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neurons have been evaluated in homogenates that contain both direct and indirect pathway cell bodies or in slices in which the two populations are not distinguished. The discovery that the weaker glutamatergic drive of indirect pathway neurons can contribute to cocaine self-administration would not have been possible without the use of this sophisticated behavioral task and the examination of each pathway independently in the context of an animal model showing inter-individual differences in behavior.

Bock *et al.*'s findings also indicate that glutamatergic activation of the indirect pathway is potentiated following cocaine self-administration only in some subjects; however, the reasons for these differences are unknown. It is possible that genetic variation results in differences in the strength of indirect pathway inputs even before drug use, and these variations may predict susceptibility to addiction. At this time, the strength of indirect pathway inputs have not been measured *in vivo*, and more studies will be necessary to determine whether this variability is a cause or a consequence of drug use and what other factors contribute to it.

The excitatory drive to nucleus accumbens neurons comes from multiple brain areas, including the prefrontal cortex, ventral hippocampus, thalamus and basolateral nucleus of the amygdala, and these inputs appear to make specific connections to accumbens neurons that in turn make connections to other structures, such as the ventral pallidum⁷. Recent studies have also identified glutamatergic neurons in the ventral tegmental area that project to the nucleus accumbens, some of which also express markers of dopamine neurons⁸. It will

be important to identify the source of the glutamate pathways that innervate indirect pathway neurons, as drug-induced changes in the activity of those neurons would be critical for synaptic plasticity that feeds back and affects motivation to seek the drug.

One consistent observation in human imaging studies of individuals who abuse addictive drugs is a decrease in the levels of D2 receptor binding in the striatum⁹. In addition, decreases in striatal D2 receptor binding have been linked in human and animal studies to increased impulsive behavior and greater likelihood of drug-taking^{9,10}. D2 receptors inhibit the activity of neurons on which they are expressed; thus, a decrease in D2 receptor activity in indirect pathway neurons would take away a layer of inhibition and would be expected to increase indirect pathway activity, thereby decreasing motivation to take cocaine. If this is the case, why would there be a decrease in D2 receptor binding in human drug addicts? One possibility is that D2 receptor number is not reduced on indirect pathway neurons in the brains of those struggling with addiction, but rather D2 levels are reduced in other neuronal subtypes, such as the large cholinergic interneurons in the nucleus accumbens or on the dopamine terminals themselves. Mice lacking D2 receptors exclusively on dopamine neuron terminals show greatly increased sensitivity to cocaine, suggesting that a decrease in presynaptic D2 receptors might increase motivation to seek cocaine¹¹. The identity of the cell types exhibiting decreased D2 receptor binding in individuals with addiction is currently an open question, and more research will be required to decipher this question.

Given the complexity of D2 signaling in the nucleus accumbens, the study by Bock and colleagues³ suggests that medications targeting indirect pathway neurons and increasing their activity may be more effective than medications targeting dopamine receptors to help treat addiction. One way to alter the activity of these neurons might be to develop pharmacological agents that target molecules expressed specifically in the indirect pathway, such as adenosine receptors¹² or targets of the opiate family peptide enkephalin¹³. These approaches are currently being tested in animal models and human subjects and hopefully will provide some long-sought clues to the complex neurobiological mechanisms that mediate addiction.

COMPETING FINANCIAL INTERESTS

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NUB1 snubs huntingtin toxicity

Rebecca Aron, Andrey Tsvetkov & Steven Finkbeiner

A screen now identifies a protein that regulates degradation of mutant huntingtin, which causes Huntington's disease, and manipulations show that promoting clearance of the toxic protein itself may be sufficient to halt disease.

Huntington's disease is a hereditary neurodegenerative disorder that is characterized by progressive motor, cognitive and behavioral deficits. The cause of Huntington's disease, an

expansion of the stretch of repeated CAG trinucleotides in the gene that encodes the protein huntingtin (HTT), was identified nearly 20 years ago, yet there are still no treatments to slow disease onset or progression. Huntington's disease is similar to other neurodegenerative disorders in which accumulation of a misfolded protein, in this case the polyglutamine-expanded mutant HTT (mHTT), is toxic and leads to neurodegeneration. Vast efforts have focused on understanding the molecular mechanisms of mHTT-mediated neurodegeneration in the hope of identifying potential

targets for therapeutic intervention. An obvious target, however, remains the mHTT protein itself. In this issue of *Nature Neuroscience*, Lu *et al.*¹ report the identification of a modulator of mHTT protein abundance, NUB1, and demonstrate that targeting the mHTT protein itself for degradation is sufficient to suppress neurodegeneration *in vivo* in a fly model of Huntington's disease.

The hypothesis is straightforward. If a disease is caused by the accumulation of a toxic protein, then lowering the amount of the protein should ameliorate the disease. Indeed, this

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is the case in Huntington's disease mouse models, where turning off the expression of a mHTT fragment that induces behavioral deficits and neurodegeneration can reverse disease². The most recent strategies aimed at selectively lowering mHTT expression used RNA silencing techniques to target mHTT at the mRNA level, and they did, in fact, reverse disease phenotypes in a mouse model of Huntington's disease³. Given the success of targeting mHTT at the mRNA level in various Huntington's disease models, targeting the degradation of mHTT protein is likely to hold similar promise as a therapeutic strategy for the treatment of Huntington's disease and may be easier to develop or deliver.

Lu *et al.*¹ set out to identify factors that regulate mHTT protein degradation by performing an unbiased, genome-wide screen for genetic modifiers of mHTT protein abundance. Screening approaches have been used to identify modulators of mHTT biology, such as aggregation or proteolytic processing. However, this is the first reported genome-wide screen specifically designed to identify modulators of mHTT amounts. In their screening approach, mHTT protein was quantified in a *Drosophila* cell line by a high-throughput immunoassay (homogeneous time-resolved fluorescence, which is based on Förster resonance energy transfer) that was previously optimized for monitoring disease progression⁴. The primary screen identified ~300 genes with human homologs as potential enhancers or suppressors of mHTT degradation, and secondary screening then confirmed 13 candidates that reproducibly modulated mHTT levels in Huntington's disease patient fibroblast cell lines as modifiers of full-length endogenous mHTT abundance.

Lu *et al.*¹ next used a well-characterized *Drosophila* model of Huntington's disease to test the remaining candidates in an *in vivo* assay. This model displays a motor-impairment phenotype that can be measured by performance in a climbing assay. *Drosophila* gain- and loss-of-function mutants available for the final candidates were tested in the climbing assay, and seven of those had a climbing activity phenotype consistent with that predicted by their effect on mHTT.

The authors went on to investigate the potential function of one of these candidates, NUB1, which consistently modified mHTT protein abundance and *in vivo* toxicity. The two main pathways that clear mHTT are the ubiquitin proteasome system and autophagy⁵. Large mHTT aggregates or inclusion bodies are thought to be cleared primarily by means of autophagy, whereas the ubiquitin proteasome system has been

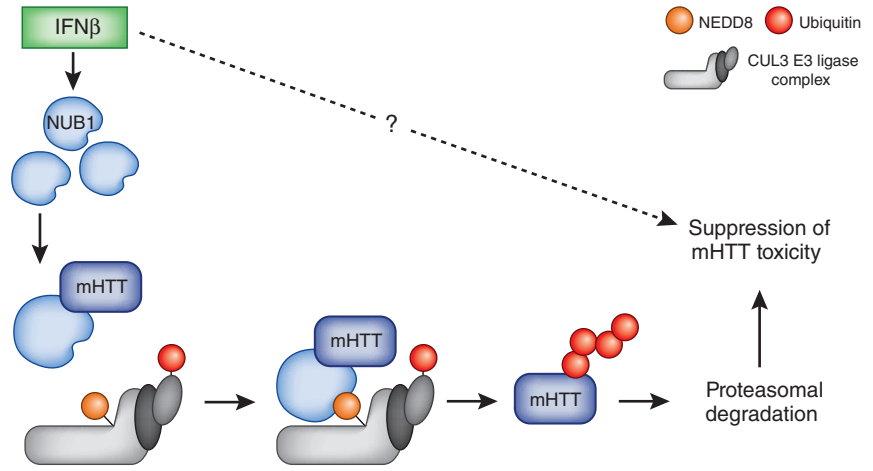


Figure 1 Potential mechanism of NUB1-mediated mHTT degradation and suppression of toxicity. NUB1 mediates targeting of mHTT to the activated, NEDD8-conjugated CUL3 E3 ubiquitin ligase complex. mHTT is then polyubiquitinated and targeted to the proteasome for degradation. IFN β can suppress mHTT-mediated cytotoxicity through induction of NUB1 or, potentially, through other pathways, such as modulation of inflammation.

suggested to be more important for clearing soluble or smaller oligomeric species of mHTT. To characterize the potential function of NUB1 in clearing mHTT by either system, Lu *et al.*¹ followed mHTT degradation in the presence of proteasome or autophagy inhibitors. The ability of NUB1 overexpression to increase the clearance of mHTT was blocked by proteasome inhibitors, but was unaffected by autophagy inhibitors, suggesting that proteasomal degradation was the primary pathway by which NUB1 mediated mHTT clearance.

Notably, NUB1 overexpression decreased both HTT and mHTT protein levels in the heterozygous Huntington's disease cell lines. In designing therapeutic strategies to lower mHTT levels, nonselective targeting of only mHTT has been an issue of some concern. HTT function is incompletely understood. It is critical in development and its deletion causes embryonic lethality. Conditional deletion of HTT in adult neurons also leads to neurodegeneration⁶. Thus, nonselective targeting of HTT could be detrimental. However, recent studies targeting *HTT* mRNA indicate that loss of up to 75% of HTT is well tolerated³. Lu *et al.*¹ tested the effect of NUB1 overexpression in neurons differentiated from heterozygous induced pluripotent stem cells derived from Huntington's disease patients, and although NUB1 overexpression reduced expression of both HTT and mHTT, it still mediated suppression of mHTT-induced toxicity. These results support the argument that reduction of both HTT and mHTT may still be more beneficial than no reduction at all.

So how might NUB1 mediate HTT clearance? Consistent with a function of NUB1 in

promoting the degradation of mHTT protein by the ubiquitin proteasome system, Lu *et al.*¹ found that NUB1 overexpression could enhance polyubiquitination of mHTT. They further demonstrated that a NUB1-interacting protein, the ubiquitin-like modifier NEDD8, is required for NUB1-mediated effects on mHTT clearance. A well-characterized NEDD8 substrate, the CUL3 E3 ubiquitin ligase, was required for NUB1 clearance of mHTT and for NUB1-induced ubiquitination of mHTT. These results suggested a model of NUB1-mediated mHTT degradation in which NUB1, through physical interaction with NEDD8 and mHTT, recruits an activated, NEDD8-conjugated CUL3 E3 ubiquitin ligase to mHTT, resulting in polyubiquitination and subsequent degradation of mHTT by the proteasome (Fig. 1).

Although these results suggest a highly plausible mechanism for NUB1-mediated degradation of mHTT, the direct targeting of mHTT to the proteasome by means of physical interaction with NUB1 has yet to be shown. NUB1 was identified by high-throughput methods as interacting with HTT⁷, although the nature of this interaction has not been further characterized. NUB1 regulates the activity of p53 (ref. 8), a loss-of-function suppressor of mHTT-mediated neurodegeneration⁹, and possible alternative or additional mechanisms of NUB1 function in mHTT toxicity could therefore still be uncovered. These and other potential disease-modifying pathways that NUB1 may be involved with could be of particular interest in future studies characterizing the potential of modulating NUB1 activity as a therapeutic strategy for Huntington's disease.

NUB1 has now been convincingly validated as a potent modulator of mHTT protein abundance and toxicity in several cell models and in *Drosophila in vivo*. But is NUB1 a viable therapeutic target for treating Huntington's disease? NUB1 itself is not an ideal target for small-molecule drugs, as it is not known to possess receptor or enzymatic activity. However, NUB1 has been reported to be inducible by interferon- β (IFN β)¹⁰, an agent approved by the US Food and Drug Administration to treat the autoimmune disorder multiple sclerosis. Lu *et al.*¹ explored this strategy of upregulating NUB1 by IFN β treatment and observed an increase in NUB1, a decrease in mHTT and suppression of mHTT-mediated toxicity in Huntington's disease cell models. Moving these studies into rodent models of Huntington's disease *in vivo* will be an important next step toward uncovering the therapeutic potential of targeting NUB1 and the use of IFN β as a treatment for Huntington's disease.

IFN β is thought to slow disease progression in multiple sclerosis by modulating inflammation. Dysregulated immune signaling and inflammation have also been reported in Huntington's disease¹¹. Although tests of IFN β treatment to modulate inflammation in Huntington's disease have not been carried

out, it is interesting to speculate that a potential mechanism of IFN β -induced suppression of inflammation in Huntington's disease might be through NUB1 or other IFN β targets involved in protein homeostasis. IFNs have been reported to induce a response to oxidative stress and to upregulate key components of protein homeostasis machinery, such as ubiquitin and immunoproteasomes¹², and immunoproteasomes have been reported to be induced in neurons from mouse models of Huntington's disease¹³. Future studies will help to more clearly define the possible link between protein degradation modifiers, such as NUB1, and inflammation in Huntington's disease.

Can targeting NUB1-mediated protein degradation be beneficial for treating other protein misfolding-related neurodegenerative diseases? NUB1 has been proposed to reduce, through regulation of glycogen synthase kinase-3 β protein levels, phosphorylation and aggregation of the protein tau, which has been implicated in neurodegenerative disorders, such as Alzheimer's disease and other tauopathies¹⁴. NUB1 may also modulate proteasomal degradation of synphilin-1, a protein that interacts with α -synuclein and is a major component of the Lewy bodies associated

with dementia and Parkinson's disease¹⁵. Although more studies are needed to determine the implications of targeting NUB1 as a therapeutic strategy for neurodegenerative diseases, targeting proteasomal degradation of selected substrates has the potential to be broadly applicable to many diseases associated with expression and accumulation of toxic proteins.

COMPETING FINANCIAL INTERESTS

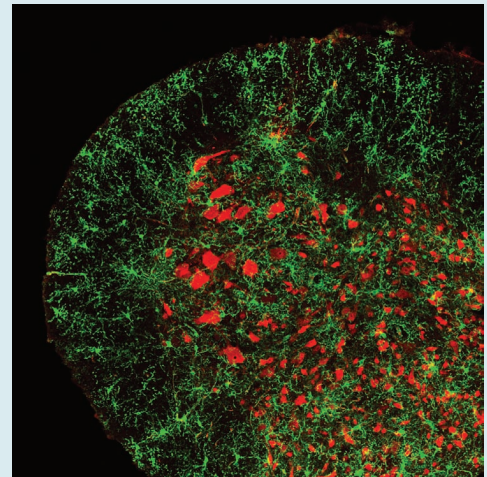
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Oligodendrocyte failure in ALS

Amyotrophic lateral sclerosis (ALS) is a debilitating neurodegenerative disease that is characterized by the death of motor neurons and a progressive loss of all motor function. The motor neuron degeneration seen in ALS has also been intimately linked to glial cell dysfunction, which includes reactive gliosis, remyelination failure and alteration in glial metabolic support. Previous studies have documented that the glial precursor cells, which generate the myelinating cells of the CNS, known as NG2⁺ oligodendrocyte progenitor cells, exhibit greatly enhanced proliferation during the end stage of disease in the spinal cord of the *SOD1(G93A)* transgenic mouse model of ALS. However, the exact extent and timing of the oligodendrocyte abnormalities in ALS patients and the mouse model are unclear. In addition, the cause of the remyelination failure, and whether this is even important for disease pathology, remains unknown. On page 571 in this issue of *Nature Neuroscience*, Kang *et al.* show that NG2⁺ progenitor proliferation is enhanced before disease onset in this ALS mouse model.

NG2⁺ oligodendrocyte progenitor cells are peppered throughout the gray matter of the spinal cord and are found in close proximity to neurons (as shown in this picture that depicts immunostaining of NG2 protein (green) and NeuN (red) in the ventral horn of mouse spinal cord). Kang *et al.* use genetic cell fate tracing techniques to show an enhanced proliferation of NG2⁺ cells in the spinal cord gray matter of young, asymptomatic *SOD1(G93A)* mice. Surprisingly, however, these NG2⁺ cells fail to mature properly or sufficiently remyelinate spinal cord motor neurons. Although the NG2⁺ cells are able to differentiate into oligodendrocytes, the newly formed oligodendrocytes show morphological abnormalities, such as irregularly shaped cell processes and soma, and do not fully mature, as assessed by the reduction in remyelination and expression of myelin-forming proteins. The authors also find similar NG2⁺ and myelination defects from postmortem spinal cord and motor cortex samples taken from ALS patients. To determine whether these defects had any effect on disease onset or progression, the authors then returned to their mouse model of ALS, and genetically inactivated the ALS-linked mutant gene *SOD1(G37R)* specifically from NG2⁺ glial progenitors. This delayed disease onset in the mice and increased their survival. These results suggest that preserving oligodendrocyte function may be a potential therapeutic target for combating this deadly disease.



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