Final Report

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Title of Project:

Mechanisms mediating the therapeutic effects of N-Acetylcysteine in neurodegenerative disease

Summary: (approximately 1,000 words)

Huntington's disease (HD) is a fatal disorder involving psychiatric symptoms, dementia and uncontrollable movements. The disease is caused by a 'genetic stutter' in a single stretch of repeated DNA. One extraordinary aspect of this disease is that HD sufferers often only have a handful of extra glutamines in their huntingtin protein, in a protein made up of over 3,000 amino acids, amongst tens of thousands of different proteins encoded by the human genome. Thus, in such inherited disorders, it is an extremely fine line between health and a devastating disease that eventually kills the patients. At present, there is no treatment available which directly slows or stops the disease progression. Therefore, research facilitating the development of new treatments is desperately needed.

Over the past decade, our laboratory has characterized a mouse model of HD which has had the gene mutation inserted into the genome. We have been able to show that cognitive and affective changes precede the movement disorder. The current project followed up our recent discovery that a drug called N-acetylcysteine (NAC) has therapeutic effects in this animal model of HD (Wright et al., 2015, Translational Psychiatry). We investigated how this drug works in the brain. In order to do this we have been using behavioural testing, combined with studies of specific cell populations and molecules, to work out how the drug exerts its beneficial effects.

We found that N-acetylcysteine modulated glutamatergic dysfunction and depressive behavior in Huntington's disease (Wright et al., Hum Mol Genet.). We demonstrated that the R6/1 transgenic mouse model of HD has lower basal levels of cystine. Furthermore, NAC was able to rescue changes in key glutamate receptor proteins related to excitotoxicity in HD, including NMDAR2B. Overall, we have shown that baseline reductions in cysteine underlie glutamatergic dysfunction and depressive-like behavior in HD and these changes can be rescued by treatment with NAC.

Hypothesis vs Findings

Our main hypothesis that NAC would rescue depressive-like behaviours was verified by our data. However, unexpectedly, it seems like NAC was not able to rescue the cognitive deficit.

Unanswered Questions

Based on our findings, we now think that NAC is also acting on a recently discovered pathway called ‘ferroptosis’. The NAC data were the basis on a NHMRC Project Grant which has been funded in 2017.

What these research outcomes mean

The success of this project could rapidly lead to clinical trials for HD, in Australia and internationally. HD is a fatal disease which is currently incurable, so any new treatment would have enormous impacts on HD families across Australia and around the world. Furthermore, HD has many aspects in common with motor neuron disease, Parkinson’s disease, Alzheimer’s disease and other forms of dementia.

Please include any appropriate photos or diagrams.

From Wright et al 2016, Hum Mol Genet.

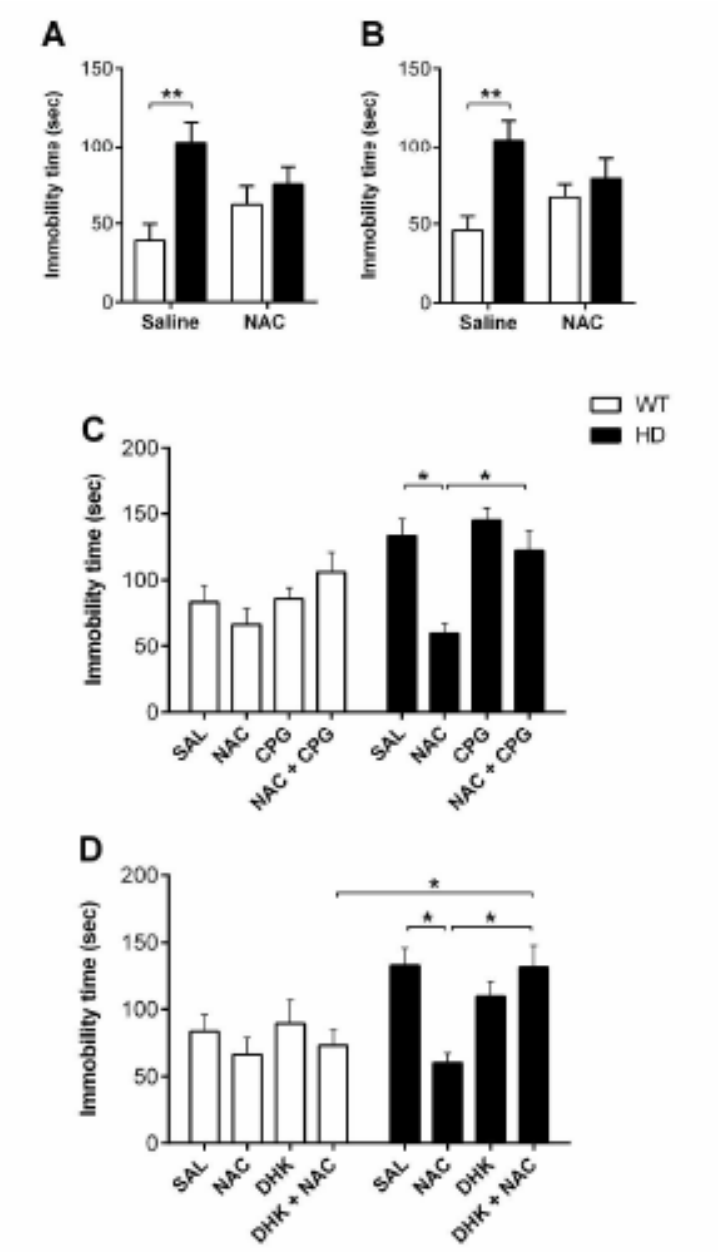

Figure 1. The antidepressant effect of NAC is mediated by glutamate transporters. A) Acute and B) chronic treatment with NAC (500 mg/kg/day) has an antidepressant-like effect on HD mice in the forced-swim test. For both acute and chronic tests, a significant interaction between genotype and treatment was found. Post-hoc tests revealed that HD-saline mice spent more time immobile than WT-saline mice. No other groups displayed a difference. C) The system xc- inhibitor, CPG (200 mg/kg) was then co-administered with NAC prior to the FST. A significant genotype-by-treatment interaction was found. Post-hoc tests revealed that NAC-HD mice showed less immobility compared to Saline-HD mice. NAC-HD mice did not differ from Saline-WT mice on levels of immobility. The addition of CPG to NAC treatment ablated much of the effect of NAC, such that the immobility of NAC+CPG-HD mice was significantly higher than the NAC-HD mice and did not differ from Saline-HD mice. D) NAC (500 mg/kg) was co-administered with the GLT-1- inhibitor, DHK (20 mg/kg) prior to administering the FST. A significant genotype effect and a significant treatment effect were found. Post-hoc tests revealed that NAC-HD mice showed less immobility compared to Saline-HD mice. NAC-HD mice showed levels of immobility that did not differ from Saline-WT mice. The addition of DHK ablated

New hypothesis resulting from our findings.

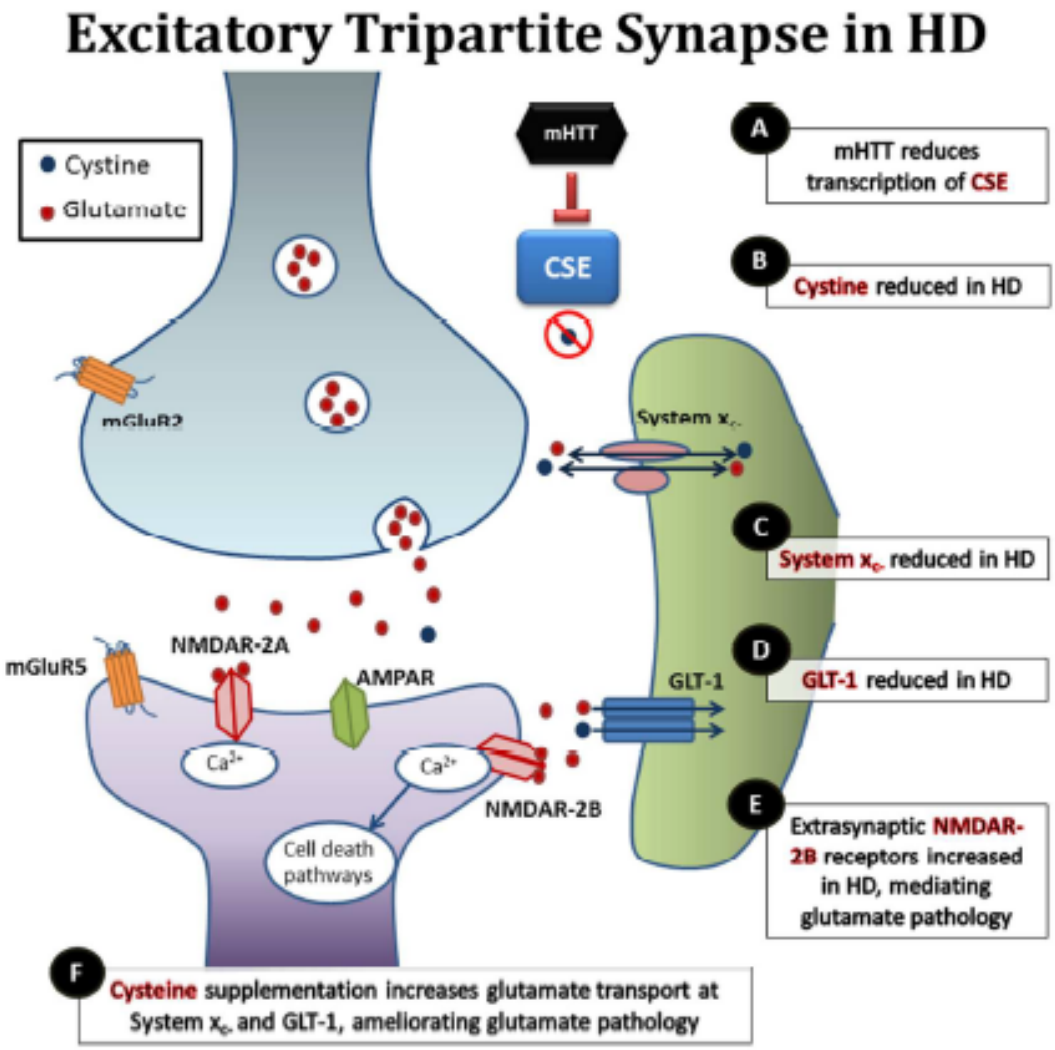

Figure 5. A proposed model of pathogenesis mediating abnormal glutamate homeostasis and dysfunction of excitatory tripartite synapses. A) Mutant huntingtin inhibits transcription of cystathionine-γ-lyase (CSE), B) leading to reductions in extracellular cysteine and cystine. Lower cystine disrupts transport of glutamate through both system xc- and GLT-1. Specifically, C) cystine is not taken up into astrocytes through system xc-, reducing export of glutamate, whilst D) glutamate is not removed from the extracellular space via GLT-1. E) Increased extrasynaptic glutamate is then able to activate aberrant NMDAR2B, leading to glutamate-related pathologies. F) Supplementing cysteine using NAC, increased synaptic glutamate transport via GLT-1 and system xc-.