**, ARND VOERSTE, ISABELLE BRABET, FRANK MAULER, CÉCILE JOLY, HORST ANTONICEK, JOËL BOCKAERT, THOMAS MÜLLER, JEAN PHILIPPE PIN, and LAURENT PRÉZEAU

▼
1st Allosteric drug – Bayer failed to appreciate

[³H](2S,4R)-4-Methylglutamate: a novel ligand for the characterization of glutamate transporters

Karina Apricó,* **Philip M. Beart**,* Andrew J. Lawrence,* Duncan Crawford† and Ross D. O'Shea*

▼
Patents for glutamate reuptake modulators

Delayed Treatment With AM-36, a Novel Neuroprotective Agent, Reduces Neuronal Damage After Endothelin-1-Induced Middle Cerebral Artery Occlusion in Conscious Rats

Jennifer K. Callaway, PhD; Melissa J. Knight, BSc; Dianne J. Watkins, BSc; **Philip M. Beart** DSc; Bevy Jarrott, PhD

▼
Multiple patents – UK company collapsed

Vampire Bat Salivary Plasminogen Activator (Desmoteplase) Inhibits Tissue-Type Plasminogen Activator-Induced Potentiation of Excitotoxic Injury

Courtney Reddrop, BSc (Hons); Randal X. Moldrich, PhD; **Philip M. Beart**, DSc; Mark Farso, BSc (Hons); Gabriel T. Liberatore, PhD; David W. Howells, PhD; Karl-Uwe Petersen, MD; Wolf-Dieter Schleuning, MD, PhD; Robert L. Medcalf, PhD

▼
Lundbeck discontinued Dec 2014

Transcriptomic Profiling of Astrocytes Treated With the Rho Kinase Inhibitor Fasudil Reveals Cytoskeletal and Pro-Survival Responses

CHEW L. LAU,¹ VICTORIA M. PERREAU,^{2,3} MINGHUI J. CHEN,⁴ HOLLY S. CATE,^{1,2} DANIEL MERLO,^{1,2} NAM S. CHEUNG,^{4,5} ROSS D. O'SHEA,^{1,6} AND **PHILIP M. BEART**^{1,7*}

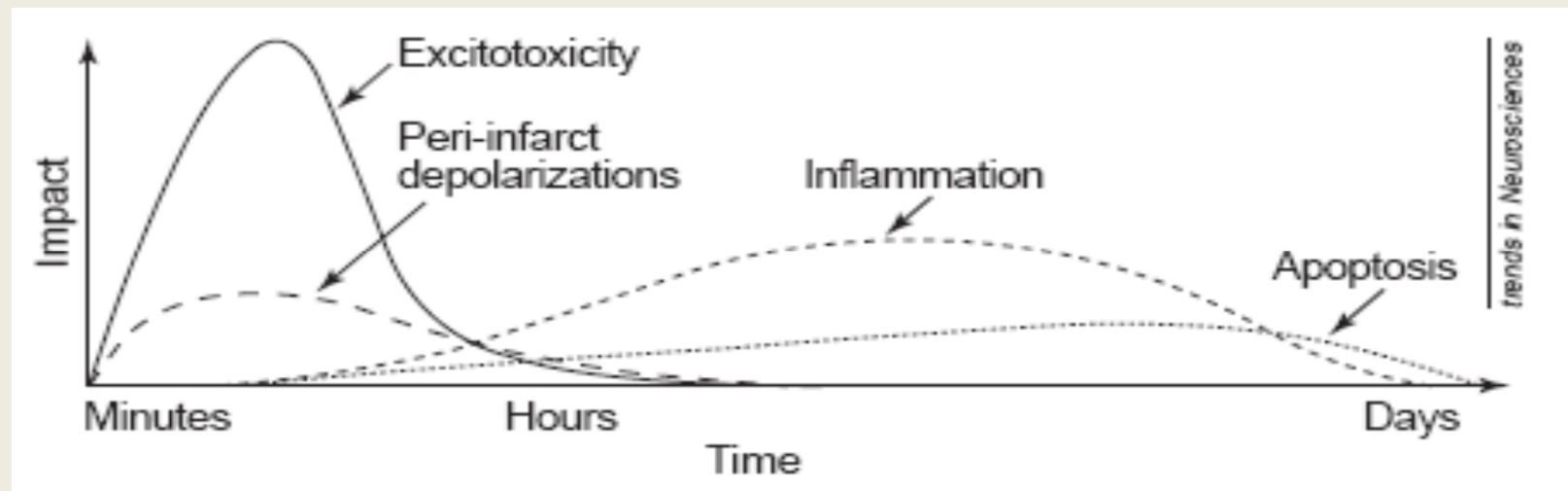
▼
Patented as anti-inflammatory ALS/MND

DISEASE BURDEN – UNMET THERAPEUTIC NEED

| PATHOLOGY | INCIDENCE | COSTS PER ANNUM |
|-----------------------------|------------------------------|---|
| Stroke | 50,000 per annum | \$2 billion |
| Perinatal hypoxia | 1-4 per 1000 infants | \$3 billion (USA) |
| Motor Neuron Disease | 1300, 532 died (2006) | Impacts carers, families & friends |
| Parkinson's Disease | 30-300 per 10,000 | \$500 million |
| Huntington's disease | 1 per 10,000 | ??? |

Sources : Access Economics, National Stroke Association, Medical News Today, www.umdj.edu

| DISEASE | APOPTOSIS | AUTOPHAGY | PROG NECROSIS |
|---------------------|------------------|------------------|--------------------------|
| <i>Stroke</i> | ✓ | ✓ | ✓ |
| <i>Parkinson's</i> | ✓ | ✓ | ✓? |
| <i>MND/ALS</i> | ✓ | ✓ | ✓? |
| <i>Huntington's</i> | ✓ | ✓ | ✓? |



Pathobiology of ischaemic stroke: an integrated view

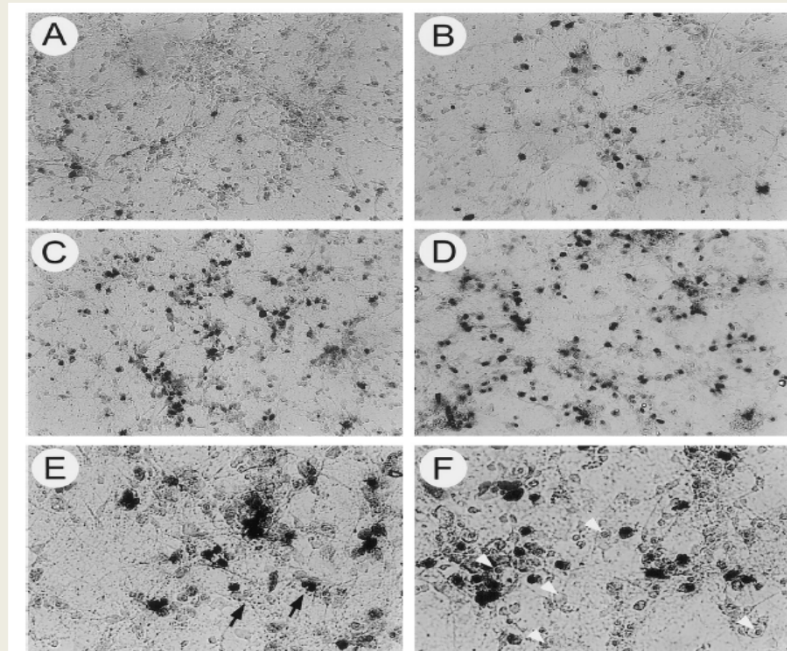
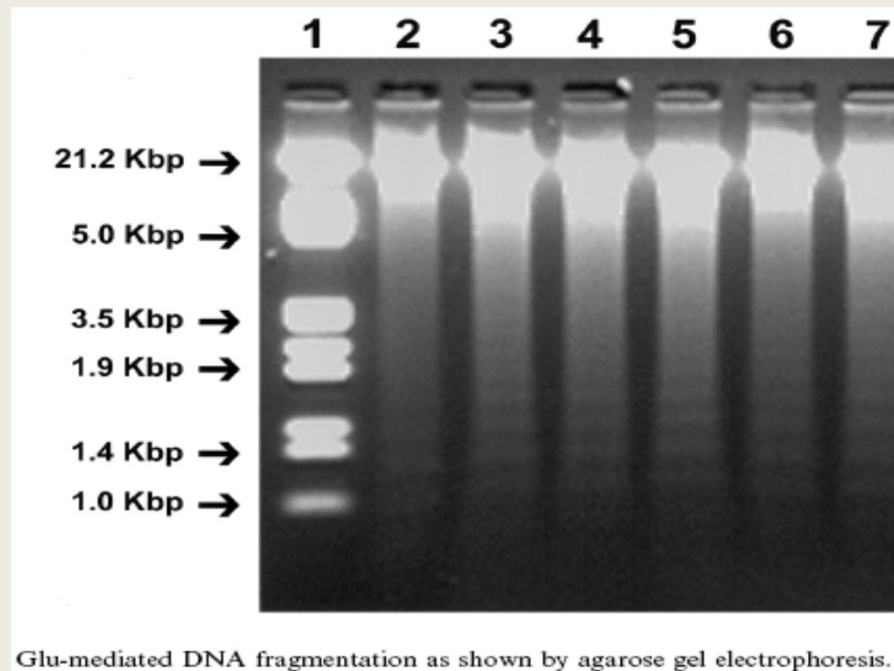
Ulrich Dirnagl, Costantino Iadecola and Michael A. Moskowitz

TINS 1999

1,103 cites

Micromolar L-glutamate induces extensive apoptosis in an apoptotic-necrotic continuum of insult-dependent, excitotoxic injury in cultured cortical neurones

Nam S. Cheung, Catherine J. Pascoe, Sarah F. Giardina, Christopher A. John, Philip M. Beart *



Neuropharmacology 1998

151 cites

DEATH IS NOT SIMPLE

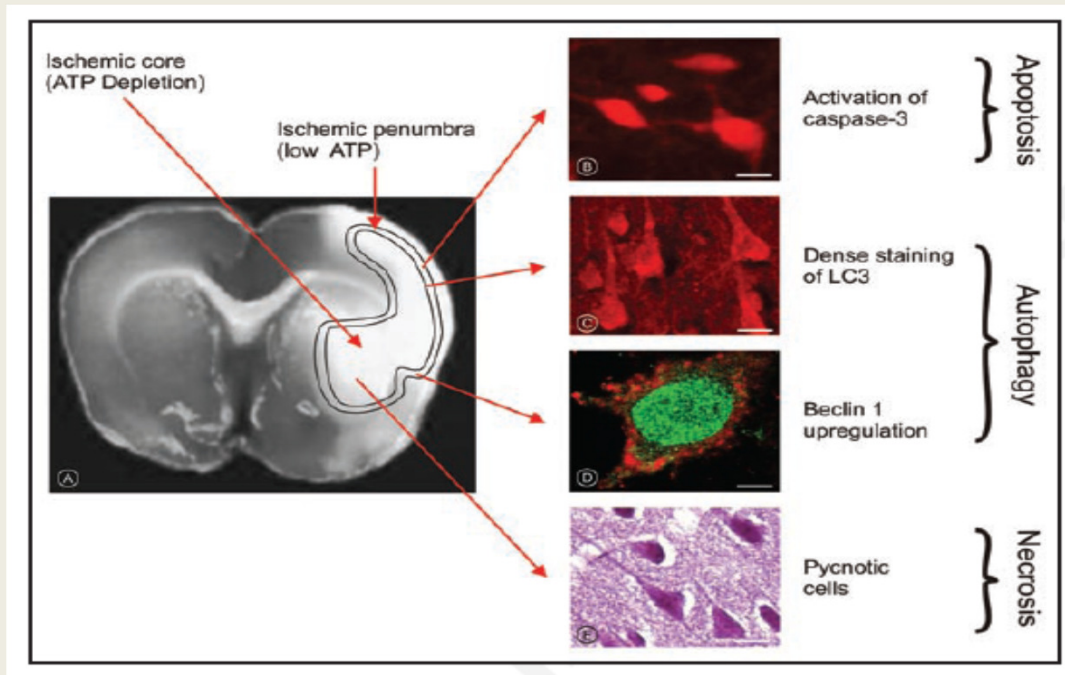
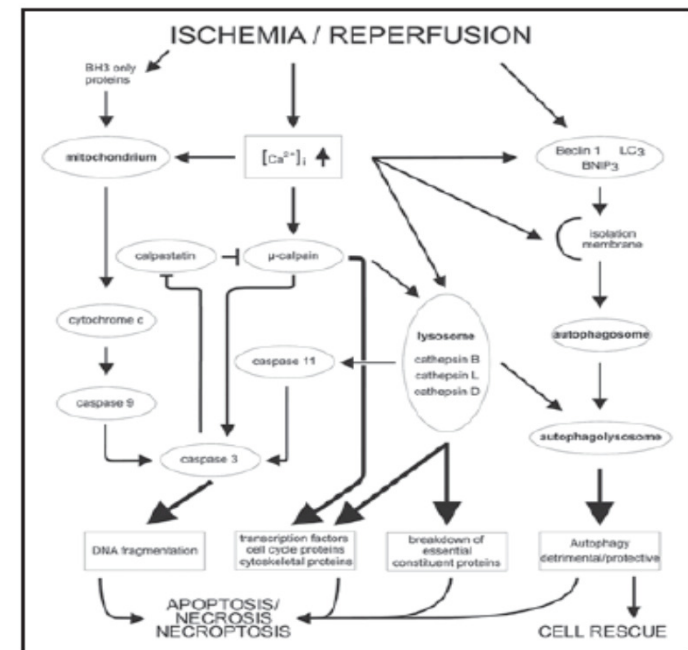


Figure 1. Necrosis predominates in the ischemic core, whereas apoptosis and autophagy are often observed in the penumbra after focal ischemia. In the ischemic core (A) necrosis refers to morphological signs seen after a cell has already died and reached equilibrium with its surroundings (E). The presence of necrosis tells that a cell has died but not necessarily how death occurred. In the penumbra, hybrid forms of cell death occur:



Rami & Kogel,
Autophagy (2008)

EDITORIAL
HIGHLIGHT

Autophagy: a common road to perdition in acute brain injuries and Alzheimer's disease

Giuseppina Tesco

Alzheimer's Disease Research Laboratory, Department of Neuroscience, Tufts University School of Medicine, Boston, Massachusetts, USA

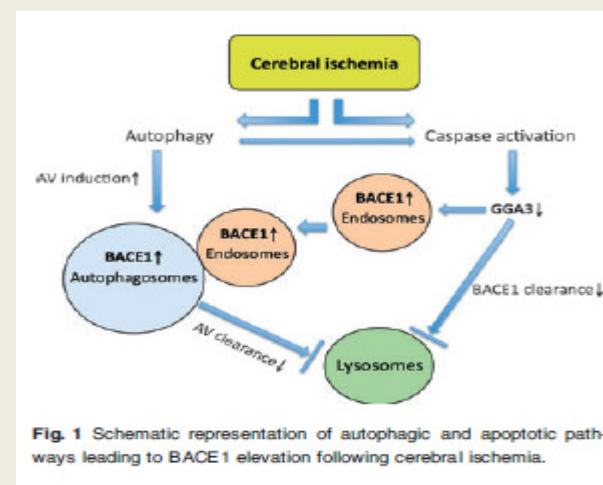
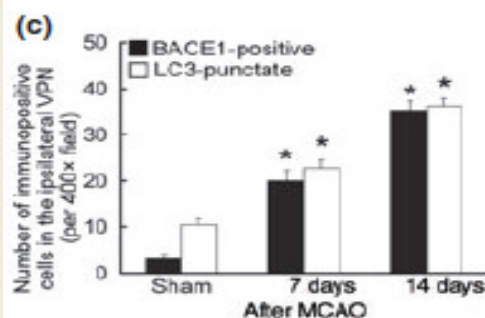


Fig. 1 Schematic representation of autophagic and apoptotic pathways leading to BACE1 elevation following cerebral ischemia.



ORIGINAL
ARTICLE



Autophagosomes accumulation is associated with β -amyloid deposits and secondary damage in the thalamus after focal cortical infarction in hypertensive rats

Jian Zhang,^{*,1} Yusheng Zhang,^{†,1} Jingjing Li,^{*,1} Shihui Xing,^{*} Chuo Li,^{*} Yiliang Li,^{*} Chao Dang,^{*} Yuhua Fan,^{*} Jian Yu,^{*} Zhong Pei^{*} and Jinsheng Zeng^{*}

Autophagy

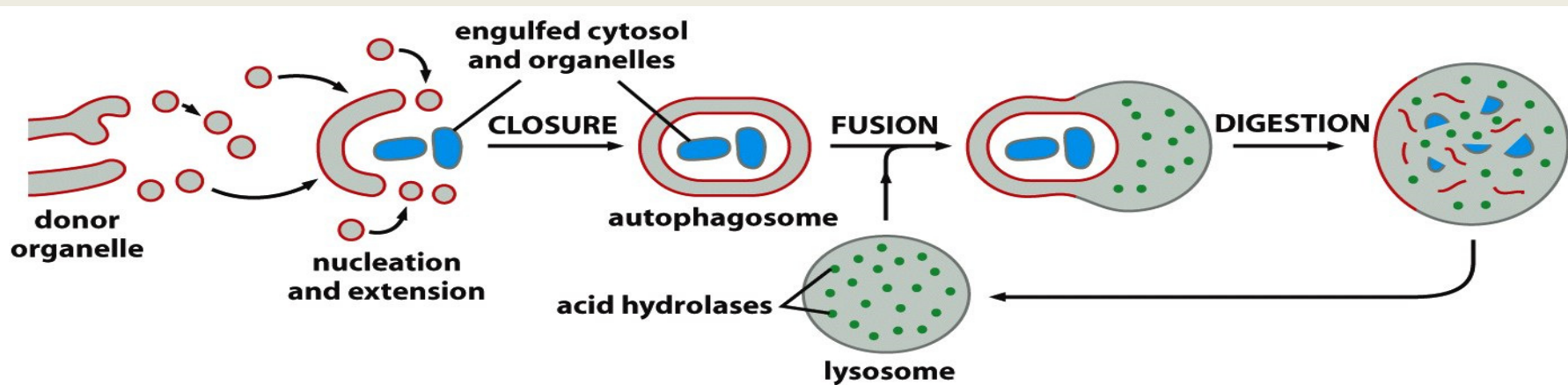
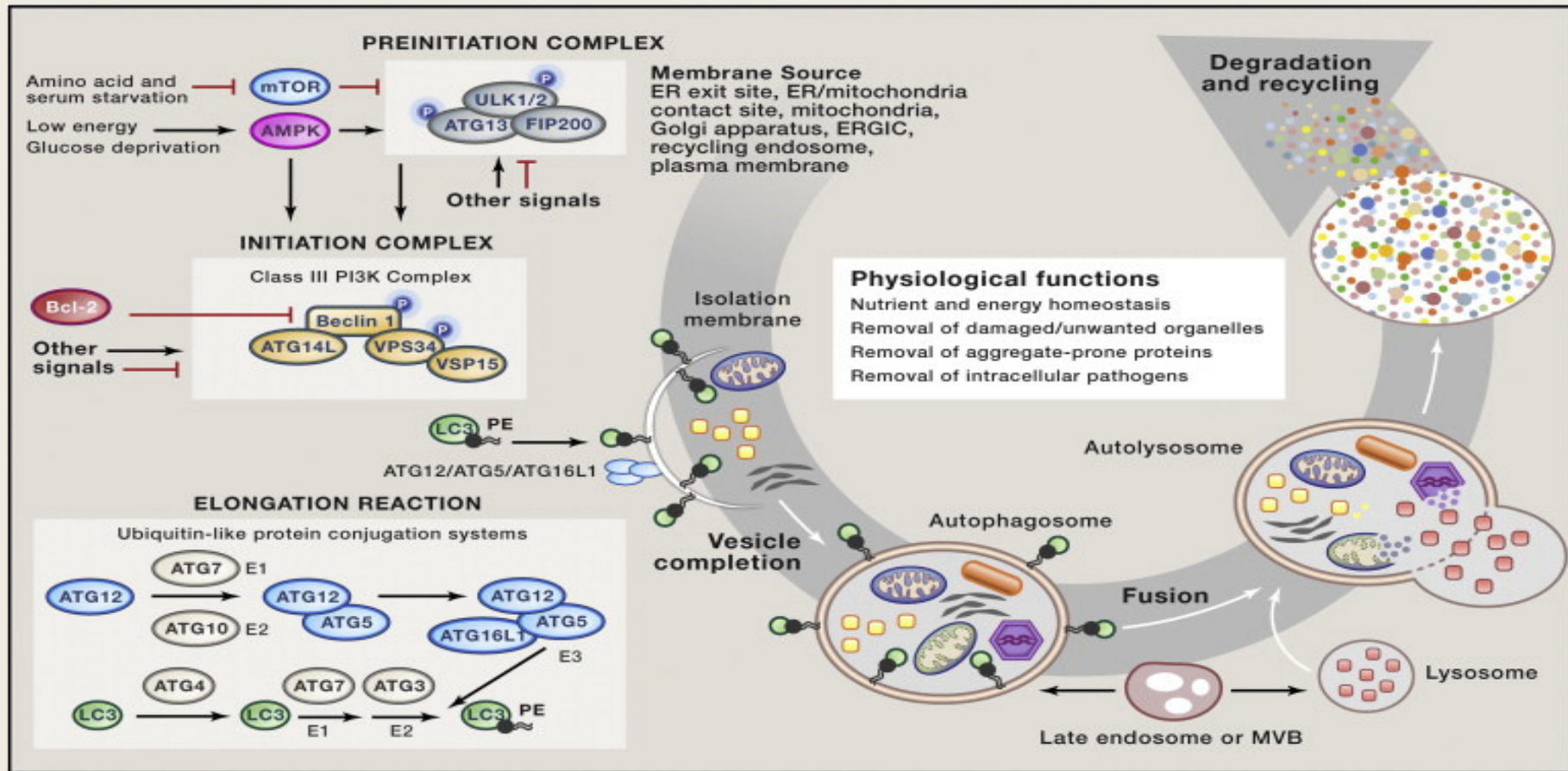


Figure 13-41 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- ❑ **Cellular homeostasis:** Autophagy recycles cellular damaged components & responds to energy deficits
- ❑ **Cellular defence:** Autophagy can help cells avoid damage and death under various stresses



Overview of the General Autophagy Pathway



Green & Levine , *Cell* (2014)



Autophagy is generally good for cells



Autophagic cell death (ACD)



Autophagy

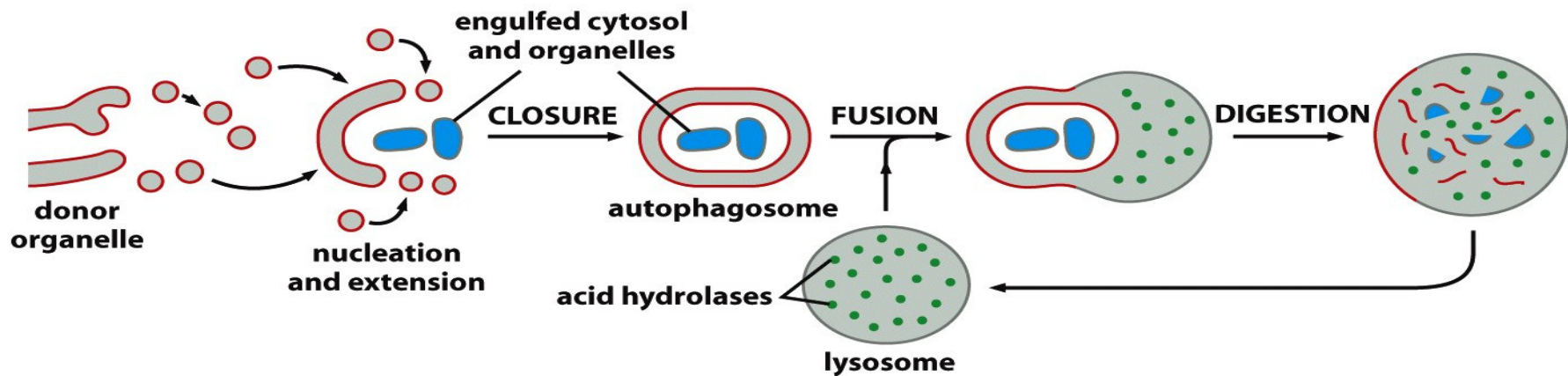
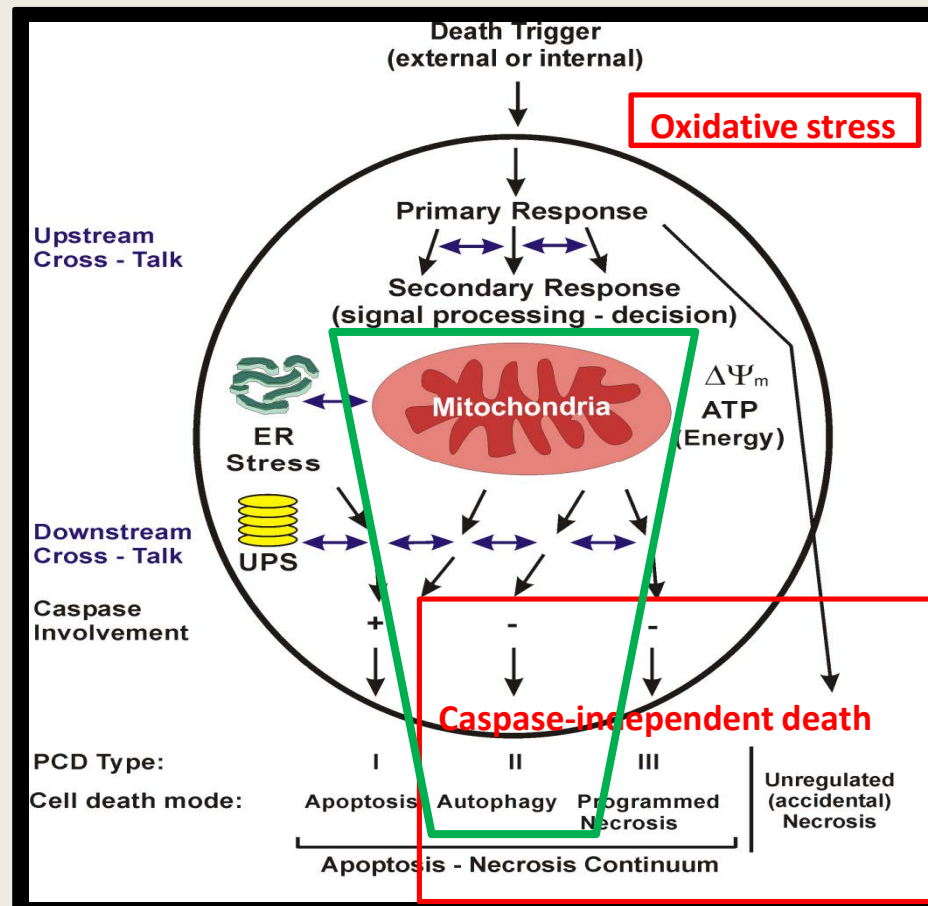


Figure 13-41 Molecular Biology of the Cell 5/e (© Garland Science 2008)

- ❑ **Cellular homeostasis:** Autophagy recycles cellular damaged components & responds to energy deficits
- ❑ **Cellular defence:** Autophagy can help cells avoid damage and death under various stresses
- ❑ **Cell death process (PCD Type II):** Autophagy directly contributes to the cell death outcome

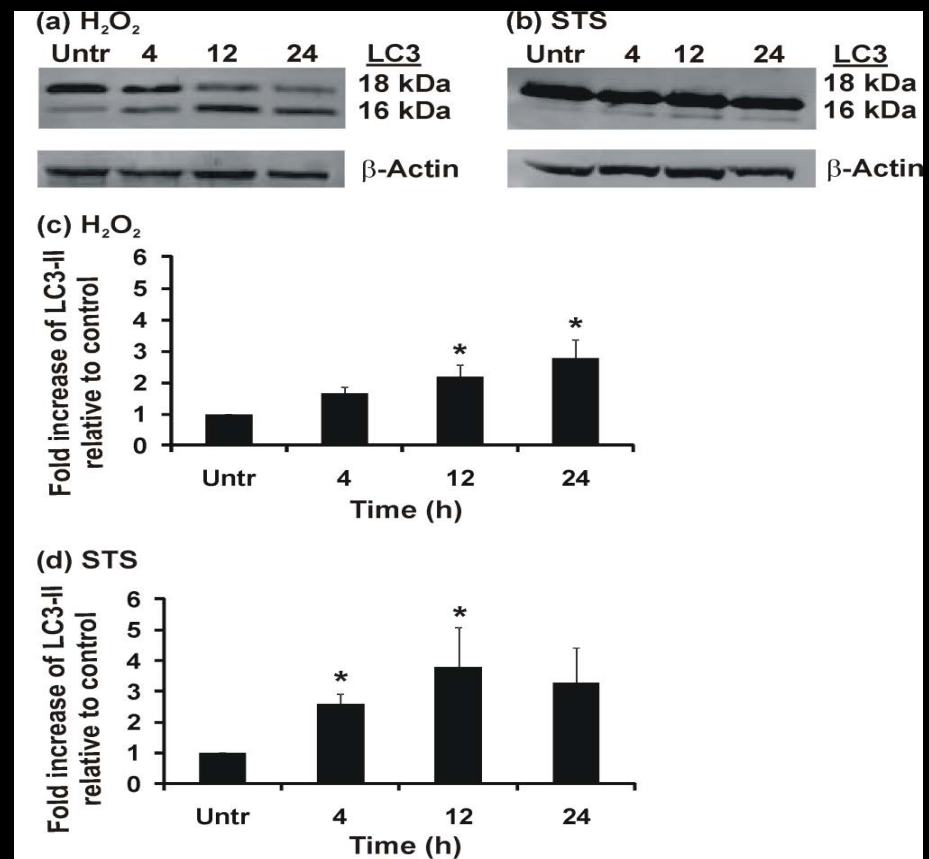
Diversity of cell death in neurons



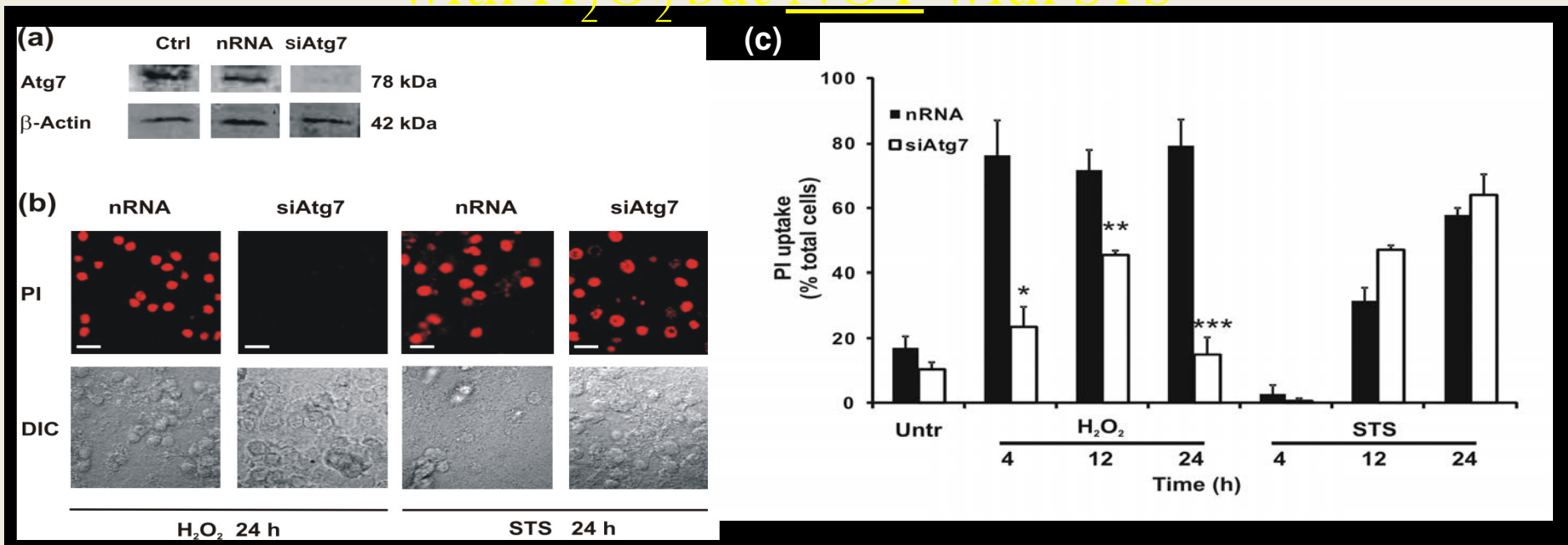
Nagley et al Beart. BBA 2010

Conversion of LC3-I to LC3-II after treatment with STS or H_2O_2 indicates an increase in autophagic activity

Autophagy is induced in these neurons treated with either H_2O_2 or STS



The progression to cell death is blocked by knockdown of Atg7 in neurons treated with H_2O_2 but NOT with STS



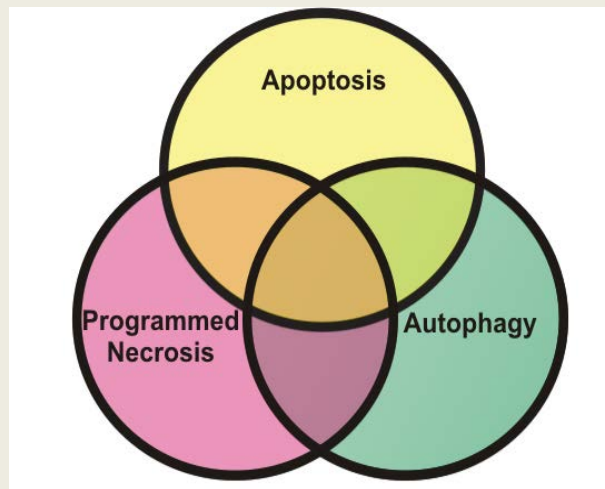
Scale bar = 10 μ m

* $P < 0.01$

** $P < 0.001$

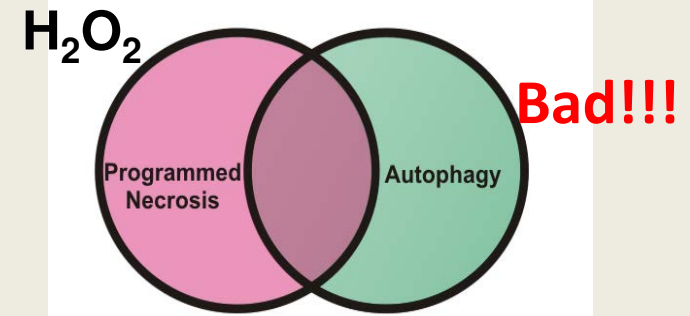
*** $P < 0.0001$

GENERAL ASPECTS OF CELL DEATH IN NEURONS



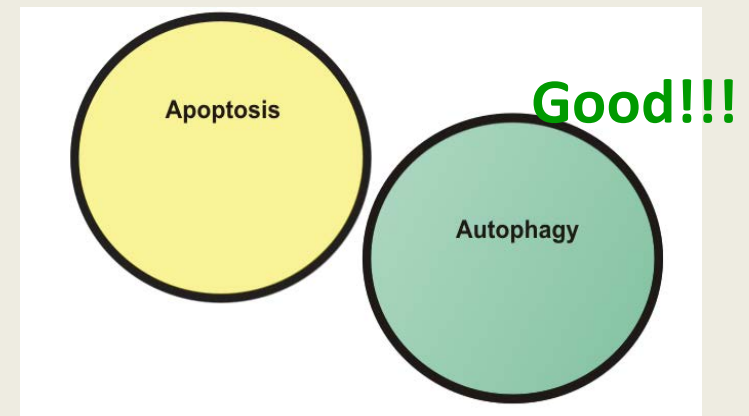
Death outcomes differ in terms of biochemical mechanisms and cellular morphologies.

Relative involvement of individual death processes depends upon the neuronal type and stressor (Nagley et al., 2010).



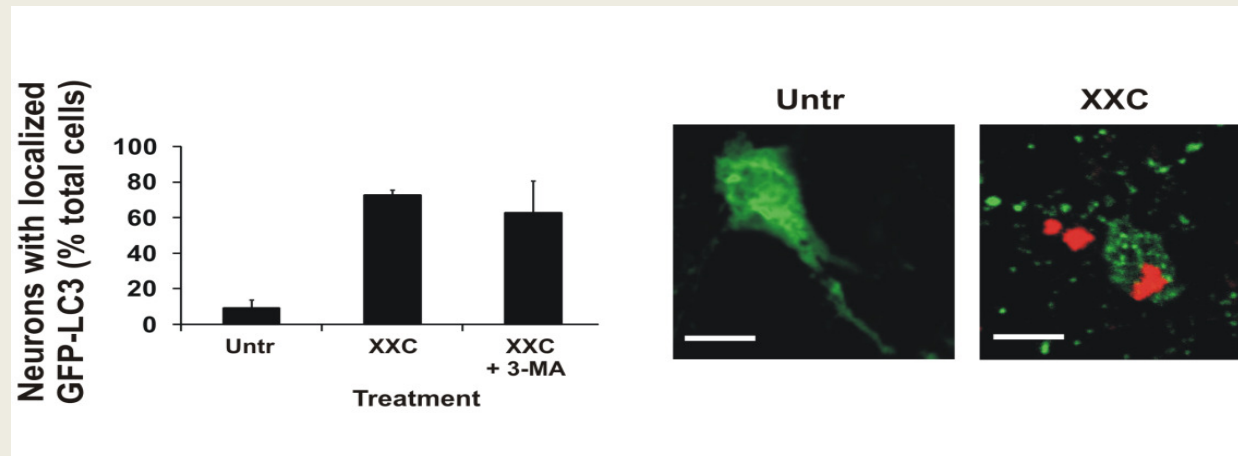
PCD-Type II and PCD-Type III. Inhibition of the autophagy pathway blocks cell death revealing a link between PCD-Type III and autophagy (PCD-Type II).

STS



Apoptosis and autophagy. Death has characteristics of apoptosis (PCD-Type I), but inhibition of autophagy fails to block death – the events are not functionally linked.

What about autophagy & death following sustained exposure to oxidative stress?



The Approach: Cortical neurons treated with superoxide ($O_2^{\cdot-}$) generated from xanthine/xanthine oxidase and catalase (XXC) alongside a reference apoptotic inducer staurosporine (STS).

Free Radical Biology and Medicine 53 (2012) 1960–1967



Contents lists available at SciVerse ScienceDirect

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journal homepage: www.elsevier.com/locate/freeradbiomed



Original Contribution

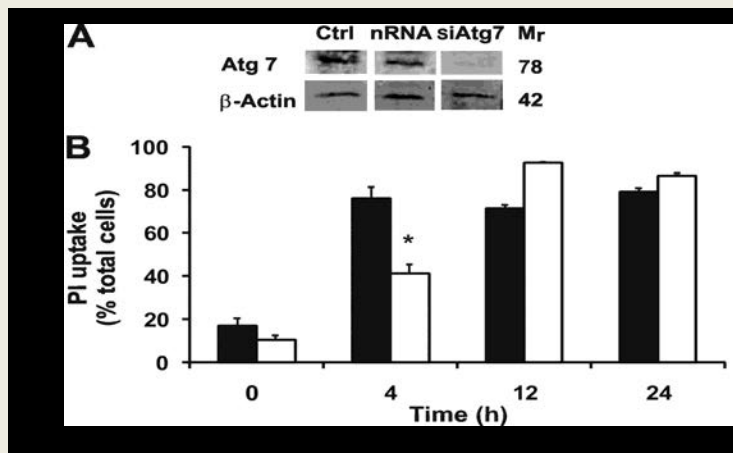
Transitory phases of autophagic death and programmed necrosis during superoxide-induced neuronal cell death

Gavin C. Higgins^a, Rodney J. Devenish^{a,b}, Philip M. Beart^{c,d}, Phillip Nagley^{a,b,*}

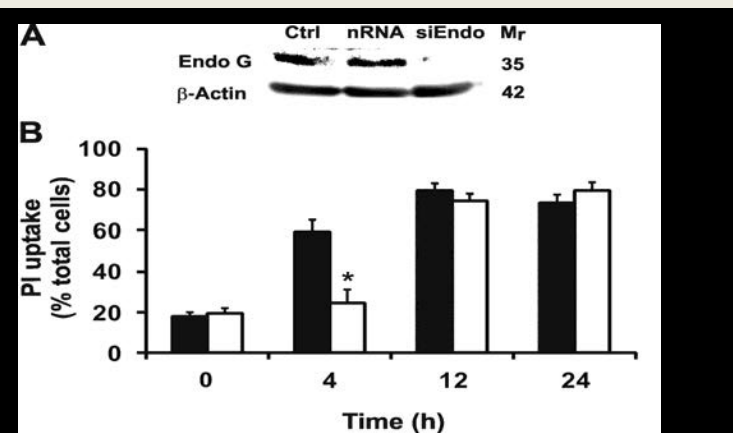
Cell death by $O_2^{\cdot-}$ flux is transiently blocked by Atg7 and Endo G knockdown

Atg7 knockdown

EndoG knockdown



Transient
autophagic cell death



Transient
Programmed necrosis

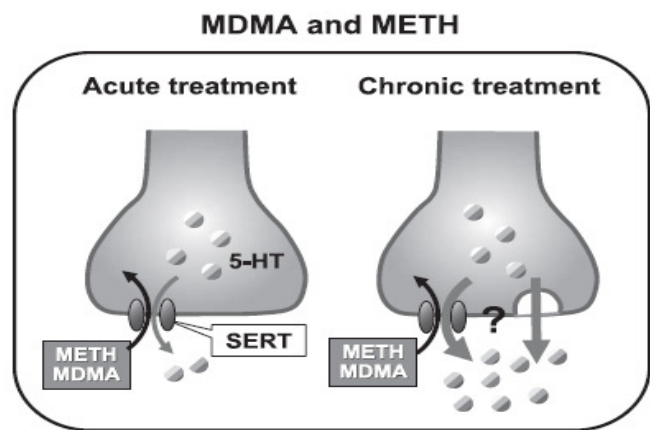
Higgins et
al. FRBM
2012

Cell death still occurs, suggesting $O_2^{\cdot-}$ overwhelms cells to undergo unregulated necrosis

ACD overwhelmed by unregulated necrosis

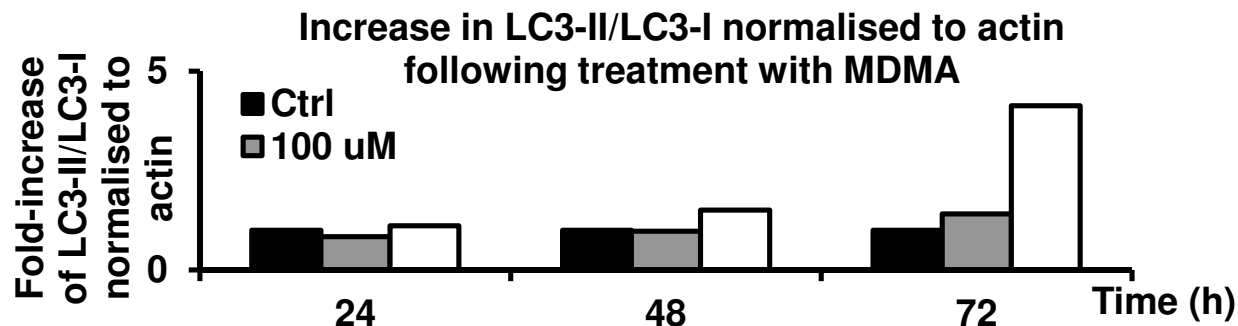
- Caspase activity in the presence of oxidative stress has been reported in other neuronal cell systems, BUT it is clear that this is not the case under our experimental conditions (i.e. no apoptosis).
- Early dissipation of $\Delta\Psi_m$ followed by rapid redistribution of cyt c, Smac/DIABLO and Endo G indicated mitochondrial involvement.
- Both autophagic cell death and programmed necrosis are activated.
- We envisage that pathways leading to autophagic death and programmed necrosis may be running in parallel in early stages.
- Complete blockade of cell death was not achieved by such disruption of either ACD or programmed necrosis , suggesting that ultimately cells default to death by unregulated necrosis.

A role for *autophagy* in ecstasy (MDMA)-induced death of serotonin neurones?



MDMA-induced brain injury

- Slow, PCD with caspase-dependent component?
- Effects include ox stress, DNA damage, inflammation
- **Ubiquitinated inclusions**
- **Disturbed energy metabolism**
- **Impaired axonal transport = damaged organelles?**
- **MDMA induces Atg5 expression in cell lines**
- **UPS recruited (autophagy?)**



Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington's disease

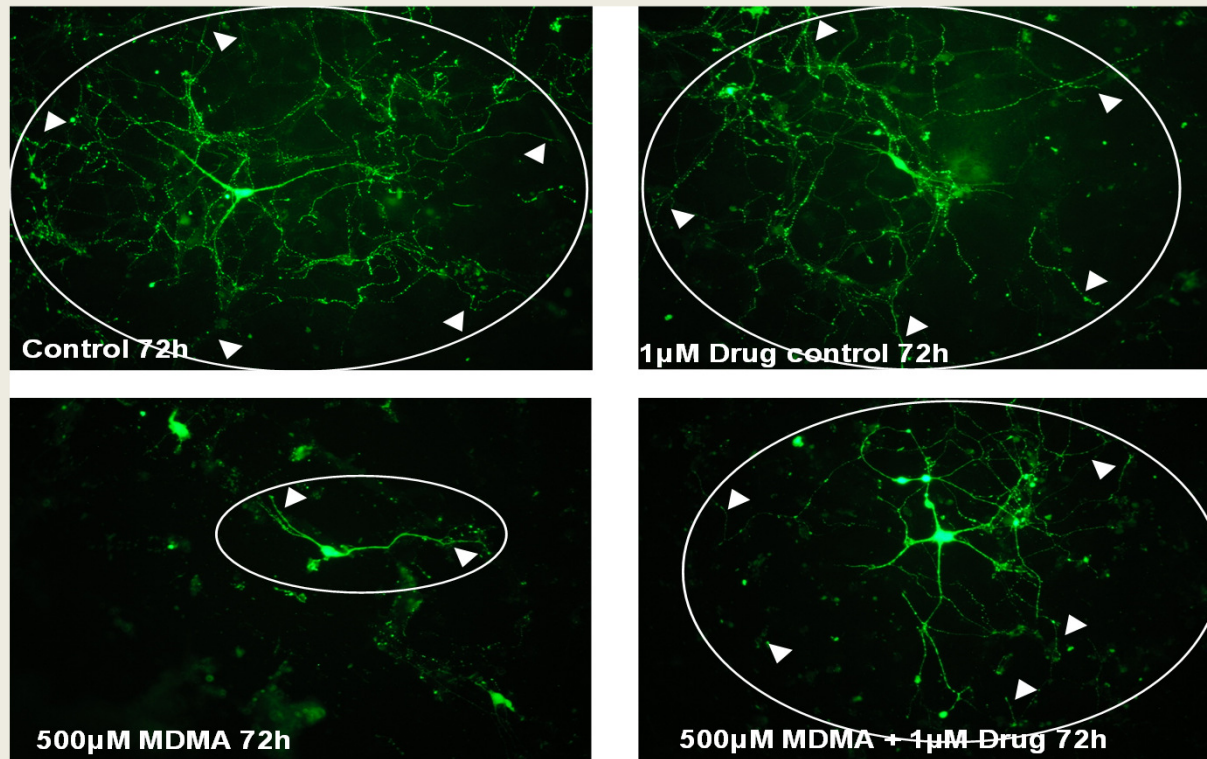
Claudia Rose¹, Fiona M. Menzies¹, Maurizio Renna¹, Abraham Acevedo-Aroza², Silvia Corrochano², Oana Sadiq¹, Steve D. Brown² and David C. Rubinsztein^{1,*}

¹Department of Medical Genetics, University of Cambridge, Cambridge Institute for Medical Research, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0XY, UK and ²Medical Research Council Mammalian Genetics Unit, Harwell, Oxfordshire, UK

Received December 11, 2009; Revised February 3, 2010; Accepted February 25, 2010

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by a polyglutamine expansion in huntingtin. There are no treatments that are known to slow the neurodegeneration caused by this mutation. Mutant huntingtin causes disease via a toxic gain-of-function mechanism and has the propensity to aggregate and form intraneuronal inclusions. **One therapeutic approach for HD is to enhance the degradation of the mutant protein.** We have shown that this can be achieved by upregulating autophagy, using the drug rapamycin. **In order to find safer ways of inducing autophagy for clinical purposes, we previously screened United States Food and Drug Administration-approved drugs for their autophagy-stimulating potential. This screen suggested that rilmenidine, a well tolerated, safe, centrally acting anti-hypertensive drug, could induce autophagy in cell culture via a pathway that was independent of the mammalian target of rapamycin.** Here we have shown that rilmenidine induces autophagy in mice and in primary neuronal culture. Rilmenidine administration attenuated the signs of disease in a HD mouse model and reduced levels of the mutant huntingtin fragment. **As rilmenidine has a long safety record and is designed for chronic use, our data suggests that it should be considered for the treatment of HD and related conditions.**

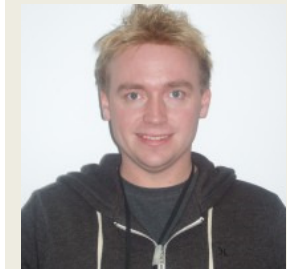
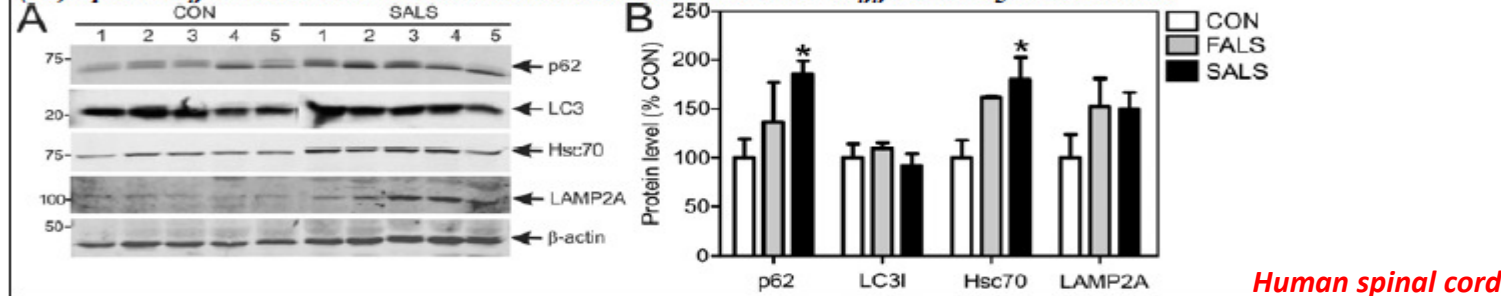
Activation of Autophagy: Neuroprotection



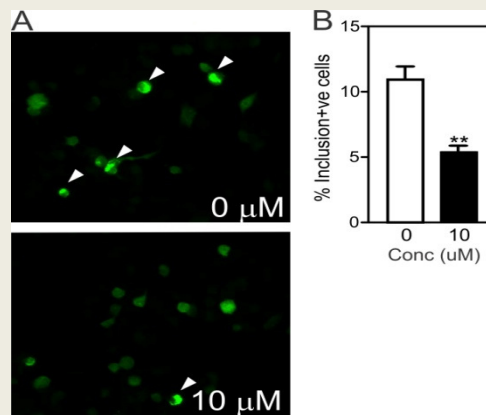
Murine serotonin (5-HT) neurones in culture

Autophagy: a valid clinical target in MND/ALS?

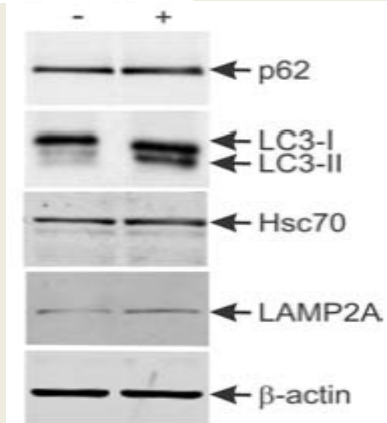
Fig. 2. (A) Autophagic markers are increased in spinal cords of familial (n=3) and sporadic (n=10) MND patients, compared to controls (n=5) by immunoblot analysis with (B) quantification. Please note LC3-II was not sufficiently resolved.



Brad Turner



**Suppression of mutant
SOD1 aggregation
stimulates autophagy in
NSC-34 MN cell line**
*Autophagy activator
provided by Servier*



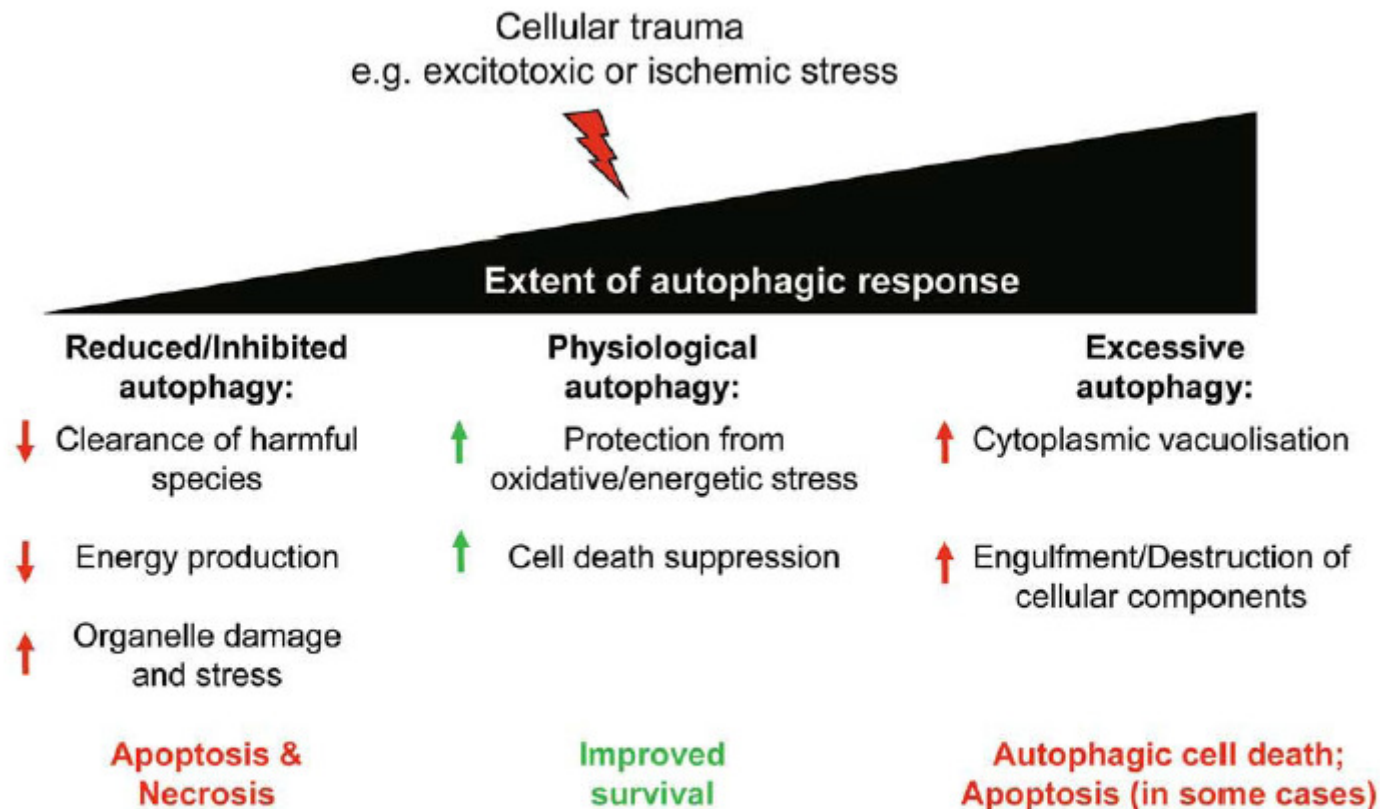


Fig. 2. One hypothetical model where the extent of autophagy dictates the fate of neurons under stress. When faced with a harsh stress, neurons rely on autophagy induction as a means of protection and damage limitation, suppressing cell death and promoting survival. This means an inefficient or inhibited level of autophagy can be detrimental to neuronal health. At the other end of the spectrum, an excessive autophagic response may result in the degradation of vital cellular components, culminating in ACD. Therefore, it seems that in order for autophagy to exert its protective effects, a balance needs to be maintained to avoid neuronal death.

What is Mitophagy???

- A form of autophagy where damaged or dysfunctional mitochondria selectively undergo degradation, can occur as a consequence of PCD when mitochondria fragment and remodel inner-membrane cristae.
- Moreover, cellular bioenergetics is entwined with mitochondrial dynamics, and mitochondrial insults, including depolarization and ETC inhibition, trigger mitochondrial fragmentation.

ACTIVATE AUTOPHAGY IN YOUR MODEL

Neuroprotection of kaempferol by autophagy in models of rotenone-mediated acute toxicity: possible implications for Parkinson's disease

Rilmenidine attenuates toxicity of polyglutamine expansions in a mouse model of Huntington's disease

Tsc1 (hamartin) confers neuroprotection against ischemia by inducing autophagy

Autophagy activators rescue and alleviate pathogenesis of a mouse model with proteinopathies of the TAR DNA-binding protein 43

Table 1. Selected strategies of autophagy flux restoration in models of neurodegeneration (mammalian where available)

| Strategy | Neurodegenerative Disease | Changes to Pathology | Reference |
|-----------------------------|---------------------------|---|-------------|
| Pharmacological | | | |
| Rapamycin | Alzheimer's disease | Autophagy induction; reductions in A β and cognitive recovery in AD mice | [71],[112] |
| | Huntington's disease | Reductions in Htt aggregate formation, improvements in behavioral tests in mice | [111] |
| | Parkinson's disease | Reductions in α -synuclein accumulation, alleviation of neurodegenerative behavior in mice | [113],[114] |
| Rilmenidine | Huntington's disease | Autophagy induction; enhanced clearance of mutant Htt, improved motor performance in mice | [110] |
| Spermidine | Parkinson's disease | Autophagy induction; Improved motor performance in fruit fly, reduced dopaminergic neuron loss in nematodes | [115] |
| Arctigenin | Alzheimer's disease | Autophagy induction; Reduction in A β plaques through inhibition of formation and enhanced clearance, improved memory in mice | [117] |
| GTM-1 | Alzheimer's disease | Autophagy induction and increased flux; removal of A β oligomers, cognitive improvements in mice | [116] |
| Glucosylceramide inhibitors | Niemann-Pick Type-C 1 | Correction of autophagic flux; improved clearance of cholesterol and autophagic vesicles in mouse and cat models, prolonged neuron survival | [119],[120] |
| Genetic | | | |
| TFEB | Alzheimer's disease | Upregulation of lysosomal and autophagy genes; | [124] |
| | Huntington's disease | enhanced clearance of tau, α -synuclein, and mutant | [25] |
| | Parkinson's disease | Htt aggregates | [67] |

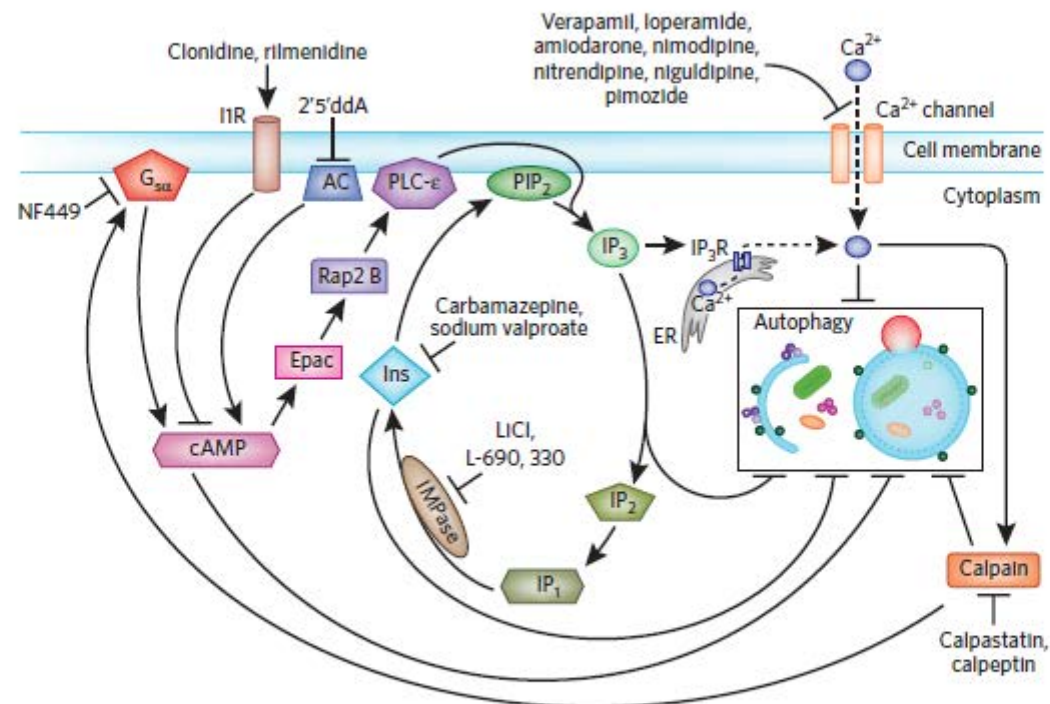


Figure 3 | mTOR-independent autophagy induction pathways. Chemical screens for new autophagy-inducing agents have identified the cyclical Ca²⁺-calpain-G α_s and cAMP-Epac-PLC- ϵ -IP₃ pathways as pharmacologically tractable for the modulation of autophagy. Inhibition of various components of these pathways results in autophagy induction. However, the precise mechanism by which levels of cAMP, Ca²⁺, calpain, inositol or IP₃ control autophagy have yet to be elucidated.

AUTOPHAGY IN HUMAN NEUROPATHOLOGIES?

Autophagy is increased in mice after traumatic brain injury and is detectable in human brain after trauma and critical illness

Robert S.B. Clark, Hülya Bayir, Charleen T. Chu, Sean M. Alber, Patrick M. Kochanek & Simon C. Watkins

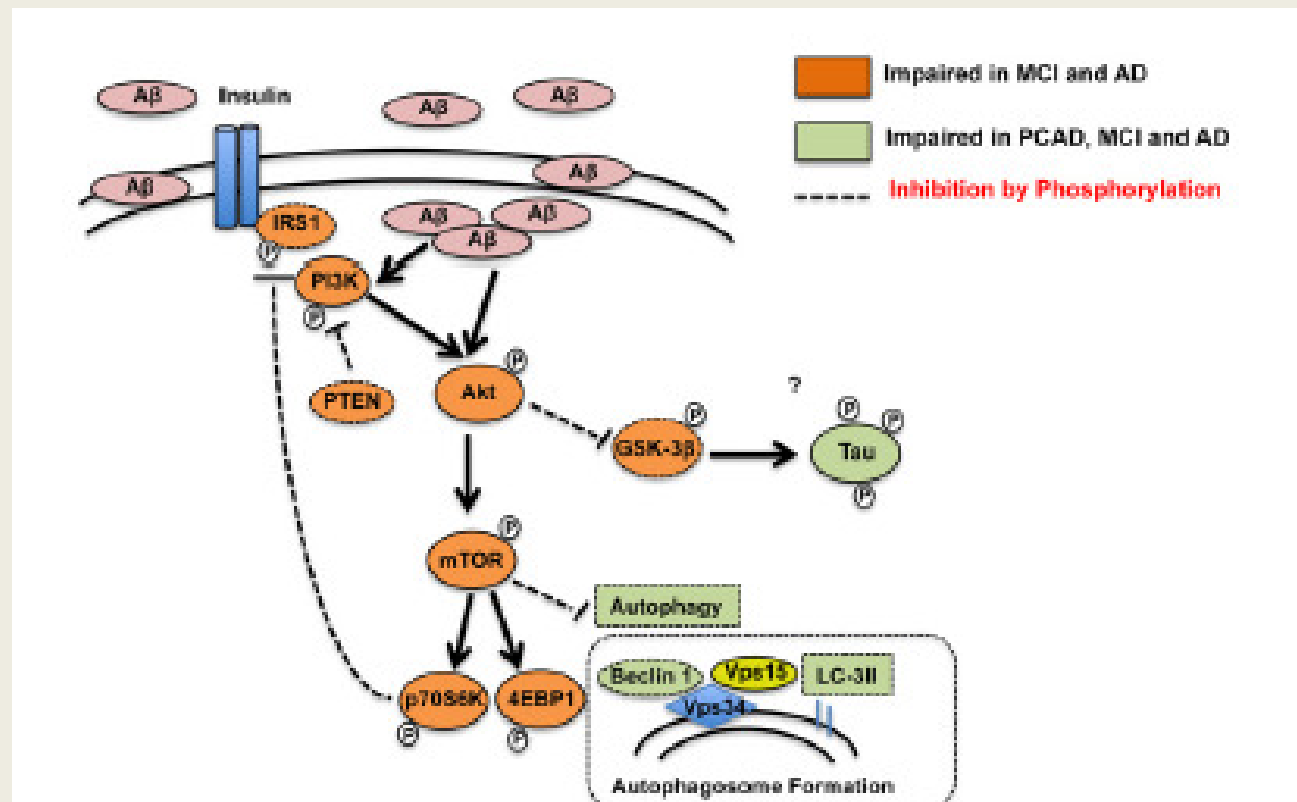
Neuropathological role of PI3K/Akt/mTOR axis in Down syndrome brain



Marzia Perluigi^{a,1}, Gilda Pupo^{a,1}, Antonella Tramutola^a, Chiara Cini^a, Raffaella Coccia^a, Eugenio Barone^a, Elizabeth Head^b, D. Allan Butterfield^{b,c}, Fabio Di Domenico^{a,*}

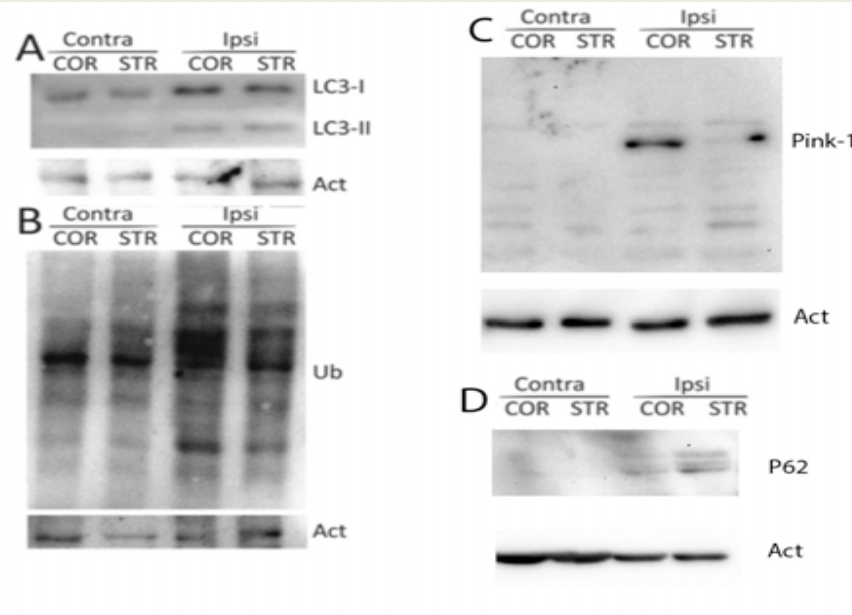
Alteration of mTOR signaling occurs early in the progression of Alzheimer disease (AD): analysis of brain from subjects with pre-clinical AD, amnesic mild cognitive impairment and late-stage AD

Antonella Tramutola,* Judy C. Triplett,† Fabio Di Domenico,* Dana M. Niedowicz,‡ Michael P. Murphy,‡ Raffaella Coccia,* Marzia Perluigi* and D. Allan Butterfield†‡



MCI = mild cognitive impairment
AD = late Alzheimer's disease
PCAD = pre-clinical AD

Autophagy, mitophagy & UPS *in vivo* stroke injury



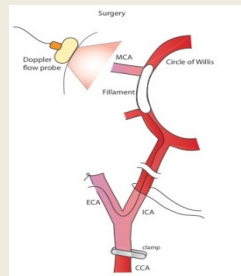
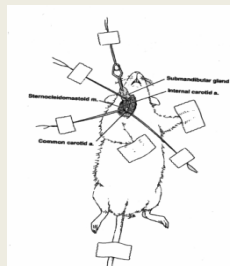
Immunoblotting of contralateral (Contra) and ipsilateral (Ipsi) hemisphere tissue, cortex (COR) and striatum (STR), of a mouse brain after 1 h occlusion & 24 h reperfusion.

Samples probed for LC3 (A), PINK1 (C) & p62 (D).
B. Samples probed for Ub.

Enhanced expression in cortex and striatum of the ipsilateral hemisphere highlighting increased autophagy flux & possible mitophagy. This pattern is mirrored by Ub labelling (B), revealing increased protein ubiquitination. Loading control: β -actin (Act).



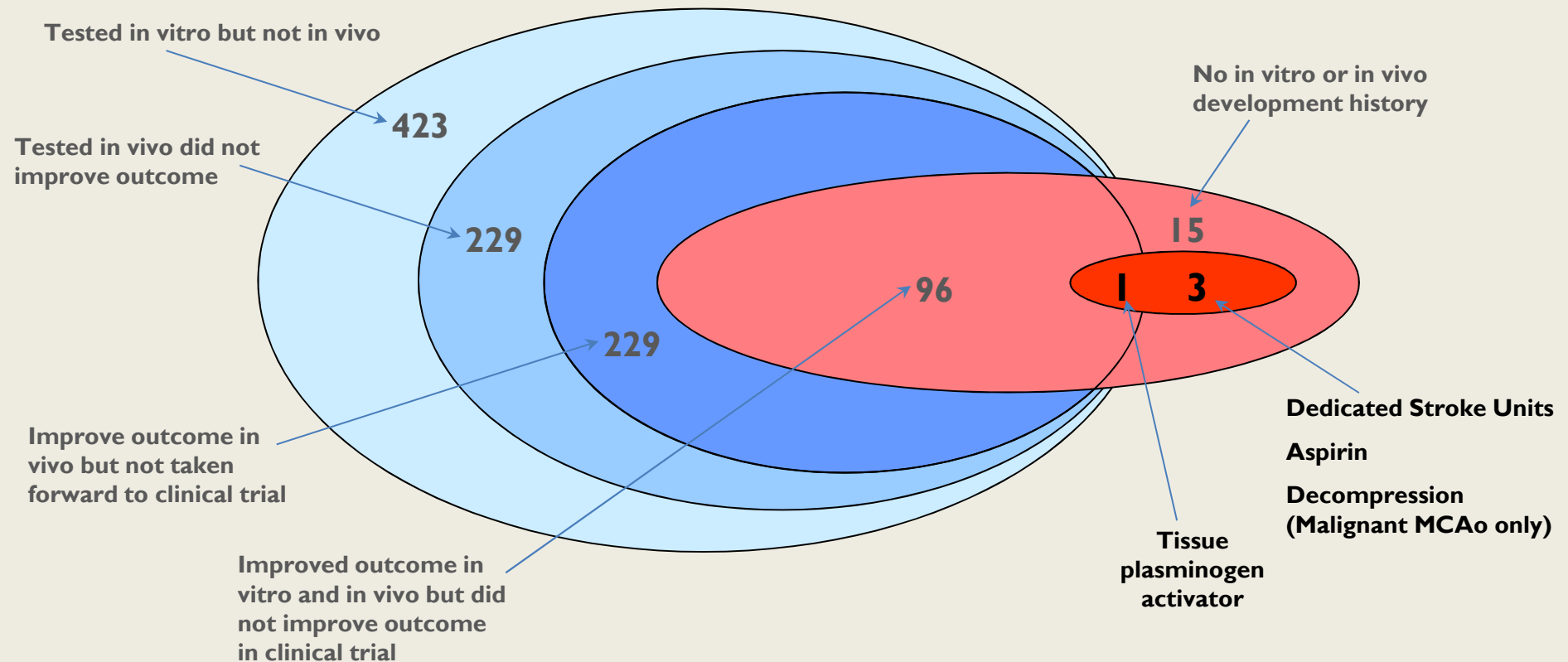
Peter Crack



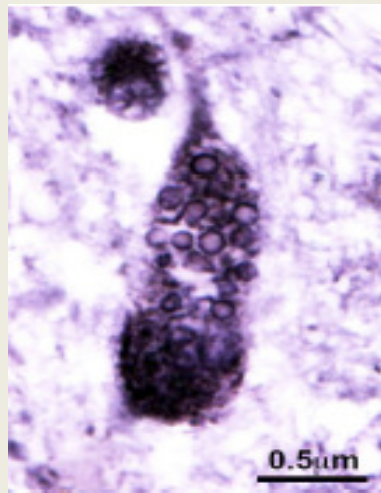
1,026 Experimental Treatments in Acute Stroke

Victoria E. O'Collins, B.Sci,¹ Malcolm R. Macleod, MRCP, PhD,³ Geoffrey A. Donnan, MD, FRACP,²
Laura L. Horky, MD, PhD,² Bart H. van der Worp, MD, PhD,⁴ and David W. Howells, PhD¹

Ann Neurol 2006;59:467–477

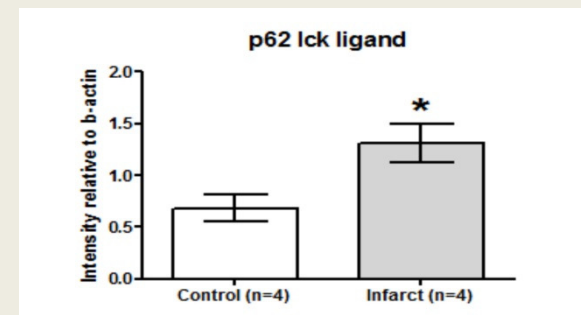
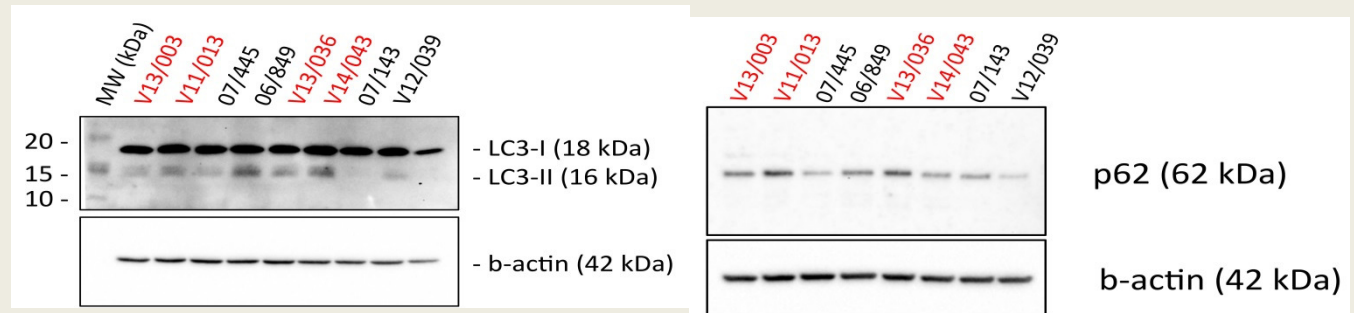


What we are talking about today matters to human brain health



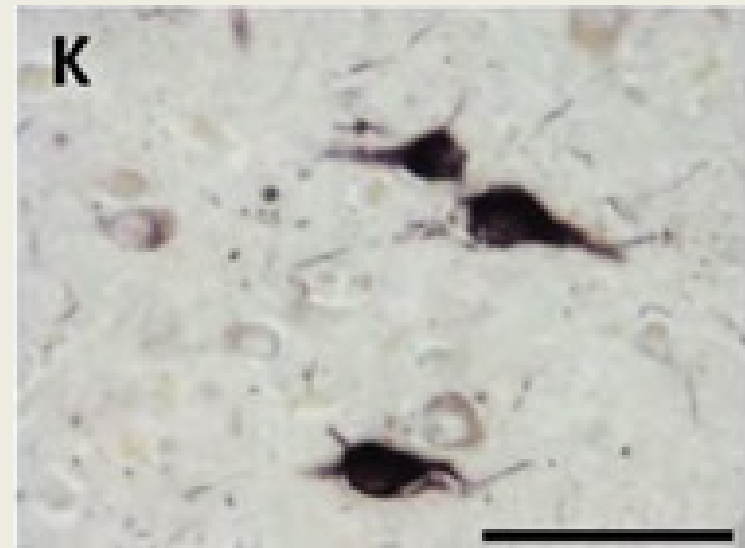
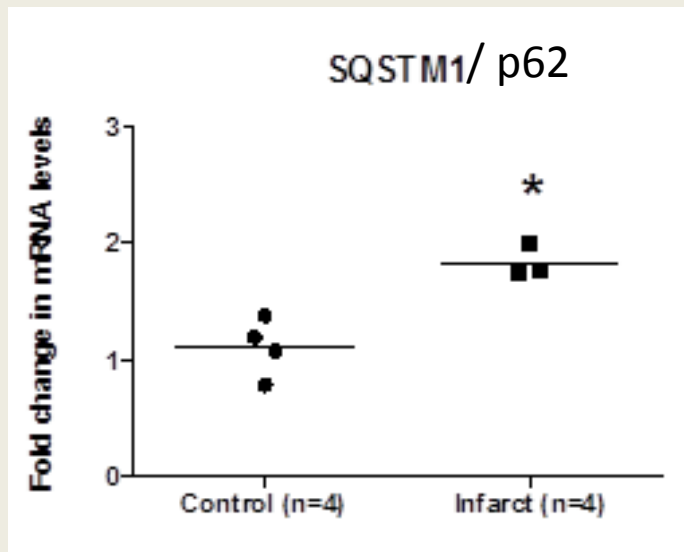
**Autophagic
marker LC3 in
human stroke
brain.**

Cortex of patients with history of stroke



Peter Crack, Catriona Maclean, Philip Beart, Tony Frugier
Unpublished observations, manuscript in revision

EVIDENCE FOR THE RECRUITMENT OF AUTOPHAGIC VESICLES IN HUMAN BRAIN AFTER STROKE



5μm

p62 immunolabelling in
stroke injury

Parkin-mediated mitophagy

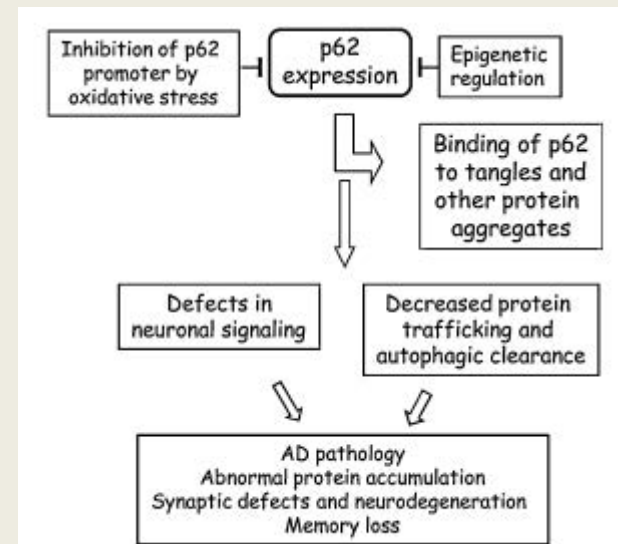
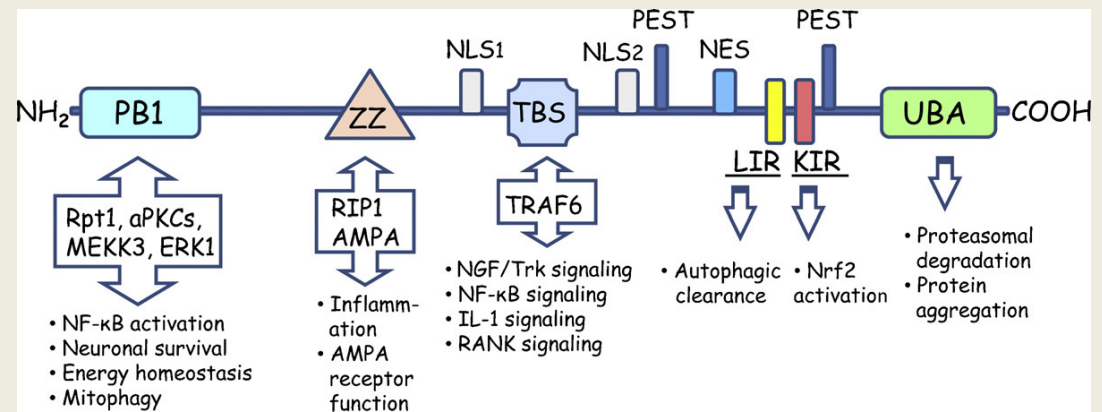
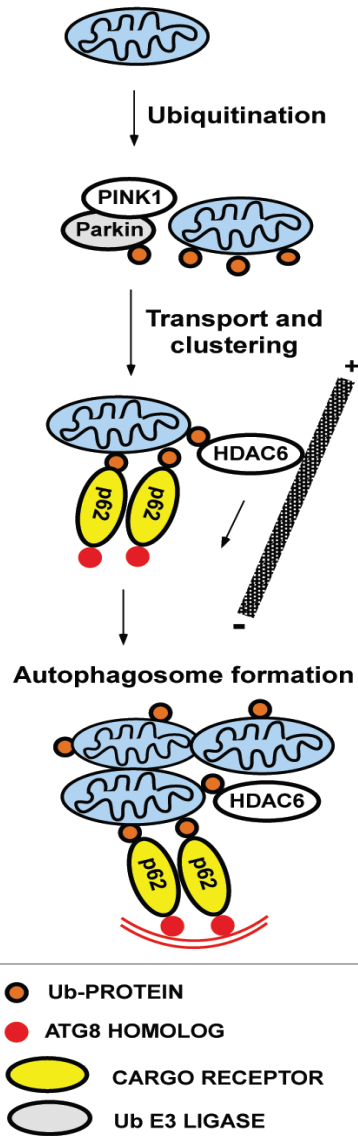


Fig. 3 A hypothetical scheme for the pathological role of p62 in the pathogenesis of

Conclusions and overview

- Paucity of work studying autophagy/mitophagy in neurons (which do not behave like immortalised cancer cells, most often studied in this field)
- Recruitment of autophagy after neuronal injury is complex
- ACD exists – is energy balance the determinant?
- “Good” autophagy that maintains a healthy cell AND can rescue the cell after some stresses
versus “bad autophagy” that can eventually kill the cell
- “Good” autophagy (= non-ACD) valid pharmacological target

