## BRAIN DEGENERATIVE DISEASES

### P01-01
### CONTROL OF NEURITE GROWTH BY S-NITROGLUTATHIONE REDUCTASE VIA DENITROSYLATION OF HDAC2

K Wu[1,2], W Su[1], B Wen[1,2], Y Zhang[1,2], T Wang[1], L Zhang, P Wang[1], J Wang[1], L Liu[3], C Chen[1]
[1]*National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China;*
[2]*University of Chinese Academy of Sciences, Beijing, China;*
[3]*The Department of Microbiology and Immunology, University of California, San Francisco, CA, United States*

Neurite growth control is central to the development and plasticity of neural circuits. By controlling intracellular level of S-nitrosoglutathione (GSNO), S-nitrosoglutathione reductase (GSNOR) has been implicated in many biological processes. However, as the sole brain alcohol dehydrogenase, its role in the nerve system is still largely a mystery. Here we report that GSNOR is significantly downregulated during developing mouse brain and the neurite growth was negatively regulated by GSNOR. Our data further show this negative regulation is mediated by denitrosylation of histone deacetylase 2 (HDAC2). Our findings define a novel role of GSNOR in neurite development via interacting with the NO signaling and provide a new molecular insight into the control of neurite growth and neurogenesis.

### P01-02
### PARKINSON'S DISEASE; TOWARDS NEW DISEASE MODIFYING THERAPIES

DI Finkelstein
*Head Parkinson's Disease Laboratory, Florey Institute of Neuroscience and Mental Health, University of Melbourne, VIC, Australia*

**Objective:** To develop a neuroprotective treatment for Parkinson's disease (PD).
**Background:** Iron dysregulation is implicated in damage to neurons of the substantia nigra pars compacta in PD. We have been developing drugs for neurodegenerative diseases designed to re-establish normal metal homeostasis (Adlard, *et al.* 2008. Neuron *59*, 43–55; Lannfelt, *et al.* 2008. Lancet Neurol *7*, 779–786). This presentation will describe methods used developing these some of these compounds.
**Methods:** Compounds were screened using *in vitro* assays to prevent metal-dependent redox activity and iron-mediated aggregation of α-syn. The most efficient compounds were further tested in two intoxication models (MPTP and 6-hydroxydopamine), knockout (Ceruloplasmin, Tau) and a transgenic (hA53T α-syn) animal models.
**Results:** PBT434 (8-hydroxyquinazolin – 4(3H)-one; Prana), was identified as a potential drug candidate as it inhibits iron mediated oxidative damage and α-syn aggregation *in vitro.* PBT434 reduced α-syn and iron accumulation, improved motor function and prevented loss of SNpc neurons in the three animal models. Restoration of neuronal function was inferred from preservation of tyrosine hydroxylase in SNpc neurons and increased terminal varicosities in the caudate putamen.
**Conclusions:** That compounds designed to target iron-dependent neurodegenerative pathways can maintain the survival of SNpc neurons and represent a plausible addition to current PD therapies.
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### P01-03
### APPROACHES TO COMBATING ALZHEIMER'S DISEASE

LA Miles, GAN Crespi, DB Asche, MW Parker
*St Vincent's Institute of Medical Research, Fitzroy, Vic, Australia*

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease in humans with age being the biggest risk factor. The mechanisms by which the disease progresses to cognitive decline in the sufferer are complex and not fully elucidated. A defining pathological feature is the deposition of extracellular plaques composed primarily of misfolded amyloid beta (A$\beta$) peptide: a proteolytic breakdown product of the much larger Amyloid Precursor Protein. While A$\beta$ peptides are the main constituents of amyloid plaques that burden the diseased brain, plaque burden correlates poorly with the severity of the disease. There is accumulating evidence that a prefibrillar or protofibrillar soluble form of A$\beta$ can compromise neuronal functions and trigger cell death.
Immunotherapy targeting Abeta is a promising direction in AD research with active and passive immunotherapies shown to lower cerebral A$\beta$ levels and rescue cognitive function in animal models. Anti-A$\beta$ immunotherapies are a significant class of AD therapeutics currently in human clinical trials. We have been examining the molecular basis of antibody engagement of A$\beta$ epitopes to inform the analysis of clinical trial data and to guide the engineering of anti-A$\beta$ antibodies with optimised specificity and affinity. We have determined the structures of three different AD antibodies in complex with A$\beta$ peptides. All these studies reveal surprising aspects of A$\beta$ peptide recognition by the antibodies and suggest new avenues for AD antibody development.
In a surprising discovery our collaborators Professor Fred Mendelsohn, Dr Siew Yeen Chai and coworkers showed that inhibition of the membrane-bound receptor called insulin-regulated aminopeptidase caused memory-enhancing effects in animals. Our structural studies were used to identify drug-like inhibitors of the enzyme that were subsequently shown to not only improve memory in rats but also have disease modifying effects in animal models of AD. The inhibitors not only prevent A$\beta$ plaques being formed but also appear to dissolve existing ones. This work is now the subject of collaboration with an international Pharmaceutical company.

## P01-04
## CLINICAL AND BIOMARKER CHANGES IN DOMINANTLY INHERITED ALZHEIMER'S DISEASE

PR Schofield[1], RJ Bateman[2], C Xiong[2], TLS Benzinger[2], AM Fagan[2], A Goate[2], NC Fox[3], DS Marcus[2], NJ Cairns[2], X Xie[2], TM Blazey[2], DM Holtzman[2], A Santacruz[2], V Buckles[2], A Oliver[2], K Moulder[2], PS Aisen[4], B Ghetti[5], WE Klunk[6], E McDade[6], RN Martins[7], CL Masters[8], R Mayeux[9], JM Ringman[10], MN Rossor[3], RA Sperling[11], S Salloway[12], JC Morris[2], for the Dominantly Inherited Alzheimer Network DIAN
[1]*Neuroscience Research Australia, Sydney, NSW, Australia;* [2]*Washington University, St Louis, MO, USA;* [3]*University College London, London, UK;* [4]*University California San Diego, San Diego, CA, USA;* [5]*Indiana University, Indianapolis, IN, USA;* [6]*University of Pittsburgh, Pittsburgh, PA, USA;* [7]*Edith Cowan University, Perth, WA, Australia;* [8]*University of Melbourne, Melbourne, VIC, Australia;* [9]*Columbia University, New York, NY, USA;* [10]*University California Los Angeles, Los Angeles, CA, USA;* [11]*Brigham and Women's Hospital, Harvard University, Boston, MA, USA;* [12]*Butler Hospital, Brown University, Providence, RI, USA*

The order and magnitude of pathologic processes in Alzheimer's disease (AD) are not well understood, partly because the disease develops over many years. Autosomal dominant AD has a predictable age at onset and provides an opportunity to determine the sequence and magnitude of pathologic changes that culminate in symptomatic disease.

In this prospective, longitudinal study, we analyzed data from 128 participants who underwent baseline clinical and cognitive assessments, brain imaging, and cerebrospinal fluid (CSF) and blood tests. We used the participant's age at baseline assessment and the parent's age at the onset of symptoms of AD to calculate the estimated years from expected symptom onset (age of the participant minus parent's age at symptom onset). We conducted cross-sectional analyses of baseline data in relation to estimated years from expected symptom onset in order to determine the relative order and magnitude of patho-physiological changes.

Concentrations of amyloid beta (A$\beta$)42 in the CSF appeared to decline 25 years before expected symptom onset. A$\beta$ deposition, as measured by positron emission tomography with the use of Pittsburgh compound B, was detected 15 years before expected symptom onset. Increased concentrations of tau protein in the CSF and an increase in brain atrophy were detected 15 years before expected symptom onset. Cerebral hypometabolism and impaired episodic memory were observed 10 years before expected symptom onset. Global cognitive impairment, as measured by the Mini–Mental State Examination and the Clinical Dementia Rating scale, was detected 5 years before expected symptom onset, and patients met diagnostic criteria for dementia at an average of 3 years after expected symptom onset.

We found that autosomal dominant AD was associated with a series of patho-physiological changes over decades in CSF biochemical markers of AD, brain amyloid deposition, and brain metabolism as well as progressive cognitive impairment. Our results require confirmation with the use of longitudinal data and may not apply to patients with sporadic AD.

## P01-05
## MOLECULAR REGULATION OF MITOCHONDRIAL QUALITY VIA A MECHANISM OF MITOPHAGY

Q Chen[1,2]
[1]*Institute of Zoology, Chinese Academy of Sciences, Beijing, China;* [2]*College of Life Sciences, Nankai University, Tianjin, China*

Mitochondrial are essential organelle that determine both cell's life and death. Accumulation of damaged mitochondria could be harmful to the cell and has been causally linked with metabolic disorders, neurodegenerative diseases and cancers. It is emerging that damaged or unwanted mitochondria can be selectively removed by a mechanism of mitophagy (or mitochondrial autophagy) to ensure the health of the cell. How individual mitochondrion as a cargo is recognized for removal and how mitochondrial stresses are sensed to activate receptor mediated-mitophagy remains poorly. We have recently identified that FUNDC1 harbors LC3-interacting region (LIR) and serves as a receptor to interact with LC3 to mediate mitophagy. We showed that hypoxia induced dephosphorylation of FUNDC1 and enhanced its interaction with LC3 for selective mitophagy. Furthermore, we found a phosphatase and kinases mediate the reversible phosphorylation of FUNDC1 upon hypoxia or FCCP treatments. Our results thus uncovered a major mechanism to control mitophagy in mammalian systems.

## P01-06
## OPEN–CLOSED MOTION OF MINT2 REGULATES APP METABOLISM

X Xie[1,2], X Yan[1,2], Z Wang[1,2], H Zhou[1,2], W Diao[1,2], W Zhou[1,2], J Long[1,2], Y Shen[1,2]
[1]*State Key Laboratory of Medicinal Chemical Biology, Nankai University, Tianjin, China;* [2]*College of Life Sciences, Nankai University, Tianjin, China*

The amyloid precursor protein (APP) plays a crucial role in the pathogenesis of Alzheimer's disease (AD). Knock-out and transgenic mouse studies of the adaptor protein Mint2 have revealed that it is a major player in regulating APP metabolism physiologically through the binding of its phosphotyrosine binding (PTB) domain to the intracellular domain of APP. However, the molecular mechanism of APP dynamically binding to Mint2 remains elusive. Here, we report the structures of APP peptide-free and APP peptide-bound C-terminal Mint2 mutants at resolutions of 2.7 and 3.3 Å, respectively. Our structures reveal that APP peptide-free Mint2 exists in a closed state in which the ARM domain blocks the peptide-binding groove of the PTB domain. In sharp contrast, APP peptide-bound Mint2 exists in an open state in which the ARM domain drastically swings away from the bound peptide. Mutants that control the open–closed motion of Mint2 dynamically regulated APP metabolism both *in vitro* and *in vivo*. Our results uncover a novel open–closed mechanism of the PTB domain dynamically binding to its peptide substrate. Moreover, such a conformational switch may represent a general

regulation mode of APP family members by Mint proteins, providing useful information for the treatment of AD.

## P01-07
## BLOCKING GSK3$\beta$-MEDIATED DRP1 PHOSPHORYLATION PROVIDES NEUROPROTECTION AND RESCUES MEMORY DEFICITS IN APPSWE/PS1DE9 TRANSGENIC MICE

J Yan, Z-Y Chen
*Department of Neurobiology, Shandong Provincial Key Laboratory of Mental Disorders, School of Medicine, Shandong University, Jinan, Shandong, China*

Mitochondrial dysfunction has been widely recognized as a feature in neurodegenaration diseases. Recent studies have provided a deeper understanding of mitochondrial dynamics, which is regulated by mitochondrial fission and fusion proteins, in the pathology of Alzheimer's disease. However, the dedicated regulation mechanism is still unclear. Here, we demonstrated that GSK3$\beta$, which is a key factor in Alzheimer's disease, could promote mitochondrial fragmentation through dynamin-related GTPase Drp1. Phosphorylation of Drp1 at $Ser^{40}$ and $Ser^{44}$ residues by GSK3$\beta$ resulted in excessive mitochondrial fragmentation and thus neuronal toxicity. Moreover, we found that blockage of GSK3$\beta$-Drp1 interaction by using a polypeptide Tat-$Drp1_{35–49}$DD could provide neuroprotection both *in vitro* and *in vivo*. Notably, microinjection of Tat-$Drp1_{35–49}$DD into hippocampus could rescue the memory deficits in APPswe/PS1dE9 transgenic mice. These findings advanced our understanding of mitochondrial dynamics in Alzheimer's disease pathology and provide a potential therapy target for Alzheimer's disease.

## P01-08
## NEURODEGENERATION STUDY: FROM MOLECULES TO BIG ANIMAL MODELS

Z-C Xiao
*Department of Anatomy and Developmental Biology, Monash University, Clayton, Vic, Australia*

Appropriate connections or interactions among different neural cell types are essential for the correct and efficient functioning of the nervous system during development and regeneration after trauma or degeneration. The aim of my research is to understand the molecular events that mediate communication among neural cells, in the nervous system during development, myelination, learning and memory, degeneration, and regeneration. These studies have yielded insights into the therapeutic potential of cell signalling molecules to ameliorate or even ablate the detrimental consequences of nervous system injury and neurodegenerative diseases, including stroke, traumatic brain injury, spinal cord injury, Alzheimer's Disease (AD), and multiple sclerosis (MS).

## P01-09
## THE ROLE OF NR2B AND NEURONAL NITRIC OXIDE SYNTHASE IN GLUTAMATE INHIBITORY EFFECT ON IGF-1 RECEPTOR SIGNALING

L Zhang[1], Q Chen[1], C Han[1], R Wang[1], F Yan[1], Y Lai[1], R Liao[1], P Little[2], W Zheng[1]
[1]*State Key Laboratory of Ophthalmology,Zhongshan Ophthalmic Center and School of Pharmaceutical Sciences and Sun Yat-Sen University, Guangzhou, China;* [2]*Health Innovations Research Institute, RMIT University, Bundoora, Australia*

Impairing neurotrophic factor receptor signaling by the activation of N-Methyl-D-aspartate receptors (NMDARs) has been suggested as a novel mechanism of glutamate-induced neurotoxicity (J Biol Chem, 2009). However, the interrelationship between these receptor signaling pathways remains to be elucidated. Using specific antagonists and siRNA to block individual NMDAR subunits, we found that the activation of NR2B-containing NMDARs was critical for glutamate to inhibit IGF-1 signaling. In addition, inhibition of nNOS blocked the attenuating effect of glutamate on IGF-1 signaling. Nitric oxide donors, L-arginine and SNAP, inhibited the tyrosine phosphorylation of the IGF-1 receptor (IGF-1R) and its downstream signaling. In nNOS null mice ($nNOS^{-/-}$), SNAP impaired IGF-1 signaling but glutamate and L-arginine had no effect. Furthermore, the NR2B subunit of the NMDAR promoted the activation of nNOS and the production of nitric oxide, while the NR2A subunit inhibited nNOS activation. These findings indicate that the glutamate-induced attenuation of IGF-1 signaling is mediated by NR2B-containing NMDARs and the nNOS pathway. Our study also suggests a novel mechanism of altering neurotrophic factor signaling by NMDA receptor in glutamate excitotoxicity.
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## P01-10
## STEREOSELECTIVE REDUCTION OF 1-O-ISOPROPYLOXYGENIPIN STABILIZED GENIPIN DERIVATIVES AND ENHANCED THEIR NEUROPROTECTIVE ACTIVITY AGAINST SODIUM NITROPRUSSIDE-INDUCED APOPTOSIS OF RETINAL GANGLION CELLS

S Liao, R Gen, Q Chen, R Wang, Y Lai, F Yan, H Chenc, W Zheng
*State Key Laboratory of Ophthalmology,Zhongshan Ophthalmic Center and School of Pharmaceutical Sciences and Sun Yat-Sen University, Guangzhou, China; Health Innovations Research Institute, RMIT University, Bundoora, Australia*

Genipin is a traditional Chinese herbal iridoid with neuritogenic and neuroprotective actions in neuronal cell lines. It is unstable and difficult to use in practice. Therefore, efforts have been made to develop more stable genipin derivatives with better biological

functions. Among all the compounds reported, (1R)-isopropyloxy-genipin (IPRG001) is more stable but less active than genipin. In this study, we developed two new stable genipin derivatives CHR20/CHR21 from IPRG001 by the acetallization of IPRG001 at C-1 and then the reduction of C6 = C7 double bond. Obtained new compounds CHR20/CHR1 are more stable than IPRG001. Cell viability and apoptosis assays showed that sodium-nitroprusside (SNP)-induced apoptosis of RGC cells while CHR20/21 significantly inhibited SNP-induced cell toxicity. These two new compounds also blocked the generation of reactive oxygen induced by SNP and induced the expression of antioxidant genes like Gclc in RGC-5. Moreover, CHR20/21 effectively blocked the negative effect of SNP in the activation of Akt and Erk1/2. Pretreatment of RGC-5 cells with PI3K inhibitor LY294002 or the MAPK pathway inhibitor PD98059 blocked the protective effect of CHR20/21. These results indicate that CHR20-21 can promote the survival of RGC by the PI3K/Akt and the MAPK pathways. They also suggest that stereoselective reduction of 1-O-Isopropyl-oxygenipin can enhances its neuroprotective activity against SNP-induced apoptosis of retinal ganglion cells.

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## P01-11
## FOXO3A ATTENUATED NGF-INDUCED NEURONAL DIFFERENTIATION OF PC12 CELLS BY INHIBITING NEUROCHONDRIN EXPRESSION

L Zhang, Y Lai, R Wang, Q Chen, H Wang, W Zheng
*State Key Laboratory of Ophthalmology, Zhongshan Ophthalmic Center and School of Pharmaceutical Sciences, and Sun Yat-sen University, Guangzhou, China*

Forkhead box O3 (FoxO3a) is a forkhead family transcription factor playing important roles in non-neuronal differentiation, metabolism, proliferation and survival, but its role in neuronal differentiation remains unclear. In this project, we study the role of FoxO3a in neuronal differentiation and its underlying mechanisms. Our results showed that overexpression of FoxO3a inhibited neuronal differentiation of PC12 cells induced by nerve growth factor (NGF) while knockdown of FoxO3a by siRNA enhanced NGF-induced differentiation. DNA microarray analysis and quantitative reverse transcription PCR (RT-PCR) showed that the overexpression of FoxO3a significantly attenuated expression of neurochondrin (NCDN), a neurite-outgrowth-related protein, in PC12 cells, while knocking down the expression of FoxO3a had the opposite effect. Bioinformatics studies found that the regulatory region of NCDN promoter contained multiple FoxO3a binding sites. Dual-Luciferase reporter assay with report gene containing NCDN promoter showed that FoxO3a significantly decreased the transcription activity of NCDN promoter. These results indicate that NCDN is a direct downstream target of FoxO3a which negatively regulates the expression of NCDN. Interestingly, NGF-induced NCDN expression and cell differentiation was blocked by the inhibition of phosphatidylinositol-3-kinase (PI3K)-protein kinase B (PKB, Akt) signal pathway (activation of FoxO3a) and overexpression of FoxO3a. Moreover, Knock-down of NCDN by siRNA blocked NGF-induced neuronal differentiation of PC12 cells while overexpression of NCDN significantly promoted neurite outgrowth. These results put together demonstrate that NCDN plays an important role in NGF-induced neuronal differentiation and suggest that FoxO3a inhibits NGF-induced neuronal differentiation, at least in part, by suppressing the expression of NCDN.

## P01-12
## DEVELOPMENT OF ANALGESIC α-CONOTOXINS FOR TREATMENT OF CHRONIC PAIN

DJ Adams[1], G Berecki[1], R Clark[2], DJ Craik[2]
[1]*Health Innovations Research Institute, RMIT University, Melbourne, VIC, Australia;* [2]*Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia*

Peptides derived from cone snail venoms (conotoxins) have attracted attention as leads for drugs to treat severe pain. They have exquisite selectivity and potency for membrane receptors and ion channels involved in pain pathways. Several disulfide-bonded α-conotoxins inhibit nicotinic acetylcholine receptors (nAChR) and are in preclinical development for use as drugs to treat chronic and neuropathic pain. We explored the primary target of α-conotoxins Vc1.1 and RgIA, and engineered conotoxins with a cyclic peptide backbone to improve their stability and oral bioavailability. We investigated the actions of the synthetic cyclized peptides Vc1.1 and RgIA using patch clamp whole-cell recording techniques and tested them in the chronic constriction injury (CCI) model of neuropathic pain in rats. Cyclic Vc1.1 and RgIA more potently inhibit N-type (Cav2.2) calcium channel currents in rat sensory neurons via the G protein-coupled GABAB receptor (GABABR) than the nAChR. Additionally, baclofen, cyclic Vc1.1 and RgIA inhibit voltage-dependent $Ca^{2+}$ currents in HEK293 cells transfected with Cav2.2 channels and human GABABR subunits, but not cells transfected with Cav2.2 channels alone. Our engineered cyclic α-conotoxin Vc1.1 is more potent than the linear (non-cyclized) peptide and at least two orders of magnitude more potent than the leading clinically used human drug, gabapentin, in the rat CCI model. Cyclic Vc1.1 and RgIA have better receptor specificity and proteolytic stability than their linear counterparts. The primary target of α-conotoxins Vc1.1 and RgIA was believed to be α9α10 neuronal nAChRs, but our findings confirm a class of α-conotoxins modulate Cav2.2 channels via GABABR activation. GABABRs are a clear therapeutic target for these and modified conotoxins.

## P01-13
## THE MECHANISMS OF THE ACCUMULATION OF MISFOLDED α-SYNUCLEIN IN PARKINSON'S DISEASE: CURRENT FINDINGS REVIEWED

J-F Li, J-P Liu
*Institute of Aging Research, School of Medicine, Hangzhou Normal University, Hangzhou, Zhejiang Province, China*

Parkinson's disease (PD) is a progressive neurodegenerative disorder that is characterized by the selective loss of dopaminergic neurons and the deposition of misfolded α-synuclein in various region of the brain. Current thinking suggests that many factors, such as α-synuclein gene mutation, lipid metabolism, oxidative stress and heavy metal exposure can lead to the accumulation of misfolded α-synuclein. However, the exact mechanism by which this occurs is not fully defined. Herein, we review recent findings on the structural characterization of α-synuclein and exploit the possible molecular and cellular mechanisms involved in the accumulation of misfolded α-synuclein.

## P01-14
## ZILEUTON, A SELECTIVE INHIBITOR OF 5-LIPOXYGENASE, PROTECTS PC12 CELLS AGAINST MICROGLIA-MEDIATED NEUROTOXICITY

X-Y Zhang[1,3], L Chen[1], X-R Wang[2], D-M Xu[2], J-F Zhang[1], Y Yang[1], W Zheng[1], Y-F Wang[1], C-T Li[1], L-H Zhang[1]
[1]*Department of Pharmacology, Hangzhou Key Laboratory of Medical Neurobiology, School of Medicine, Hangzhou Normal University, Hangzhou, China;* [2]*Department of Pharmacology, School of Medicine, Zhejiang University, Hangzhou, China;* [3]*Biotherapy Center, The General Hospital of Beijing Military Command, Beijing, China*

Neuroinflammation characterized mainly by microglial activation is one of the pathological processes of many neurodegenerative diseases, including Parkinson disease (PD). Increasing evidence has suggested the involvement of the cysteinyl leukotriene (CysLT) signal system in brain inflammation. The 5-lipoxygenase (5-LOX) products CysLTs (LTC4, D4 and E4) are potent pro-inflammatory mediators closely associated with neuroinflammation and neuronal injury through the activation of CysLT receptors. We have recently reported that zileuton, a selective inhibitor of 5-LOX, attenuated neuronal injury induced by high concentrations of rotenone (0.3–30 μM) in PC12 cells. We have also found that zileuton inhibited microglial phagocytosis as well as release of the pro-inflammatory cytokines induced by low concentration rotenone (0.001–0.01 μM) in BV2 cells, a mouse microglial line. The objective of this study was to examine the effect of zileuton on microglia-mediated rotenone neurotoxicity. Microglia-mediated neurotoxicity was evaluated by the sequential linking of two autonomously growing cell lines, mouse microglial BV2 cells and neuron-like PC12 cells. The supernatant from rotenone-stimulated BV2 cells was used as the conditioned media for PC12 cells. The viability of PC12 cells was determined by 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Cell death was observed by lactate dehydrogenase (LDH) release and double fluorescence staining with Hoechst /propidiumiodide (PI). Rotenone at 0.001–0.01 μM was nontoxic to PC12 cells directly, but the conditioned media from rotenone-treated BV2 cells induced cytotoxicity in PC12 cells. The viability of PC12 cells was reduced, and LDH release and cell necrosis were increased by 0.001–0.01 μM rotenone-stimulated BV2 conditioned media. Pretreatment of BV2 cells with the 5-LOX inhibitor zileuton (0.01–1 μM) protected PC12 cells from microglia-mediated rotenone toxicity. These results indicate that the 5-LOX inhibitor zileuton effectively attenuates microglia-mediated rotenone neurotoxicity. This may represent a novel approach for the treatment of microglial-mediated neurotoxicity of PD via inhibition of CysLT signaling pathway.

## P01-15
## EXTENSIVE INVOLVEMENT OF AUTOPHAGY IN EXPERIMENTAL NEURITIC DEGENERATION

Y Yang[1,2], Y-H Sun[2], X-X Zheng[2], L-H Zhang[1]
[1]*Department of Pharmacology, Hangzhou Key Laboratory of Medical Neurobiology, School of Medicine, Hangzhou Normal University, Hangzhou, China;* [2]*Department of Biomedical Engineering, Zhejiang Provincial Key Laboratory of Cardio-Cerebral Vascular Detection Technology and Medicinal Effectiveness Appraisal, Zhejiang University, Hangzhou, China*

Autophagy is a cellular degradation pathway involving lysosomal-dependent recycling of intracellular constituents. It plays an essential role in the maintenance of neuronal homeostasis and dysfunctional autophagy is associated with a variety of neurodegenerative diseases. Axons and dendrites, collectively known as neurites, are particularly vulnerable to various insults due to their unique structures. Neuritic degeneration, with the appearance of growth cone collapse, synaptic dysfunction, neuritic retraction, and accumulative focal beading or swelling formation, occurs early in many neurodegenerative disorders and may contribute to the disease progression and clinical symptoms. Using *in vitro* cultured neurons and PC12 cells, we observed extensive involvement of autophagy in diverse experimental models of neuritic degeneration, including mechanical injury (transection), pharmacological toxin exposure (e.g. amyloid β peptide [Aβ], zinc depletion), nutrition withdrawal (e.g. nerve growth factor [NGF] or serum deprivation) and lysosomal inhibition (e.g. vinblastine, bafilomycin A1, leupeptin and pepstatin). During autophagy induction, autophagosomes and autolysosomes accumulated along neurites, especially in focal swellings and distal ends. These autophagic vesicles moved along neurites in both anterograde and retrograde directions depending on microtubules as well as motor proteins. In addition, administration of autophagy inhibitor 3-methyladenine or knocking down the key autophagy-related genes atg7 and beclin1 significantly delayed axonal and dendritic degeneration after transection or NGF deprivation, suggesting over-activated autophagy may lead to the aggravation of neuritic degeneration. In contrast, autophagy seems to serve as a self-defense mechanism in neurites exposed to Aβ, as pharmacologi-

cal inhibition of autophagy by 3-methyladenine, chloroquine or LY294002 had no influence on A$\beta$-induced neuritic degeneration, whereas autophagy stimulator rapamycin significantly suppressed neuritic dystrophy, indicating insufficient autophagy may cause the pathogenesis of neuritic injury in Alzheimer's disease. Collectively, our findings demonstrate that insufficient or deregulated autophagy both contribute to axonal and dendritic death, highlighting the crucial role of autophagy in the regulation of neuritic degeneration.

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