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# Intracellular $Ca^{2+}$ Signaling and Human Disease: The Hunt Begins with Huntington's

**Huntingtin, a protein altered by polyglutamine expansion in Huntington's disease ($Htt^{exp}$), forms a signaling complex with the $InsP_3R$, an intracellular calcium channel, and Htt-associated protein 1A (HAP1A). The addition of $Htt^{exp}$ increases the $InsP_3R$ sensitivity to $InsP_3$, which subsequently makes neurons hyperresponsive to stimulation and presumably more prone to neurodegenerative processes.**

Despite the recent advances in molecular neuroscience, the molecular bases for most neurological diseases are poorly understood. In this issue of *Neuron*, Tang and coworkers show for the first time a direct link between intracellular calcium signaling and the pathogenesis of Huntington's disease. The authors utilize a multipronged approach, combining biochemical and electrophysiological tools with calcium imaging to show that there are functional interactions at the molecular and cellular levels between Huntingtin (the protein altered in Huntington's disease) and the intracellular calcium release channel, the inositol 1,4,5 trisphosphate receptor ($InsP_3R$). The results of this paper suggest a pathophysiological mechanism for Huntington's disease, which provides insights for the development of new therapies against the progression of the disorder (Tang et al., 2003). The promise presented by the approaches used in this study bodes well for future investigations into the mysteries of a host of neurological diseases.

In 1872, the American physician George Huntington described an illness that he called "an heirloom from generations away back in the dim past" (Durbach and Hayden, 1993). He was not the first to describe the disorder, which has been traced as far back as the Middle Ages. One of its earliest names was *chorea*, which, as in "choreography," is the Greek word for dance. The term chorea describes how people affected with the disorder wriggle, twist, and turn in a constant, uncontrollable dance-like motion. In modern medical practice, this highly complex neuronal disorder is called Huntington's disease.

Huntington's disease, a fatal, autosomal-dominant neurological illness, causes involuntary movements, severe emotional disturbance, and cognitive decline. Huntington's disease usually strikes in mid-life, in the thirties or forties, although it can also attack children and the elderly. Because it is an autosomal-dominant disorder, each child of a parent with Huntington's disease has a 50% risk of inheriting the illness. The prevalence of the disease is approximately 1 in every 10,000 persons, which translates to 30,000 afflicted people in the United States alone. Approximately 250,000 people in United States are "at risk" to inherit the disease from an affected parent, making it one of the most common genetic disorders, on par with hemophilia, cystic fibrosis, or muscular dystrophy. Unfortunately, there is no treatment to halt the inexorable progression, which leads to death after 10 to 25 years.

The exact mechanisms underlying neuronal death in Huntington's disease are still unknown; however, the molecular basis of Huntington's disease has been shown to be the polyglutamine (polyQ) expansion (exp) in the N terminus of Huntingtin (Htt), a cytosolic protein expressed in almost all cells of the body. For a decade, the leading models of neurodegeneration in this disease have involved mitochondrial dysfunction and subsequent excitotoxic injury, oxidative stress, and apoptosis. Recent studies have lent support to these models (see Bates, 2003; Feigin and Zgaljardic, 2002, for detailed reviews), but additional experimental data is required to understand the initiation and development of the pathophysiological pattern of Huntington's disease in neurons.

The generation and propagation of membrane excitability is central to neuronal functions. Ion channels and their associated proteins are the molecular players of cell physiology and have been targeted in many neurological disorders. Indeed, molecular mapping of several neurological diseases has identified alterations in a number of voltage-gated cationic channels on the plasma membrane (see http://www.neuro.wustl.edu/neuromuscular/mother/chan.html for a comprehensive account of channelopathies).

Interestingly, there are fewer instances where human diseases have been attributed to the malfunctioning of intracellular channels. The primary examples of diseases explained by altered intracellular calcium signaling rely upon modifications in the ryanodine receptor (RyR). RyR type 1, a calcium release channel of the sarcoplasmic reticulum of skeletal muscle, has been implicated in Malignant Hyperthermia, Central Core disease, and Granulomatous Myopathy (Dirksen and Avila, 2002). RyR type 2 has been shown to play a critical role in several cardiovascular diseases, such as ventricular tachycardia, stress-induced polymorphic and right ventricular dilated (ARVD) cardiomyopathy (Scoote and Williams, 2002). More recently, two additional proteins associated with human disease have been proposed to function as intracellular calcium channels: polycystic kidney disease protein 2 (PKD2) (Somlo and Ehrlich, 2001) and the protein modified in mucolipidosis, mucolipin-1 (LaPlante et al., 2002). Quite surprisingly, none of these proteins are associated with neurological disorders, at least not yet.

Even more curious is that the $InsP_3R$, although implicated in many physiologically important processes and thought to be an essential component of long-term depression (Inoue et al., 1998), has not been associated with any human neuronal pathology. However, recent reports are beginning to highlight the importance of the $InsP_3R$ in human diseases of both nonneuronal and neuronal origin. The first demonstration in nonneuronal disease is in bile duct cholestasis, where there is a selective

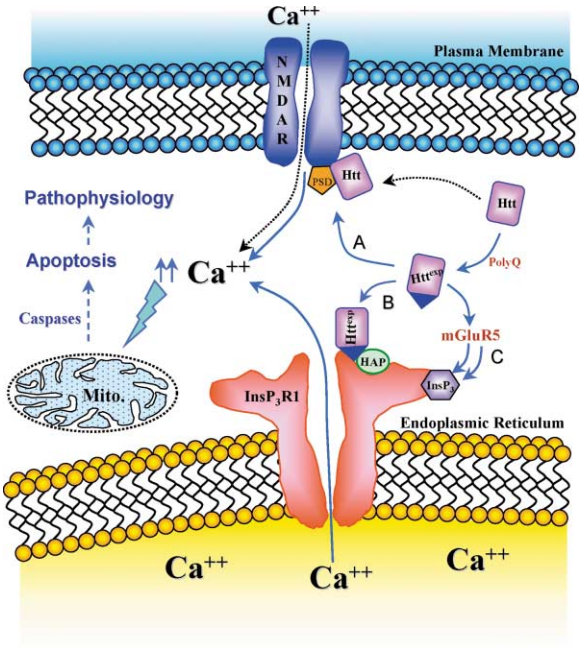


Figure 1. A Schematic Representation of the Role of Huntingtin in Medium Spiny Neuronal Calcium Signaling

Normally, Htt interacts with the NMDAR to increase intracellular calcium (black dotted arrows). In the pathogenesis of Huntington's disease, Htt expands ($Htt^{exp}$) and has effects on innate calcium signaling pathways (blue arrows); (A) $Htt^{exp}$ further enhances NMDAR function, possibly through an altered interaction with the PSD95-NR1A/NR2B complex, and (B) $Htt^{exp}$ interacts with the $InsP_3R1$ and HAP1A to sensitize the $InsP_3R1$. (C) In addition, although not directly tested, it is possible that $Htt^{exp}$ increases the amount of $InsP_3$ produced by mGluR5 stimulation. The combination of these effects radically increases the intracellular calcium concentration, which triggers the neuronal apoptotic program. The final outcome of the apoptotic program in medium spiny neurons leads to the pathophysiological symptoms of Huntington's disease.

degradation of all isoforms of the $InsP_3R$ with a concomitant decrease in intracellular calcium signaling (Shibao et al., 2003). The present study of the molecular basis of Huntington's disease by Tang et al. is the first report of the importance of the $InsP_3R$ in human neurological disease and its role in a calcium signaling complex.

As the first step in identifying the cellular components responsible for establishing a signaling microdomain, Tang et al. found that Htt-associated protein-1 (HAP1A) interacts directly with the $InsP_3R$ type 1 ($InsP_3R1$) using yeast two-hybrid techniques. They further show that the complex also contains Htt. Importantly, the expanded version of huntingtin ($Htt^{exp}$) binds to $InsP_3R1$ much stronger than the wild-type Htt. The biochemical association is then correlated with an increased sensitivity of the $InsP_3R$ to $InsP_3$ in single channel recordings of isolated receptors incorporated into planar lipid bilayers and exposed to $Htt^{exp}$ (but not to wild-type Htt) and in measurements of calcium transients in medium spiny neurons transfected with $Htt^{exp}$ (but not with wild-type Htt). HAP1A is primarily localized in axonal terminals, a subcellular milieu that affords a maximal impact on neuronal function. The story becomes even more complex as other pieces of the calcium signaling puzzle get assembled. In previous studies (e.g., Zeron et al., 2001), it was discovered that $Htt^{exp}$ preferentially enhances the activity of the isoforms of the NMDA receptor (NR1A/NR2B) found in medium spiny neurons, a primary locus for Huntington's disease neurodegeneration (Figure 1). Furthermore, in medium spiny neurons the expression levels of metabotropic glutatmate receptor type 5 (mGluR5) are high. Therefore, modest stimulation of the mGluR5 in the presence of $Htt^{exp}$ would then impact on two different components of the calcium signaling cascade resulting in increased calcium signaling: enhanced signaling through $InsP_3$ and enhanced activation of NMDAR, as type 1 mGluRs are known to potentiate the activity of this receptor. Thus, the signaling complex with the altered Htt is poised to make the cells more responsive to stimulation by glutamate receptor agonists, eventually leading to neuronal degeneration and Huntington's disease.

The functional association of HAP1A, $Htt^{exp}$, and the $InsP_3R$ elegantly demonstrates the importance of spatial patterns in signaling complexes. In other cells, signaling complexes have been used to show specificity in responses to diverse agonists. For example, in sympathetic ganglion cells, bradykinin acts through $InsP_3$-induced calcium release, whereas muscarinic M1 receptors use an alternative pathway which is relatively inefficient at releasing calcium via $InsP_3R$ (Delmas et al., 2002). In medium spiny neurons, the sensitivity of the response is selectively altered in the progression of Huntington's disease. It is now possible to imagine that specificity of a signaling domain can be altered by developmental processes, the physiological state of a cell, or by pharmacological agents. In addition, it is expected that temporal patterns will also be regulated by many of the same parameters. All these conditions will become critical components in the analysis of intracellular signaling.

In sum, the ability to go from a molecular interaction to a clinical problem and then to use this information to propose a mechanism to explain an aspect of the disease and a potential therapeutic approach is the direction needed for the future. It is hoped that the experimental advances gleaned from investigations into each of the neurological disorders will be relevant to the understanding of other neuronal pathophysiologies such as Schizophrenia, Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis. In that way, progress toward our understanding of the pathogenesis will lead to the development of treatments and cures of some of the most devastating medical mysteries. One of the more practical lessons from the study by Tang et al. is that mGluR5 should be considered as a potential drug target for Huntington's disease. It follows from the model in their paper that blockage of mGluR5 should result in lower amounts of $InsP_3$ generated in medium spiny neurons and may offset the sensitizing influence of $Htt^{exp}$ on $InsP_3R1$. If this hypothesis holds, the work of Tang et al. may finally open a path toward the long-awaited cure of Huntington's disease. The hunt must now continue!

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# Serotonin and Whisking

**Rhythmic whisker movements, called "whisking," are produced by a brainstem central pattern generator (CPG) that uses serotonin to induce periodic firing in facial motorneurons. During active touch, motor cortex could regulate whisking frequency by controlling the rate of firing of the serotonergic neurons.**

Who among the thousands of neuroscientists that daily work with mice or rats has not wondered "what motor makes those whiskers go"? In this issue of *Neuron*, Hattox et al. (2003) examine this question in detail, employing a range of experimental approaches to identify the brain mechanisms that mediate and regulate the characteristic rhythmic movements of facial whiskers, called "whisking." Whisking behavior is becoming particularly significant in light of rapid advancements in our understanding of the development, function, and plasticity of the whisker sensory system. At each level of the whisker-to-cortex pathway, whisker-related groups of neurons, termed "barrels" in the somatosensory cortex (Jones and Diamond, 1995; Woolsey and Van der Loos, 1970), constitute identifiable neural circuits whose secrets are becoming increasingly amenable to detailed study via a host of powerful in vivo and in vitro methodologies. Fascinating in its own right, the study of whisking may provide a powerful model for understanding other important rhythmic behaviors, including breathing, walking, chewing, and suckling.

Like other mammalian sensorimotor behaviors, whisking is a carefully regulated motor action linked intimately to the acquisition and processing of sensory information. During exploratory behavior, rats repetitively sweep their whiskers through the sensory environment in a rhythmic $\sim$8 Hz pattern that is finely coordinated with body and head movements and with the respiration cycle (Welker, 1964). This allows objects of interest to be inspected not only with mechanical sensors on the face, including the whiskers, but also with taste receptors in the mouth and olfactory receptors in the nose.

Rats can use their whiskers to perform subtle texture discriminations at a level comparable to human and nonhuman primates using their fingertips (Carvell and Simons, 1990). During discriminative behavior, whisking produces relative motion between the palpated object and the sensory apparatus, a key feature of active touch in all mammals (see Lederman and Klatzky, 1987). The velocity range over which this occurs is similar to the speed of finger movements used by humans during texture discrimination. This range of relative motion velocities has also been found to be optimal for detection, by human observers and monkey somatosensory cortical neurons, of the direction of stimuli moving across the skin surface. Rats employ subtly different combinations of whisker velocity and amplitude depending on the nature of the textured surfaces they are palpating.

Not surprisingly, the neural mechanisms involved in coordinating the motor and sensory functions of the whiskers are located throughout the brain and involve nearly every major neural center. The whisker system itself is perhaps best viewed as an overlaid system of multiple closed anatomical/functional loops (Kleinfeld et al., 1999). Afferent sensory pathways originate in the whisker hair follicles and terminate in sensory areas of the cerebral cortex. Motor pathways, including those arising from the motor cortex, eventually terminate in the brainstem facial motor nucleus whose motorneurons directly innervate muscles responsible for whisker movement. Linkages between sensory and motor structures at many levels of the pathways provide for integration of sensory and motor processing centers, enabling animals to adjust whisking and sniffing movements based on the ongoing barrage of acquired sensory information.

The complexity of the system notwithstanding, whisking is rapidly emerging as an important model for the study of motor rhythms and sensorimotor integration. The mechanical apparatus itself is relatively simple (Dorfl, 1982). Each whisker follicle is enveloped by a sling of striated muscle that wraps around the base of the follicle rostrally and attaches to the immediately caudal follicle nearer the skin surface (Figure 1). Contraction of the sling muscles pull the base of the follicle backward and, due to the lever-like mechanical coupling of the follicle to the overlying skin, the whisker moves forward, or "protracts." Retraction is more rapid and is thought to reflect largely the viscoelastic properties of mystacial pad tissue. Whisking thus occurs within a single plane (horizontal with respect to the face) and does not involve load-bearing, articulated joints and coordination of complexly organized agonist and antagonist muscle groups. The sling muscles themselves are anatomically and functionally homogeneous, and whiskers on the mystacial pad move in unison with each other and in synchrony with whiskers on the other side of the face. All of these features greatly simplify the measurement and analysis of whisking behavior.

Whisking, like other rhythmic motor acts, has been thought to reflect the operations of small networks of