# Proteomics and phosphoproteomics reveal insights into metformin protection of selective dopamine neurons from methamphetamine–induced damage in nonhuman primates: implications for Parkinson's disease

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## Introduction

The antidiabetic drug metformin (MF) has gained increasing interest due to the possible beneficial effects in different pathological conditions, including Parkinson's disease[1,2]. Rodent studies have indicated that metformin rescues dopaminergic neuron loss and motor phenotypes in PD models, but the precise mechanisms of metformin is still under debate and needs further characterization. Here, we used proteomics and bioinformatic methods to elucidate metformin's protective actions against methamphetamine (METH) in African green monkeys[3].

## Methods

Adult Male African green monkeys (Chlorocebus sabeus) were housed and studied at the AAALAC-accredited St Kitts Biomedical Research Foundation animal facility. This study was approved by IACUC and performed according to the "Guide for the Care and Use of Laboratory Animals". Tissues collected from these animals have been and will be used in multiple other studies.

Monkeys were treated with metformin orally each day (12.5 mg/kg for 1 week, then 25 mg/kg for 3 weeks) or vehicle (Sunquick juice) and were administered meth (0.5 mg/kg twice on day 22, 1.0 mg/kg i.m. twice on day 23) or matching saline injections (days 22-23). Tissues were collected one week after final METH dose.

Dopamine (DA) and homovanillic acid (HVA) concentrations were measured by HPLC with electrochemical detection.

Metformin and phenytoin (internal standard) were detected by high performance liquid chromatography with ultraviolet detection (HPLC-UV) based on [4].

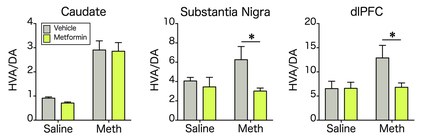
Substantia nigra (SN) samples were subjected to label-free quantification of bulk proteome and Ti-O2 enriched phosphoproteome by tandem MS-MS. In the bulk proteome, 3102 proteins were detected with a false discovery rate <1% and two peptide per protein filter. In phospho-enriched samples, 14499 phosphopeptides were detected corresponding to 2933 proteins.

Functional enrichment (gene ontology; GO) analysis on significantly regulated proteins in ClueGO (version 2.5.7), a biological networks plugin for Cytoscape (version 3.5.1)[5,6]. Pathway analysis was performed in IPA (QIAGEN Inc.).

## 1: Metformin protected dopamine terminals against METH in selective brain regions

Plasma MF concentration was 2.03 ± 0.39 μg/mL at time of sacrifice

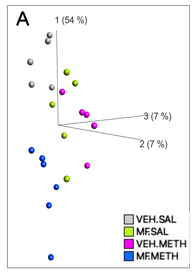
MF protected dlPFC and SN, but did NOT protect striatum, VTA, prelimbic CTX or NAcc.

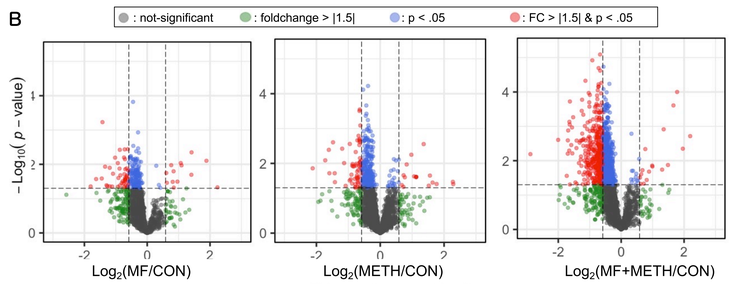



## 2: Metformin and METH trigger few but distinct changes in protein abundance in SN

A: Principal component analysis shows that MF+METH proteome is distinct from other treatment groups. Interaction between MF and METH treatment aligns along PC1.

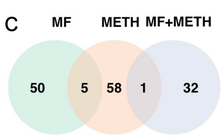
B: Change in expression relative to control group (VEH+SAL) for MF, METH, and MF+METH groups (left to right). Vertical line indicates expression ratio >|1.5|. Horizontal line indicates p=.05 (two-group comparison).

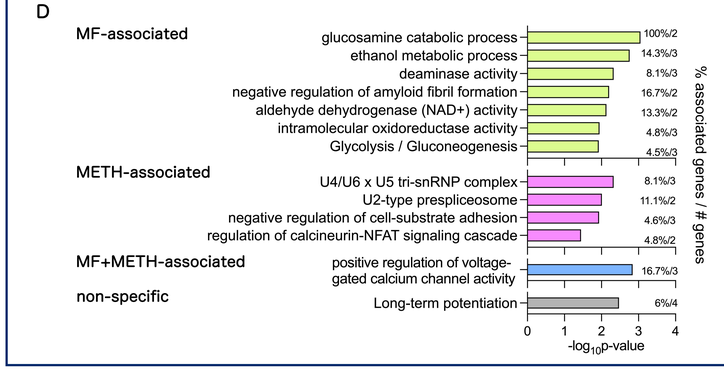



C: 146 were differentially expressed (foldchange > ±1.5 and p<.05).

D: Gene ontology (GO) terms associated with differentially expressed proteins.

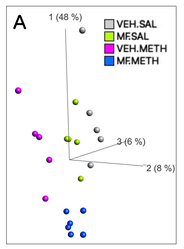


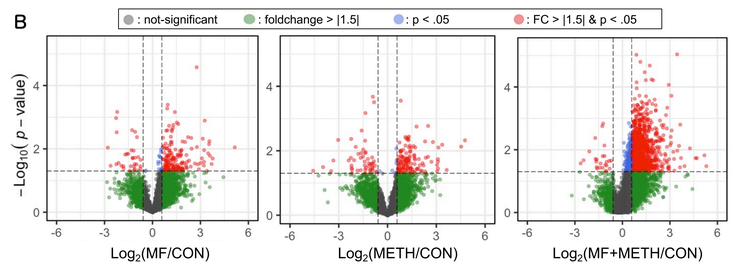




## 3: Phosphoproteome analysis revealed extensive effects of metformin that depend on METH

A: Phosphorylation changes in MF+METH group are distinct from other groups. The interaction between MF and METH treatment aligns along PC1. PC2 aligns with VEH/SAL against other 3 groups.

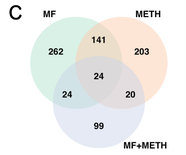
B: Change in expression relative to control group (VEH_SAL) for MF, METH, and MF+METH groups (left to right). Vertical line indicates expression ratio >|1.5|. Horizontal line indicates p=.05 (two-group comparison).

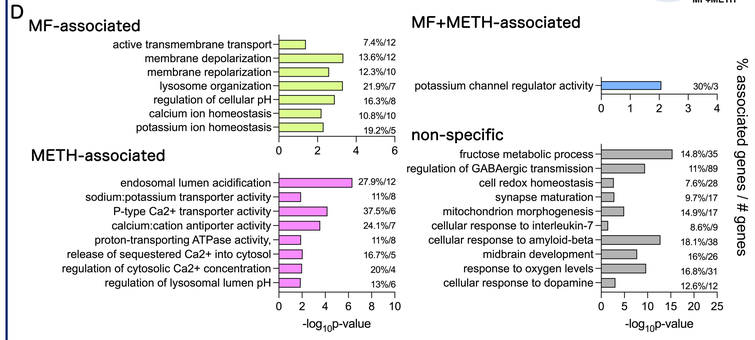


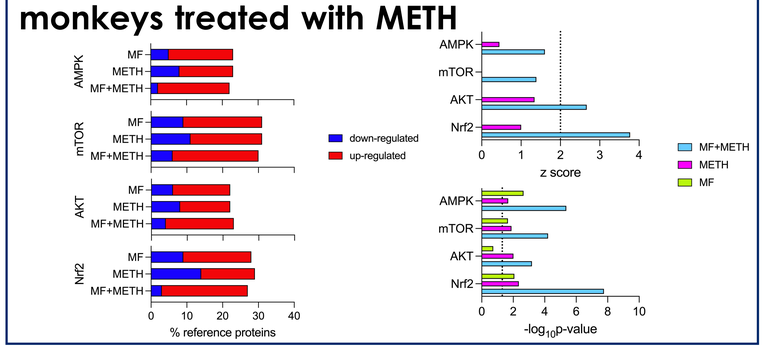

C: 773 proteins contained 1116 phosphopeptides that were differentially regulated (DR; foldchange > ± 1.5 and p < 0.05).

D: GO terms associated with DR proteins.

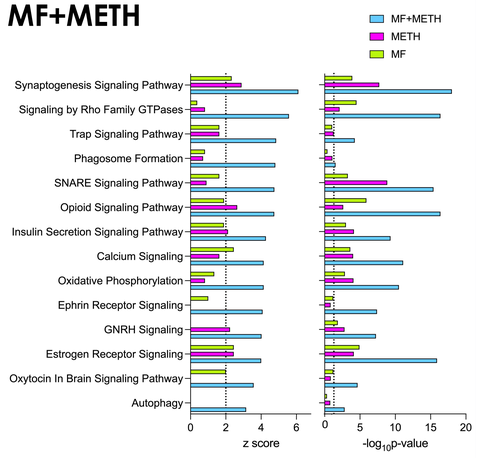



## 4: Metformin activated key pathways only in monkeys treated with METH




## 5: Pathways most activated by MF+METH




## Conclusions

Metformin's neuroprotective mechanisms depends on brain region and oxidative stress status.

These data suggest that Nrf2 and AKT signaling pathways contributed to protection against METH-induced oxidative stress in SN.

Other mechanisms that could be involved in metformin's neuroprotective activity include ephrin signaling, estrogen signaling, phagosome formation.

## References

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