

Submitter Name: Srivatsan Kidambi
Submitted email: skidambi2@unl.edu

METH elicits dose- and time-dependent autophagic cytotoxicity in primary astrocytes

Christina Wilson¹, Kimberly Stanke¹, Rodrigo Franco Cruz², Rick Bevins³, Oleh Khalimonchuk⁴,
and Srivatsan Kidambi¹

¹Department of Chemical and Biomolecular Engineering, University of Nebraska-Lincoln;

²School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln;

³Department of Psychology, University of Nebraska-Lincoln; ⁴Department of Biochemistry,
University of Nebraska-Lincoln

Methamphetamine (METH) induces inflammation, alters glutamate uptake, and brain energy utilization resulting in pathogenesis of neurodegeneration. It is also neurotoxic and broadly cytotoxic, leading to overdose deaths. The effect of METH on microglia and neurons has been extensively studied; however its impact on astrocytes is under explored. We demonstrate that the cytotoxic actions of METH in primary astrocytes via selective enhancement of autophagy, a process that physiologically degrades metabolites and cellular organelles, and that uncontrolled autophagy can also lead to cell death. In primary astrocytes cultures, METH induced a concentration dependent decrease in cell viability. We found that METH exposure resulted in significantly higher glycolytic activity and higher mitochondrial stress in astrocytes using Seahorse Experiments. We observed that METH reduced glutamate uptake, key function of astrocytes, in a dose- and time-dependent manner. We found that METH caused an increase in LC3-II and p62 level (both actions characteristic of autophagy) in astrocytes at concentration as low as 1 μ M. We also validated the presence of autophagy using phase images of cytoplasmic vacuoles. Furthermore, transmission electron microscope images showed higher formation of autophagosomes, amphisomes, and lysosomes in astrocytes in a dose- and time-dependent manner. We also observed that pharmacologic inhibition of autophagy using 3-methyladenine (3-MA) protects astrocytes against METH-induced cell death. This suggests that autophagy functions as a key player in astrocytes apoptosis caused by METH. These data indicates possible unexplored role of autophagy and astrocytes dysfunction during METH exposure. Treatments that selectively influence METH-associated autophagy may afford therapeutic benefit.