Huntington disease (HD) is an inherited neurodegenerative disorder with no cure or effective palliative treatment. An ideal therapy would arrest pathogenesis at early stages before neuronal damage occurs. However, although the genetic mutation that causes HD is known, the molecular chain of events that leads from the mutation to disease is not well understood. Accumulating evidence suggests that synaptic dysregulation may be involved, and the earliest known deficit is hyperfunction of glutamate-type N-methyl-D-aspartate receptors (NMDARs) in the selectively vulnerable medium spiny neurons of the striatum. A previous study found that the mutant Htt protein interferes with downregulation of juvenile NMDAR subtypes that contain GluN3A subunits by sequestering the endocytic adaptor PACSIN1 and preventing their removal from the cell surface. Loss of PACSIN1 and consequent gain of GluN3A function reactivated a synapse pruning mechanism that is important during development but harmful when active at later stages. Suppressing the GluN3A reactivation corrected the NMDAR hyperfunction and prevented the full range of HD signs and symptoms in mouse models, encouraging efforts to develop GluN3A-selective antagonists and/or explore alternative therapeutic approaches to block GluN3A expression.

Huntington disease (HD) is an inherited autosomal dominant neurodegenerative disorder characterized by progressive motor disability, decline of cognitive functions, and psychiatric disturbances. Onset usually occurs in middle adult life, and subsequent disease progression is inexorable for 15 to 20 years, eventually leading to death. Clinical diagnosis is based mainly on the presence of involuntary movements, especially chorea. However, subtle to severe cognitive and psychiatric symptoms often emerge beforehand and are major burdens for patients with HD and their families. Neuregeneration involves early synapse dysfunction and loss followed by massive cell death, especially in the striatum, where medium spiny neurons (MSNs) that make up 90% to 95% of all neurons are selectively vulnerable.

At the genetic level, HD is caused by expansion of a sequence of CAG nucleotide repeats in the first exon of the gene that encodes the Htt protein. The expansion codes for an abnormally long polyglutamine tract in the N-terminal region. The number of CAG repeats is inversely correlated with age at disease onset and severity. The clinically defined threshold is 36, but individuals with 27 to 35 repeats are considered at risk and can develop subtle emotional or behavioral abnormalities. Juvenile cases have been reported in which the expansion includes more than 100 repeats.

Although the genetic mutation was discovered more than 2 decades ago, there is no cure or treatment that slows disease progression. Even palliative treatments are rudimentary, and full-time care is required at late stages. The goal, not only for HD but also for other neurodegenerative diseases, is to find treatments that slow (ideally prevent) progression before neuronal damage is irreversible. Key challenges for basic research have been an incomplete knowledge of mechanisms that mediate mutant Htt toxic effects, especially at early disease stages before behavioral symptoms or cell death are manifest, and a related lack of sensitive assays for identifying compounds targeted to these early disease mechanisms.

The native function of Htt is not well understood, but there seems to be something inherently neurotoxic about the expanded polyglutamine tract because other neurodegenerative diseases are caused by analogous expansions in unrelated proteins. Nevertheless, the protein context matters because the vulnerable neuronal populations vary for the different polyglutamine diseases. A widespread hypothesis has been that toxic effects are caused by aberrant protein–protein interactions promoted by domains that are exposed in the mutant due to protein misfolding. In support of this, the expanded polyglutamine tract causes mutant Htt and other proteins to fold incorrectly and aggregate in inclusions throughout the neuronal soma and dendrites, and screens for protein–protein interactions that are either enhanced or disrupted by the polyglutamine mutation have yielded promising new targets for another polyglutamine disease, spinocerebellar ataxia.

In the case of HD, recent work has linked one of the Htt interactors to hyperfunction of glutamate-type N-methyl-D-aspartate receptors (NMDARs). This finding is particularly salient because NMDAR hyperfunction is one of the earliest alterations in HD mouse models and has long been thought to be the pathogenic driver in a range of acute and chronic neurologic diseases, including stroke, HD,
and Alzheimer disease. In this article, we discuss the rationale for targeting NMDARs to treat HD and examine prospects for translating the new findings from basic research into therapies.

**NMDAR Dysfunction as a Molecular Basis for HD Neurodegeneration**

The main targets of extrinsic synaptic inputs to the striatum are the MSNs. Major incoming afferents originate in the cerebral cortex and thalamus, making up to 10,000 excitatory synapses onto spines of the dendrites of a single MSN. Spines are small dendritic protrusions that form the postsynaptic compartment of synaptic contacts and include a structure known as postsynaptic density (PSD), where neurotransmitter receptors are clustered. The neurotransmitter used at the incoming synapses is glutamate, which is the main excitatory neurotransmitter in the central nervous system. The glutamate receptors in the PSD include several types with distinct functions, including NMDARs. Studies in HD mouse models have found that NMDAR hyperfunction can be detected in MSNs at the earliest stages—long before synapse and spine loss, neuronal death, or behavioral deficits—motivating the hypothesis that NMDARs are key players in the pathogenic cascade.

Specifically, NMDAR hyperfunction presents as an enhancement in the amplitude and time course of MSN responses to neurotransmitter released from afferent synaptic connections. The enhancement has been attributed to increases numbers of NMDARs located at extrasynaptic regions, away from the PSD and outside the synaptic cleft, where the neurotransmitter concentration is highest during synaptic transmission.

Extrasynaptic receptors are thought to be activated by excess glutamate that spills out of the synaptic cleft during heavy synaptic activity. A current concept is that the extrasynaptic receptors are toxic because they are coupled to signaling pathways that trigger cell death, whereas NMDARs located within the PSD are trophic, important for learning, memory, and neuronal survival. On the basis of this concept, strategies directed to preserving synaptic NMDAR function while selectively blocking extrasynaptic receptors are viewed as candidate therapeutic options not only for HD but also for other neurodegenerative diseases, such as Alzheimer disease, and acute brain insults, such as ischemia. Indeed, a widely prescribed current treatment for Alzheimer disease is memantine, an NMDAR blocker that at low concentrations is a widely prescribed current treatment for Alzheimer disease. In this article, we discuss the rationale for targeting NMDARs to treat HD and examine prospects for translating the new findings from basic research into therapies.

One possibility is that the trophic vs toxic signaling properties of synaptic and extrasynaptic NMDARs are due to differences in subunit composition that determine both receptor localization and coupling to specialized signaling microdomains. The NMDAR subunits have long intracellular C-terminal domains with sequences that have diverged over evolution and could mediate association with pro-death (in the case of extrasynaptic receptors) or trophic (in the case of synaptic receptors) signaling complexes. If so, NMDAR toxic effects might be preventable with antagonists selectively targeted to the subunits that are linked to prodeath signaling pathways.

Individual NMDARs are heterotetrameric complexes made from a menu of 8 subunits. Each complex includes at least 1 GluN1 subunit, 1 or 2 GluN2 subunits (A-D), and sometimes a GluN3 subunit (A or B). Extrasynaptic NMDARs are thought to contain GluN2B subunits and/or GluN3A subunits. Increased levels of GluN2B have been reported in HD mouse models, where NMDAR hyperfunction could be blocked using the selective GluN2B antagonist ifenprodil. However, neither the neurodegeneration nor the behavioral deficits have been cleanly eliminated by GluN2B antagonists or in a GluN2B genetic knockout. Moreover, blocking GluN2B-containing NMDARs, even if the block was selective, might cause significant secondary effects because GluN2B-containing NMDARs are located within synaptic clefts in addition to the extrasynaptic expression, and broad-spectrum NMDAR blockers, such as the anesthetic ketamine and the recreational drug phencyclidine, have major effects on cognition.

In contrast, GluN3A subunits are preferentially located at extrasynaptic sites and have already been partially validated for HD. The HD screens identified the GluN3A-selective endocytic adaptor PACSIN1—also known as syndapin1—as an Htt interactor that binds with higher affinity to the mutant Htt. A separate study found that PACSIN1 targets GluN3A-containing NMDARs (GluN3ARs) for removal from mature excitatory synapses, providing a disease mechanism linking mutant Htt to NMDAR hyperfunction that had been missing. That is, GluN3ARs are normally expressed during early postnatal and juvenile stages, and PACSIN1 plays a role in keeping levels low during adulthood (Figure 1A); thus, sequestration of PACSIN1 by mutant Htt was predicted to cause a developmentally inappropriate increase (Figure 1B). The mechanism was verified in vitro, with mutant Htt causing GluN3ARs to accumulate at the neuronal surface by impairing PACSIN1 function. Elevated GluN3A expression was further confirmed in a variety of HD mouse models and in patients. Finally, knocking out GluN3A reversed the NMDAR hyperfunction, progressive synapse loss, motor deficits, and much of the neuronal death that occurs in a standard mouse model (Figure 2), indicating that GluN3A reactivation is an important factor in HD pathogenesis.

The GluN3AR reactivation hypothesis suggests an explanation for why neuronal death is preceded by synapse and spine loss, which is an important step for refining the interconnectivity of neuronal circuits during critical periods of postnatal brain development. aberrant reactivation would drive continued synapse elimination beyond the developmentally appropriate time window. In support of this, preventing GluN3A downregulation by overexpressing a transgene or by interfering with GluN3A endocytosis caused developmentally inappropriate synapse elimination in the hippocampus, a brain region where development has been studied intensively. In addition, postmortem neuropathologic analyses suggested that striatal synapses are more labile in HD brains, which is reminiscent of adult mouse synapses expressing transgenic GluN3A in which synapse elimination was partially counterbalanced by new synapse formation, particularly when PACSIN1-mediated endocytosis was impaired. The loss of synapses could explain subsequent neuronal death because MSN survival requires continual trophic sustenance generated by afferent synaptic activity. Indeed, a longitudinal study in patients destined to develop HD found that the loss
of gray matter at premanifest stages, likely signifying synapse elimination, was highly correlated with the age at onset and later severity of HD symptoms. Notably, abnormal GluN3A levels\(^\text{26}\) and elimination of excitatory synapses\(^\text{27}\) are also associated with schizophrenia, although a causal role has not yet been established.

### Downstream Mechanisms

In the future, it will be important to determine how GluN3ARs drive synapse elimination and neuronal death because elucidating the mechanisms that operate downstream of GluN3A could provide additional disease modifiers and guide the design of alternative early therapeutic strategies. The NMDARs are ionotropic receptors (meaning that ligand binding opens an integrated ion channel) that flux Ca\(^{2+}\) along with other cations. Excessive Ca\(^{2+}\) influx can be toxic and has been implicated in neurodegeneration, but GluN3ARs admit less Ca\(^{2+}\) than standard NMDAR subtypes (composed of GluN1 and GluN2 subunits), and GluN3A overexpression was paradoxically neuroprotective in acute models of brain injury where Ca\(^{2+}\) is known to be the toxic intermediary.\(^\text{28,29}\) At a minimum, results such as these indicate that there are important mechanistic differences in NMDAR-dependent toxic effects in acute models, in which Ca\(^{2+}\) influx is massive and the damage occurs in minutes to hours, compared with neurodegenerative diseases in which excess Ca\(^{2+}\) accumulation would cause damage for years to decades if it occurs at all.

GluN3A-driven toxic effects might instead reflect the absence of enough trophic Ca\(^{2+}\) influx at synaptic sites, where it is essential for activating mechanisms that stabilize synapses and counteract synapse destabilization and elimination mechanisms operating in the background. Reduced synaptic Ca\(^{2+}\) influx in HD would occur if GluN3ARs replaced standard NMDAR subtypes at synaptic sites, because of the lower Ca\(^{2+}\) permeability, and/or drove NMDARs away from PSDs into extrasynaptic locations. Supporting the latter theory, investigators have suggested that GluN3ARs may form part of a mobile receptor pool with higher propensity to diffuse in the plane of...
GluN3A in Huntington Disease

Figure 2. GluN3A Suppression Prevents a Wide Spectrum of Pathologic Events in Huntington Disease (HD)

A. High magnification of dendritic segments from striatal medium spiny neurons (MSNs) of control and YAC128 mice showing degeneration of dendritic spines (original magnification ×63). B. Genetic suppression of GluN3A in YAC128 mice expressing full-length mutant Htt with 128 CAG repeats prevents not only prodromal synaptic defects, including enhanced GluN3A-containing N-methyl-D-aspartate receptor (NMDAR) currents and synapse and spine loss, but also later neurodegeneration and behavioral deficits.

the membrane between the synaptic and extrasynaptic sites.20 In addition, GluN3A overexpression impairs the stabilization of GluN2B subunits at PSDs in striatal tissue.26

Alternatively, toxicity might be an intrinsic property of GluN3A subunits, and the synaptic vs extrasynaptic location might be of secondary importance or epiphenomenal. It is increasingly recognized that intracellular domains of a variety of ionotropic glutamate receptors, including NMDARs, can activate second messenger signaling pathways on ligand binding in the absence of ion permeation (metabotropic signaling).30 To date, the carboxy-terminal domain of GluN3A has been found to bind several actin regulatory proteins that could be involved in synaptic destabilization and elimination by regulating cytoskeletal rearrangements.22,31 To test the relevance of location, it will be interesting to see whether inducing standard NMDARs to move to extrasynaptic sites by disrupting cytoskeletal anchoring motifs will increase toxic effects and, conversely, whether artificially anchoring GluN3ARs more firmly to the PSD will lessen their effect on spine stability and elimination.

Finally, multiple other factors, such as defective neurotrophin signaling, dysregulation of potassium pumps, or activation of apoptotic signaling pathways, have been implicated in HD,32-34 and it will be important to determine whether and how these factors are related to GluN3A reactivation. That is, although the GluN3A deletion experiments10 suggested that GluN3A expression is necessary or permissive for HD pathogenesis, it did not rule out the parallel involvement of other factors. One intriguing possibility is that the other factors might be downstream of the mutant Htt interaction with PACSIN1 because, although PACSIN1 seems to be a selective adapter for GluN3A among glutamate receptors, it is additionally known to adapt other proteins for endocytosis and to be involved in synaptic vesicle-trafficking mechanisms that could alter synaptic transmission and neuronal network excitability.

Limitations and Therapeutic Perspectives

Limiting secondary effects is particularly important for a disease, such as HD, for which any therapy would likely need to be lifelong. GluN3A might prove to be a better target with less secondary effects than other NMDAR subunits because programmed downregulation largely eliminates expression by adulthood in healthy individuals. However, GluN3A expression is maintained in some populations of interneurons, and the emerging concept that PACSIN1 is required to keep expression levels low in excitatory neurons suggests that GluN3A may continue to play a role in adult brains. On the other hand, an analysis35 of GluN3A knockout mice revealed only mild behavioral phenotypes, suggesting that secondary effects of GluN3AR antagonists might be tolerable.

Although the prevention of HD deficits achieved by knocking out GluN3A is promising (Figure 2B), true target validation will require further experiments because the gene was deleted from the germline, which could be a confounding factor because GluN3A was absent during early developmental stages in multiple brain regions not affected in HD. Follow-up experiments using conditional knockout mice in whom aberrant GluN3A expression is not knocked out until after normal downregulation has occurred could resolve some outstanding caveats. Reduction of GluN3A levels using small interfering RNA delivered with viral vectors is another option that might achieve the same sort of result with more flexibility and additionally provide information about dosage. In both cases, the efficiency of GluN3A suppression could be tested at a variety of disease stages and in selected neuronal populations. The outcome of these experiments should allow determination of whether GluN3ARs had to be blocked during a critical time window and whether blockade should be targeted exclusively to HD-vulnerable cell types. If successful, it might be possible to extrapolate small interfering RNA-based strategies to humans.

An alternative intervention would be to restore normal PACSIN1 levels with gene therapy approaches or to inhibit the pathologic Htt-PACSIN1 interactions using brain-penetrant small molecules or peptides. Of these options, our own attempt at restoring PACSIN1 function in an ex vivo slice model of HD was complicated by toxicity issues, raising the concern that overexpressing PACSIN1 might be excessively sensitive to dosage owing to dynamin sequestration and consequent nonspecific inhibition of endocytosis.

Ultimately, we anticipate that a selective GluN3A antagonist will be required. None is yet available, but an investment in screening for one is warranted. This development of a selective GluN3A antagonist should be possible because neurotransmitter receptors of-
ten make good targets compared with other molecular players that are not exposed to the extracellular space. Indeed, a wide range of antagonists against other subtypes of glutamate receptors or ion channels have already been produced.

Conclusions

Key translational goals in HD research are the development of a noninvasive assay that could track and predict disease progression from early (premanifest or prodromal) stages and delineation of the full sequence of events that lead from the genetic mutation to the disease. Assay development is arguably more important because a range of ideas for creating new drugs has already emerged, but the currently available strategy of evaluating candidate medicines during the decades of disease progression might be too costly to be tenable, and shorter-term measures are needed. Imaging techniques to test for compounds that reverse or slow gray matter loss seem promising, but detecting NMDAR hyperfunction or related events at a much earlier stage could possibly be even better. Further information on the events that play causative as opposed to epiphenomenal roles early in disease progression might help identify biomarkers that could be used to facilitate clinical trials. Such information should additionally provide insights into neuronal vulnerability factors that could be modified to prevent or delay disease onset.

ARTICLE INFORMATION
Accepted for Publication: October 29, 2014.
Published Online: February 16, 2015.

Author Contributions: Drs Pérez-Otaño and Wesseling had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: All authors.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: All authors.
Critical revision of the manuscript for important intellectual content: All authors.
Obtained funding: All authors.
Administrative, technical, or material support: Pérez-Otaño.
Study supervision: All authors.

Conflict of Interest Disclosures: None reported.
Funding/Support: This study was funded by the Unión Temporal de Empresas project at the Centro de Investigación Médica Aplicada, the Hereditary Disease Foundation (Dr Pérez-Otaño), and grants CS02008-00005 (Dr Pérez-Otaño), BFU2009-1216 (Dr Wesseling), SAF2010-20636 (Dr Pérez-Otaño), and SAF2013-48933R (Drs Pérez-Otaño and Wesseling) from the Spanish Ministry of Science.

Role of the Funder/Sponsor: The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

Additional Contributions: Julio Artieda, MD, and Jose Obejo, MD (University Clinic and Medical School, University of Navarra, Pamplona, Spain), provided critical readings of the manuscript. The contributors did not receive financial compensation.

REFERENCES


