

# Effect of caffeine on cell proliferation and key elements of neurotransmitter pathways in human neuroblastoma SH-SY5Y cell line

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## Introduction

- Caffeine is the most widely consumed stimulant in the world. It acts as a stimulant to the central nervous system mainly by antagonism of adenosine receptors.
- It has been detected in wastewater, surface water and groundwater worldwide, so there is a concern for its adverse impact on nontarget organisms, including wildlife and humans.
- Effects of wide range of concentrations of caffeine, including environmentally relevant, were investigated on human neuroblastoma cell line (SH-SY5Y) by:
  - ✓ sulforhodamine B (SRB) cytotoxicity/proliferation assay
  - ✓ MTT assay - activity of mitochondrial dehydrogenase
  - ✓ TMRE assay - mitochondrial membrane potential
  - ✓ gene expression analysis (RQ-PCR) – key elements of neurotransmitter pathways

## Results

### SRB and MTT assay

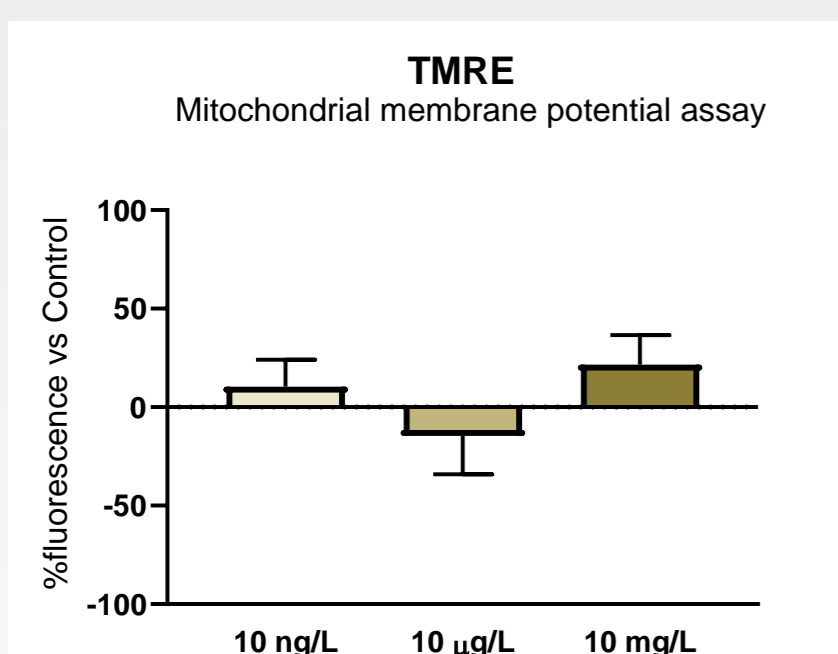
In a concentration range 1pg/L – 10mg/L (24h and 72h treatment):

- ✓ Caffeine did not disturb cell proliferation (SRB assay).
- ✓ Caffeine did not disturb the activity of mitochondrial dehydrogenase (MTT assay).

72h-treatment by 100 mg/mL induced 40% cytotoxicity and 48% inhibition of mitochondrial dehydrogenase vs control.



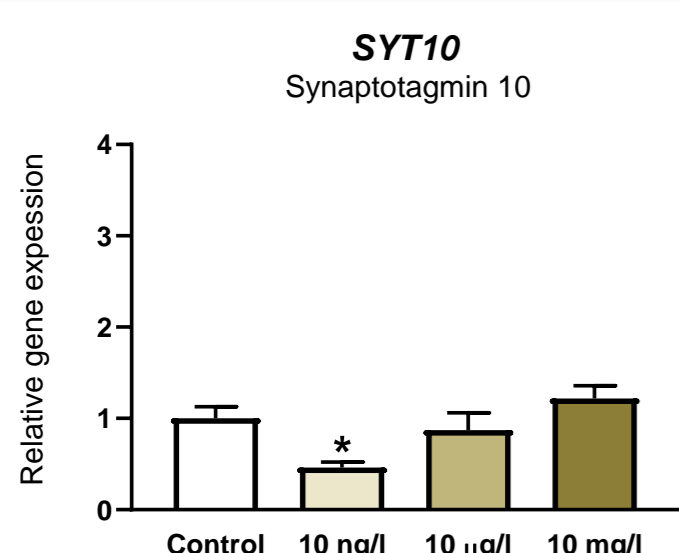
### TMRE assay



- ✓ Caffeine did not significantly disturb mitochondrial membrane potential (TMRE assay).

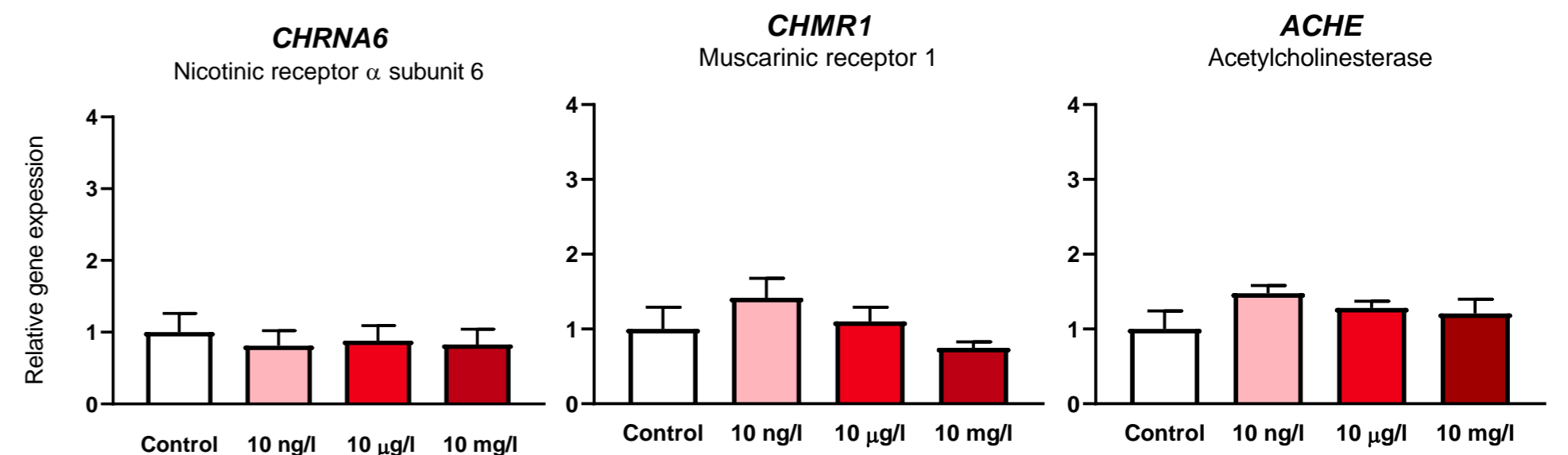
### Gene expression analysis – RQ-PCR

#### EXOCYTOSIS OF NEUROTRANSMITTERS



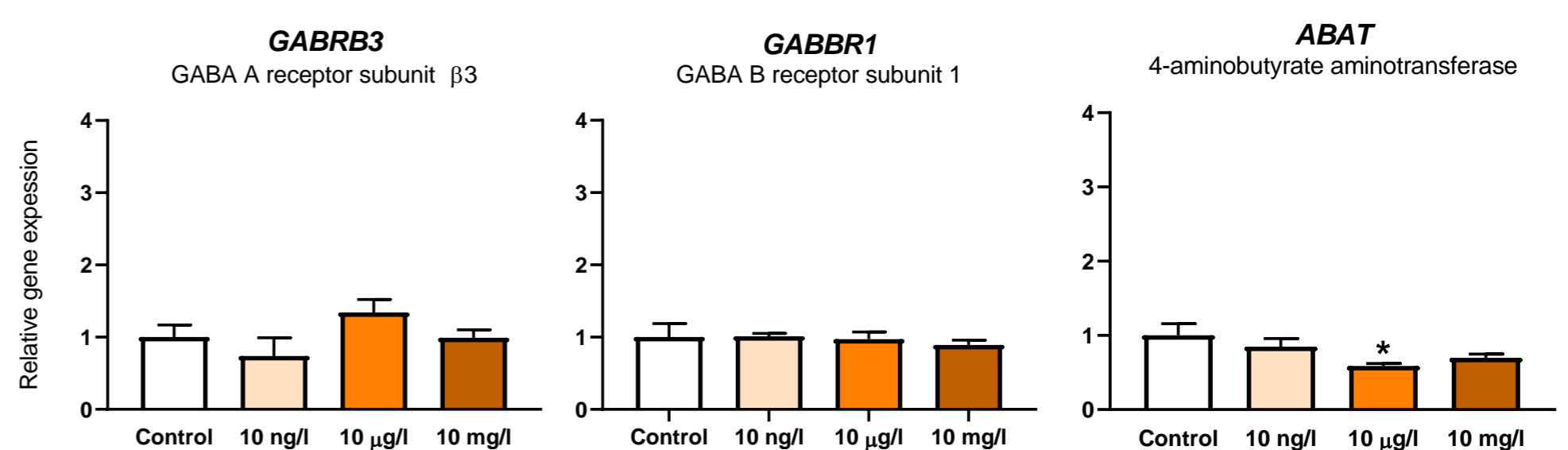
- ✓ Significant downregulation at 10 ng/L of genes encoding protein involved in exocytosis of neurotransmitters – synaptotagmin 10 (SYT10).

#### ACETYLCHOLINE PATHWAY



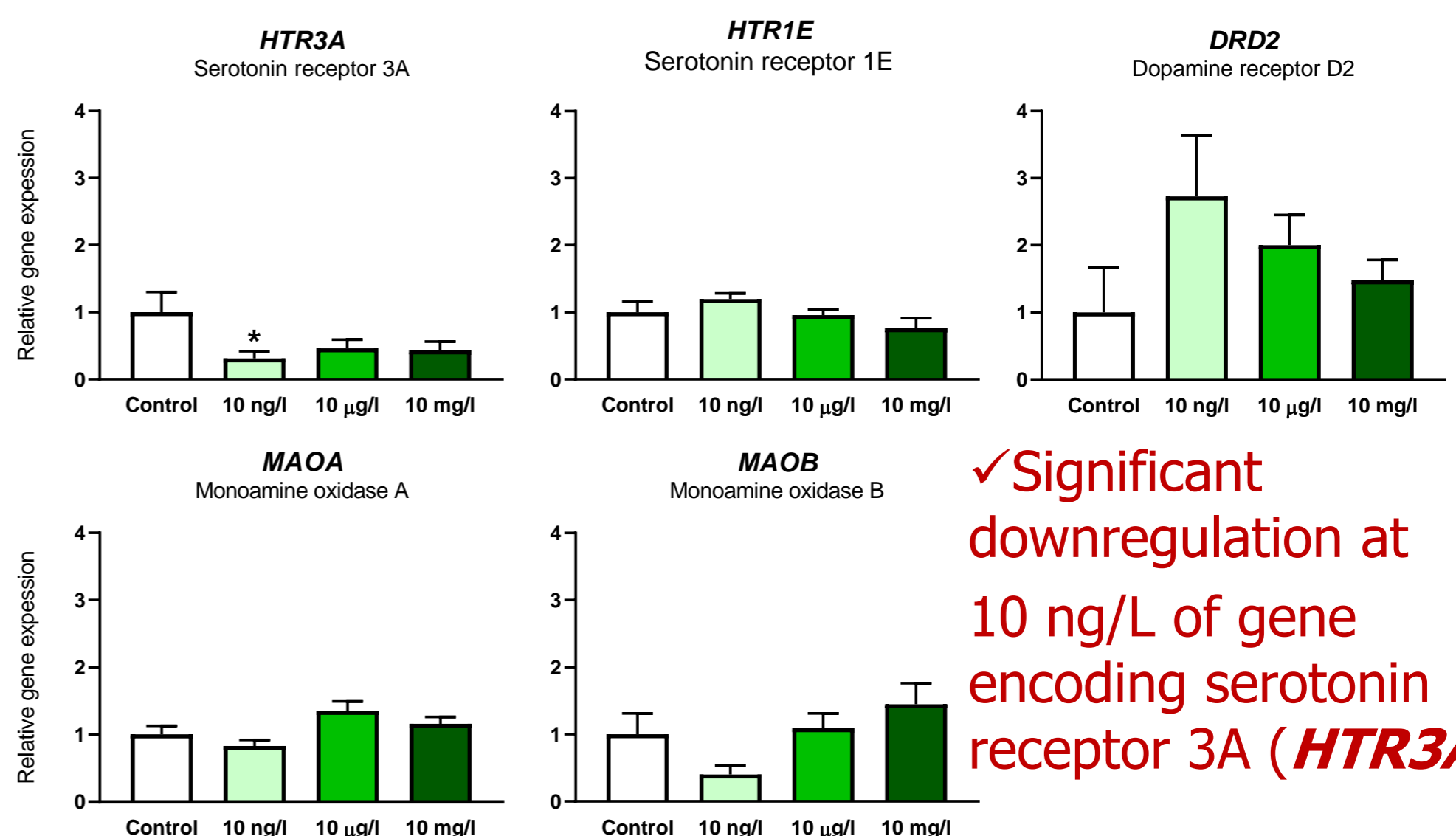
- ✓ Caffeine showed no effect on gene expression of acetylcholine pathway key elements.

#### GABA PATHWAY



- ✓ Significant downregulation at 10 µg/L of gene encoding enzyme 4-aminobutyrate aminotransferase (ABAT), responsible for the removal of GABA from the synaptic cleft.

#### SEROTONIN AND DOPAMINE PATHWAY



- ✓ A trend of upregulation of dopamine receptor D2 (DRD2) and downregulation of monoamine oxidase B (MAOB) was also noticed.

## Concluding remarks

The results imply to the elements of neurotransmitter pathways responsive to caffeine exposure (SYT10, ABAT, HTR3A) and represent a contribution to the mechanistic knowledge on caffeine effects on humans, other than its primary mode of action. They also represent a contribution to the development of new biomarkers of effects of neuroactive compounds that could be used in characterisation of contaminants in complex environmental mixtures.

## Acknowledgements

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