Genes, Brain, and Behavior 2022, University of Tennessee Health Science Center, May 23-27th, 2022, Memphis, TN USA

Genetic screen and proteomic analysis: complementary approaches for studying methamphetamine-induced behaviors in *D. melanogaster*

Franka Rigo, Ana Filošević Vujnović and Rozi Andretić Waldowski

University of Rijeka, Department of Biotechnology, Laboratory for behavioral genetics, Radmile Matejčić 2, 51 000 Rijeka, Croatia, e-mail: randretic@biotech.uniri.hr

Introduction

Exposure to stimuli (addictive drugs) → Structural changes in the brain → Functional changes in the brain → Behavioral changes (LS)

Locomotor sensitization (LS) is an easily quantifiable behavior evident as an increased locomotor response that develops after repeated administration of psychostimulants. LS relates to intense craving in humans and identifying genes involved in LS in *Drosophila* has a big translational potential. Cellular mechanisms underlying LS are only partially elucidated and include neuroplastic processes which have lately been connected to redox modulation.

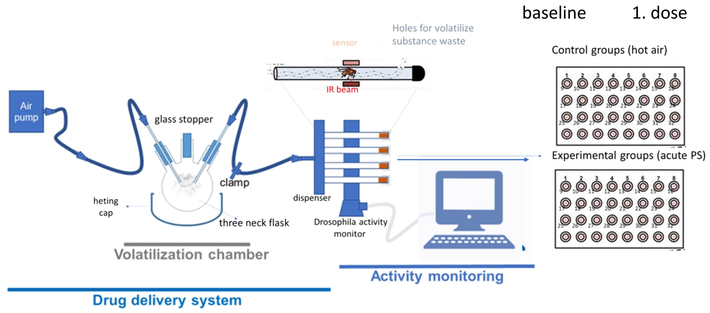
Aim:

To identify genes that regulate LS to methamphetamine (METH), with emphasis on genes that regulate redox homeostasis. To validate our results, we combined two approaches: genetic screen and proteomic analysis.

Methods

FlyBong

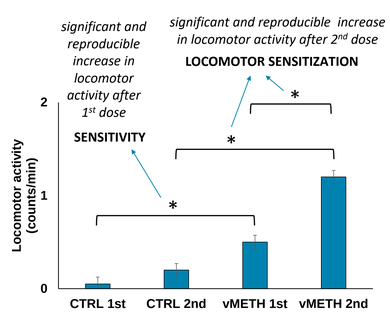
Measuring motor-activating effects of METH:



2 doses: **75 µl of vMETH (10mg/ml)**
Time interval between doses: **10 h**

Output:

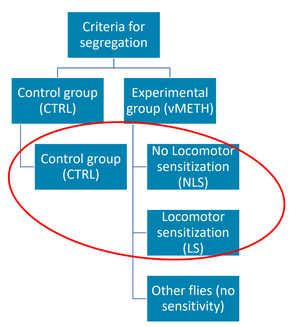
Change in locomotor activity measured 10 min after each dose



Approach1: Proteomics

phenotype LS or NLS → discover genes underlying phenotype

1. FlyBong test



2. Proteomic analysis

- Brain dissection
- Protein isolation and quantification
- Transport in Laemmli buffer
- 1D SDS-PAGE
- InGel digestion
- LC-MS/MS

Approach2: Genetic screen

known genes redox-related (UAS-RNAi) → phenotype Locomotor sensitization

1. UAS/Gal4 expression system

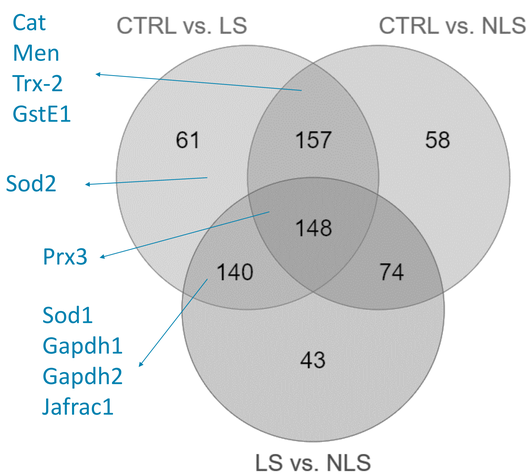
Tissue specific silencing of genes involved in redox regulation

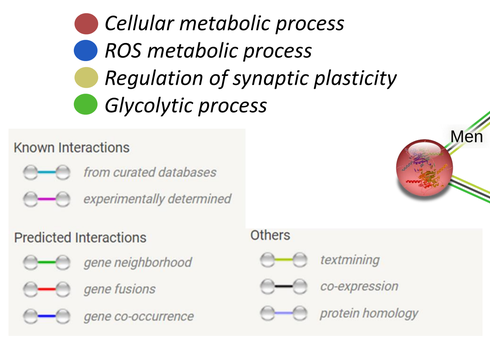
2. FlyBong test

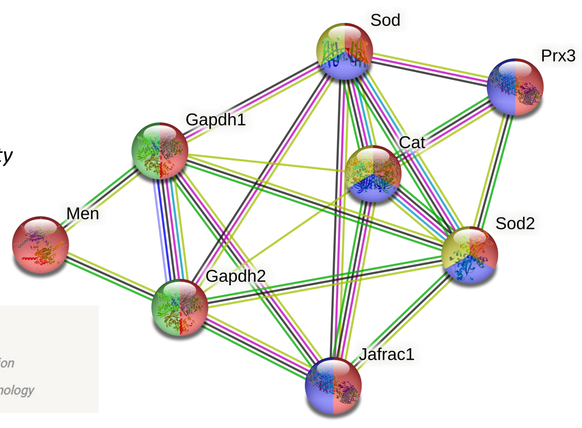
Results

Proteomics

Total proteins detected: 886
Significant: 681



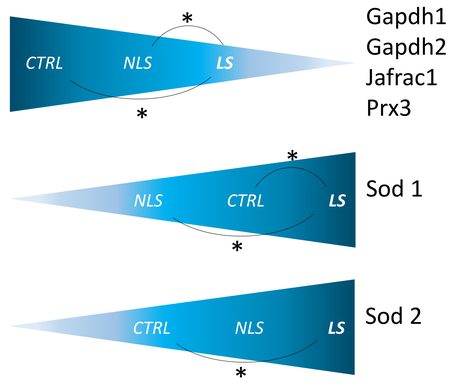




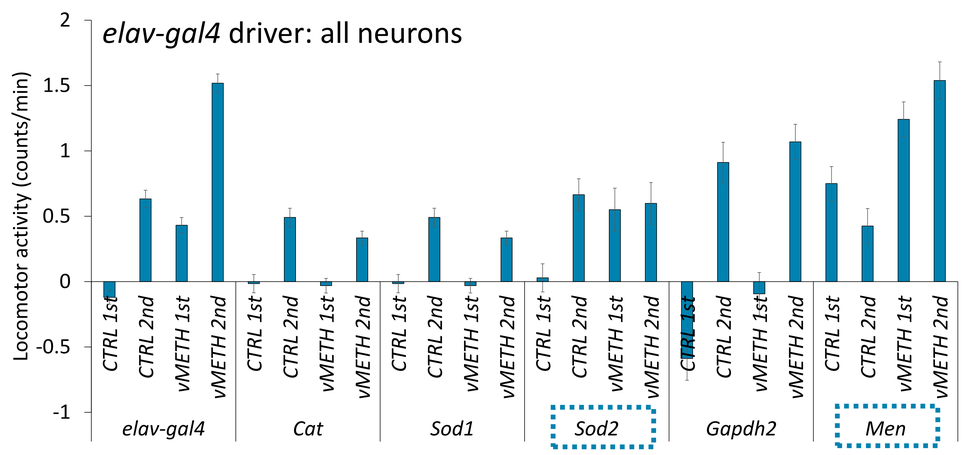
Drug administration:
↓ Cat, Men
↑ Trx-2, GstE1

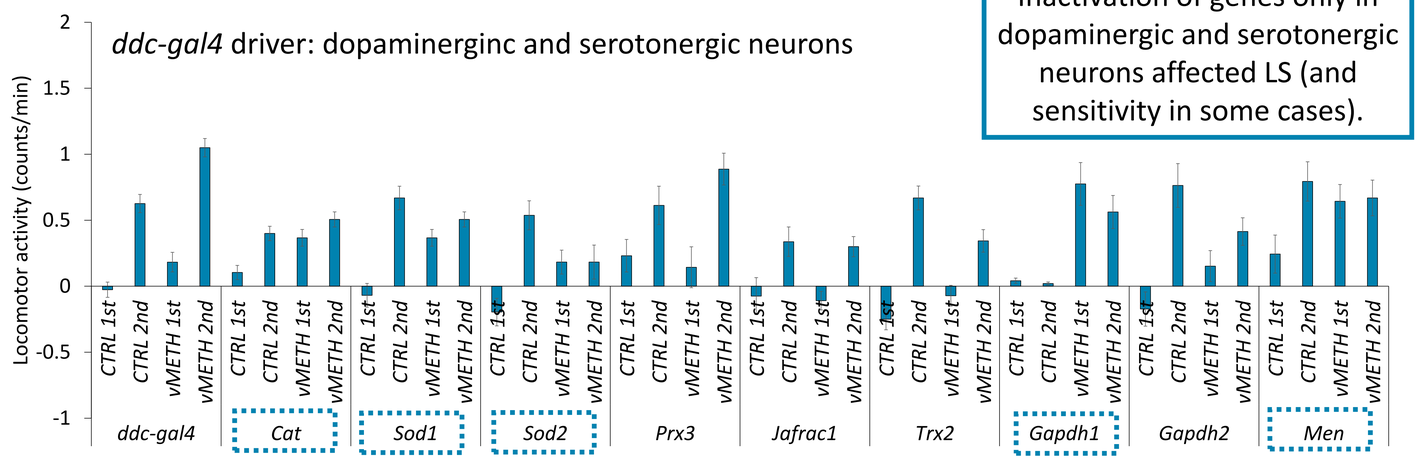
*no difference between LS and NLS

Locomotor sensitization (LS):
↓ Gapdh1, Gapdh2, Jafrac1, Prx3
↑ Sod1, Sod2



Genetic screen





Genes of interest

- Involved in LS
- When silenced, flies show sensitivity, but do not develop LS

Gene inactivation in the whole brain often resulted in lethality or other systemic effects.

Inactivation of genes only in dopaminergic and serotonergic neurons affected LS (and sensitivity in some cases).

This combined approach resulted in several predicted candidate genes, such as malic enzyme, glyceraldehyde-3-phosphate dehydrogenase, catalase, and superoxide dismutase.

Conclusion

We show involvement of redox-related genes in LS which suggests a possibility of targeting redox pathways in prevention or for the therapeutic intervention of addiction. Our next steps will include testing RNAi lines with the driver for only dopaminergic neurons and verification of other redox-related proteins identified by proteomics using Flybong

FINANCIAL SUPPORT: Croatian Science Foundation Research Grant # 2794; Doctoral Support Croatian Science Foundation 2019-22; University of Rijeka Research support # 1127. MENTOR: prof. Rozi Andretić Waldowski, PhD