Homocysteine and homocysteine thiolactone contribute to Alzheimer's disease via TAU modifications in N2A-APPswe cells

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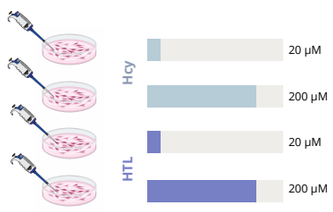
Introduction

Homocysteine (Hcy), derived from methionine, is a risk factor for several diseases, including Alzheimer's disease (AD). Hcy can be converted to homocysteine thiolactone (HTL), which modifies proteins and may promote their damage and misfolding.

Patients with AD show elevated Hcy[1], reduced HTL-hydrolyzing activity[2], and characteristic amyloid-β and TAU deposition. TAU hyperphosphorylation and acetylation are closely linked to its aggregation, but the mechanistic roles of Hcy and HTL in AD development and progression remain unclear.

Aim & methods

Aim of study: to test how Hcy and HTL promote TAU accumulation *via* hyperphosphorylation and acetylation of TAU in mouse neuroblastoma N2a-APPswe cells.



Cell model: N2A-APPswe mouse neuroblastoma cells expressing mutant human APP (Swedish mutation)

Treatment: 20 or 200 µM Hcy or HTL

Incubation: 24 h, methionine-free medium

Analysis: confocal microscopy using anti-Phospho-TAU (Ser396, Thr205), anti-Acetyl-Tau (Lys174), anti-TAU; Western blot (anti-GSK3α, anti-GSK3β, anti-CDK5)

Results

HCY AND HTL LEAD TO AGGREGATION OF HYPERPHOSPHORYLATED TAU

I) at **Ser396**

- The strongest Phospho-TAU (Ser396) accumulation was observed after treatment with 200 µM Hcy, followed by 200 µM HTL *(Fig. 1)*.
- Higher metabolite concentrations led to increased Phospho-TAU (Ser396) signal count, area and size, indicating dose-dependent Phospho-TAU (Ser396) accumulation in cells after Hcy and HTL treatment *(Fig. 1)*.

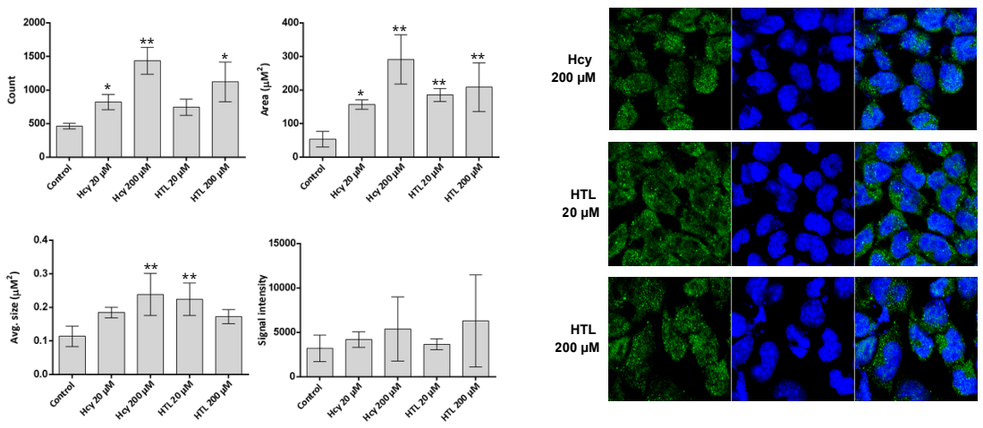


*Fig. 1 Confocal microscopy images of Phospho-TAU (Ser396) signals from N2a-APPswe cells treated with Hcy or HTL. Bar graphs show quantification of signals. Data are mean ± standard deviation (SD) values from three biologically independent experiments. * p<0.05, **p<0.001*

II) at **Thr205**

- At Thr205, the number of Phospho-TAU (Thr205) signals is reduced at high Hcy and both HTL concentrations *(Fig. 2)*.
- Three treatments (Hcy 200 µM and both HTL concentrations) increase the average signal size, with the strongest enlargement observed for 200 µM Hcy. This results from signals blending together and indicates strong Phospho-TAU (Thr205) aggregation *(Fig. 2)*.

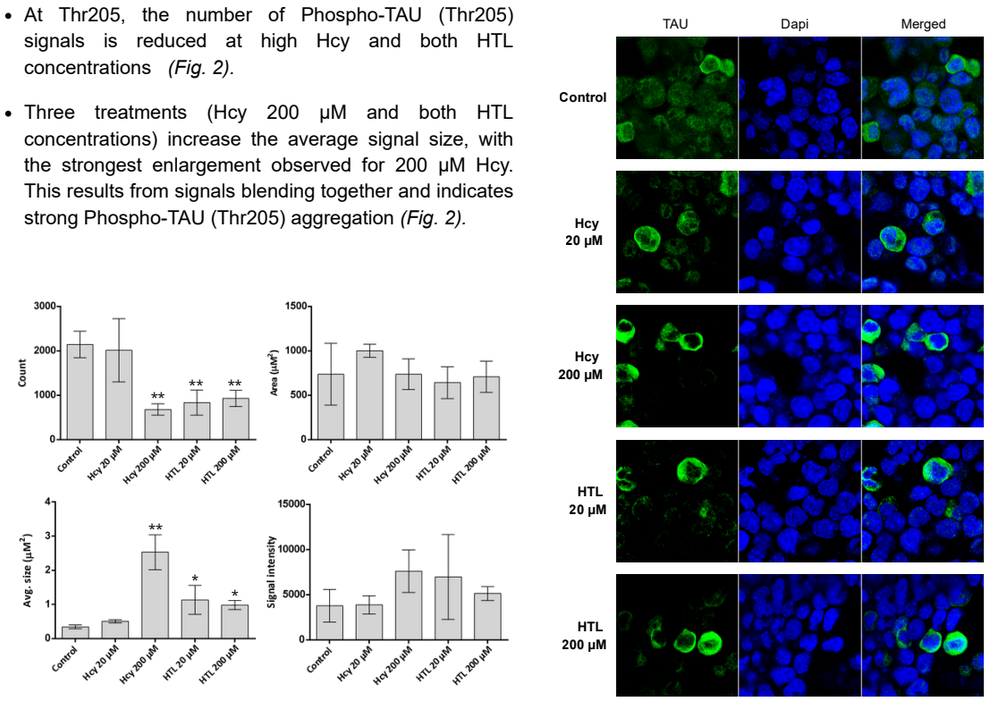
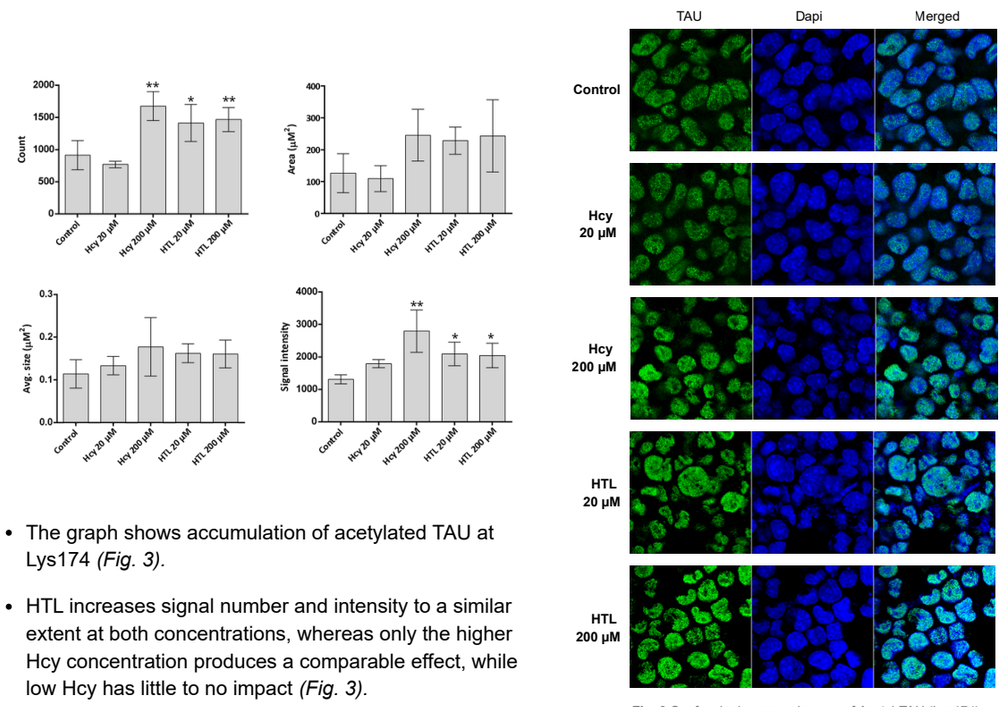


*Fig. 2 Confocal microscopy images of Phospho-TAU (Thr205) signals from N2a-APPswe cells treated with Hcy or HTL. Bar graphs show quantification of signals. Data are mean ± standard deviation (SD) values from three biologically independent experiments. * p<0.05, **p<0.001*

HCY AND HTL LEAD TO AGGREGATION OF ACETYLATED TAU



- The graph shows accumulation of acetylated TAU at Lys174 *(Fig. 3)*.
- HTL increases signal number and intensity to a similar extent at both concentrations, whereas only the higher Hcy concentration produces a comparable effect, while low Hcy has little to no impact *(Fig. 3)*.

*Fig. 3 Confocal microscopy images of Acetyl-TAU (Lys174) signals from N2a-APPswe cells treated with Hcy or HTL. Bar graphs show quantification of signals. Data are mean ± standard deviation (SD) values from three biologically independent experiments. * p<0.05, **p<0.001*

TOTAL TAU PROTEIN

Hcy nad HTL did not affect total TAU protein level *(Fig. 4)*.

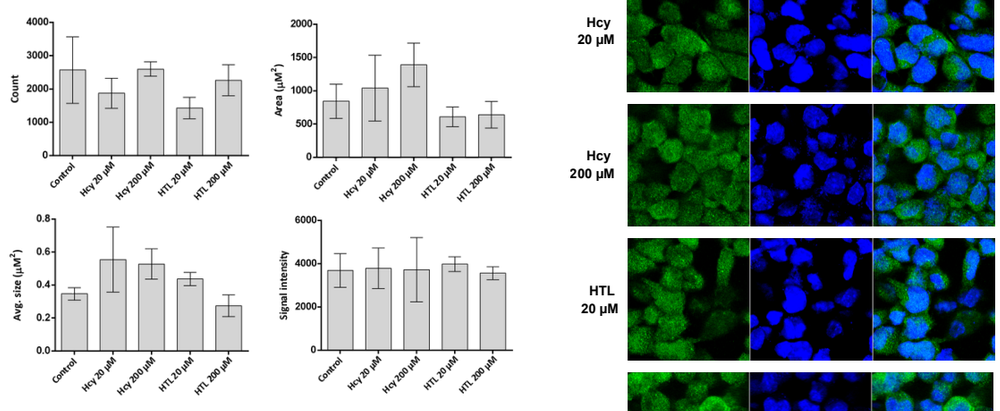
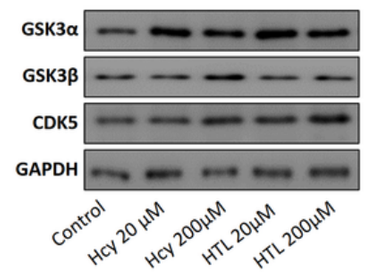


*Fig. 4 Confocal microscopy images of TAU signals from N2a-APPswe cells treated with Hcy or HTL. Bar graphs show quantification of signals. Data are mean ± standard deviation (SD) values from three biologically independent experiments. * p<0.05, **p<0.001*

AFFECTED ENZYMES INVOLVED IN TAU MODIFICATIONS



- GSK3α levels were elevated the most across all treatments *(Fig. 5)*.
- GSK3β levels were highest after treatment with 200 µM Hcy (Fig. 5).
- CDK5 levels were increased by both Hcy and HTL, with no significant differences between the two metabolites, apart from a minor effect at 20 µM Hcy *(Fig. 5)*.

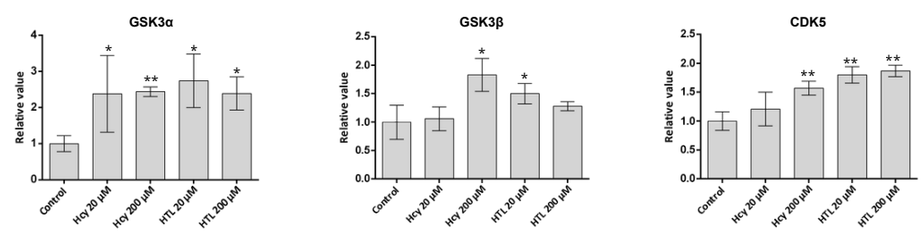


*Fig. 5 Representative western blot pictures and quantifications of western blot analysis of selected TAU kinases after treatments of N2A-APPswe cells with Hcy and HTL. * p<0.05, **p<0.001*

Conclusions

- Treatment with **Hcy or HTL** affects **TAU modifications**, thereby promoting **Phospho-TAU (Ser396, Thr205) and Acetyl-TAU (Lys174) aggregation**.
- Hyperphosphorylation of TAU results from **elevated levels of TAU kinases (CDK5, GSK3α)**, which are upregulated by Hcy and HTL treatments.

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