ZINC ACCUMULATION IN THE MIDBRAIN FOLLOWING METHAMPHETAMINE EXPOSURE AS A POTENTIAL BIOMARKER FOR NEURODEGENERATION

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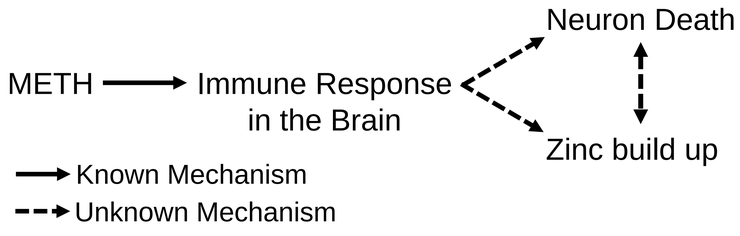
Introduction

Illicit use of methamphetamine (METH) is a rising concern due to the well-established link between METH intoxication and neuron death. Many studies have suggested that the death of these neurons is in part due to an overzealous response of the immune system following METH exposure. Notably, excessive buildup of zinc, a common co-factor of the immune system, has also been implicated in many neurodegenerative disorders.

Hypothesis

Neuron death following METH exposure is due to the accumulation of zinc in the brain from over activation of the immune system.



Methods

Day 1:
- Weigh mice
- Injections:
  -METH (5mg/kg)
  -Saline (0.9% NaCl)

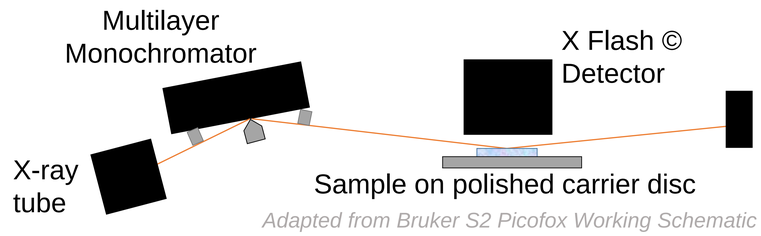
Days 2-9:
- Weigh mice
- Injections

Day 10:
- Weigh mice
- Final injection
- Harvest tissues:
  -Midbrain
  -Striatum

- Tissues were sonicated and analyzed for elemental content via Total Reflection X-Ray Fluorescence and CuZn superoxide dismutase activity.

Methods (cont'd)

Total Reflection X-Ray Fluorescence:



Adapted from Bruker S2 Picofox Working Schematic

Results

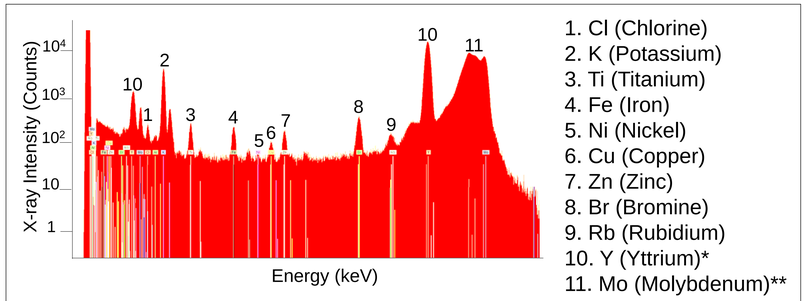


Figure 1. Representative Total Reflection X-Ray Fluorescence readout following analysis of trace metal content in brain regions. Elemental amounts were determined by secondary X-ray fluorescence using an internal standard (Y) by which all other relative metal amounts are measured. *Internal Standard added before analysis. **Large amounts found inside instrument.

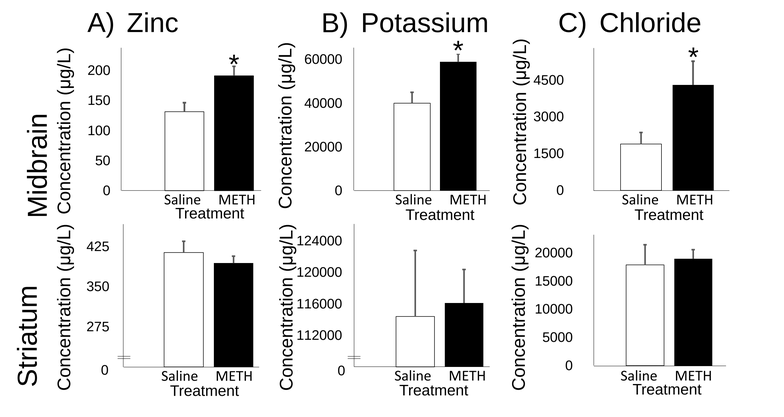


Figure 2. A) Zinc, B) Potassium, and C) Chlorine deposition as determined by Total Reflection X-Ray Fluorescence in the midbrain and striatum of C56BL/6 mice following 10 days of METH (n=6) or Saline (n=6) treatment. Data are expressed as means±SE, *p<0.05 vs. saline; two sample *t*-test.

Results (cont'd)

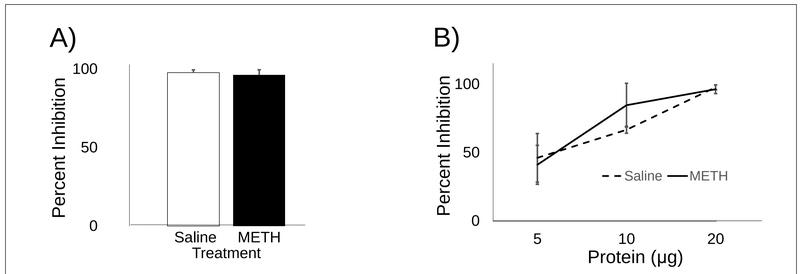


Figure 3. Relative levels of CuZn Superoxide Dismutase in the midbrain of C56BL/6 mice following 10 days of METH (n=3) or Saline (n=3) treatment. Inhibition rate is reported following coloremetric methodologies. A) Total protein concentrations were standardized at 20 ug of protein per sample. Data are expressed as means±SE; two sample *t*-test; p=0.36 vs. saline. B) Protein concentration-dependent responses of CuZn Superoxide Dismutase activity. Data are expressed as means±SE; two-way ANOVA.

Future Directions

- Analyze genes of interest relating to inflammation and neurodegeneration, including nitric oxide synthase (eNOS/nNOS).
- Continue our search for alternate sources of zinc, including brain specific zinc transporters (MT3).

Summary and Conclusions

- An increase in zinc deposition was observed in the midbrain of METH treated mice compared to saline.
- While zinc is an important co-factor for superoxide dismutase, this enzymatic activity does not appear to be the source of zinc deposition following METH treatment.
- Although we do not have a known mechanism, we have established that METH exposure leads to zinc accumulation in the midbrain.

Acknowledgements

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