OXFORD

Human Molecular Genetics, 2016, Vol. 25, No. 14 2923–2933

doi: 10.1093/hmg/ddw144
Advance Access Publication Date: 14 May 2016
Original Article

ORIGINAL ARTICLE

N-acetylcysteine modulates glutamatergic dysfunction and depressive behavior in Huntington's disease

Dean J. Wright[1,2], Laura J. Gray[1,2,*], David I. Finkelstein[1], Peter J. Crouch[1,3], David Pow[4], Terence Y. Pang[1], Shanshan Li[1], Zoe M. Smith[5], Paul S. Francis[5], Thibault Renoir[1,†] and Anthony J. Hannan[1,6,†]

[1]Florey Institute of Neuroscience and Mental Health, University of Melbourne, 30 Royal Pde, Parkville, Victoria 3010, Australia, [2]School of Medicine, Faculty of Health, Deakin University, Locked Bag 20000, Geelong, Victoria 3220, Australia, [3]Department of Pathology, University of Melbourne, Parkville, Victoria 3010, Australia, [4]The University of Queensland Centre for Clinical Research, Queensland 4029, Australia, [5]Centre for Chemistry and Biotechnology, School of Life and Environmental Sciences Faculty of Science, Engineering and Built Environment, Deakin University, Geelong, Victoria 3220, Australia and [6]Department of Anatomy and Neuroscience, University of Melbourne, Parkville, Victoria 3010, Australia

*To whom correspondence should be addressed at: School of Medicine, Faculty of Health, Deakin University, Locked Bag 20000, Geelong, VIC 3220, Australia. Tel: +61 3 5227 2852; Email: l.gray@deakin.edu.au

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

Abstract

Glutamatergic dysfunction has been implicated in the pathogenesis of depressive disorders and Huntington's disease (HD), in which depression is the most common psychiatric symptom. Synaptic glutamate homeostasis is regulated by cystine-dependent glutamate transporters, including GLT-1 and system x_c^-. In HD, the enzyme regulating cysteine (and subsequently cystine) production, cystathionine-γ-lygase, has recently been shown to be lowered. The aim of the present study was to establish whether cysteine supplementation, using *N*-acetylcysteine (NAC) could ameliorate glutamate pathology through the cystine-dependent transporters, system x_c^- and GLT-1. We demonstrate that the R6/1 transgenic mouse model of HD has lower basal levels of cystine, and showed depressive-like behaviors in the forced-swim test. Administration of NAC reversed these behaviors. This effect was blocked by co-administration of the system x_c^- and GLT-1 inhibitors CPG and DHK, showing that glutamate transporter activity was required for the antidepressant effects of NAC. NAC was also able to specifically increase glutamate in HD mice, in a glutamate transporter-dependent manner. These *in vivo* changes reflect changes in glutamate transporter protein in HD mice and human HD post-mortem tissue. Furthermore, NAC was able to rescue changes in key glutamate receptor proteins related to excitotoxicity in HD, including NMDAR2B. Thus, 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. These findings have implications for the development of new therapeutic approaches for depressive disorders.

[†]These authors contributed equally.

Received: April 2, 2016. **Revised:** April 2, 2016. **Accepted:** May 9, 2016

© The Author 2016. Published by Oxford University Press.
All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

Introduction

Huntington's disease (HD) is a severe neurodegenerative disorder, inflicting a triad of psychiatric, cognitive and motor symptoms for over half the patient's life. The most common psychiatric symptom is depression (1). There are currently no available disease-modifying treatments for HD. Glutamate-related pathology has been implicated as one of the major sources of cell dysfunction and death in HD (2). However, the mechanism whereby neurons in HD are more prone to glutamate toxicity remains unclear. By elucidating this mechanism, we can seek out therapeutic targets that alleviate this core pathology in HD.

Animal models of HD display abnormally increased excitation in response to exogenous *N*-methyl-D-aspartate (NMDA), but not AMPA administration, implying that the HD genotype confers a selective NMDA receptor (NMDAR) sensitivity (3,4). This NMDAR sensitivity is dependent on 'extrasynaptic' receptor activation (5). In HD models, over-activation and subsequent toxicity was seen when extrasynaptic NMDARs, primarily those containing the 2B subunit, were stimulated (6).

Preclinical and clinical data indicates that NMDAR dysregulation is also an important pathological mechanism in depression (7). Accumulating evidence points to the acute and sustained antidepressant effects of agents such as ketamine, which act at NMDA receptors (8). This effect is mediated by 2B-containing receptors (9), thus providing a convergence in mechanisms between major depressive disorder and HD. However, in the context of HD, it is still unclear what drives the spread of glutamate beyond the synapse to activate extrasynaptic NMDARs.

Glutamate is removed from the synapse through a variety of Na^+-dependent and independent transporters, including GLAST, GLT-1 and system x_c^-. Dysfunction of these transporters, expressed on neurons and glial cells, may contribute to extrasynaptic spill-over of glutamate. GLAST mRNA or expression has not been shown to change in HD mice (10). However, GLT-1 mRNA and protein expression have been found to be reduced in the cortex and striatum of R6 mice after the onset of motor abnormalities (10–13). Although HD mice did not show baseline deficits in extracellular glutamate, they displayed decreased uptake through GLT-1 (10,11). Decreased expression of the light chain of system x_c^- has been shown in the striatum of 6-week-old R6/2 HD mice, and this was associated with decreased glutamate uptake in HD striatal cells (14). Thus, dysfunctional glutamate uptake through GLT-1 and system x_c^- may lead to glutamate spill-over during times of increased glutamate release. The mechanism underlying dysfunction of these transporters has not yet been characterized.

Both system x_c^- and GLT-1 transport cystine into astrocytes and therefore changes in cystine concentration can affect their function (15). This is particularly so for system x_c^-, which exchanges glutamate for cystine in a 1:1 ratio, whereas GLT-1 has a lower affinity to cystine (16). Transcriptional changes in HD may cause decreases in cysteine. Specifically, there is evidence that mutant Huntingtin (mHTT) can inhibit transcription of the cystathionine-γ-lygase (CSE) gene (17). CSE generates cysteine from cystathionine (18). Reductions in CSE were found in both human and rodent models of HD, and this was associated with lower levels of cysteine (17). Cysteine is oxidized to become cystine. Cystine is more favourably found in the extracellular matrix and cysteine within intracellular compartments, due to the redox status of their molecular environment (19). Thus, by inhibiting CSE, mHTT lowers the availability of extracellular cystine, which may impair activity of GLT-1 and system x_c^-. Dysfunction of astrocytic transport of glutamate can therefore account for the excess spill-over of glutamate at the synapse.

Dysfunction of system x_c^- and GLT-1 may also play a part in depression. System x_c^- knockout mice display decreased depressive-like behaviors in the forced-swim test (FST) and tail-suspension test (20), whilst the antidepressant effect of ketamine was found to be mediated by changes in GLT-1 (21). Given that ketamine has strong potential for abuse, and may not have optimal efficacy, other therapeutics affecting these transporters need to be investigated. The cysteine pro-drug, *N*-acetylcysteine (NAC) is one such candidate therapeutic agent.

We have recently shown that by treating HD mice with NAC we can delay the onset and severity of motor symptoms (22). A combination of NAC and dietary cysteine supplementation has also been shown to decrease motor deficits and neurodegeneration in HD mice (17). By donating cysteine, NAC can regulate glutamate flux through GLT-1 and system x_c^-. This action of NAC is therapeutic in clinical and animal models of addiction (23). NAC also donates cysteine for the production of glutathione, thus acting as an antioxidant. It was originally hypothesized that NAC was acting by increasing cysteine for glutathione production. We were able to show that NAC decreased oxidative stress and increased mitochondrial function in the striatum of HD mice (22). However, we found that glutathione redox was unchanged and thus, the mechanism by which NAC is acting remains unclear. The aim of this study was to assess whether the therapeutic effect of NAC in HD is due to the restoration of glutamate flux through astrocyte transporters, GLT-1 and system x_c^-. Given the potential for glutamate receptor dysfunction to contribute to the pathophysiology of depression, we hypothesized that NAC would also have antidepressant-like effects in HD mice.

Results

NAC rescues a depression-like endophenotype in HD mice

The FST is used as a measure of depressive-like behavior in rodents (24) with higher levels of immobility being consistently found in female HD mice (25). We have previously found that acute NAC treatment (500 mg/kg) is able to lower the duration of immobility in the FST in both HD and WT male mice (22). A significant interaction between genotype and treatment was found in FST immobility time in response to a single injection of NAC (i.p. 500 mg/kg; $F_{(1,47)} = 4.686$, $P = 0.036$; Fig. 1A and B). *Post-hoc* comparisons show that saline-HD mice had higher immobility time than saline-WT mice ($P < 0.01$). NAC-HD mice showed immobility times intermediate to saline-HD and saline-WT mice, although no statistical differences were found. After 4 weeks of daily injections of NAC, an interaction between treatment and genotype was also found [$F_{(1,51)}=4.703$, $P = 0.035$]. Similarly, *post-hoc* comparisons show that saline-HD mice had higher immobility time than saline-WT mice ($P < 0.01$), with no other differences found.

The antidepressant-like effect of NAC is dependent on glutamate transport

In order to assess the contribution of glutamate transporters to the acute antidepressant effects of NAC, we co-administered either S-4-carboxyphenylglycine (CPG) or dihydrokainic acid (DHK), inhibitors of GLT-1 and system x_c^-, respectively (Fig. 1C

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

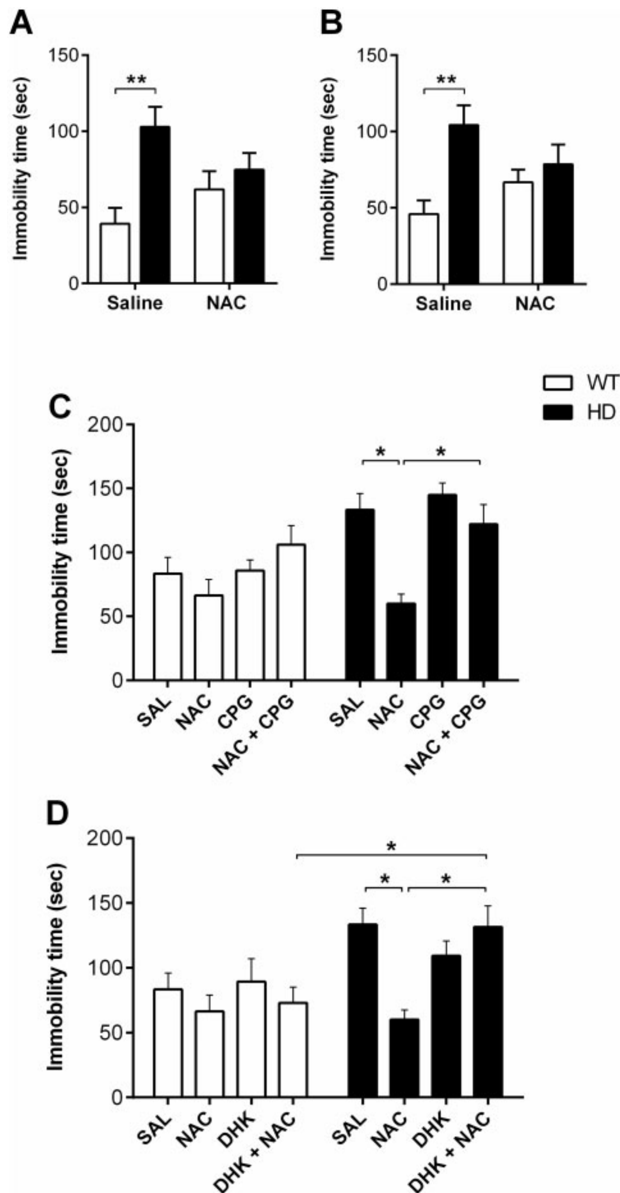

Figure 1. The antidepressant effect of NAC is mediated by glutamate transporters. (**A**) Acute and (**B**) chronic treatment with NAC (500 mg/kg/d) has an antidepressant-like effect on HD mice in the FST. For both acute and chronic tests, a significant interaction between genotype and treatment was 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 x_c^- 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 with 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 with Saline-HD mice. NAC-HD mice showed levels of immobility that did not differ from Saline-WT mice. The addition of DHK ablated the effect of NAC, such that NAC + DHK-HD mice were significantly more immobile than NAC-HD mice and did not differ from Saline-HD mice. Error bars represent mean ± SEM; $n = 9$-15; *Post-hoc* comparisons $^{*}P < 0.05$, $^{**}P < 0.01$.

and D). A significant treatment by genotype interaction was found when looking at the effects of CPG and NAC on immobility in the FST [$F_{(3,83)}$=2.961, $P = 0.037$]. *Post-hoc* comparisons revealed that NAC-HD mice had lower immobility than saline-HD mice ($P < 0.05$). HD mice co-administered with CPG and NAC showed higher immobility times than NAC-HD mice and did not differ from saline-HD mice. This demonstrates that co-administration of CPG with NAC ablates the antidepressant-like effects of NAC in the HD mice.

A main effect of genotype [$F_{(1,82)} = 9.482$, $P = 0.003$] and treatment [$F_{(3,82)}$=4.201, $P = 0.008$] was found when looking at the effects of DHK and NAC on FST performance. NAC-HD mice exhibited lower immobility than Saline-HD mice ($P < 0.05$), confirming the antidepressant-like effect of NAC. HD mice co-administered DHK with NAC showed higher immobility times than NAC-HD mice, and were not significantly different to saline-HD mice. This reveals that co-administration of DHK is also able to negate the antidepressant-like effects of NAC in HD mice.

Expression of glutamate transporters in HD mice and human post-mortem brain tissues

Western blot analysis was carried out on brain samples from mice at 12 weeks of age, after 4 weeks of daily treatment with NAC or vehicle. Protein levels of GLT-1 and the light chain of system x_c^-, xCT, were quantified (Fig. 2). A significant treatment-by-genotype interaction was found in xCT expression within the striatum [$F_{(1,26)} = 4.223$, $P = 0.049$]. No significant *post-hoc* differences were found, however there was a trend for Saline-HD expression to be lower than NAC-HD ($P = 0.055$) and Saline-WT expression ($P = 0.074$). Increased xCT protein levels were found in the hippocampus of HD mice [$F_{(1,26)} = 4.241$, $P = 0.049$], whilst no changes were found in the frontal cortex. A significant treatment-by-genotype interaction was found in the hypothalamus [$F_{(1,23)} = 10.20$, $P = 0.004$], with saline-HD mice expressing higher levels of xCT than saline-WT ($P < 0.05$) and NAC-HD mice ($P < 0.05$).

Levels of xCT were also measured in human HD post-mortem tissue (Fig. 2I–L), demonstrating xCT deficits in the caudate [$t_{(22)} = 5.736$, $P < 0.001$] and hippocampus [$t_{(12)} = 2.620$, $P = 0.022$] of HD patients relative to matched controls.

In the HD mouse brain, GLT-1 expression was decreased in the hippocampus [$F_{(1,25)} = 5.831$, $P = 0.023$] and frontal cortex [$F_{(1,25)} = 5.659$, $P = 0.025$], relative to WT control littermates. There was a trend toward a decrease in the striatum of HD mice [$F_{(1,25)} = 4.111$, $P = 0.054$]. In human post-mortem tissue, no changes in GLT-1 were found in any regions (Supplementary Material, Figure S1A). The large variance present within groups in the GLT-1 analysis led us to postulate that perhaps changes may occur selectively to specific splice variants of GLT-1. This corresponds with changes in GLT-1a and GLT-1b described previously in (26). However, no changes in GLT-1a protein levels were observed in any brain regions (Supplementary Material, Figure S1B).

Extracellular cystine is decreased in HD

No-net-flux analysis of cystine was conducted via *in vivo* microdialysis in the HD hippocampus (Fig. 3). This region was selected for several reasons: As can be seen in Figure 2, the hippocampus was the only region to show genotype changes in both xCT and GLT-1 protein levels. Although NAC treatment did not rescue these changes, it is still likely that NAC can change the activity of these transporters. Furthermore, decreased adult neurogenesis, volume, and function of the hippocampus are pathological hallmarks of major depression and HD (27,28).

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

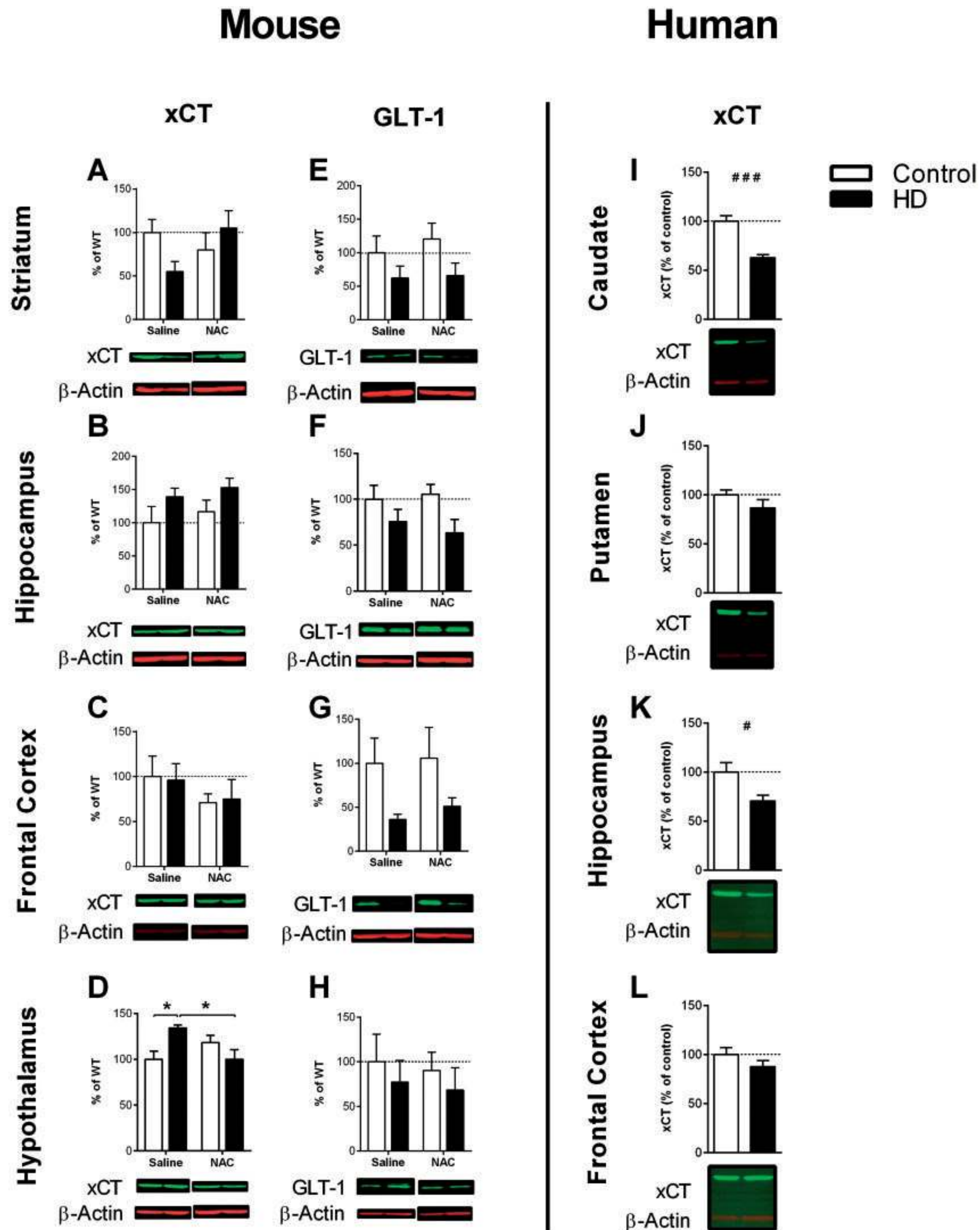

Figure 2. Human post-mortem and mouse protein levels of xCT (system x_{c-}) and GLT-1. (A) A significant genotype-by-treatment interaction was found in the expression of xCT in the striatum. *Post-hoc* differences were not significant; (B) An increase in xCT expression was seen in the hippocampus of HD mice; (C) no differences between groups were found in the frontal cortex; and (D) a significant genotype-by-treatment interaction was found in xCT expression in the hypothalamus. *Post-hoc* tests revealed Saline-HD mice had higher expression than Saline-WT and NAC-HD mice. (E) No difference in GLT-1 between groups was observed in the striatum; (F) a decrease in GLT-1 was seen in the hippocampus and (G) frontal cortex of HD mice; and (H) no difference in GLT-1 was seen between groups in the hypothalamus. In human HD post-mortem tissue, xCT was lower in the (I) caudate and (K) hippocampus. No changes in xCT were found in the (J) putamen or (L) frontal cortex. Error bars represent mean ± SEM; $n = 6$–12; genotype effects: $^{\#}P < 0.05$; $^{\#\#\#}P < 0.001$. *Post-hoc* comparisons: $^{*}P < 0.05$.

Cystine was infused in aCSF via reverse-dialysis at concentrations ranging from 1 to 3000 nM. The dialysate recovered is then a homeostatic balance of endogenous and infused cystine. Using linear regression a decreased net-flux was seen in HD mice, indicating lower basal levels of extracellular cystine in HD mice [$F_{(1,100)} = 7.177$; $P = 0.009$].

NAC increases extracellular glutamate and this is dependent on system x_c^- and GLT-1

In vivo microdialysis was performed to measure extracellular levels of glutamate in response to NAC in the hippocampus (Fig. 3C). A significant effect of time was found [$F_{(2,32)} = 4.508$, $P = 0.019$], with *post-hoc* tests revealing that HD mice had

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

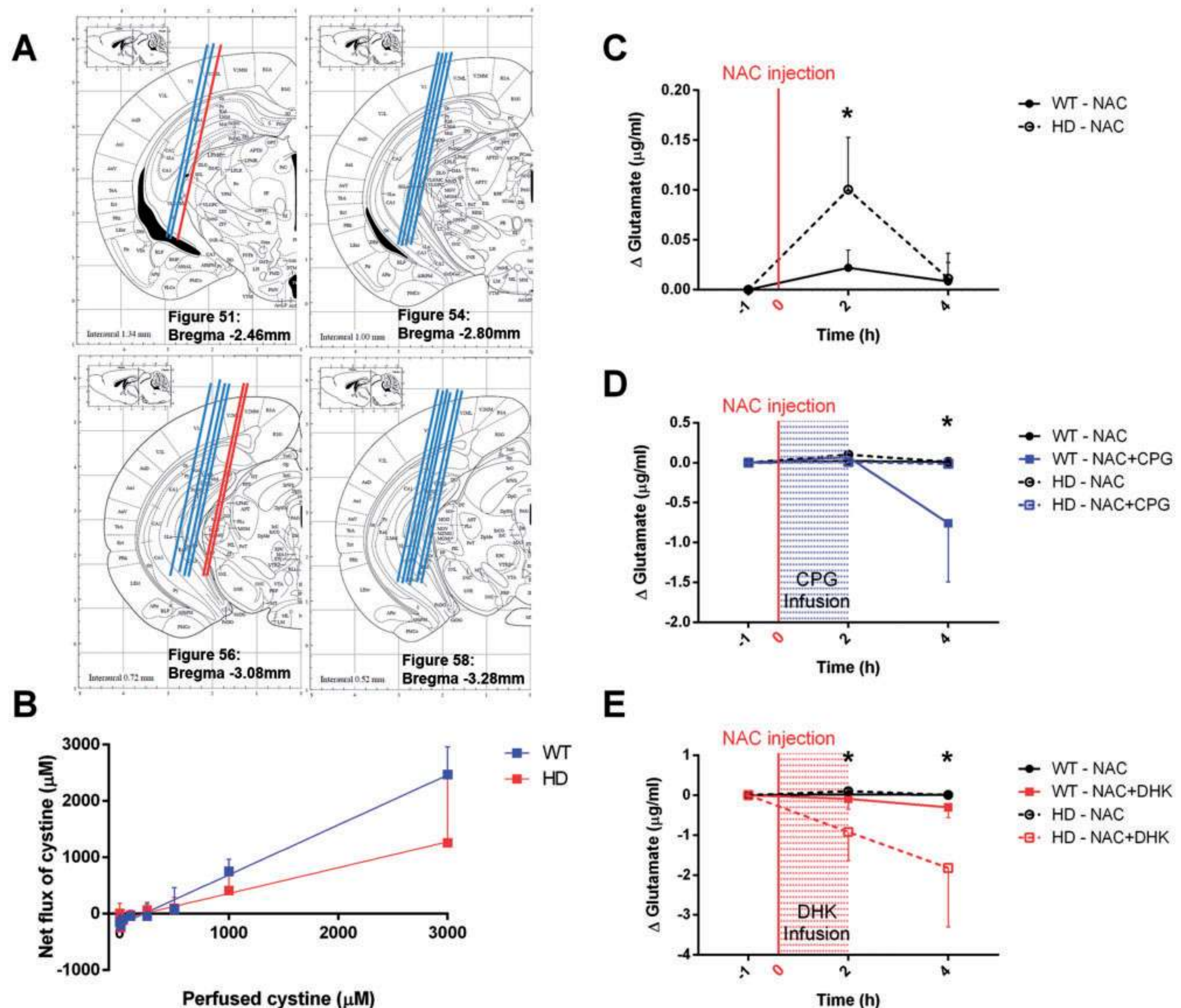

Figure 3. *In vivo* microdialysis for cystine and glutamate. **(A)** Probe insertion sites were validated within perfused slices. All probe insertion sites occurred 2.46–3.30 mm posterior of bregma (figure 51–58 of Franklin and Paxinos mouse brain atlas[27]). Mice whose probe insertion site fell outside of the hippocampus (red lines) were removed from statistical analyses. **(B)** Cystine no-net-flux analysis was conducted to assess basal levels of cystine within the hippocampus. HD mice have a significantly lower gradient than WT mice indicating lower levels of cystine ($P < 0.01$; $n = 7$). **(C)** HD mice show increased extracellular glutamate in response to NAC (i.p. 500 mg/kg). A significant effect of time was found with *post-hoc* tests revealing that HD mice show higher extracellular glutamate compared with WT, 2 h after injection. CPG (100 µM) and DHK (50 µM) were infused after systemic NAC administration (i.p. 500 mg/kg). **(D)** A significant effect of time was found with *post-hoc* tests revealing that WT mice given a CPG infusion had lower extracellular glutamate at 4 h post NAC administration, compared with all other groups. **(E)** A significant effect of time was found with *post-hoc* tests revealing that HD mice given a DHK infusion had lower extracellular glutamate at 2 and 4 h post NAC administration, compared with all other groups. Error bars represent mean ± SEM; $n = 4$–10; *post-hoc* comparisons: $^*P < 0.05$.

significantly higher glutamate 2 hours post NAC injection ($P < 0.05$) with no difference at 4 h post-injection.

To assess whether the increase in extracellular glutamate was due to the effect of NAC on glutamate transporters, GLT-1 and system x_c^- inhibitors (DHK and CPG, respectively) were infused into the hippocampus via reverse dialysis (Fig. 3D and E). A significant effect of time was seen with CPG infusion [$F_{(2,32)} = 3.830$, $P = 0.021$]. *Post-hoc* tests revealed that WT animals co-treated with NAC and CPG had a larger reduction in glutamate at 4 h after injection compared with NAC and CPG co-treated HD animals ($P < 0.05$) and both WT and HD NAC-treated groups ($P < 0.01$). A significant time-by-group interaction was found with DHK infusions [$F_{(6,52)} = 3.381$, $P = 0.007$]. *Post-hoc* comparisons revealed that HD animals co-treated with NAC and DHK had a greater reduction in glutamate at 4 h after injection compared with NAC and DHK co-treated WT animals ($P < 0.01$) and both WT and HD NAC-treated groups ($P < 0.001$).

Effects of NAC on glutamate receptor expression

Given the modulatory effect of NAC on extracellular glutamate, we examined downstream effects of chronic NAC on glutamate receptors expression (Fig. 4). No changes were found in the expression of NMDAR2A receptor subunits in the striatum, frontal cortex or hypothalamus. However, NMDAR2A was decreased in the hippocampus of HD mice [$F_{(1,25)} = 6.465$, $P = 0.018$]. In contrast, the 2B subunit was not changed in the hippocampus, or the frontal cortex. A significant interaction was found in

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

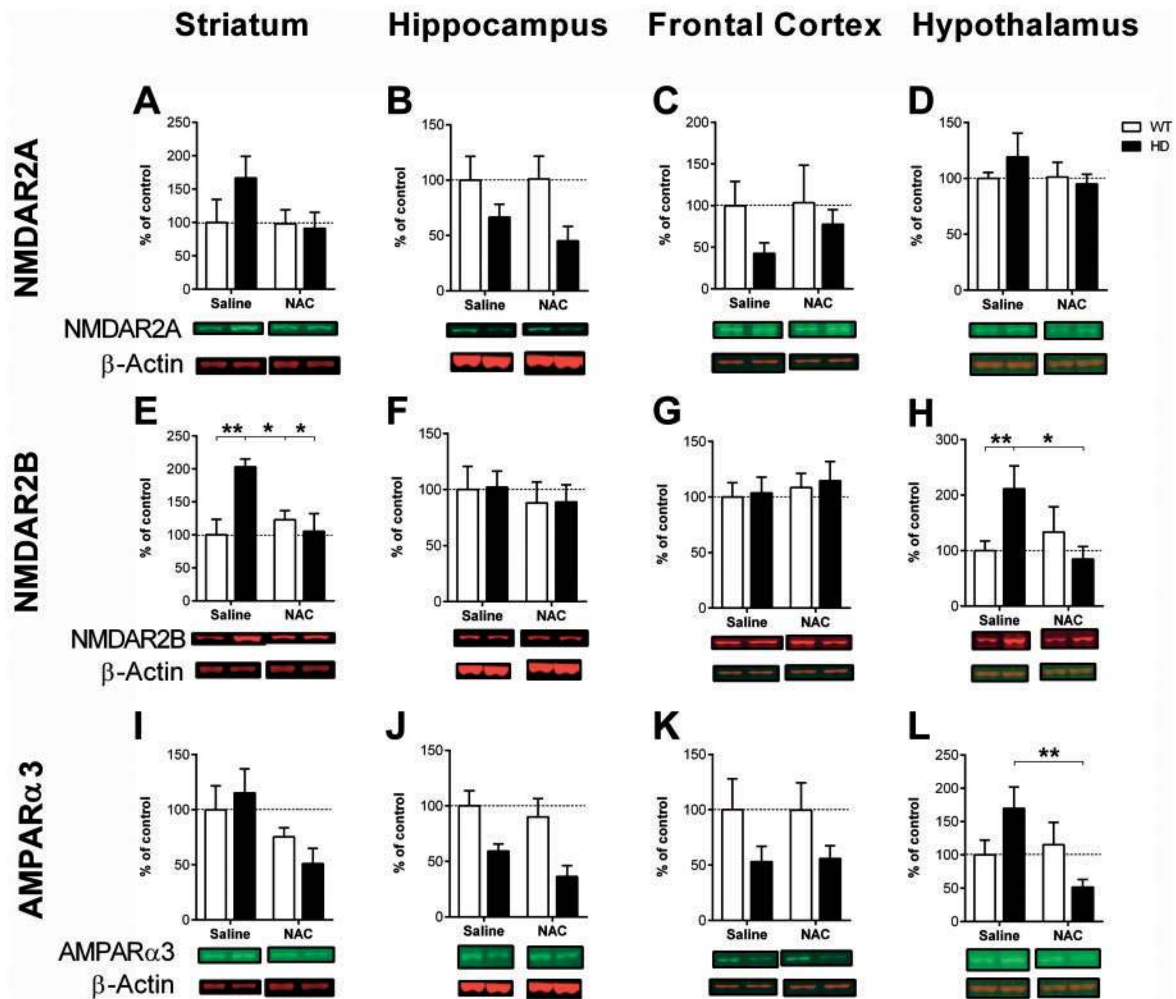

Figure 4. Protein levels of glutamate receptors in mice. No changes in NMDAR2A expression were seen in the (A) striatum, (C) frontal cortex or (D) hypothalamus. (B) However, a reduction was seen in the hippocampus of HD mice. (E) A significant genotype-by-treatment interaction was found in levels of NMDAR2B in the striatum. *Post-hoc* tests revealed that HD-saline mice had higher expression than all other groups; (F) No differences in NMDAR2B expression were seen in the hippocampus or (G) frontal cortex. (H) A significant treatment-by-genotype interaction was found in the hypothalamus, with *post-hoc* tests revealing that Sal-HD mice showed higher levels of NMDAR2B relative to both Sal-WT and NAC-HD. (I) AMPAα3 expression was lower in the striatum of NAC-treated mice. HD mice showed decreased expression in both the (J) hippocampus and (K) frontal cortex. (L) A significant treatment-by-genotype interaction was found in the hypothalamus, with *post-hoc* tests revealing a difference between HD-saline mice and HD-NAC mice. Error bars represent mean ± SEM; $n = 6$-8; *post-hoc* comparisons: *$P < 0.05$, **$P < 0.01$.

expression of NMDAR2B protein within the striatum [$F_{(1,24)} = 8.814$, $P = 0.007$] and hypothalamus [$F_{(1,24)} = 6.003$, $P = 0.022$]. *Post-hoc* comparisons revealed that expression was higher in saline-HD than saline-WT ($P < 0.01$), NAC-HD ($P < 0.05$) and NAC-WT ($P < 0.05$) in the striatum. NMDAR2B expression was higher in Saline-HD than Saline-WT ($P < 0.01$) and NAC-HD ($P < 0.05$) in the hypothalamus.

Expression of a specific AMPA receptor subunit (AMPARα3) was decreased in the striatum of both WT and HD animals treated with NAC [$F_{(1,24)} = 6.003$, $P = 0.022$]. AMPARα3 expression was lowered in the hippocampus [$F_{(1,25)} = 13.71$, $P = 0.001$] and frontal cortex of HD mice [$F_{(1,24)} = 4.326$, $P = 0.048$]. A significant genotype-by-treatment interaction was found in AMPARα3 expression in the hypothalamus [$F_{(1,24)} = 6.537$, $P = 0.017$], with *post-hoc* comparisons revealing that Saline-HD mice have higher expression than NAC-HD mice ($P < 0.01$). Finally, no changes were found in the expression of either mGluR2 or mGluR5 (Supplementary Material, Figure S2) in any brain regions.

Discussion

NAC shows antidepressant effects dependent on system x_c^- and GLT-1 in HD

Our results show that NAC exerts a rapid antidepressant-like effect, which is maintained after 4 weeks of treatment. Although we have previously demonstrated beneficial effects of NAC on oxidative stress and mitochondrial function in HD mice, these are longer-term processes (22). We therefore hypothesized that the mechanism of action underpinning the acute behavioral response to NAC in the FST was more likely to be through rapid modulation of glutamate flux. System x_c^- and GLT-1 are both necessary for the homeostasis of glutamate and cystine in astrocytes and at excitatory synapses (16,29,30). We were able to block the acute antidepressant-like effects of NAC by co-administering CPG and DHK, specific inhibitors of these transporters. Thus, the therapeutic effects of NAC are dependent on GLT-1 and system x_c^-. These therapeutic effects reflected *in vivo*

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

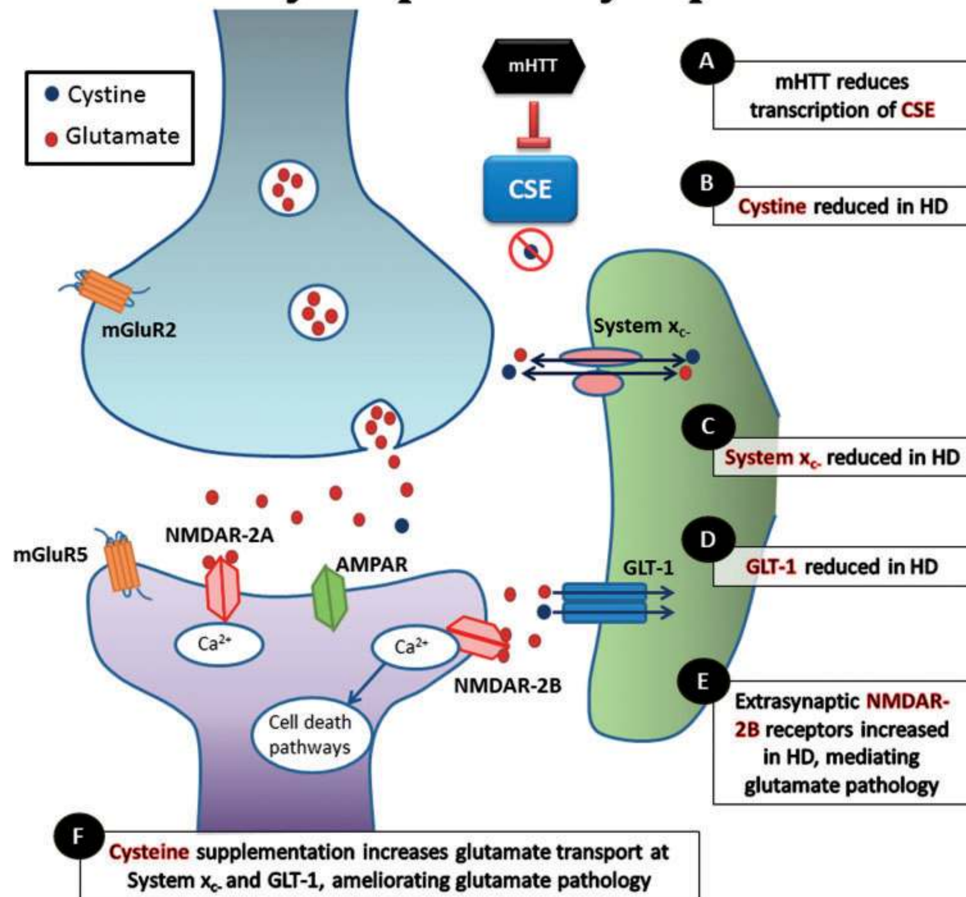

Figure 5. A proposed model of pathogenesis mediating abnormal glutamate homeostasis and dysfunction of excitatory tripartite synapses. (**A**) mHTT inhibits transcription of CSE, (**B**) leading to reductions in extracellular cysteine and cystine. Lower cystine disrupts transport of glutamate through both system x_c^- and GLT-1. Specifically, (**C**) cystine is not taken up into astrocytes through system x_c^-, 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 x_c^-.

changes in glutamate homeostasis (see Fig. 5 for a summary diagram).

Cystine regulates glutamate homeostasis through system x_c^-

We have shown that extracellular cystine is decreased in the hippocampus of HD mice. This is likely due to mHTT-induced reductions in the transcription of CSE, the enzyme that regulates cysteine production in the brain (17). Considering cystine is a rate-limiting substrate required for activity of system x_c^-, these reductions are likely to decrease the export of glutamate. By supplementing cysteine with NAC, there was a HD-specific increase in glutamate. Furthermore, infusion of the system x_c^- inhibitor, CPG, blocked the NAC-induced spike in glutamate. These functional changes mirror the reductions in xCT. xCT is the light-chain of system x_c^-, which conveys substrate specificity. Depletion of this protein was found in the striatum of HD mice and in the caudate and hippocampus of human post-mortem tissue, supporting the theory that reductions in cystine dysregulates glutamate export through system x_c^-. This correlates with a previous independent study which showed lowered xCT in the striatum of HD mice (14). Furthermore, NAC was able to restore xCT levels in the HD mouse striatum, indicating that chronic cysteine supplementation rescues HD-induced reductions in system x_c^-. However, increases in xCT were found in the mouse HD hippocampus. The regional disparity in xCT may represent differential pathological progressions. The striatum is known to show degenerative changes prior to the hippocampus in HD (31). At this stage of the disease progression, xCT may be compensating for the lack of cystine in the hippocampus, whilst this capacity has been lost in the striatum due to the more advanced neurodegeneration. This may also explain why xCT was lowered in both the hippocampus and caudate of HD post-mortem tissue, which was from a comparatively later stage in the disease.

Decreased uptake of glutamate through GLT-1 is mediated by cystine supplementation

GLT-1 inhibitors also blocked the NAC-induced spike in glutamate, and further lowered glutamate in HD mice. This may be secondary to increased glutamate release from system x_c^-, providing more substrate for GLT-1 uptake of glutamate. Alternatively, it may represent direct actions of cystine as a substrate for GLT-1. Reductions in cystine may be causing the

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

decreased levels of GLT-1 observed in the hippocampus and frontal cortex of HD mice (with a trend towards a decrease in the striatum). This aligns with previous research showing decreased GLT-1 protein in the striatum and cortex of transgenic mice (10,11,32), with one study showing a decrease in GLT-1 protein in the striatum of human HD post-mortem tissue (33). This allows excess glutamate to accumulate at extrasynaptic sites. It is through the activation of glutamate at extrasynaptic receptors, that glutamate-related pathology in HD is mediated (6). However, we were unable to replicate the GLT-1 reduction in human post-mortem tissue.

We found no reductions in either total GLT-1 or the GLT-1a splice variant. Analysis of western blots in a previous study shows a thin clear band representing GLT-1 protein (33). This is similar to the detection of GLT-1 in mouse brains in our present study. However, our human antibodies detect a thick smear, which is similar to previous studies [for review see (34)]. Future studies need to clarify the differences in these antibodies to help elucidate the precise GLT-1 changes occurring in HD.

To the authors' knowledge, the pattern of lowered GLT-1 in the hippocampus and increased GLT-1 in the hypothalamus has not been reported elsewhere. Importantly, the increase in hypothalamic GLT-1 was ablated by chronic NAC treatment. This may relate to hyper-activation of the HPA-axis which has been found to be associated with depression-like behaviors in HD mice (35). Specifically, glutamatergic inputs to the paraventricular nucleus (PVN) of the hypothalamus regulate the excitation and release of corticotrophin releasing hormone (36). Thus, over-excitation in the PVN may represent a chronically activated stress response. Considering NAC administration did not rescue changes in hippocampal GLT-1 or xCT expression, it may be that chronic NAC exerts antidepressant effects through changes in the hypothalamus.

Effects on glutamate receptors

HD is associated with changes in expression of glutamate receptors, particularly the NMDA 2A and 2B receptor subunits (6). Furthermore, gene expression profiling (data not shown) isolated further glutamate receptors that may be of interest, including AMPARα3 and mGluR3. Other targets were identified based on studies which have shown that modulation of groups I and II metabotropic glutamate receptor (mGluR1/5 and mGluR2/3, respectively) was therapeutic in mouse models of HD (37–39). These receptors have also been shown to be modulated by NAC in animal models of addiction, leading to therapeutic metaplastic changes (40).

Glutamate toxicity in HD is dependent on the NMDARs containing the 2B subunit (6). We replicated this finding in an independent model, showing the 2B subunit was increased in the HD striatum. Chronic NAC rescued this change, indicating that aberrant NMDAR signalling was ameliorated in the striatum. Thus, by supplementing cysteine, we were able to increase the levels of this species in the extrasynaptic space. This increased the activity of both GLT-1 and system x_c^-, re-establishing synaptic glutamate homeostasis, preventing excess extrasynaptic glutamate inducing aberrant NMDAR-2B activation. This is likely to have downstream consequences on mitochondrial function, as observed in our previous study (22).

Increased NMDAR activation leads to increased calcium ion influx into the cell and subsequently, into mitochondria. HD mitochondria have impaired capacity to deal with increased calcium load (41) and we have previously shown that NAC rescues mitochondrial dysfunction and oxidative stress in HD mice (22). Thus, it is likely that through restoration of synaptic glutamate homeostasis via transporters, system x_c^- and GLT-1, NAC decreases activation and expression of NMDA-2B receptors, rescuing mitochondrial dysfunction and oxidative stress.

These changes in glutamate receptor tone may be caused by activation of specific mGluRs, as observed in models of addiction (40). However, there were no changes in group I or II mGluR (i.e. mGluR2 and mGluR5) levels in HD mice, indicating that the change in glutamatergic receptor tone was more likely due to changes in synaptic glutamate.

NAC did not modulate the reductions in NMDAR2A or AMPAR in the hippocampus or frontal cortex of HD mice. This implies that although NAC can rescue changes in pathological NMDAR activation through 2B containing-receptors, it is unable to rescue changes in synaptic plasticity associated with NMDAR2A and AMPAR. This may explain why NAC has not been able to rescue a hippocampal-dependent cognitive deficit in this mouse model (data not shown).

Implications for depression

Our findings have wider implications for research into depression. We highlight the importance of cysteine/cystine in regulating glutamate flux in the hippocampus of HD, with associated changes in depressive-like behavior. This was regulated by glutamate transporters, GLT-1 and system x_c^-.

GLT-1 has recently been implicated in the pathogenesis of depression, with preclinical models showing lowered GLT-1 function in the amygdala and hippocampus associated with depression-like behaviors (42,43), whilst RNA and protein levels are also decreased in the post-mortem brain of depressed patients (44). Increasing GLT-1 is thought to exert therapeutic effects via reducing NMDAR activation, in a manner akin to the acute antidepressant action of ketamine.

NAC has previously been shown to ameliorate depressive symptoms in bipolar patients (45). The therapeutic mechanism was hypothesized to be related to glutathione redox and antioxidant activity. Our findings indicate regulation of glutamate homeostasis through cystine-dependent transporters is a more likely downstream mechanism underlying the antidepressant effect of NAC.

Glutamate-related pathology has long been implicated in HD. We provide evidence that reductions in cysteine/cystine cause glutamate dyshomeostasis in HD transgenic mice. We show these changes underlie depressive-like behavior in these mice, and by increasing cysteine (using NAC) we were able to rescue depressive-like behavior and glutamate homeostasis. These were reflected in long-term changes of glutamate transporters and receptors, including NMDAR2B. Taken together with previous studies showing therapeutic effects in preclinical models of HD and clinical studies of depression, NAC is a strong candidate for clinical trials in HD.

Materials and Methods

Mice

R6/1 transgenic hemizygote males (46) were originally obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and bred with CBB6 (CBA–C57/B6) F1 females to establish the R6/1 colony at the Florey Institute of Neuroscience and Mental Health. Standard housing conditions have been described previously in (22). All animal experiments were approved by the Florey

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

Institute Animal Ethics Committee, following the guidelines of the National Health and Medical Research Council (NHMRC).

Behavioral pharmacology experiments

Forced-swim test

The FST was conducted as described previously in (25). Three separate FST experiments were performed: (i) Acute effects of NAC were assessed at 8 weeks of age around the time of phenotype onset and prior to motor dysfunction ($n = 12$–15); (ii) Chronic effects of NAC, at 12 weeks of age, after 4 weeks of treatment ($n = 12$–15); and (iii) acute co-administration of glutamate transport inhibitors with NAC at 12 weeks of age ($n = 9$–15). Experiments 1 and 2 were performed on the same cohort of mice. Experiment 3 was performed on two separate cohorts, with groups balanced between cohorts.

Pharmacological challenges

For experiment 1, NAC (500 mg/kg) or vehicle (saline) were injected (i.p.) 1 h prior to the FST. Mice were 8-weeks old and behaviorally naïve. For experiment 2, daily injections began on the following day and continued until mice were culled at 12 weeks.

For experiment 3, the GLT-1 inhibitor dihydrokainic acid (20 mg/kg) and the system xc- inhibitor (S)-4-carboxyphenylglycine (200 mg/kg) were injected (i.p.) 1.5 h prior to FST (i.e. 30 min prior NAC). Doses were taken from previous studies (47–51).

Microdialysis

Surgery was conducted on behaviorally naïve female mice aged 8–9 weeks. Mice were allowed to recover for 1 week prior to experiments beginning. Coordinates for the ventral hippocampus were, from bregma: −3.3 mm posterior, 2.0 mm right, 0.5 mm deep, at an angle of 12.5° (Fig. 3) (52).

Microdialysis was conducted as described previously in (53). For systemic NAC experiments (i.p.; 500 mg/kg), aCSF was infused at a rate of 0.5 μl/min, with samples collected in 1 h bins. Two-hour infusions of either CPG (100 μM) or DHK (50 μM) were co-administered.

For no-net-flux experiments, increasing concentrations of cystine (Sigma no. C7602; 10, 30, 100, 250, 500, 1000 and 3000 nM) were infused at a rate of 1 μl/min. Concentrations increased every hour and samples were collected in 30 min bins.

HPLC and mass spectrometry

Glutamate

An Agilent Technologies 1260 HPLC was coupled with Agilent Technologies 1100 fluorescence detector for quantification of glutamate, based on previous procedures (54,55) with some modifications. A Phenomonex Gemini-C18 (5 μm × 4.6 mm × 150 mm) reversed phase column was used with a gradient mobile phase of 40 mM phosphate buffer (pH 5.88) containing 30% MeOH increasing to 50% MeOH over 5.5 min, which was held for a further 4.5 min. The autosampler was temperature controlled at 4 °C. Derivatization was completed using an injector program to react sample and borate buffer (75 mM; pH 10.4) with the OPA derivatizing reagent (5 mg/ml containing 0.1% β-mercaptoethanol). The mixture was allowed to react for 2 min before injection.

Cystine

Agilent Technologies 6495 triple quadrupole LC-MS was used for the quantification of cystine. Samples were derivatized and cystine was reduced using TCEP prior to analysis, as described previously in (56).

Western blotting

Mouse and human brains were prepared and immunoblots performed on 10% acrylamide/bis-tris gels as described previously in (22). Human brain tissue was donated by the Victorian Brain Bank Network (see Supplementary Material, Table 1, for patient characteristics). The following primary antibodies were used: β-Actin (Cell Signaling Technologies, no. 3700; 1:5000), GLT-1 (Millipore no. ABN102; 1:1000), mGluR2 (Abcam no. Ab15672; 1:500), mGluR5 (Abcam no. Ab76316; 1:500), NMDAR2a (Cell Signaling Technologies no. 4205; 1:500), NMDAR2b (BD Bioscience no. 610417; 1:500), xCT (Abcam no. Ab175186; 1:1000). PAN-GLT-1, GLT-1a and GLT-1b were produced by David Pow, at RMIT University, Melbourne, Australia. Analyses of results were carried out as ratio of protein-of-interest:β-actin, before being converted to percentages compared with controls.

Statistics

Statistical analyses were performed using IBM SPSS statistics Version 21.0 (IBM, Armonk, NY, USA) and GraphPad Prism 6 (GraphPad software, Inc., LA Jolla, CA). Microdialysis no-net-flux data was analyzed by comparing slopes and intervals using linear regression, whilst drug responses were measured using two-way repeated-measures ANOVA. If sphericity was violated, Greenhouse-Geisser corrections were used. If significant interactions were found, alpha-levels for *post-hoc* comparisons were corrected using Bonferroni's method. All other data were analyzed using two-way ANOVAs. Alpha levels for *post-hoc* comparisons were corrected using Fisher's method. In all cases significance was set at $P < 0.05$.

Supplementary Material

Supplementary Material is available at *HMG* online.

Acknowledgements

We thank Blaine Roberts, from the Neuroproteomics Facility at the Florey Institute of Neuroscience and Mental Health for use and training with the HPLC-MS; Damien Callahan and Berin Boughton, for providing the standards used to complete the amino acid analysis; Sean Klinkradt from the School of Health and Biomedical Sciences, RMIT, for his help with GLT-1 antibodies; and Lisa Bray from the Florey Institute for her training in performing stereotaxic surgery and microdialysis. The Florey Institute of Neuroscience and Mental Health acknowledges the support from the Victorian Government's Operational Infrastructure Support Grant.

Conflict of Interest statement. None declared.

Funding

This work was supported by an Australian Research Council (ARC) FT3 Future Fellowship (A.J.H.) and National Health and Medical Research Council (NHMRC) Project Grant (A.J.H., T.R.

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

and P.J.C.), as well as a philanthropic grant from the Macquarie Foundation (A.J.H.). A.J.H. is an NHMRC Senior Research Fellow, T.R. is an ARC DECRA Fellow and P.J.C. is an NHMRC Career Development Fellow.

References

1. Ossig, C. and Storch, A. (2015) Depression in Huntington's Disease. In: Reichmann H (ed). *Neuropsychiatric Symptoms of Movement Disorders*. Springer International Publishing, pp. 201–209.
2. Tabrizi, S.J., Cleeter, M.W., Xuereb, J., Taanman, J.W., Cooper, J.M. and Schapira, A.H. (1999) Biochemical abnormalities and excitotoxicity in Huntington's disease brain. *Ann. Neurol.*, **45**, 25–32.
3. Cepeda, C., Ariano, M.A., Calvert, C.R., Flores-Hernandez, J., Chandler, S.H., Leavitt, B.R. *et al.* (2001) NMDA receptor function in mouse models of Huntington disease. *J. Neurosci. Res.*, **66**, 525–539.
4. Starling, A.J., Andre, V.M., Cepeda, C., de Lima, M., Chandler, S.H. and Levine, M.S. (2005) Alterations in N-methyl-D-aspartate receptor sensitivity and magnesium blockade occur early in development in the R6/2 mouse model of Huntington's disease. *J. Neurosci. Res.*, **82**, 377–386.
5. Okamoto, S.I., Pouladi, M.A., Talantova, M., Yao, D.D., Xia, P., Ehrnhoefer, D.E. *et al.* (2009) Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin. *Nat. Med.*, **15**, 1407–U1408.
6. Milnerwood, A.J., Gladding, C.M., Pouladi, M.A., Kaufman, A.M., Hines, R.M., Boyd, J.D. *et al.* (2010) Early increase in extrasynaptic NMDA receptor signaling and expression contributes to phenotype onset in Huntington's disease mice. *Neuron*, **65**, 178–190.
7. Sanacora, G., Treccani, G. and Popoli, M. (2012) Towards a glutamate hypothesis of depression: An emerging frontier of neuropsychopharmacology for mood disorders. *Neuropharmacology*, **62**, 63–77.
8. Fond, G., Loundou, A., Rabu, C., Macgregor, A., Lancon, C., Brittner, M. *et al.* (2014) Ketamine administration in depressive disorders: a systematic review and meta-analysis. *Psychopharmacology*, **231**, 3663–3676.
9. Miller, O.H., Yang, L., Wang, C.-C., Hargroder, E.A., Zhang, Y., Delpire, E. *et al.* (2014) GluN2B-containing NMDA receptors regulate depression-like behavior and are critical for the rapid antidepressant actions of ketamine. *eLife*, **3**, e03581.
10. Lievens, J.C., Woodman, B., Mahal, A., Spasic-Boscovic, O., Samuel, D., Kerkerian-Le Goff, L. *et al.* (2001) Impaired glutamate uptake in the R6 Huntington's disease transgenic mice. *Neurobiol. Dis.*, **8**, 807–821.
11. Behrens, P.F., Franz, P., Woodman, B., Lindenberg, K.S. and Landwehrmeyer, G.B. (2002) Impaired glutamate transport and glutamate-glutamine cycling: downstream effects of the Huntington mutation. *Brain*, **125**, 1908–1922.
12. Rebec, G.V., Barton, S.J., Marseilles, A.M. and Collins, K. (2003) Ascorbate treatment attenuates the Huntington behavioral phenotype in mice. *Neuroreport*, **14**, 1263–1265.
13. Sari, Y., Prieto, A.L., Barton, S.J., Miller, B.R. and Rebec, G.V. (2010) Ceftriaxone-induced up-regulation of cortical and striatal GLT1 in the R6/2 model of Huntington's disease. *J. Biomed. Sci.*, **17**, 62.
14. Frederick, N.M., Bertho, J., Patel, K.K., Petr, G.T., Bakradze, E., Smith, S.B. *et al.* (2014) Dysregulation of system xc(-) expression induced by mutant huntingtin in a striatal neuronal cell line and in R6/2 mice. *Neurochem. Int.*, **76**, 59–69.
15. McBean, G.J. and Flynn, J. (2001) Molecular mechanisms of cystine transport. *Biochem. Soc. Trans.*, **29**, 717–722.
16. McBean, G.J. (2002) Cerebral cystine uptake: a tale of two transporters. *Trends Pharmacol.*, **23**, 299–302.
17. Paul, B.D., Sbodio, J.I., Xu, R., Vandiver, M.S., Cha, J.Y., Snowman, A.M. *et al.* (2014) Cystathionine gamma-lyase deficiency mediates neurodegeneration in Huntington's disease. *Nature*, **509**, 906–100.
18. Paul, B.D. and Snyder, S.H. (2012) H2S signalling through protein sulfhydration and beyond. *Nat. Rev. Mol. Cell Biol.*, **13**, 499–507.
19. Banerjee, R. (2012) Redox outside the Box: Linking Extracellular Redox Remodeling with Intracellular Redox Metabolism. *J. Biol. Chem.*, **287**, 4397–4402.
20. Bentea, E., Demuyser, T., Van Liefferinge, J., Albertini, G., Deneyer, L., Nys, J. *et al.* (2015) Absence of system xc- in mice decreases anxiety and depressive-like behavior without affecting sensorimotor function or spatial vision. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **59**, 49–58.
21. Liu, W.X., Wang, J., Xie, Z.M., Xu, N., Zhang, G.F., Jia, M. *et al.* (2015) Regulation of glutamate transporter 1 via BDNF-TrkB signaling plays a role in the anti-apoptotic and antidepressant effects of ketamine in chronic unpredictable stress model of depression. *Psychopharmacol (Berl)*, **233**, 405–415.
22. Wright, D.J., Renoir, T., Smith, Z.M., Frazier, A.E., Francis, P.S., Thorburn, D.R. *et al.* (2015) N-Acetylcysteine improves mitochondrial function and ameliorates behavioral deficits in the R6/1 mouse model of Huntington's disease. *Transl. Psychiatry*, **5**, e492.
23. Reissner, K.J. and Kalivas, P.W. (2010) Using glutamate homeostasis as a target for treating addictive disorders. *Behav. Pharmacol.*, **21**, 514–522.
24. Porsolt, R.D., Le Pichon, M. and Jalfre, M. (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature*, **266**, 730–732.
25. Renoir, T., Zajac, M.S., Du, X., Pang, T., Leang, L., Chevarin, C. *et al.* (2011) Sexually Dimorphic Serotonergic Dysfunction in a Mouse Model of Huntington's Disease and Depression. *PLoS One*, **6**, e22133.
26. Petr, G.T., Schultheis, L.A., Hussey, K.C., Sun, Y., Dubinsky, J.M., Aoki, C. *et al.* (2013) Decreased expression of GLT-1 in the R6/2 model of Huntington's disease does not worsen disease progression. *Eur. J. Neurosci.*, **38**, 2477–2490.
27. Ransome, M.I., Renoir, T. and Hannan, A.J. (2012) Hippocampal neurogenesis, cognitive deficits and affective disorder in Huntington's disease. *Neural Plast.*, **2012**, 874387.
28. Sahay, A. and Hen, R. (2007) Adult hippocampal neurogenesis in depression. *Nat. Neurosci.*, **10**, 1110–1115.
29. Bjornsen, L.P., Hadera, M.G., Zhou, Y., Danbolt, N.C. and Sonnewald, U. (2014) The GLT-1 (EAAT2; slc1a2) glutamate transporter is essential for glutamate homeostasis in the neocortex of the mouse. *J. Neurochem.*, **128**, 641–649.
30. Bridges, R., Lutgen, V., Lobner, D. and Baker, D.A. (2012) Thinking outside the cleft to understand synaptic activity: contribution of the cystine-glutamate antiporter (system xc−) to normal and pathological glutamatergic signaling. *Pharmacol. Rev.*, **64**, 780–802.
31. Waldvogel, H.J., Kim, E.H., Tippett, L.J., Vonsattel, J.P. and Faull, R.L. (2015) The neuropathology of Huntington's disease. *Curr. Top. Behav. Neurosci.*, **22**, 33–80.
32. Estrada-Sanchez, A.M., Montiel, T., Segovia, J. and Massieu, L. (2009) Glutamate toxicity in the striatum of the R6/2

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022

Huntington's disease transgenic mice is age-dependent and correlates with decreased levels of glutamate transporters. *Neurobiol. Dis.*, **34**, 78–86.

33. Faideau, M., Kim, J., Cormier, K., Gilmore, R., Welch, M., Auregan, G. *et al.* (2010) In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport: a correlation with Huntington's disease subjects. *Hum. Mol. Genet.*, **19**, 3053–3067.
34. Danbolt, N.C. (2001) Glutamate uptake. *Prog. Neurobiol.*, **65**, 1–105.
35. Du, X., Leang, L., Mustafa, T., Renoir, T., Pang, T.Y. and Hannan, A.J. (2012) Environmental enrichment rescues female-specific hyperactivity of the hypothalamic-pituitary-adrenal axis in a model of Huntington's disease. *Transl. Psychiatry*, **2**, e133.
36. Herman, J.P., Mueller, N.K. and Figueiredo, H. (2004) Role of GABA and glutamate circuitry in hypothalamo-pituitary-adrenocortical stress integration. *Annals NY Acad. Sci.*, **1018**, 35–45.
37. Kang, H.J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C.A., Licznerski, P. *et al.* (2012) Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nat. Med.*, **18**, 1413–1417.
38. Ribeiro, F.M., Paquet, M., Ferreira, L.T., Cregan, T., Swan, P., Cregan, S.P. *et al.* (2010) Metabotropic glutamate receptor-mediated cell signaling pathways are altered in a mouse model of Huntington's disease. *J. Neurosci.*, **30**, 316–324.
39. Uher, T. and Bob, P. (2012) Cerebrospinal fluid S100B levels reflect symptoms of depression in patients with non-inflammatory neurological disorders. *Neurosci. Lett.*, **529**, 139–143.
40. Moussawi, K., Pacchioni, A., Moran, M., Olive, M.F., Gass, J.T., Lavin, A. *et al.* (2009) N-Acetylcysteine reverses cocaine-induced metaplasticity. *Nat. Neurosci.*, **12**, 182–189.
41. Tang, T.S., Slow, E., Lupu, V., Stavrovskaya, I.G., Sugimori, M., Llinas, R. *et al.* (2005) Disturbed $Ca2+$ signaling and apoptosis of medium spiny neurons in Huntington's disease. *Proc. Natl Acad. Sci. USA*, **102**, 2602–2607.
42. Chen, J.X., Yao, L.H., Xu, B.B., Qian, K., Wang, H.L., Liu, Z.C. *et al.* (2014) Glutamate transporter 1-mediated antidepressant-like effect in a rat model of chronic unpredictable stress. *J. Huazhong Univ. Sci. Technolog. Med. Sci.*, **34**, 838–844.
43. John, C.S., Smith, K.L., Van't Veer, A., Gompf, H.S., Carlezon, W.A., Jr., Cohen, B.M. *et al.* (2012) Blockade of astrocytic glutamate uptake in the prefrontal cortex induces anhedonia. *Neuropsychopharmacology*, **37**, 2467–2475.
44. Rajkowska, G. and Stockmeier, C.A. (2013) Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr. Drug Targets*, **14**, 1225–1236.
45. Berk, M., Copolov, D.L., Dean, O., Lu, K., Jeavons, S., Schapkaitz, I. *et al.* (2008) N-acetyl cysteine for depressive symptoms in bipolar disorder–a double-blind randomized placebo-controlled trial. *Biol Psychiatry*, **64**, 468–475.
46. Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C. *et al.* (1996) Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell*, **87**, 493–506.
47. Arriza, J.L., Fairman, W.A., Wadiche, J.I., Murdoch, G.H., Kavanaugh, M.P. and Amara, S.G. (1994) Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. *J. Neurosci.*, **14**, 5559–5569.
48. Garzon-Muvdi, T., Pradilla, G., Ruzevick, J.J., Bender, M., Edwards, L., Grossman, R. *et al.* (2013) A glutamate receptor antagonist, S-4-carboxyphenylglycine (S-4-CPG), inhibits vasospasm after subarachnoid hemorrhage in haptoglobin 2-2 mice corrected. *Neurosurgery*, **73**, 719–728.
49. Gunduz, O., Oltulu, C., Buldum, D., Guven, R. and Ulugol, A. (2011) Anti-allodynic and anti-hyperalgesic effects of ceftriaxone in streptozocin-induced diabetic rats. *Neurosci. Lett.*, **491**, 23–25.
50. Gunduz, O., Oltulu, C. and Ulugol, A. (2011) Role of GLT-1 transporter activation in prevention of cannabinoid tolerance by the beta-lactam antibiotic, ceftriaxone, in mice. *Pharmacol. Biochem. Behav.*, **99**, 100–103.
51. Hobo, S., Eisenach, J.C. and Hayashida, K-i. (2011) Up-regulation of spinal glutamate transporters contributes to anti-hypersensitive effects of valproate in rats after peripheral nerve injury. *Neurosci. Lett.*, **502**, 52–55.
52. Franklin, K.B.J. and Paxinos, G. (2008) *The Mouse Brain in Stereotaxic Coordinates*, 3rd edn. New York: Academic Press/Elsevier, 2008.
53. Adlard, P.A., Cherny, R.A., Finkelstein, D.I., Gautier, E., Robb, E., Cortes, M. *et al.* (2008) Rapid restoration of cognition in Alzheimer's transgenic mice with 8-hydroxy quinoline analogs is associated with decreased interstitial Aβ. *Neuron*, **59**, 43–55.
54. Therrien, G. and Butterworth, R.F. (1991) Cerebrospinal fluid amino acids in relation to neurological status in experimental portal-systemic encephalopathy. *Metab. Brain Dis.*, **6**, 65–74.
55. Zapata, A., Chefer, V.I., Parrot, S. and Denoroy, L. (2013) Detection and quantification of neurotransmitters in dialysates. In C.R. Gerfen *et al.* (eds). *Current Protocols in Neuroscience*, Chapter 7: Unit 7.4.
56. Boughton, B.A., Callahan, D.L., Silva, C., Bowne, J., Nahid, A., Rupasinghe, T. *et al.* (2011) Comprehensive profiling and quantitation of amine group containing metabolites. *Anal. Chem.*, **83**, 7523–7530.

Downloaded from https://academic.oup.com/hmg/article/25/14/2923/2525769 by guest on 21 August 2022