Results: Immobilisation time (IT) in FST after the administration of imipramine was shorter than the control, same as for subgroups treated with AE I, II and VS. In the subgroup treated with fluoxetine, IT in TST was shorter than the control time, and the same was observed in subgroups treated with AE I, II and VS.

Significant binding energies were found for Serotonin Reuptake Transporter (SERT) and verbenalin (−7.20 kcal/mol) and verbascoside (−6.61 kcal/mol), and for the Leucine Transporter (LeuT), the homologue of the noradrenaline reuptake transporter, and verbenalin (−6.27 kcal/mol) and caffeic acid (−5.85 kcal/mol).

Conclusion: In both pharmacodynamic tests the antidepressive effect of AE and VS has been confirmed. Verbenalin and verbascoside binding energies and poses in interaction with SERT were similar to those of paroxetine. For LeuT, verbenalin showed both a similar binding energy and pose to that of imipramine, whereas caffeic acid showed only a similar binding energy.1-4

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PS213
Thermal denaturation profiles of proteome and blood serum of rats with drug-induced dementia. A DSC study
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Aim: The aim of this study is to evaluate the effect of scopolamine on different brain segments using DSC.

Introduction: In this work, probes from different brain segments of rats with drug-induced dementia were characterized by differential scanning calorimetry (DSC) and their thermodynamic properties were determined.

Methods: Male Wistar rats were injected with scopolamine for 14 consecutive days in order to induce drug model of dementia. After being decapitated, their brains were divided into the following segments: telencephalon, mesencephalon and cerebellum. Afterwards, the brain supernatants of the latter 3 segments were examined by DSC and compared with the controls.

Results: The DSC measurements revealed large differences between the denaturation profiles of rat brain supernatants and blood serum. The thermograms of brain tissues displayed clearly expressed low-temperature exothermic transitions with peaks in the range 35–45 °C which are missing in blood serum samples. There were differences between the thermograms of the separate brain segments as well. The thermodynamic parameters of the denaturation profiles were also determined.

Conclusion: These measurements show that DSC is an appropriate method with great potential for detection and characterization of the changes taking place at molecular level in different tissues, especially in brain tissues affected by neurodegenerative disorders.

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PS231
Effects of Vitamin D on the expression of markers of principal neurons, interneurons and astrocytes in cerebral cortex and hippocampus in gerbils exposed to transient global cerebral ischemia
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Aim: Examination of the effects of vitamin D pretreatment on the expression of markers of principal neurons (NeuN), inhibitory interneurons (PV) and astrocytes (GFAP) in cerebral cortex and hippocampus in gerbils who were exposed to transient global cerebral ischemia.

Introduction: Brain ischemia may cause serious damage to the cells in the central nervous system. Vitamin D has an important role in brain injury treatment due to its neuroprotective effects.

Methods: Gerbils were divided in 5 groups: control group; two groups that underwent ischemia and then reperfusion for three (I/R3d) and seven days (I/R7d) and two groups that were treated with vitamin D before I/R (vitD + I/R3d and vitD + I/R7d). Complete blood supply to the brain was cut off for 10 minutes and reperfusion lasted 3 and 7 days. They were daily treated with vitamin D for 7 days prior ischemia. Expression of proteins was detected using Western blot.

Results: No changes were detected in expression of NeuN markers in cortex of experimental groups, while there was increase in expression in hippocampus in groups I/R7d and vitD + I/R7d in comparison to the control group and group vitD + I/R3d. Expression of PV in cortex was significantly reduced in group I/R7d in comparison to group I/R3d, whereas in hippocampus the expression was significantly higher in group vitD + I/R3d than in group I/R3d. Expression of GFAP has significantly risen in all groups in comparison to the control group whereas in hippocampus there was a rise in groups vitD + I/R3d, I/R7d and vitD + I/R7d in comparison to the control group. There was also a rise of GFAP expression in groups treated with vitamin D (vitD + I/R3d and vitD + I/R7d) in comparison to those that have not been treated (I/R3d, I/R7d).

Conclusion: Vitamin D has positive effect on astrocytes in both structures of gerbils that underwent global cerebral ischemia, especially in hippocampal region.

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PS238
Identification of genetic modifiers of somatic CAG instability in Huntington’s Disease by in vivo CRISPR – Cas9 genome editing
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Introduction: In this work, probes from different brain segments of rats with drug-induced dementia were characterized by differential scanning calorimetry (DSC) and their thermodynamic properties were determined.

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Results: The DSC measurements revealed large differences between the denaturation profiles of rat brain supernatants and blood serum. The thermograms of brain tissues displayed clearly expressed low-temperature exothermic transitions with peaks in the range 35–45 °C which are missing in blood serum samples. There were differences between the thermograms of the separate brain segments as well. The thermodynamic parameters of the denaturation profiles were also determined.

Conclusion: These measurements show that DSC is an appropriate method with great potential for detection and characterization of the changes taking place at molecular level in different tissues, especially in brain tissues affected by neurodegenerative disorders.

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Methods: Gerbils were divided in 5 groups: control group; two groups that underwent ischemia and then reperfusion for three (I/R3d) and seven days (I/R7d) and two groups that were treated with vitamin D before I/R (vitD + I/R3d and vitD + I/R7d). Complete blood supply to the brain was cut off for 10 minutes and reperfusion lasted 3 and 7 days. They were daily treated with vitamin D for 7 days prior ischemia. Expression of proteins was detected using Western blot.

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Conclusion: Vitamin D has positive effect on astrocytes in both structures of gerbils that underwent global cerebral ischemia, especially in hippocampal region.

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PS238
Identification of genetic modifiers of somatic CAG instability in Huntington’s Disease by in vivo CRISPR – Cas9 genome editing
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2 Center for Genomic Medicine, Massachusetts General Hospital
Aim: To develop an experimental platform for in vivo investigation of candidate genetic modifiers of somatic CAG instability in Huntington’s disease.

**Introduction:** Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG repeat expansion within the huntingtin gene (HTT). Despite being a monogenic disorder, for which the mutation has been known for some time now, no cure or disease-modifying therapy is available, indicating that novel approaches are critical.

Somatic CAG repeat instability, characteristic of mutant HTT alleles, is inversely correlated with patient age of onset and may contribute to HD pathogenesis. This phenotype, common to other trinucleotide repeat disorders, was previously shown to be DNA mismatch repair (MMR) dependent. The DNA repair machinery was further implicated as a modifier of HD age of motor onset in a recent genome wide association study, underlining its promise as a relevant disease mechanism that could potentially be therapeutically targeted.

In this study, we are developing a CRISPR/Cas9-based approach that will enable the investigation of candidate genetic modifiers of HD age of onset as potential modifiers of somatic CAG repeat instability in a HD mouse model.

**Methods:** We have developed CRISPR reagents against known and candidate genetic modifiers of somatic CAG instability in Huntington’s disease. In preliminary experiments, we treated HD mice with CRISPR reagents against Mlh1 and investigated the level of gene editing achieved as well as the impact on liver CAG instability.

**Results:** We were able to significantly suppress the CAG expansion process in the liver of HD mice by knocking out the Mlh1 gene in our in vivo CRISPR platform. The efficiency achieved in modifying the instability phenotype makes us very confident that we will be able to test and validate additional candidate modifiers. To that end, we have already validated reagents for efficient knockout of a subset of known and candidate modifier genes and we have developed assays that will allow detailed characterization of gene editing at these sites.

**Conclusion:** We have successfully developed an in vivo CRISPR-Cas9-based platform that allows for knocking out genes of interest in the liver of adult mice, and consequently perturb the somatic CAG expansion process. We will next use this tool to test the role that candidate genes might play in that disease-relevant process. While the scope of this project was liver oriented, future work will also be aimed at targeting the striatum which is the main site of HD-related pathology.

**References**


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**PS024**

The effect of prenatal Vitamin C deficiency on endochondral ossification in guinea pigs

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Aim: The aim of the research is to investigate the effect of prenatal vitamin C deficiency on endochondral ossification in guinea pigs.

**Introduction:** Vitamin C is an essential nutrient which inter alia enables the synthesis of collagen and therefore endochondral ossification. Throughout years a lot of research has been published investigating the exact role of vitamin C and the impairment developed due to its deficiency. However there is insufficient data about the effect of prenatal deficit of vitamin C on the developing bone structures.

**Methods:** The study encompassed 14 fertilized female albino guinea pigs. Their diet was comprised of vitamin C-free food and ad libitum water enriched with vitamin C. The 10th day of fertilization, experimental group was depleted of vitamin C. Deprivation lasted until the 50th day, after which the females were sacrificed and their fetuses were taken out. Forelegs of fetuses were fixed and dehydrated, after which they were embedded in paraffin and