Huntingtin and Huntingtin-Associated Protein 1 Influence Neuronal Calcium Signaling Mediated by Inositol-(1,4,5) Triphosphate Receptor Type 1

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Summary
Huntington’s disease (HD) is caused by polyglutamine expansion (exp) in huntingtin (Htt). The type 1 inositol (1,4,5)-triphosphate receptor (InsP3R1) is an intracellular calcium (Ca2+) release channel that plays an important role in neuronal function. In a yeast two-hybrid screen with the InsP3R1 carboxy terminus, we isolated Htt-associated protein-1A (HAP1A). We show that an InsP3R1-HAP1A-Htt ternary complex is formed in vitro and in vivo. In planar lipid bilayer reconstitution experiments, InsP3R1 activation by InsP3 is sensitized by Httexp, but not by normal Htt. Transfection of full-length Httexp, but not by normal Htt. Transfection of full-length Httexp or caspase-resistant Httexp, but not by normal Htt, into medium spiny striatal neurons facilitates Ca2+ release in response to threshold concentrations of the selective mGlurR1/5 agonist 3,5-DHPG. Our findings identify a novel molecular link between Htt and InsP3R1-mediated neuronal Ca2+ signaling and provide an explanation for the derangement of cytosolic Ca2+ signaling in HD patients and mouse models.

Introduction
Huntington’s disease (HD) has onset usually between 35 and 50 years with chorea and psychiatric disturbances and gradual but inexorable intellectual decline to death after 15–20 years (Vonsattel and DiFiglia, 1998). Neuropathological analysis reveals selective and progressive neuronal loss in the striatum (Vonsattel et al., 1985), particularly affecting the GABAergic medium spiny striatal neurons (MSNs). At the molecular level, the cause of HD is a polyglutamine (polyQ) expansion (exp) in the amino terminus of huntingtin (Htt), a 350 kDa ubiquitously expressed cytoplasmic protein (HDCRG, 1993; Nasir et al., 1996). A number of transgenic HD mouse models have been generated, which reproduce many HD-like features (Menalled and Chesselet, 2002; Rubinsztein, 2002). Despite significant progress, cellular mechanisms that link the mutation with the disease remain controversial (Tobin and Signer, 2000).

A number of Htt binding partners have been identified in yeast two-hybrid (Y2H) screens with an Htt amino-terminal fragment (Gusella and MacDonald, 1998; Kalchman et al., 1997; Singaraja et al., 2002). Htt-associated protein-1 (HAP1) was the first identified Htt binding partner (Li et al., 1995; Gutekunst et al., 1998; Page et al., 1998). Importantly, the HD-causing polyQ expansion of Htt (Httexp) promotes Htt-HAP1 association (Li et al., 1995, 1998b). In rodents, two HAP1 protein isoforms differing in their carboxy termini are expressed via alternative splicing—HAP1A and HAP1B—both of which bind Htt (Li et al., 1995; Nasir et al., 1998). Only one HAP1 isoform has been identified in humans, and this is most similar to rodent HAP1A (Li et al., 1998b). Association of HAP1 with hepatocyte growth factor-regulated tyrosine kinase substrate (Hrs) (Li et al., 2002), the p150Glued subunit of dynactin (Engelender et al., 1997; Li et al., 1998a), and the Rac1 guanine nucleotide exchange factor Kalirin-7/Duo (Colomer et al., 1997) has been discovered in Y2H screens. Targeted disruption of the HAP1 gene in mice results in postnatal death and depressed feeding behavior, suggesting an important role of HAP1 in hypothalamic function (Chan et al., 2002). Despite all these data, the role of HAP1 in neuronal signaling and the pathogenesis of HD remain unclear (Bertaux et al., 1998).

The inositol (1,4,5)-triphosphate receptor (InsP3R) is an intracellular calcium (Ca2+) release channel that plays an important role in neuronal Ca2+ signaling (Berridge, 1998). Three isoforms of InsP3R have been identified (Furuichi et al., 1994). The type 1 receptor (InsP3R1) is the predominant neuronal isoform. Mice lacking InsP3R1 display severe ataxic behavior (Matsumoto et al., 1996), and mice with a spontaneous mutation in the InsP3R1 gene experience convulsions and ataxia (Street et al., 1997), suggesting a major role of InsP3R1 in neuronal function. To identify novel InsP3R1 neuronal binding partners, we performed a Y2H screen of a rat brain cDNA library and isolated neuronal cytoskeleton 4.1N protein (Maximov et al., 2003) and HAP1A. In the present manuscript, we describe biochemical and functional interactions of InsP3R1 with HAP1A and Htt. The discovered association of InsP3R1 with HAP1A and Htt provides additional insight into HAP1 function in the brain and to our knowledge for the first time links neuronal InsP3R1 function with Htt and HD.

Results
InsP3R1 Binds to HAP1A in the Yeast Two-Hybrid System
We aimed to identify novel proteins that bind to the carboxy-terminal cytosolic region of the InsP3R1. We used the carboxy-terminal region of rat InsP3R1 in the pLexN vector (IC bait, amino acids D2590–A2749) for Y2H screening of a rat brain cDNA library and isolated 16 positive clones. The clones were rescued and tested...
Figure 1. The InsP₃R₁ Binds HAP1A and Htt in the Yeast Two-Hybrid System and In Vitro

(A) The strength of interaction between each InsP₃R₁ IC bait and clone 11 (Q273–L599 of rat HAP1A) was determined by a liquid Y2H assay. The β-galactosidase activity is plotted as a percentage relative to the IC/clone 11 interaction (mean ± SE, n = 3). On the top, predicted secondary structure elements within the IC sequence are shown. The predicted minimal fragment of the rat InsP₃R₁ carboxy terminus (F2627–G2736) required for association with HAP1A is shaded. The minimal 4.1N binding domain (F2627–R2676) (Maximov et al., 2003) and minimal PP₁β binding region (R2731–A2749) (Tang et al., 2003) are also shown.

(B) The strength of interaction between full-length rat HAP1A, rat HAP1B, and clone 11 with IC10 InsP₃R₁ bait was determined by liquid Y2H assay. Data are presented in percentages relative to the observed interaction between clone 11 and IC10 (mean ± SE, n = 3). The carboxy-terminal regions unique to HAP1A and HAP1B isoforms are depicted by striped boxes. Also shown are the Htt binding region of HAP1 (L277–K370) (Li et al., 1995, 1998b) and motifs identified in HAP1 by computer analysis.

(C) GST-IC8/IC10 pull-down experiments of HA-HAP1A or HA-HAP1B from COS7 cells extracts.

(D) Lysates from RT1-infected Sf9 cells and COS7 cells overexpressing either HA-HAP1A or HA-HAP1B were mixed for 2 hr and analyzed by immunoprecipitation with anti-InsP₃R₁ polyclonal antibodies or corresponding preimmune sera (IP/S).

(E) GST-IC8/IC10 pull-down experiments of Htt-23Q or Htt-82Q from HEK293 cells extracts. COS7 lysate containing overexpressed HA-HAP1A was added to GST-IC10 pull-down reactions as indicated. The input lanes on (C)–(E) contain 1/50 of the COS7 and HEK293 cell lysates used in GST pull-down or immunoprecipitation experiments. The precipitated fractions were analyzed by Western blotting with anti-HA monoclonal antibodies (G and D), or anti-Htt monoclonal antibodies (E).

for their strength of association with the IC bait in a liquid Y2H assay. Three of the 16 isolated clones displayed especially strong interaction with the IC bait. Two of the strongly interacting clones (clones 7 and 8) corresponded to neuronal cytoskeleton protein 4.1N (Maximov et al., 2003). The third strongly interacting clone (clone 11) corresponded to a partial fragment of HAP1A.

To systematically map the HAP1A-interacting domain, a number of InsP₃R₁ carboxy-terminal fragments were cloned into pLexN vector, and the strength of interaction with clone 11 (partial HAP1A) was measured in a liquid Y2H assay (Figure 1A). From the obtained results we concluded that the minimal InsP₃R₁ region required for interaction with HAP1A corresponds to amino acids F2627–G2736 (Figure 1A, shaded). Thus, the HAP1A-interacting domain in the InsP₃R₁ carboxy-terminal region partially overlaps with the minimal 4.1N-interacting region (F2627–R2676; Figure 1A) and largely complements the minimal protein phosphatase 1α (PP₁α) binding region (R2731–A2749; Figure 1A) mapped in our previous studies (Maximov et al., 2003; Tang et al., 2003). Two carboxy-terminal splice variants of HAP1 protein are expressed in the rodent brain (Li et al., 1995). Clone 11 corresponds to a carboxy-terminal portion of the rat HAP1A isoform. To address whether both HAP1 isoforms bind InsP₃R₁ with similar affinity, we cloned full-length rat HAP1A and HAP1B cDNAs into the Y2H vectors and tested their ability to associate with IC10 bait. We observed strong interaction between IC10 and either full-length HAP1A or clone 11 (Figure 1B). In contrast, the HAP1B isoform did not display any detectable interaction with IC10. Thus, unique carboxy-terminal sequences present in HAP1A protein (K578–L599) appear to robustly modulate association with the IC10 bait.
InsP$_3$R$_1$-HAP1A-Htt Association In Vivo

We performed additional GST pull-down experiments with rat cerebellar and cortical lysates. In agreement with data using cultured cells, GST-IC10, but not GST-IC8, precipitated endogenous Htt from rat brain cerebellar and cortical lysates (Figure 2A). To address whether InsP$_3$R$_1$ and Htt interacted in vivo, we performed coimmunoprecipitation experiments with rat brain cerebellar and cortical lysates using anti-InsP$_3$R$_1$ polyclonal antibodies. In agreement with the pull-down data, endogenous Htt protein is coimmunoprecipitated with the InsP$_3$R$_1$, even in the presence of GST-IC8 protein, which
Figure 3. The Httexp Amino Terminus Sensitizes the InsP3R1 to InsP3
(A) Lysates from full-length InsP3R1 (RT1)-infected Sf9 cells were used in pull-down experiments with GST, Htt-N-15Q, and Htt-N-138Q GST-fusion proteins as indicated. The precipitated fractions were analyzed by Western blotting with anti-InsP3R1 polyclonal antibodies.
(B) Effects of GST, Htt-N-15Q, and Htt-N-138Q on activity of recombinant InsP3R1 in planar lipid bilayers at 100 nM InsP3. Each current trace corresponds to 10 s (2 s for expanded traces) of current recording from the same experiment.
(C) The average InsP3R1 open probability (P_o) in the presence of 100 nM InsP3 is calculated for a 5 s window of time and plotted for the duration of an experiment. Data from the same experiment are shown on (B) and (C). Similar results were obtained in four independent experiments.
(D) The average InsP3R1 P_o plot for the experiment performed in the presence of 2 μM InsP3. Similar results were obtained in two independent experiments. The times of InsP3, GST, Htt-N-15Q, and Htt-N-138Q additions on (C) and (D) are shown above the P_o plot.

was added in the reaction (Figure 2B). In contrast to GST-IC8, inclusion of GST-IC10 protein into anti-InsP3R1 immunoprecipitation reactions significantly reduced the amounts of precipitated Htt (Figure 2B). These results are consistent with the formation of an InsP3R1-HAP1-Htt ternary complex in neurons within rat cerebellum and cortex regions. Similar results were obtained in experiments with rat striatal lysates (data not shown).

To determine the requirement of HAP1 for the InsP3R1-Htt association, we studied brain extracts from mice with targeted disruption of HAP1 (Chan et al., 2002). In these experiments, brain extracts from HAP1+/− mouse pups and wild-type control littermates were used in immunoprecipitation experiments with anti-InsP3R1 polyclonal antibodies. The precipitated fractions were blotted with anti-Htt monoclonal antibodies. In agreement with the rat brain coimmunoprecipitation results, we found that anti-InsP3R1 polyclonal antibodies specifically precipitated Htt from wild-type mouse cortical lysates (Figure 2C, left panel, third lane). Slightly reduced amounts of Htt protein were precipitated by anti-InsP3R1 polyclonal antibodies from HAP1+/− cortical lysates (Figure 2C, right panel, third lane). To investigate preferentially strong protein-protein interactions, an additional 0.5 M KCl washing step was employed. The 0.5 M KCl wash did not appreciably decrease the amount of Htt precipitated by anti-InsP3R1 antibodies from wild-type mouse brain extract (Figure 2C, left panel, fourth lane). In contrast, the high-salt wash drastically reduced the amount of Htt precipitated from HAP1−/− brain extracts (Figure 2C, right panel, fourth lane). From these results we concluded that the presence of HAP1 proteins is required for the formation of a high-affinity salt-resistant InsP3R1-Htt association. Western blotting of anti-InsP3R1 immunoprecipitates with HAP1 monoclonal antibodies confirmed the preferential binding of InsP3R1 to the HAP1A isoform (Figure 2D, left panel) and the absence of both HAP1 isoforms in HAP1−/− samples (Figure 2D, right panel). The binding of InsP3R1 to HAP1 was not significantly affected by the 0.5 M KCl wash (Figure 2D, left panel).

Httexp, but Not Normal Htt, Activates InsP3R1
In Vitro
The HAP1 binding site and the site of polyQ expansion are localized to the most amino-terminal region of Htt protein (Li et al., 1995). We expressed amino-terminal fragments (Htt-N, amino acids 1–158) of Htt-15Q and Htt-138Q as GST-fusion proteins and confirmed association of Htt-N-15Q and Htt-N-138Q with HA-HAP1A.
(data not shown). We further demonstrated that bacterially expressed Htt-N-15Q and Htt-N-138Q proteins specifically precipitated recombinant full-length InsP$_3$R$_1$ from Sf9 cell lysates (Figure 3A). Since Sf9 lysates do not contain any HAP$_1$ (data not shown), this Htt-N interaction with full-length InsP$_3$R$_1$ appears to be direct. Our GST-IC10 pull-down experiments (Figure 1E) and immunoprecipitation experiments from HAP$_1^{-/-}$ cortical lysates (Figure 2C) also support the existence of a direct Htt-InsP$_3$R$_1$ interaction. InsP$_3$R$_1$ associated with Htt-N-138Q more strongly than with Htt-N-15Q (Figure 3A), although this difference was less dramatic than the difference between Htt-23Q and Htt-82Q in GST-IC10 pull-down experiments (Figure 1E).

What are the functional consequences of InsP$_3$R$_1$ association with HAP$_1$ and Htt? To address this, we expressed InsP$_3$R$_1$ in Sf9 cells and reconstituted recombinant InsP$_3$R$_1$ into planar lipid bilayers as previously described (Tang et al., 2003; Tu et al., 2002). Addition of 100 nM InsP$_3$ to the cis (cytosolic) chamber induced low levels of InsP$_3$R$_1$ activity (Figure 3B, second trace, and Figure 3C). Further addition of GST protein or repetitive additions of Htt-N-15Q protein directly to the bilayer had no effect on InsP$_3$R$_1$ activity (Figure 3B, traces 3–5, and Figure 3C). In contrast, addition of Htt-N-138Q protein to the same bilayer resulted in facilitation of InsP$_3$R$_1$ activity (Figure 3B, trace 6, and Figure 3C). On average, InsP$_3$R$_1$ open probability was equal to 0.020 ± 0.008 (n = 16) in the presence of 100 nM InsP$_3$, 0.02 ± 0.01 (n = 6) after addition of Htt-N-15Q, and 0.19 ± 0.05 (n = 4) after addition of Htt-N-138Q. The facilitation of InsP$_3$R$_1$ activity by Htt-N-138Q was most pronounced at low InsP$_3$ concentrations. When InsP$_3$R$_1$ was activated by 2 μM InsP$_3$, additions of GST, Htt-N-15Q, and Htt-N-138Q proteins did not have a significant effect on InsP$_3$R$_1$ open probability (Figure 3D). From these results, we concluded that Htt-N-138Q, but not Htt-N-15Q, sensitizes InsP$_3$R$_1$ to activation by submaximal doses of InsP$_3$.

To evaluate the role of HAP$_1$ in InsP$_3$R$_1$ activation by Htt, we expressed and purified full-length HAP$_1$A as a GST-fusion protein. Addition of HAP$_1$A to the bilayer had no effect on the activity of InsP$_3$R$_1$ in the presence of 100 nM InsP$_3$ (Figure 4A, fourth trace, and Figure 4B). In contrast to previous experiments (Figures 3B and 3C), addition of Htt-N-15Q to the bilayer pre-exposed to HAP$_1$A facilitated InsP$_3$R$_1$ activity (Figure 4A, fourth trace, and Figure 4B). The following addition of Htt-N-138Q to the same membrane had an additional potentiating effect (Figure 4A, trace 5, and Figure 4B). Similar results were obtained in the experiments when HAP$_1$A was premixed with Htt-N-15Q or Htt-N-138Q prior to addition to the bilayer (data not shown). On average, InsP$_3$R$_1$ open probability was equal to 0.020 ± 0.008 (n = 16) in the presence of 100 nM InsP$_3$, 0.14 ± 0.04 (n = 5) after addition of HAP$_1$A + Htt-N-15Q, and 0.26 ± 0.11 (n = 5) after addition of HAP$_1$A + Htt-N-138Q. From these in vitro functional experiments and our biochemical data, we concluded that HAP$_1$A facilitates activation of InsP$_3$R$_1$ by Htt, most likely by promoting Htt association with InsP$_3$R$_1$ carboxy terminus (Figure 1E).

Since amino-terminal Htt fragments were used in bilayer experiments shown above, we determined the effect of full-length Htt on InsP$_3$R$_1$ activity. We generated baculoviruses expressing full-length Htt-23Q and Htt-82Q and used them in coinfection experiments with RT1 baculovirus encoding InsP$_3$R$_1$. In immunoprecipitation experiments we found that InsP$_3$R$_1$ and Htt-23Q/82Q formed a complex when coexpressed in Sf9 cells (Figure 5A), which do not contain any detectable HAP$_1$. As such, interaction of the InsP$_3$R$_1$ with Htt-23Q/82Q in Sf9 cells appears direct, consistent with our earlier findings. Similar to pull-down data gathered with the GST-Htt-N protein (Figure 3A), binding of Htt to full-length InsP$_3$R$_1$ was only modestly modulated by polyQ expansion.

Microsomes prepared from Sf9 cells coinfected with RT1 and Htt-23Q or Htt-82Q baculoviruses were fused to planar lipid bilayers. No channel activity was observed in control experimental conditions (Figures 5B and 5D, Figure 1E).
Figure 5. Full-Length Httexp Sensitizes InsP3R1 to Activation by InsP3

(A) Microsomes from Sf9 cells coinfected with RT1 and Htt-23Q or Htt-82Q baculoviruses were solubilized in 1% CHAPS, precipitated with anti-InsP3R1 polyclonal antibodies, and blotted with the anti-Htt monoclonal antibodies. The input lanes contain 1/10 of lysates used in the immunoprecipitation experiments.

(B) Activity of InsP3R1 coexpressed in Sf9 cells with Htt-23Q and reconstituted into planar lipid bilayers. Responses to application of 100 nM InsP3 and 2 μM InsP3 are shown. Each current trace corresponds to 10 s (2 s for expanded traces) of current recording from the same experiment.

(C) The average InsP3R1 open probability (P0) was calculated for a 5 s window of time and plotted for the duration of an experiment. The times of 100 nM and 2 μM InsP3 additions to the bilayer are shown above the P0 plot. Data from the same experiment are shown on (B) and (C). Similar results were obtained in four independent experiments.

(D) Same as (B) and (C) for InsP3R1 coexpressed in Sf9 cells with Htt-82Q. Similar results were obtained in six independent experiments.

first trace). Addition of 100 nM InsP3 induced only low levels of channel activity in InsP3R1 coexpressed with Htt-23Q (Figure 5B, second trace, and Figure 5C), but resulted in dramatic activation of InsP3R1 coexpressed with Htt-82Q (Figure 5D, second trace, and Figure 5E). Elevation of InsP3 concentration to 2 μM increased activity of InsP3R1 coexpressed with Htt-23Q (Figure 5B, third trace, and Figure 5C) and had no additional effect on activity of InsP3R1 coexpressed with Htt-82Q (Figure 5D, third trace, and Figure 5E). From these experiments we concluded that sensitivity of InsP3R1 to activation by InsP3 is increased by formation of a complex with Htt-82Q full-length protein, but not with Htt-23Q full-length protein.

Httexp Activates InsP3R1 in Cultured MSNs

Striatal MSN neurons are the most selectively and severely affected in HD (Vonsattel et al., 1985). To test the in vivo functional effects of Httexp on InsP3R1, we established primary MSN cultures from E18 embryonic rats (Mao and Wang, 2001). Over 90% of striatal neurons are projection GABAergic MSNs (Gerfen, 1992). A large fraction of cells in our cultures (>90%) were strongly positive for GAD65 marker in immunostaining experiments (data not shown), confirming their identity as MSNs (Chesselet et al., 1993; Mao and Wang, 2001). We transfected MSNs at 20 days in vitro (DIV) with full-length Htt-23Q, Htt-82Q, or Htt-138Q expression plasmids. To identify transfected cells, the Htt plasmids were cotransfected with enhanced green fluorescent protein (EGFP)-expressing plasmid. During transfections, the Htt:EGFP plasmid ratio was kept at 3:1 to ensure that every GFP-positive cell was transfected with Htt-expressing plasmid. In control experiments, MSNs were transfected with the EGFP plasmid alone. Only GFP-positive cells were compared in our analysis of different Htt constructs.

In contrast to striatal interneurons, MSNs abundantly express phospholipase C (PLC)-linked mGluR1/5 receptors (Mao and Wang, 2001; Tallaksen-Greene et al., 1998). To stimulate InsP3R1-mediated Ca2+ release, we challenged Fura-2-loaded MSN neurons with 10 μM 3,5-dihydroxyphenylglycine (DHPG), a specific mGluR1/5 receptor agonist (Mao and Wang, 2002; Schoepp et al., 1999). To exclude the contribution of N-methyl-D-aspartate receptors (NMDAR) and L-type Ca2+ channels to the observed Ca2+ signals and to simplify the analysis, the imaging experiments were performed in Ca2+-free media containing 100 μM EGTA (see Experimental Pro-
Figure 6. Effects of Htt<sup>exp</sup> on DHPG-Induced Ca<sup>2+</sup> Release in Transfected Medium Spiny Neurons

Representative images showing Fura-2 340/380 ratios in transfected rat medium spiny neurons (MSNs). The pseudocolor calibration scale for 340/380 ratios is shown on the right. Ratio recordings are shown for 10 μM DHPG-induced Ca<sup>2+</sup> transients in MSN neurons transfected with EGFP (first row), EGFP + Htt-23Q (second row), EGFP + Htt-82Q (third row), and EGFP + Htt-138Q (fourth row). The recordings were performed in Ca<sup>2+</sup>-free ACSF containing 100 μM EGTA. GFP images (1st column) were captured before Ca<sup>2+</sup> imaging to identify transfected cells (arrowheads). 340/380 ratio images are shown for MSN neurons 1 min before (2nd column), and 8 s, 30 s, 1 min, 2 min, and 3 min after application of 10 μM DHPG as indicated. The GFP-negative cell that responds to 10 μM DHPG in the third row (triangle arrow) is interpreted to correspond to a neuron transfected with Htt-82Q plasmid alone. We used Htt:EGFP plasmids at a 3:1 ratio during transfections, which probably resulted in the expression of Htt in some GFP-negative MSNs. Only GFP-positive MSNs were considered for quantitative analyses shown on Fig 7.

cedures for details). The local Ca<sup>2+</sup> concentration in these experiments is estimated from the ratio of Fura-2 signals at 340 nm and 380 nm excitation wavelengths as shown by pseudocolor images. Representative data with EGFP, EGFP + Htt-23Q, EGFP + Htt-82Q, and EGFP + Htt-138Q transfected MSN neurons are shown on Figure 6. The transfected cells were identified by GFP imaging (Figure 6, first column, arrows) prior to collecting quantitative Fura-2 340/380 ratio data. We noticed that prior to application of DHPG, the basal Ca<sup>2+</sup> levels were slightly elevated in Htt-transfected cells when compared to control cells (Figure 6, second column). On average, the basal 340/380 ratio was equal to 0.43 ± 0.02 (n = 14) for EGFP-transfected cells (Figures 7A and 7I), 0.51 ± 0.02 (n = 18) for EGFP + Htt-23Q, 0.55 ± 0.02 (n = 29) for EGFP + Htt-82Q, and 0.54 ± 0.015 (n = 21) for EGFP + Htt-138Q (Figures 7C, 7E, 7G, and 7I). The basal Ca<sup>2+</sup> levels were significantly (p < 0.01, unpaired t test) higher in Htt-transfected MSNs as compared to control MSNs. These results are in agreement with elevated basal Ca<sup>2+</sup> levels observed in hippocampal neurons from YAC46 transgenic HD mice (Hodgson et al., 1999).

Ten micromolar DHPG corresponds to a threshold concentration for mGluR1/5 receptor activation in MSN neurons, and only a small response to DHPG application at this concentration was observed in control MSNs transfected with EGFP plasmid alone (Figure 6, first row, and Figures 7A and 7B) and in MSN neurons transfected with Htt-23Q plasmid (Figure 6, second row, and Figures 7C and 7D). In contrast, significant response to 10 μM DHPG was observed in MSNs transfected with Htt-82Q
Figure 7. Htt exp Facilitates DHPG-Induced Ca\textsuperscript{2+} Release in Medium Spiny Neurons

Basal and peak 340/380 ratios are shown for individual MSN neurons transfected with (A), EGFP, (C), EGFP + Htt-23Q, (E), EGFP + Htt-82Q, (G), EGFP + Htt-138Q. The experiments were performed as described in Figure 6. Only GFP-positive MSNs were considered for quantitative analysis for each group of cells. The basal ratios were determined 1 min prior to DHPG application (−1 min). The peak ratios were measured from maximal signals observed within 30 s after DHPG application. 340/380 ratio traces for representative cells (marked *) are shown in (B), (D), (F), and (H). Time of DHPG application is shown. Similar results were obtained in four independent transfections. (I) Summary of MSN Ca\textsuperscript{2+} imaging experiments with Htt and Htt(R) constructs. Average basal and DHPG-evoked peak 340/380 ratios from four independent transfections are shown as mean ± SEM (n number of cells). These averages represent the data shown in this figure in addition to data from other experiments. The peak ratios in MSNs transfected with EGFP + Htt-82Q, EGFP + Htt-138Q, or EGFP + Htt(R)-138Q are significantly (**p < 0.001, paired t test) higher than the basal ratios in the same cells.

Even stronger response to this agonist was observed in MSNs transfected with Htt-138Q plasmid (Figure 6, third row, and Figures 7E and 7F). Paired t test analysis revealed that peak 340/380 ratios were significantly (p < 0.001) higher than basal 340/380 ratios in Htt-82Q and Htt-138Q transfected neurons, but not in Htt-23Q and EGFP-only transfected neurons (Figure 7I). Thus, we concluded that overexpression of full-length Htt exp sensitizes InsP\textsubscript{3}R\textsubscript{1} to InsP\textsubscript{3} in MSN neurons. The effects of Htt exp on InsP\textsubscript{3}R\textsubscript{1} function were most pronounced at threshold levels of stimulation, as control, Htt-, and Htt exp-transfected MSN neurons responded in a similar manner to 500 \textmu M DHPG (data not shown).

Htt is a substrate for cleavage by caspases, and Htt proteolysis may be an early step in the pathogenesis of HD (Wellington et al., 2000, 2002). Removal of caspase 3 and 6 cleavage sites in the Htt quintuple caspase-resistant mutants [Htt(R)] reduces Htt exp proteolysis and toxicity in apoptotically stressed neurons (Wellington et al., 2000). Does sensitization of InsP\textsubscript{3}R\textsubscript{1} by Htt exp depend on prior cleavage by caspases? To answer this question, we cotransfected MSN neurons with EGFP + Htt(R)-15Q or EGFP + Htt(R)-138Q and analyzed responses of transfected cells to 10 \textmu M DHPG. We discovered that Htt(R)-138Q, but not Htt(R)-15Q, significantly potentiated DHPG-induced Ca\textsuperscript{2+} release in MSNs (Figure 7I). Importantly, the potentiating effects of caspase-resistant Htt(R)-138Q and the cleavable form of Htt-138Q were similar (Figure 7I). From these results, we concluded that Htt exp does not require proteolytic cleavage by caspases 3 and 6 to have potentiating effects on InsP\textsubscript{3}R\textsubscript{1}-mediated Ca\textsuperscript{2+} release in MSN neurons.

Discussion

Huntington’s Disease and Abnormal Ca\textsuperscript{2+} Signaling

Programmed neuronal death (apoptosis) underlies the symptoms of many neurodegenerative disorders, including Alzheimer’s, Parkinson’s, and Huntington’s disease (Mattson, 2000). Ca\textsuperscript{2+} plays an important role in neuronal signaling (Berridge, 1998), and perturbed Ca\textsuperscript{2+} homeostasis is one of the key steps during initiation of the apoptotic program in affected neurons (Mattson and Chan, 2001). Abnormalities in ER-mediated Ca\textsuperscript{2+} signaling due to a mutation of presenilin 1 have been linked to the development of Alzheimer’s disease (Mattson and Chan, 2001). Several lines of experimental evidence
Figure 8. Proposed Mechanism Linking Httexp to Increased InsP3R1- and NMDAR-Mediated Neuronal Ca2+ Signaling in Medium Spiny Neurons of HD Patients

Glutamate released from corticostriatal projection neurons stimulates NMDARs and mGluRs on striatal medium spiny neurons (MSNs). (A) In normal MSNs, NMDAR activation triggers Ca2+ influx from extracellular sources. Activation of mGluRs stimulates phospholipase C (PLC) via a heterotrimeric G protein (G) pathway to generate diacylglycerol (DAG) and inositol triphosphate (InsP3) second messengers. Activation of mGluR5 cooperates with NMDAR activation largely through the actions of DAG, which activates PKC. The InsP3 generated downstream of mGluR5 does not trigger robust Ca2+ release from the endoplasmic reticulum (ER) in response to low levels of glutamate, as InsP3R1 sensitivity to InsP3 is low. The association of Htt with the cytosolic carboxy-terminal tail of the InsP3R1 is weak and with the PSD95-1NR1A/NR2B is strong. In this schematic, only the interaction of Htt with the InsP3R1 carboxy-terminal tail is represented. This interaction appears to be stabilized by the presence of HAP1 (labeled as HAP1) in normal MSNs. (B) In MSNs of Huntington’s disease (HD) patients, Httexp alters mitochondrial Ca2+ homeostasis by directly interacting with the mitochondrial membrane (Panov et al., 2002). While this interaction likely contributes to mitochondrial dysfunction, it remains unclear how intracellular Ca2+ is affected by Httexp at the mitochondria. An alternative molecular mechanism has been suggested by the recent description of a Htt-PSD95-NMDAR complex (Sun et al., 2001) and the ability of Httexp to potentiate NMDAR function (Chen et al., 1999; Sun et al., 2001; Zeron et al., 2002). In addition to influx via NMDAR and voltage-gated Ca2+ channels, neuronal Ca2+ is also largely influenced by release from intracellular stores in the endoplasmic reticulum (ER) upon activation of class 1 metabotropic glutamate receptors (mGluR1/5) (Pin and Duvoisin, 1995). The alterations in ER enzymes that have been observed in HD brains (Cross et al., 1985) might be caused by abnormal Ca2+ release from the ER (Korkotian et al., 1999). Here, we describe a novel mechanism linking Httexp to InsP3R1-mediated Ca2+ release from the ER in striatal MSNs.

InsP3R1-HAP1A-Htt Functional Complex

We have identified a protein complex that contains InsP3R1, Htt, and HAP1. This complex was discovered through the identification of HAP1A as a binding partner of the InsP3R1 cytosolic carboxy-terminal tail in the Y2H screen (Figures 1A and 1B). In biochemical experiments, we found that Htt directly interacts with the InsP3R1 cytosolic carboxy-terminal tail and that binding to this limited region of InsP3R1 was highly dependent on both levels of glutamate, as InsP3R1 sensitivity to InsP3 is low. The association of Htt with the cytosolic carboxy-terminal tail of the InsP3R1 is weak and with the PSD95-NR1A/NR2B is strong. In this schematic, only the interaction of Htt with the InsP3R1 carboxy-terminal tail is represented. This interaction appears to be stabilized by the presence of HAP1 (labeled as HAP1) in normal MSNs. In addition, Httexp strongly binds to InsP3R1 carboxy terminus and sensitizes the InsP3R1 to activation by InsP3. As a result, low levels of glutamate released from corticostriatal projection neurons lead to supranormal Ca2+ influx via NMDAR and Ca2+ release via the InsP3R1. The net result is elevated intracellular Ca2+ levels in MSNs that eventually trigger pathogenic Ca2+-dependent downstream pathways such as increased caspase and calpain activity, increased Htt proteolysis, activation of the apoptotic program, and MSN degeneration.
planar lipid bilayer experiments, the Htt<sup>exp</sup> amino terminus or full-length Htt<sup>exp</sup>, but not wild-type Htt, greatly facilitated Ins<sub>P3</sub>RI activity at 100 nM Ins<sub>P3</sub> (Figures 3B, 3C, 3D, and 5E). However, the amino terminus of wild-type Htt facilitated Ins<sub>P3</sub>RI activity in the presence of HAP1A (Figures 4A and 4B). Thus, Ins<sub>P3</sub>RI was immobilized to Ins<sub>P3</sub> in conditions when Htt binds to the Ins<sub>P3</sub>RI carboxy terminus (Htt<sup>exp</sup> or Htt with HAP1A), but not in conditions when Htt does not bind to the Ins<sub>P3</sub>RI carboxy terminus (Htt alone). From these results we concluded that association of Htt<sup>exp</sup> or Htt-HAP1A with Ins<sub>P3</sub>RI carboxy terminus sensitizes Ins<sub>P3</sub>RI to activation by Ins<sub>P3</sub>.

Sensitization of Ins<sub>P3</sub>RI and NMDAR by Htt<sup>exp</sup> and Huntington’s Disease

The molecular and cellular basis for selective vulnerability of MSNs in HD remains elusive. One proposed mechanism is centered on the finding that MSNs primarily express the NR1A/NR2B subtype of NMDAR (Kuppenbender et al., 1999; Landwehrmeyer et al., 1995; Zeron et al., 2002). Importantly, Htt<sup>exp</sup> appears to selectively enhance the activity of NR1A/NR2B channels, relative to other NMDAR subtypes, suggesting that increased NMDAR function might be specifically occurring in MSNs of HD patients (Chen et al., 1999; Sun et al., 2001; Zeron et al., 2001). Our results suggest another possible explanation for selectivity in HD involving the enrichment of mGluR5, a member of the group I mGluRs, in MSNs (Kerner et al., 1997; Mao and Wang, 2001; 2002; Tallaksen-Greene et al., 1998). Stimulation of group I mGluR in MSNs leads to the generation of Ins<sub>P3</sub> and release of Ca<sup>2+</sup> via Ins<sub>P3</sub>RI (Mao and Wang, 2002). In addition, stimulation of group I mGluRs is known to potentiate NMDAR activity in neurons, most likely via a PKC-dependent pathway (Calabresi et al., 1999; Pisani et al., 2001; Skeberdis et al., 2001).

In striatal MSNs, the presence of full-length Htt<sup>exp</sup> sensitizes Ca<sup>2+</sup> release in response to subthreshold concentrations of the mGluR1/5 agonist DHPG (Figures 6 and 7). We propose that sensitizing influences of Htt<sup>exp</sup> on Ins<sub>P3</sub>RI and the NR1A/NR2B subtype of NMDAR (Chen et al., 1999; Sun et al., 2001; Zeron et al., 2001) have a synergistic effect on glutamate-induced Ca<sup>2+</sup> signals in MSNs of HD individuals (Figure 8). In MSNs of HD patients, the activation of both mGluR5 and NMDAR by low concentrations of glutamate released by corticostriatal projection neurons leads to supranormal Ca<sup>2+</sup> signals (Figure 8B) when compared to normal MSNs (Figure 8A), resulting in neuronal dysfunction and apoptosis. Overall, our findings provide support for the hypothesis that perturbation of neuronal Ca<sup>2+</sup> signaling mediated by NMDAR and Ins<sub>P3</sub>RI-mediated Ca<sup>2+</sup> signals in MSN neurons (Figure 8B). In addition, our model points to mGluR5 as potential target for pharmacological treatment of HD.

Experimental Procedures

Yeast Two-Hybrid Methods
The carboxy-terminal region of rat Ins<sub>P3</sub>RI (Mignery et al., 1990) (amino acids D2590–A2749) was amplified by PCR and cloned into pLexN vector (IC bait). The yeast two-hybrid screen of a rat brain (P8-P9) cDNA library (kind gift of Dr. T. Südhof) with the IC bait and liquid yeast two-hybrid assays were performed as previously described (Maximov et al., 2003).

Plasmons
The following rat Ins<sub>P3</sub>RI (Mignery et al., 1990) baits in pLexN vector were generated by PCR (listed by encoded amino acid and residue numbers): IC<sup>D</sup>2590–R2676, IC<sup>G</sup>2627–A2749, IC<sup>I</sup>2674–Q2714, IC<sup>H</sup>2738–L2646, IC<sup>C</sup>2750–K2797, IC<sup>R</sup>2767–A2749, IC<sup>Q</sup>2714–A2749, IC<sup>S</sup>2725–Q2714, IC<sup>E</sup>2590–R2676, IC<sup>I</sup>2750–L2646, IC<sup>C</sup>2750–F2627, IC<sup>R</sup>2766–A2749, IC<sup>I</sup>2710–F2627–A2749. The Ins<sub>P3</sub>RI expression constructs in pGEX-KG are GST–IC8 – F2627–A2749 and GST–IC10 – F2627–A2749. The full-length clones of rat HAP1A and HAP1B (Li et al., 1995) were amplified by RT-PCR from rat brain mRNA and cloned into the pVp16-3 yeast two-hybrid prey vector, pCMV-HA mammalian expression vector, and pGEX-KG bacterial expression vector. Full-length Htt plasmids Htt–230 (HD–FL–230) and Htt–82Q (HD–FL–82Q) in pRC/CMV expression vector were kindly provided by Dr. Christopher A Ross (Cooper et al., 1998). The full-length Htt–15Q and Htt–138Q plasmids (Wellington et al., 2000) were cloned into the pCI expression vector (Promega). The caspase 3 and 7-resistant Htt quintuple mutant plasmids Htt(R)-15Q and Htt(R)-138Q in pRC/CMV vector have been previously described (Wellington et al., 2000). The Htt-N expression constructs in pGEX-KG are Htt–N–15Q/138Q – M1–K158 of human Htt.

GST Pull-Down Assays
GST–IC8 and GST–IC10 proteins were expressed in the BL21 E. coli strain and purified on glutathione-agarose beads. HA–HAP1A and HA–HAP1B were expressed in COS7 cells by DEAE-dextran transfection (Sambrook et al., 1989). Htt–23/82Q proteins were expressed in HEK293 cells by calcium-phosphate transfection (Sambrook et al., 1989), 48 hr after transfection, COS7 or HEK293 cells were collected with ice-cold PBS and solubilized for 30 min at 4°C in extraction buffer A (1% (v/v) NaCl, 30 mM Tris–HCl, pH 7.2, 1 mM EDTA, 1 mM EGTA, and protease inhibitors). Extracts were clarified by centrifugation for 20 min at 100,000 × g and incubated for 1 hr at 4°C with GST–IC8 or GST–IC10 proteins. Beads were washed three times with the extraction buffer A. Attached proteins were analyzed by Western blotting with anti-Htt or anti-HA monoclonal antibodies.

In Vitro Binding Assay
Full-length rat Ins<sub>P3</sub>RI (RT1)-encoding baculoviruses were previously described (Tu et al., 2002). Full-length Htt plasmids Htt–230 and Htt–82Q (Cooper et al., 1998) were subcloned into pFastBac vector (Invitrogen), and Htt–23Q/82Q baculoviruses were generated using Bac-to-Bac system (Invitrogen). The Spodoptera frugiperda (SF9) cells (100 ml) were infected with RT1 viruses or cocultured with RT1:Htt–23Q/82Q viral mixtures (3:1) and collected by centrifugation 72 hr postinfection. The RT1-infected cells were solubilized in extraction buffer A, cleared by centrifugation (100,000 × g), and mixed with an equal volume of HA–HAP1A or HA–HAP1B–expressing COS7 lysates prepared as described above for 2 hr at 4°C. The mixture was precipitated with anti-Ins<sub>P3</sub>RI polyclonal antibody (T443) attached to protein A-Sepharose beads and analyzed by Western blotting with anti-HA monoclonal antibodies. The cells coinfected with RT1 and Htt–23Q/82Q baculoviruses were used to prepare microsomes for bilayer experiments as previously described (Tu et al., 2002). Obtained microsomes were solubilized in extraction buffer A, cleared by centrifugation (100,000 × g in TL–100), used in immunoprecipitation experiments with anti-Ins<sub>P3</sub>RI polyclonal antibodies, and analyzed by Western blotting with anti-Htt monoclonal antibodies.

Brain Pull-Downs and Immunoprecipitations
Rat and mouse brain tissues were isolated, homogenized, and solubilized for 1.5 hr at 4°C in extraction buffer A. The lysate was clarified by 20 min centrifugation at 100,000 × g and utilized in pull-down experiments with GST–IC8 and GST–IC10 proteins or in the immunoprecipitation experiments with anti-Ins<sub>P3</sub>RI polyclonal antibodies (T443) performed as described above. GST–IC8 and GST–IC10 pro-
teins (200 μg/ml final concentration) were included in the immunoprecipitation reactions as indicated. An additional 0.5 M KCl wash step was included in the immunoprecipitation experiments with mouse samples as indicated in the text. The precipitated fractions were analyzed by Western blotting with anti-Htt and anti-HAP1 monoclonal antibodies.

Planar Lipid Bilayer Experiments

Single-channel recordings of recombinant InsP3R1 (RT1) expressed in isolation or coexpressed with Htt-23Q/82Q proteins were performed as previously described (Tang et al., 2003; Tu et al., 2002) at 0 mV transmembrane potential using 50 mM Bz

(trans) as a charge carrier. The cis (cytosolic) chamber contained 110 mM Tris dissolved in HEPES (pH 7.35), 0.5 mM Na2ATP, pCa 6.7 (0.2 mM EGTA + 0.14 mM CaCl2) (Bezprozvanny et al., 1991). InsP3R1 were activated by addition of 100 nM InsP3, or 2 μM InsP6 (Alexis) to the cis chamber as indicated in the text. GST, Htt-N-15Q, Htt-N-138Q, and HAP1A proteins were expressed in BL21 E. coli, purified on glutathione beads, eluted with reduced glutathione, dialyzed overnight against cis recording buffer (110 mM Tris/HEPES [pH 7.35]), and added in 1 μL volume (0.3 mg/ml protein with addition of 0.02 mM ruthenium red) directly to the cis side of the bilayer containing washing reactions as indicated. An additional 0.5 M KCl wash was included in the immunoprecipitation experiments with We thank Thomas C. Südhof for advice with yeast two-hybrid screen and monoclonal antibodies.

Ca2+ Imaging Experiments

The rat medium spiny neuronal (MSN) cultures on poly-D-lysine (Sigma) coated 12 mm round glass coverslips were established by following published procedures (Mao and Wang, 2001). The 5 μM of cytosine arabinoside (AraC, Sigma) was added at 2–4 DIV to inhibit glial cell growth. At 20 DIV the MSN cultures were transfected by the calcium-phosphate method (Maximov and Bezprozvanny, 2002) with EGFP-C3 plasmid (Clontech) or a 1:3 mixture of EGFP-Htt plasmids as indicated in the text. 48 hr after transfection, the MSN neurons were loaded with 5 μM Fura2-AM (Molecular Probes) in artificial cerebrospinal fluid (ACSF) (140 mM NaCl, 5 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 10 mM HEPES [pH 7.3]) for 45 min at 37°C. For imaging experiments the coverslips were mounted onto a recording/perfusion chamber (RC-26G, Warner Instrument) maintained at 37°C (PH1, Warner Instrument), positioned on the movable stage of an Olympus IX-70 inverted microscope, and perfused with ACSF media by gravity flow. Following GFP imaging, the coverslip was washed extensively with Ca2+-free ACSF (omitted CaCl2 from ACSF and supplemented with 100 μM EGTA). In Ca2+ imaging experiments the MSN cells were intermittently excited by 340 nm and 380 nm UV light (DeltaRAM illuminator, PTI) using a Fura-2 dichroic filter cube (Chroma Technologies) and 60× UV-Grade oil-immersed objective (Olympus). The emitted light was collected by an IC-300 camera (PTI), and the images were digitized by ImageMaster Pro software (PTI). Baseline (6 min) measurements were obtained prior to bath application of 10 μM or 500 μM 3,5-DHPG (Tocris) dissolved in Ca2+-free ACSF. The DHPG solutions were prewarmed to 37°C before application to MSNs. Images at 340 and 380 nm excitation wavelengths were captured every 5 s and shown as 340/380 image ratios at time points as indicated. Background fluorescence was determined according to manufacturer’s (PTI) recommendations and subtracted.

Antibodies

The following monoclonal antibodies were used: anti-HA (HA.11) from Covance, anti-Htt (mAB2166) from Chemicon International, monoclonal anti-HAP1 B16 is a kind gift of Dr. Claire-Anne Gutekunst (Chan et al., 2002), GAD65 antibodies from BD Pharmingen. Polyclonal anti-InsP3R1 T443 antibody was previously described (Kaznecheeva et al., 1998). Secondary HRP-conjugated anti-rabbit and anti-mouse antibodies were from Jackson ImmunoResearch.

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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\(_3\)R, an intracellular calcium channel, and Htt-associated protein 1A (HAP1A). The addition of Htt\(^{exp}\) increases the InsP\(_3\)R 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\(_3\)R). 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, uncontrolled 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\(_3\)R, 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\(_3\)R 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
tested, it is possible that Htt expression increases the amount of InsP3 pro- will also be regulated by many of the same parameters.

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Selected Reading


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 ~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 palpat ing.

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 overlay system of multiple closed anatomical/functional loops (Kleinfield 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 motoneurons 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 and attaches to the immediately caudal follicle nearer the skin surface (Figure 1). Contraction of the sling muscles pulls the base of the follicle rostrally and attaches to the immediately caudal 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 pads. 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