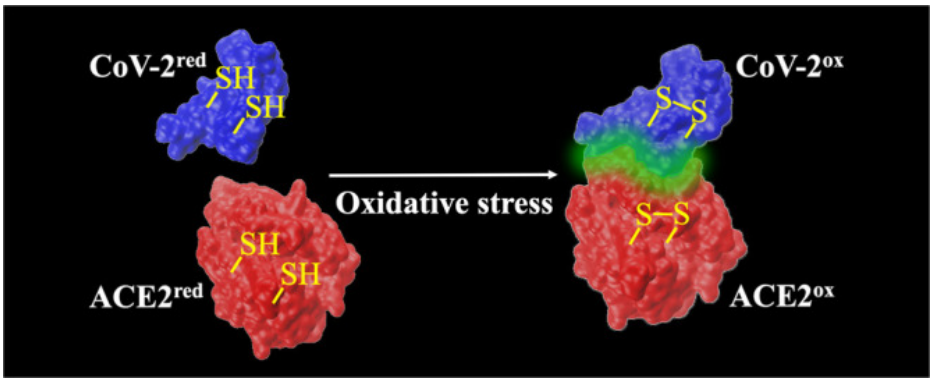
N-acetylcysteine and SARS-Cov-2 spike



Structurally, SARS-CoV-2 contains surface spike proteins, membrane proteins and envelope proteins, as well as internal nucleoproteins that package RNA. The spike protein is a homotrimeric glycoprotein complex with diverse roles realized through dynamic conformational modifications, based in part on disulfide bonds[1]. It enables infection of target cells by binding to receptors of human angiotensin-converting enzyme (ACE2), among others, which triggers proteolysis by the transmembrane protease serine 2 (TMPRSS2), furin and possibly other proteases, leading to fusion of the virion to the host cell membrane. [2]

Virus entry into mammalian cells, or "virus internalization," is a key mechanism of enveloped virus infection and is based on dynamic conformational changes in their surface glycoproteins mediated by disulfide bond reduction and regulated by cell surface oxidoreductases and proteases [3].

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7346263/

It has been shown that entry of SARS-CoV-2 into host cells begins with destabilization of the spike protein through allosteric mechanical transition, which induces a conformational change from the closed "down" to the open "up" state of the receptor binding domain (RBD) of the spike protein[4]. The conformational changes in the RBD and virus binding are induced by TMPRSS2 or cathepsin L, which trigger the transition from the pre-fusion to the post-fusion state.

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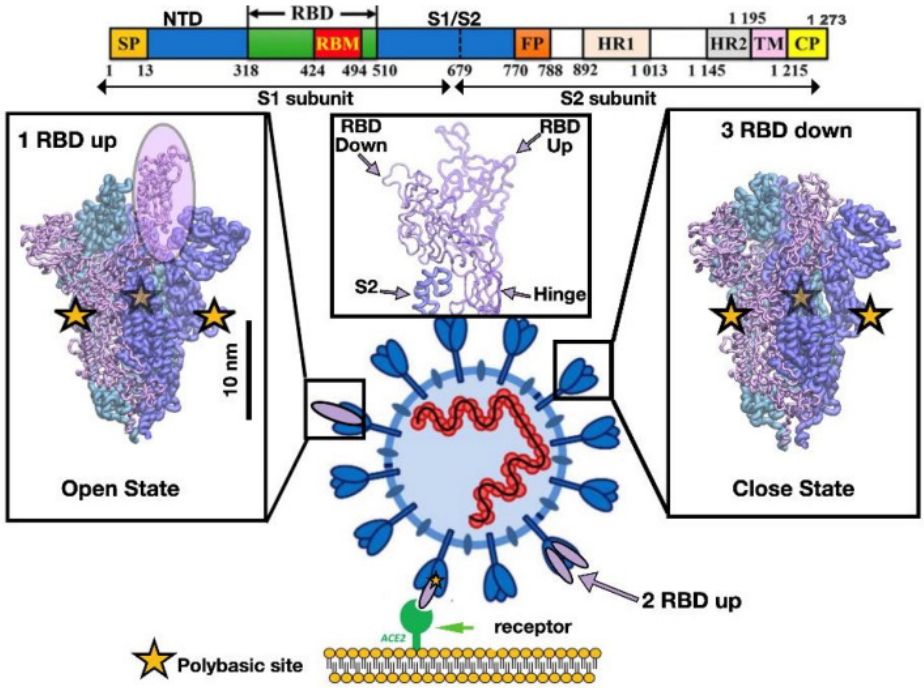

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7730245/
Representation of the different conformations of the receptor-binding domain (RBD) in the acute respiratory syndrome-new spike protein of Coronavirus 2 (SARS-CoV-2). Cellular recognition is initiated by the transition of RBD from the downward (down) to the upward (up) conformation involving RBD detachment from S2 mediated by the hinge region as illustrated in the middle panel. Then, due to the high affinity between RBD in the up conformation and the angiotensin-converting enzyme 2 (ACE2) receptor, binding occurs. The sequence of a spike protein chain is shown above as well as the residue numbers for different protein domains. The bar shows the typical length scale for the entire system. Fusion of the viral and cell membrane occurs via surface proteases that cleave each chain at the polybasic sites (yellow stars) located at the interface of the S1/S2 subunits

The energy released by the reduction of the disulfide bond increases protein flexibility, which is maximal when the reduced state is complete, thus allowing fusion of host virus membranes, which is otherwise impossible due to the repulsive hydration forces present prior to reduction. [5]

The use of acetylcysteine as an antiviral therapy [6]

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Impact of Thiol-Disulfide Balance on the Binding of Covid-19 Spike Protein with Angiotensin-Converting Enzyme 2 Receptor.
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N-Acetylcysteine for COVID-19: A Potential Adjuvant Therapy.
Journal of Health Sciences, (2021) 11(1), 1-6. https://doi.org/10.17532/jhsci.2020.1156
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Rushworth GF, Megson IL.
Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits.
Pharmacol Ther. 2014 Feb;141(2):150-9. doi: 10.1016/j.pharmthera.2013.09.006. epub 2013 Sep 28. PMID: 24080471.
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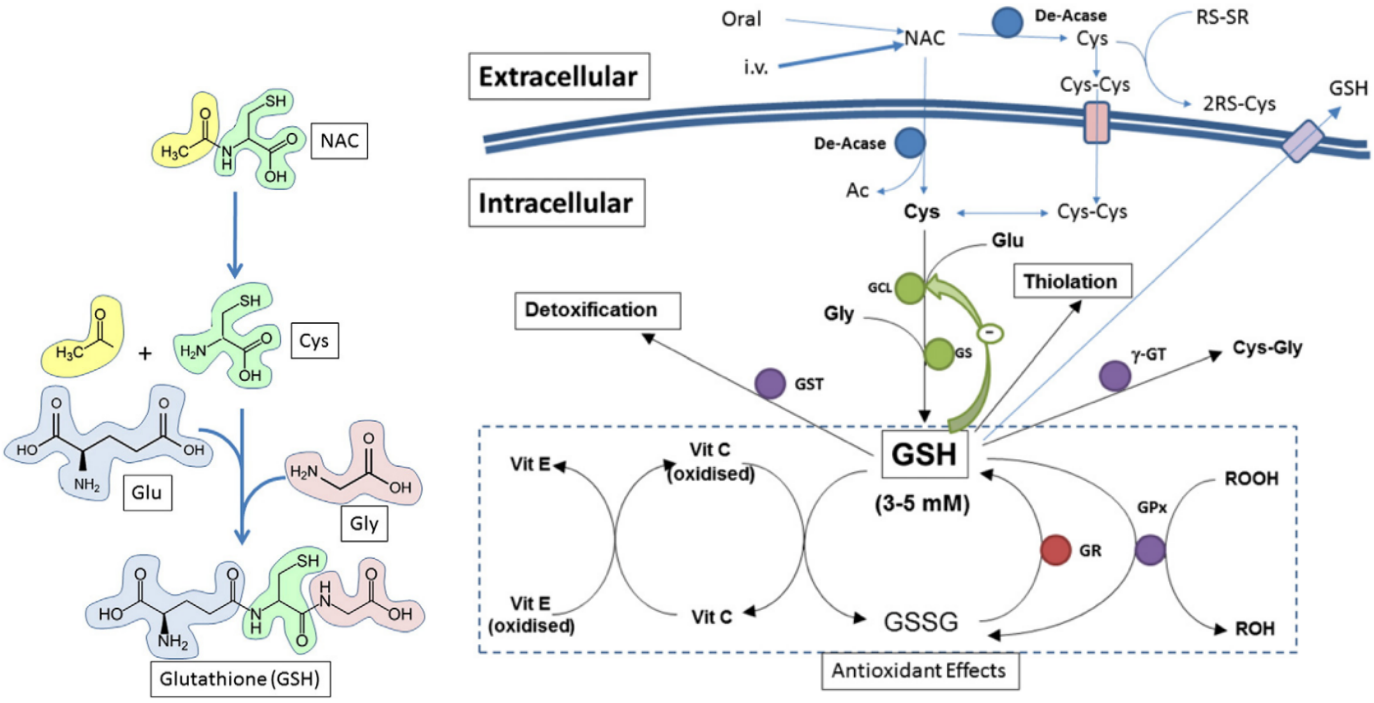
GSH, a tripeptide compound γ-L-glutamyl-L-cysteinyl-gli-cine or GSH, is the most important antioxidant produced by living cells. Severe cases of COVID-19 are associated with lower GSH levels, higher ROS levels and higher redox status (ROS/GSH ratio) than mild-to-moderate cases[7]. Cysteine in GSH has a sulfhydryl/thiol group (-SH), which has the ability to reduce and conjugate in the removal of other peroxides and xenobiotics [8]. Cysteine is also a substrate that determines the rate of GSH synthesis, i.e. when there is oxidative stress in COVID-19, the synthesis of GSH will increase through the Nrf2 activator and requires the availability of an adequate amount of cysteine [9]. NAC, the GSH precursor, functions as an oxygen-deprived radical scavenger and also replenishes depleted GSH stores, enhancing endogenous antioxidant defense. In experimental animals infected with influenza, NAC can promote the production of GSH [10]. N-acetylcysteine acts as an antioxidant directly or indirectly by releasing its cysteine or thiol groups or by breaking sulfide bonds. NAC easily penetrates into cells where it is deacetylated to L-cysteine so that it can be a precursor to GSH in the cell. [11]



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N-acetylcysteine synergizes with oseltamivir in protecting mice from lethal influenza infection.
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N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why.
Free Radic Res. 2018 Jul;52(7):751-762. doi: 10.1080/10715762.2018.1468564. Epub 2018 May 9. PMID: 29742938.
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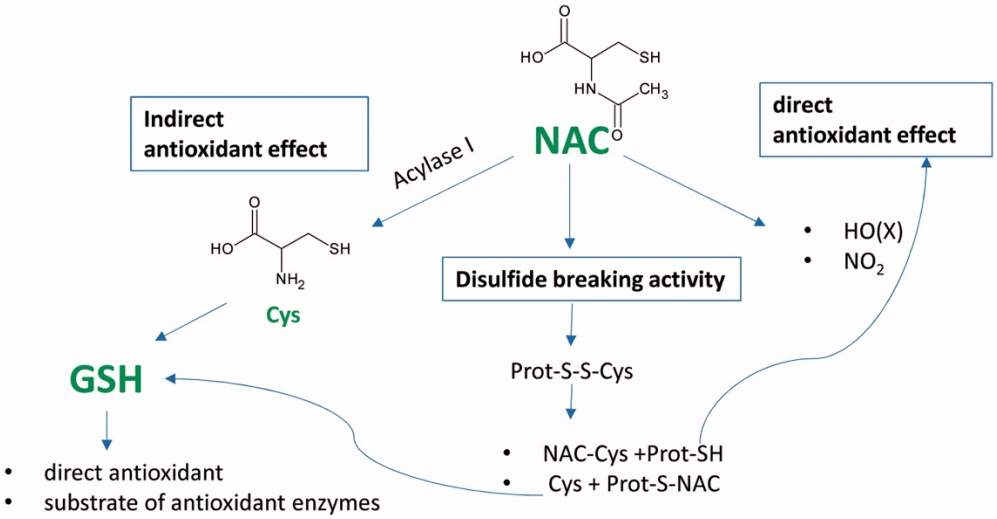
Impact of NAC on GSH synthesis and utilization pathways. De-ACase - deacetylase; GCL - glutathione cysteine ligase; GS - glutathione synthase; GPx - glutathione peroxidase; GR - glutathione reductase; GST - glutathione-S-transferase; -GT - glutamyl transpeptidase.

Human clinical trials have shown that NAC (N-acetylcysteine) improves cellular redox status. NAC has been reported to inhibit gene expression of TNF-α and IL-6, and [12]has also been used to prevent and treat microvascular thrombosis events in COVID-19. [13]
The vonWillebrandfactor multimers are disintegrated by NAC, acting in "medical revascularization" in COVID-19 patients with intravascular thrombosis.
Based on its antioxidant and anti-inflammatory mechanism, Poe et. al, hypothesized that NAC could be a potential therapeutic molecule for the treatment of COVID-19.[12]

Recently, Debnath et al [14]proposed a different mechanism of action of NAC against SARS-CoV-2. Reduction of solvent-accessible disulfide bonding followed by NAC conjugation leads to disruption of the functionally active structure of the spike protein of SARS-CoV-2 and thus reduces the infectivity of the virus.
This effect could work in synergy with the other activities reported by NAC such as antioxidant and anti-inflammatory activity in combating COVID-19.



https://www.tandfonline.com/doi/full/10.1080/10715762.2018.1468564
Overview of the antioxidant action of NAC. The antioxidant effect is due to indirect (GSH synthesis) and direct antioxidant activity, as well as disulfide breaking activity. The indirect activity refers to NAC's ability to act as a precursor to GSH, which in turn is a known direct antioxidant and a substrate of numerous antioxidant enzymes. When a state of oxidative stress depletes SH pools, NAC can act as a direct scavenger of certain oxidants such as NO(X) and NO2. NAC breaks down thiolate proteins thus releasing free thiols, which have better antioxidant activity than NAC and enhance the synthesis of GSH and reduced proteins, which in some cases, as with mercaptoalbumin, have important direct antioxidant activity.

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Conformational Perturbation of SARS-CoV-2 Spike Protein Using N-Acetyl Cysteine, a Molecular Scissor: A Likely Strategy to Combat COVID-19.
ChemRxiv. Cambridge: Cambridge Open Engage; 2020;
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N-acetyl cysteine: A tool to perturb SARS-CoV-2 spike protein conformation.
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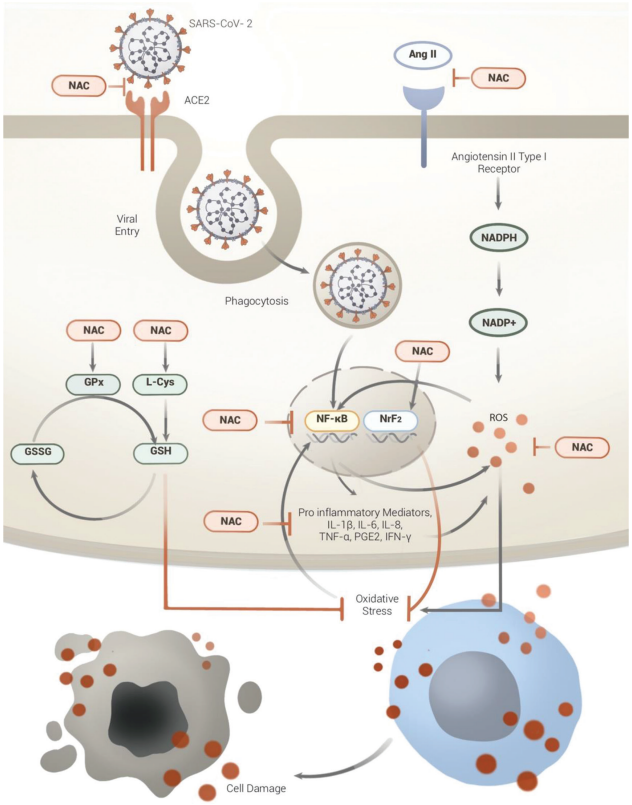
Interestingly, almost all SARS-Cov-2 variants discovered to date retain cysteine residues in their spike protein, so it can be expected that all SARS-CoV-2 variants have an identical pattern of disulfide bonds. In addition, the observed inhibition in viral replication indicates that it might be worth investigating the role of NAC in the pharmacoprevention and treatment of COVID-19 in a clinical setting.



https://www.jhsci.ba/ojs/index.php/jhsci/article/view/1156

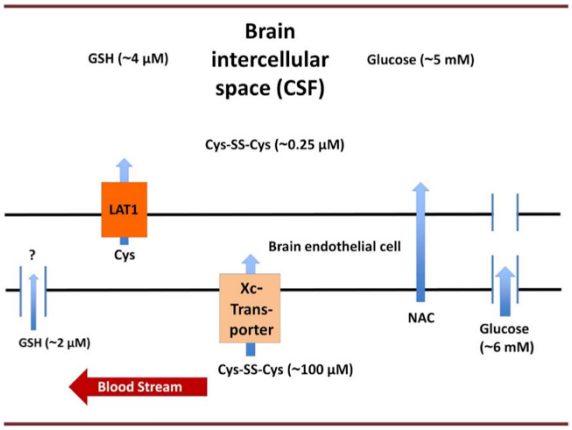
Schematic representation of the potential mechanisms of NAC as an antioxidant and anti-inflammatory in SARS-CoV2 infection. NAC: N-acetylcysteine, SARS-CoV2: severe acute respiratory syndrome coronavirus 2, ACE2: angiotensin-converting enzyme 2, Ang II: angiotensin II, GPx: glutathione peroxidase, GSSG: glutathione disulfide, GSH: glutathione, L-Cys: L -Cysteine, NF-κB: Nuclear factor-κB, NrF2: Nuclear factor 2 related to erythroid, NADPH: Reduced nicotinamide-adenine dinucleotide phosphate, ROS: Reactive oxygen species, TNF-α: Tumor necrosis factor-alpha, IL: Interleukin, PGE2: Prostaglandin E2, IFN-γ: Interferon-gamma

TABLE 1. Clinical trials using systemic NAC as a therapeutic agent for COVID-19

Study	Clinical trial ID	Intervention	Primary outcome
A study of NAC in patients with COVID-19 infection (57)	NCT04374461 Phase 2	NAC IV 6 g/day	Number of patients who are successfully extubated and/or transferred out of critical care due to clinical improvement and discharged from the hospital due to clinical improvement
Efficacy of NAC in preventing COVID-19 from progressing to severe disease (58)	NCT04419025 Phase 4	Inpatients: • NAC 25 mg/kg oral q 4 h until discharge • NAC 1200 mg oral Outpatients: NAC 2400 mg oral then 1200 mg oral twice a day × 2 weeks	• Decrease in dyspnea measured by respiratory rate • Hospital length of stay • Need for mechanical ventilation • Length of time intubated • Need for hospitalization • Outpatients on NAC needing admission to the hospital • Recovery disposition
Inflammatory regulation effect of NAC on COVID-19 treatment (INFECT-19) (59)	NCT04455243 Phase 3	NAC 150 mg/kg every 12 h for 14 days (oral/intravenous)	Time to recovery
A study to evaluate OP-101 (Dendrimer N-acetyl-cysteine) in severe coronavirus disease 2019 (COVID-19) patients (PRANA) (60)	NCT04458298	OP-101 (Dendrimer N-acetyl-cysteine) 2-8 mg/kg	Number of participants with treatment-emergent adverse events

NAC: N-acetylcysteine

The effect of NAC on the SARS-Cov-2 spike can also be exploited to treat possible central and peripheral toxic reactions due to receptor binding of the spike to cells in various tissues. Of particular interest in the case of COVID and SARS-Cov-2 vaccine damage is its "antidotal" action towards spike neurotoxicity, both due to its disulfide bridge breaking effect and antioxidant action.



Indeed, it is known that GSH depletion is a feature of a wide range of neurological and [15]neuropsychiatric disorders. [16]
Although there are subtle differences in GSH metabolism within the CNS compared to other tissues, the basic concepts are the same: GSH is synthesized predominantly in the cytoplasm of cells and depends on cysteine influx to drive the rate-limiting step in GSH synthesis.
The major player in cysteine transport in neurons is the excitatory amino acid transporter C1 (ECAAC1); astrocytes also employ the antiporter cystine/glutamate (Xc-) to supplement the intracellular thiol pool.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3497002/
The schematic describes potential modes of delivery of GSH (and the rate-limiting amino acid cyst(e)ina) through the BBB. These are considered in the studies of [37], but an explicit and quantitative estimate of the net transport of GSH from plasma to CSF is not known. From the limited data provided for GSH influx and efflux with endothelial cell and astrocyte models, it seems unlikely that direct GSH transport would contribute substantially to brain GSH content, especially since most of the GSH uptake studies by Kannan et al. were conducted with an extracellular GSH concentration of 1 mM, almost 3 orders of magnitude higher than the typical GSH concentration in plasma or cerebrospinal fluid [38,39]. The molecules cross the BBB (here represented only by the endothelial cell) at various concentrations and through different mechanisms. The drug N-acetylcysteine (NAC) can cross the BBB by passive non-ionic diffusion without a transporter.
The estimated concentrations of cystine [40,41], GSH [42] and glucose [43] in plasma and cerebrospinal fluid (CSF) are shown in the diagram. Due to its hydrophilic nature, glucose must cross the BBB using GLUT transporters even though the chemical gradient favors diffusion through the BBB. Importantly, cystine is transported into endothelial cells via the Xc- transporter and the corresponding cysteine is transported into the cerebrospinal fluid via LAT1. Some studies report that intact GSH can pass through the BBB through the use of a transport mechanism, however, characterization of the actual transporter is limited and the relative contribution of this putative mechanism to the delivery of GSH to brain cells is likely to be very small

Because cysteine is critical for neuronal synthesis of GSH, NAC has been tested in a number of neurological and neuropsychiatric disorders in which redox imbalance has been implicated in the etiology. [17]

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Disease	Mechanism
Neurodegenerative disorders: SCD, tardive dyskinesia, myoclonus epilepsy, Unverricht–Lundbor type	Antioxidant effect by free-radical scavenging and increased levels of glutathione (Arakawa and Ito 2007)
Down syndrome	Increase and modulation of the level of super oxidase dismutase (Busciglio and Yankner 1995; Behar and Colton 2003)
Multiple sclerosis	Free-radical scavenging and inhibition of TNF toxicity (Lehmann et al. 1994; Stanislaus et al. 2005)
Amyotrophic lateral sclerosis	Increasing the level of glutathione peroxidase and free-radical scavenging (Rosen et al. 1993; Louwerse et al. 1995)
Parkinson's disease	Increasing the level of glutathione and free-radical scavenging (Schapira et al. 1990; Martínez et al. 1999)
Huntington's disease	Free-radical trapping and preventing mitochondrial dysfunction (Fontaine et al. 2000; Stanislaus et al. 2005)
Alzheimer's disease	Increasing the level of glutathione (Adair et al. 2001; Tchantchou et al. 2005; Tucker et al. 2005)
Focal cerebral ischemia	NOS inhibition, regeneration of endothelium-derived relaxing factor, increasing glutathione levels, improving microcirculatory blood flow, and tissue oxygenation (Dawson and Dawson 1997; Cuzzocrea et al. 2000b)
Subarachnoid hemorrhage	Free-radicals scavenger, endothelial apoptosis inhibition, lipid peroxidation reduction, increasing glutathione levels, and SOD enzymatic activities, endothelial integrity protection (Findlay et al. 1989; Sen et al. 2006)
Traumatic brain injury	Repair of TBI-induced mitochondrial dysfunction, increasing the reduced antioxidant enzyme and glutathione levels, inhibition of the activation of NF-κB and TNF-α (Hoffer et al. 2002; Akca et al. 2005; Hsu et al. 2006; Chen et al. 2008)

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3967529/
Summary of the mechanisms of action of NAC in different neurological disorders.

In some cases, it has been suggested that NAC might be useful in this context, not only because of GSH repletion, but also because NAC-derived cystine has the potential to lead to increased glutamate release from astrocytes via Xc- antiporters, resulting in activation of neuronal glutamate receptors and dopamine release. [18]

A further positive effect has been found against proteinopathies. It is known that postmortem tissues from patients with neurodegenerative diseases demonstrate protein misfolding by oxidative stress (transition from alpha helix to beta leaflet conformation) and reduced proteasome activity that degrades misfolded proteins. This wide-ranging effect of proteotoxic stress has led to the term "proteinopathies" for neurodegenerative diseases. Unnithan and his team believe that toxicity-related proteinopathies with GSH loss have a good response to NAC as it can restore GSH loss and prevent toxicity associated with proteotoxic stress. [19]

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