Mitogen- and stress-activated protein kinase 1-induced neuroprotection in Huntington’s disease: role on chromatin remodeling at the PGC-1-alpha promoter

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Huntington’s disease (HD) is a neurodegenerative disorder due to abnormal polyglutamine expansion in huntingtin protein (Exp-Htt). This expansion causes protein aggregation, leading to neuronal dysfunction and death. We have previously shown that mitogen- and stress-activated kinase (MSK-1), a nuclear protein kinase involved in chromatin remodeling through histone H3 phosphorylation, is deficient in the striatum of HD patients and model mice. Restoring MSK-1 expression in cultured striatal cells prevented neuronal dysfunction and death induced by Exp-Htt. Here we extend these observations in a rat model of HD based on striatal lentiviral expression of Exp-Htt (LV-Exp-HTT). MSK-1 overexpression attenuated Exp-Htt-induced down-regulation of DARPP-32 expression 4 and 10 weeks after infection and enhanced NeuN staining after 10 weeks. LV-MSK-1 induced constitutive hyperphosphorylation of H3 and cAMP-responsive element binding protein (CREB), indicating that MSK-1 has spontaneous catalytic activity. MSK-1 overexpression also upregulated peroxisome proliferator-activated receptor γ coactivator alpha (PGC-1α), a transcriptional co-activator involved in mitochondrial biogenesis. Chromatin immunoprecipitation indicated that transcriptional regulation of PGC-1α is directly linked to increased binding of MSK-1, along with H3 and CREB phosphorylation of the PGC-1α promoter. MSK-1 knock-out mice showed spontaneous striatal atrophy as they aged, as well as higher susceptibility to systemic administration of the mitochondrial neurotoxin 3-NP. These results indicate that MSK-1 activation is an important and key event in the signaling cascade that regulates PGC-1α expression. Strategies aimed at restoring MSK-1 expression in the striatum might offer a new therapeutic approach to HD.

INTRODUCTION

Huntington’s disease (HD) is the most frequent neurodegenerative disease due to expansion of glutamine repeats. The main clinical manifestations of HD are chorea, cognitive impairment and psychiatric disorders (1). The mutation responsible for HD, an unstable expansion of the CAG-repeat sequence, is located in the 5’ terminal part of HTT, the gene encoding Huntingtin (Htt). One intriguing feature of HD is the particular vulnerability of the striatum, despite similar expression of the mutated protein in other brain areas (2).
Striatal cell death in HD may be due to aggregation of expanded-Htt, transcriptional dysregulation, altered energy metabolism, excitotoxicity, impaired axonal transport and/or altered synaptic transmission (2). Among these non-exclusive mechanisms, transcriptional dysregulation is an early and important event. Studies of rodent models and human HD brain tissues have shown up- or down-regulation of multiple genes encoding neurotransmitter receptors, enzymes and proteins involved in neuron structure, stress responses and axonal transport (3–6). This transcriptional dysregulation affects large genomic regions, occurs in a coordinated fashion and is associated with disease progression (7).

One important dysregulated gene in HD is peroxisome proliferator-activated receptor γ coactivator alpha (PGC-1α)—the gene encoding the peroxisome proliferator-activated receptor-γ, a transcriptional coactivator involved in energy homeostasis, adaptive thermogenesis, α-oxidation of fatty acids and glucose metabolism (8,9). PGC-1α mRNA levels are decreased in autopsy samples of human HD striatum, as well as in the striatum of HD knock-in mice and the HD striatal cell line STHdhQ111 (10,11). PGC-1α knock-out mice exhibit impaired mitochondrial functions, a hyperkinetic movement disorder and striatal degeneration, features also observed in HD patients (12,13). PGC-1α overexpression in the striatum of R6/2 transgenic HD mice prevents neuronal atrophy and improves these animals’ motor performance and survival (10). Muscle tissue of HD patients exhibits decreased expression of PGC-1α and its target genes, and low oxygen consumption (14). Interestingly, PGC-1α polymorphisms detected in HD patients explain some of the observed symptom variability (15). Together, these findings indicate that PGC-1α is a key player in HD pathogenesis, and that PGC-1α activation might have therapeutic potential. The pathways of PGC-1α activation or transcription are therefore a major focus of attention in this setting.

Mutant Htt, in its soluble or aggregated form, interacts with transcription factors and interferes with the transcriptional machinery, thereby disrupting transcriptional responses and affecting cell viability (2). Chromatin remodeling, especially in the striatum, is also likely to play a key role in the transcriptional dysregulation observed in HD. Chromatin remodeling can be defined as an ‘above the genome’ cellular process that integrates diverse environmental stimuli to exert potent and long-lasting changes in gene expression. Chromatin decompaction and access to specific genomic loci are controlled by post-translational histone modifications, which occur in a dynamic, cell-specific manner in response to external stimuli and affect histone-DNA linkage (16,17). The main post-translational histone modifications described so far include acetylation of histone H3 Lys9 and Lys14, and acetylation of histone H4 Lys5 (18,19) by histone acetyltransferases. In contrast, histone deacetylases (HDACs) catalyze deacylation. Other post-translational modifications include histone methylation, and particularly di- or tri-methylation of H3 Lys9, which represses transcription, and phosphorylation of histone H3 on Ser10, which enhances transcription (19,20).

Phosphorylation of histone H3 is crucial for inducing the nucleosomal response at some loci (21,22). The kinase responsible for H3 phosphorylation is mitogen- and stress-activated protein kinase 1 (MSK-1). MSK-1 is activated downstream of the MAPkinase/ERK signaling pathway (23), a pro-survival pathway specifically activated downstream of synaptic but not extra-synaptic N-methyl-D-aspartate (NMDA) receptors (24) and exhibiting neuroprotective properties in HD models (25). H3 phosphorylation by MSK-1 plays a key role in neuronal plasticity and related behavioral modifications (21,22,26–30). MSK-1 plays a dual role in gene regulation, as it is also a kinase targeting Ser133 of the cAMP-responsive element binding protein (CREB) transcription factor (31). Cells from MSK-1 knock-out mice show altered CREB phosphorylation in response to mitogens, growth factors and cellular stresses (32,33) and also in response to cocaine in vivo (26). Although CREB phosphorylation has been shown to directly influence PGC1-α expression levels, no direct link with MSK-1 signaling has yet been found.

We recently showed that MSK-1 is deficient, specifically in the striatum, in R6/2 HD model mice and also in caudate autopsies specimens from HD patients (34). Restoration of MSK1 expression and, subsequently, striatal H3 phosphorylation in an in vitro HD model system totally protects against neuronal disorders induced by mutated Htt, including neuritic retraction, aggregate formation and death (34). Here we examined the neuroprotective effects of MSK-1 overexpression in vivo, in a rat model of HD (35,36), and investigated the possible link between MSK-1 expression and PGC-1α transcription.

**RESULTS**

**Effect of MSK-1 overexpression on Exp-Htt-induced neuronal dysfunction**

The left striatum (Fig. 1A) was infected with a lentiviral vector encoding the 171 N-terminal amino acids of human Htt with a 82 CAG-repeat expansion (LV-Exp-Htt), while the right striatum of the same animals was infected with both LV-Exp-Htt and LV-MSK-1 (Fig. 1A). Controls were infected with an LV encoding the 171 N-terminal amino acids of human Htt with a 18 CAG-repeat expansion (LV-Htt) and with both LV-Htt and LV-MSK-1, in the right and left striata, respectively (Supplementary Material, Fig. S1). A third group received LV-MSK-1, on only one side (Supplementary Material, Fig. S1). In all groups, the volume of MSK-1 expression was measured in the whole rostro-caudal extension of the striatum, and showed similar values (Supplementary Material, Fig. S1). Four weeks after LV-Exp-Htt infection, endogenous MSK-1 expression was slightly down-regulated (Fig. 1B, left panel, asterisk). No such down-regulation was observed in control animals infected with normal Htt (see Supplementary Material, Fig. S1). MSK-1 expression was strongly upregulated by LV-MSK-1, even after LV-Exp-Htt co-infection (Fig. 1B, right panel).

DARPP-32 is a 32 kDa dopamine- and cAMP-regulated phosphoprotein important for dopamine neurotransmission (37). DARPP-32 down-regulation is an early marker of neuronal dysfunction induced by Exp-Htt in HD transgenic mice, as well as in the LV-based HD rat model (5,35,38,39). Here, striata infected with LV-Exp-Htt alone (Fig. 1B, left panel) or co-infected with LV-Exp-Htt and LV-MSK-1 (Fig. 1B, right panel) showed a clear loss of DARPP-32 immunoreactivity. Nevertheless, the total volume of DARPP-32 depletion...
was significantly smaller in the presence of MSK-1 (Fig. 1C and E) (Exp-Htt: 0.89 mm$^3$ versus Exp-Htt plus MSK-1: 0.56 mm$^3$; **$P < 0.01$): MSK-1 overexpression reduced the volume of Exp-Htt-induced DARPP-32 depletion by 37.1% at 4 weeks. Of importance, the volume of Exp-Htt did not vary in the presence of MSK-1 (Fig. 1B and D). No change in DARPP-32 immunoreactivity was detected in Htt controls (Supplementary Material, Fig. S2). Simultaneous MSK-1 and Htt overexpression did not modify DARPP-32 expression (Supplementary Material, Fig. S2).

Ten weeks post-infection, the loss of MSK-1 and DARPP-32 protein expression was even more pronounced in the Exp-Htt striata (Fig. 2A and B, left panels). On the co-infected side (Fig. 2A, right panel), this down-regulation was evident in the core of the injection, while the surrounding area showed MSK-1 expression and DARPP-32 around the injection point on the left side infected with both LV-Exp-Htt and LV-MSK-1. The right panels show a higher magnification of the area surrounding the LV-Exp + MSK-1 injection point (see square box in the middle panels). Note that all three markers, Exp-Htt, MSK-1 and DARPP-32, are expressed in this area (white arrow). (C) The Exp-Htt volume expression in the rostro-caudal extension of the striatum and (D) the DARPP-32 loss/Exp-Htt infected area were measured as described in Fig. 1. Statistics: means ± SEM; eight rats per group. ns, not significant; **$P < 0.001$ Student’s paired $t$-test, LV-Exp-Htt versus LV-Exp-Htt + LV-MSK-1 infection.
higher with Exp-Htt + MSK-1 than with Exp-Htt alone (Exp-Htt + MSK-1, 71.7 μm² versus Exp-Htt, 59.47 μm²; \(***P < 0.0001\)) (Fig. 3D), suggesting that MSK-1 overexpression prevented the nuclear atrophy induced by Exp-Htt.

**Figure 3.** MSK-1 overexpression prevents Exp-Htt-induced neuronal dysfunction. (A) LV-Exp-Htt was injected in the left striatum and both LV-Exp-Htt and LV-MSK-1 were injected in the right striatum. Double staining for MSK-1 (red) and NeuN (green) was performed 10 weeks later. Left panels (Exp-Htt): note the disappearance of both MSK-1 and NeuN labeling within the dotted lines (injection point). Right panels (Exp-Htt + MSK-1): note the down-regulation of both markers within the dotted lines, despite the presence of some immunoreactive cells. Note also the overexpression of MSK-1 and NeuN in the area surrounding the injection point (outside the dotted lines). (B) Confocal micrograph showing the co-localization of aggregated Exp-Htt (green) and MSK-1 (red) in the area surrounding the injection point. (C) Cells overexpressing NeuN were quantified by image analysis, with a threshold based on mean endogenous NeuN expression. Cells exhibiting a fluorescence level above this threshold were considered positive, and were quantified in eight animals. (D) The mean nuclear area of cells in the area surrounding the injection point was analyzed with Hoechst staining and Image-ProPlus software. \(**P < 0.01\), \(***P < 0.001\); two-way ANOVA followed by Bonferroni’s post hoc test (eight animals per group).

**Striatal MSK-1 infection induces overexpression of a catalytically active kinase**

In order to determine whether LV-MSK-1 infection induced overexpression of a catalytically active kinase, we analyzed phosphorylation levels of histone H3 and CREB and two nuclear targets of MSK-1 (41). These experiments were done 4 weeks post-infection, i.e. early during the neurodegenerative process. We found very low phospho-H3 (P-H3) immunoreactivity on both the LV-Htt-infected side and the LV-Exp-Htt-infected side (Fig. 4A), as well as in the LV-only and uninfected controls (data not shown). In contrast, MSK-1 infection, which lead to similar levels of MSK-1 overexpression in both the Htt and the Exp-Htt groups (Fig. 4A and C), induced strong P-H3 immunoreactivity (Fig. 4A). The number of P-H3-immunoreactive cells was strongly and significantly increased on the LV-Htt + MSK-1-infected side compared with the LV-Htt-infected side (Fig. 4D) (Htt, 17.0 versus Htt + MSK-1, 180.2; \(***P < 0.0001\)). Similarly, MSK-1 overexpression was associated with an increase in the number of P-H3-positive neurons on the LV-Exp-Htt/MSK-1 co-infected side when compared with Exp-Htt alone (Exp-Htt, 16.6 versus Exp-Htt + MSK-1, 129.6; \(**P < 0.01\)) (Fig. 4D). No difference was found between the LV-Htt + MSK-1 and LV-Exp-Htt + MSK-1 co-infected sides (Htt + MSK-1, 180.25; Exp-Htt + MSK-1, 129.63; ns), showing that MSK-1 overexpression induced H3 phosphorylation independent of Htt and Exp-Htt. No change in Lys9-H3 or Lys5-H4 acetylation levels was observed (data not shown).

MSK-1 activation was also analyzed by studying CREB phosphorylation at Ser-133, a key step in the stimulation of CRE-dependent gene transcription (42). In LV-Htt- and LV-Exp-Htt-infected striata, we found significant basal levels of P-CREB immunoreactivity (Fig. 4B), similar to those observed in uninfected striata (data not shown). This immunoreactivity was significantly higher on the LV-Htt + MSK-1- and LV-Exp-Htt + MSK-1-coinfected sides (Fig. 4B). P-CREB-immunoreactive cells were counted, using a threshold based on the value in LV-Htt-infected striata. P-CREB-immunoreactive cells were enhanced by MSK-1 overexpression (Htt, 80.6 versus Htt + MSK-1, 170.9; \(*P < 0.05\)) (Fig. 4E). On the LV-Exp-Htt-infected side, P-CREB immunoreactivity was down-regulated, whereas it was enhanced on the LV-Exp-Htt + MSK-1-coinfected side (Exp-Htt, 30.7 versus Exp-Htt + MSK-1, 72.5) (Fig. 4E).

**MSK-1 overexpression induces PGC-1α expression**

CREB phosphorylation is critically involved in the transcriptional events that upregulate PGC-1α, which is known to be neuroprotective in HD (10). We thus reasoned that CREB phosphorylation by MSK-1 might be a critical step in the transcriptional regulation of PGC-1α. As already shown in other models of HD (10), Exp-Htt decreased PGC-1α expression...
at weeks 4 and 10 post-infection (Fig. 5A and C, left panels, asterisk). In the LV-Exp-Htt/MSK-1-coinfected striata, PGC-1α expression was strongly increased in the core of the injection at 4 weeks (Fig. 5A, right panels), and also in the surrounding area at 10 weeks (Fig. 5B, right panels, triangle). The number of PGC-1α-immunoreactive cells was drastically reduced in the LV-Exp-Htt-infected striatum when compared with the Htt-infected and uninfected striata at 4 weeks (Exp-Htt, 6.14 versus Htt, 180.57; ***P < 0.001) (Fig. 5B). MSK-1 overexpression significantly enhanced PGC-1α expression on the LV-Exp-Htt/MSK-1-coinfected side when compared with the sides infected with LV-Htt or LV-Exp-Htt alone (Htt, 180.57 versus Htt + MSK-1, 554.57; ***P < 0.001; Exp-Htt, 6.14 versus Exp-Htt + MSK-1, 566.86; ***P < 0.001) (Fig. 5B). At 10 weeks, PGC-1α expression was strongly reduced in the core of the injection on the Exp-Htt side compared with the Htt side (Exp-Htt, 2.29 versus Htt, 139.43; ***P < 0.001) (Fig. 5D). On the LV-Exp-Htt/MSK-1-coinfected side, we dissected out the core of the injection and the surrounding area and found a slight but significant increase in PGC-1α expression in the core of the injection (Exp-Htt, 2.29 versus Exp-Htt + MSK-1, 36.14; ***P < 0.0001) and a strong increase in the surrounding area [Exp-Htt, 18.29 versus Exp-Htt + MSK-1, 299.57; ***P < 0.0001 (Fig. 5D)].

**MSK-1 controls histone H3 and CREB phosphorylation at the PGC-1α promoter**

We then attempted to unravel the molecular events that drive transcriptional regulation of PGC-1α by MSK-1. For this purpose, we used an *in vitro* model system based on primary...
cultures of striatal neurons transiently transfected with GFP-tagged MSK-1 cDNA. We have previously shown that MSK-1 is spontaneously active in this model system, as histone H3 phosphorylation is induced by LV-MSK-1 infection (34). Here we reproduced the increased PGC-1α expression induced by MSK-1 overexpression in vivo (illustrated in Fig. 6A). In a separate set of experiments, we applied glutamate, an excitatory neurotransmitter that is known to activate MSK-1 in cultured striatal neurons (43), and then measured PGC-1α mRNA levels. PGC-1α mRNA levels were significantly increased 2 h after glutamate treatment (Fig. 6B). Chromatin immunoprecipitation assays were performed with striatal neurons stimulated with 100 μM glutamate for 30 min, a time point that precedes mRNA expression and could correspond to molecular events driving chromatin remodeling and transcriptional events at the PGC-1α promoter. The mouse PGC-1α promoter region used here encompasses the CRE consensus sequence located between positions −146 and −129 from the transcriptional start site and necessary for PGC-1α induction by cAMP (44). As expected, we observed increased P-CREB binding to the PGC-1α promoter upon glutamate treatment (Fig. 6C). P-H3 binding was also increased, showing that this nucleosomal response is involved, at least in part, in chromatin remodeling on the PGC-1α promoter. Binding of MSK-1, the kinase for H3 in striatal neurons (reviewed in 41), was also increased at the PGC-1α promoter by glutamate treatment (Fig. 6C). We used a different set of primers encompassing a 3′ region to the CRE consensus site, located between positions −77 and −176 from the transcriptional start site, and failed to observe any difference in P-CREB, P-H3 or MSK-1 binding upon glutamate stimulation.
Similarly, total CREB binding did not vary upon glutamate stimulation (data not shown). Altogether, these data indicate that the signaling events induced by glutamate culminate in the recruitment of MSK-1 to the PGC-1α promoter, where it controls both chromatin remodeling and CREB phosphorylation.

Striatal degeneration in MSK-1 KO mice

We have previously found that MSK-1 expression is deficient in the striatum of R6/2 HD model mice and also in HD patients. Here we show that MSK-1 overexpression slows down neuronal dysfunction in the LV-based model of HD. Importantly, MSK-1 drives PGC1-α expression, via molecular mechanisms that we unraveled in vitro. We assumed that MSK-1 knock-out mice might display, similar to PGC1-α knock-out mice, spontaneous striatal neuron degeneration (10). We assessed ventricular volumes in MSK-1 KO mice and wild-type (WT) littermates after cresyl violet staining. Compared with WT littermates, MSK-1 KO mice displayed significant ventricular enlargement (MSK-1 KO, 0.978 mm³ versus MSK-1 WT, 0.456 mm³; **P < 0.01) (Fig. 7A and B). Intrastriatal injection of the mitochondrial neurotoxin 3-NP produces significantly larger striatal lesions and a larger number of degenerating neurons in PGC-1α KO mice bred with HD mice than in HD mice (10). We thus investigated whether MSK-1 KO mice were also more susceptible to 3-NP. We used an intoxication protocol that does not produce striatal lesions in WT mice (45). In young (6–7 week) animals, NeuN staining showed marked striatal neuron loss in MSK-1 KO mice only (759.38 versus 1028.94 in WT mice; **P < 0.01) (Fig. 7C and D). Cresyl violet staining also showed neuron loss, gliosis and nuclear fragmentation/compaction in MSK-1 KO mice (Fig. 7E). These results show that striatal dysfunction and death induced by 3-NP are exacerbated in the absence of MSK-1.
We show that MSK-1 overexpression protects lentiviral HD model rats from Exp-Htt-induced neuronal dysfunction, and that this neuroprotection is mediated by histone H3 and CREB phosphorylation at the PGC-1α promoter. These phosphorylation events induce chromatin remodeling and transcriptional regulation of PGC-1α, a protein exhibiting neuroprotective properties in various HD models. We also found that MSK-1 knock-out mice exhibit spontaneous striatal atrophy as they age, and are more susceptible to systemic administration of the mitochondrial neurotoxin 3-NP. Together, these data support a key role of MSK-1 in the regulation of genes that are dysregulated in HD, including those involved in mitochondrial biogenesis.

In the rat lentiviral model of HD, we reproduced the down-regulation of MSK-1 expression that we had previously observed in R6/2 mice and in striatal autopsy samples from HD patients (34). In this model system, in which extrastriatal brain circuitries are preserved and can exert their normal influence on cell signaling, we show that Exp-Htt expression in striatal neurons is sufficient to down-regulate MSK-1 expression. This rules out an influence of Exp-Htt expression in cortical circuits and abnormal levels of brain derived neurotrophic factor transcription or axonal transport in the regulation of striatal MSK-1 expression (46,47). We also confirm our in vitro findings obtained with primary cultures of striatal neurons, in which the MSK-1 mRNA level was decreased by Exp-Htt overexpression (34). The molecular mechanisms underlying the transcriptional control of MSK-1 by Exp-Htt were not addressed in this work, but a precise analysis of the promoter of MSK-1 indicates multiple DNA regulatory elements, including a CRE site that could be under the negative control of Exp-Htt.

**DISCUSSION**

In the rat lentiviral model of HD, we reproduced the down-regulation of MSK-1 expression that we had previously observed in R6/2 mice and in striatal autopsy samples from HD patients (34). In this model system, in which extrastriatal brain circuitries are preserved and can exert their normal influence on cell signaling, we show that Exp-Htt expression in striatal neurons is sufficient to down-regulate MSK-1 expression. This rules out an influence of Exp-Htt expression in cortical circuits and abnormal levels of brain derived neurotrophic factor transcription or axonal transport in the regulation of striatal MSK-1 expression (46,47). We also confirm our in vitro findings obtained with primary cultures of striatal neurons, in which the MSK-1 mRNA level was decreased by Exp-Htt overexpression (34). The molecular mechanisms underlying the transcriptional control of MSK-1 by Exp-Htt were not addressed in this work, but a precise analysis of the promoter of MSK-1 indicates multiple DNA regulatory elements, including a CRE site that could be under the negative control of Exp-Htt.
Our findings are further evidence that MSK-1 overexpression can mitigate the neuronal dysfunction induced by Exp-Htt, as first observed in vitro (34). At early stages, coinfection with LV-MSK-1 reduced DARPP-32 depletion induced by LV-expHtt infection. The modulation of DARPP-32 expression induced by MSK-1 overexpression may reflect an overall improvement in striatal neuron function. At late stages, MSK-1, DARPP-32 and NeuN staining was preserved in rare neurons located in the core of the injection. These neuronal markers were upregulated in the surrounding area, while nuclear shrinkage of MSN was attenuated, despite Exp-Htt expression. Finally, DARPP-32 loss over Exp-Htt was reduced at this stage, indicating that MSK-1 overexpression prevented the extension of the lesion produced by very high expression of Exp-Htt in the core of the injection.

One important result of this study concerns the role of MSK-1 in PGC-1α regulation. PGC-1α activity can be regulated by both post-translational and transcriptional events. Post-translational events include direct phosphorylation or deacetylation, which can increase PGC-1α activity or expression in various tissues (48–52). In particular, overexpression of SIRT1 deacetylase and suppression of GCN5 acetylation increase the transcriptional activity of PGC-1α and prevent Exp-Htt-induced mitochondrial loss in neurons (53). Transcriptional regulation of PGC-1α expression is known to be regulated by CREB (54–56). A direct link between CREB phosphorylation and transcriptional regulation at the PGC-1α promoter has been observed in neuronal cells (10). Impairment of the CREB/PGC-1α signaling cascade by suppression of excitatory synaptic activity or by stimulation of extracellular NMDA receptors increases the vulnerability of HD neuronal cells (57). In HD striatal neurons, inhibition of transglutaminase 2 was found to derepress PGC-1α and cytochrome C and to normalize the expression of 40% of dysregulated genes, including mitochondrial and nuclear genes (58). TG2 interacted physically with histone H3, independent of HDAC inhibition. It was thus proposed (59,60) that TG2 hyperactivation in HD could contribute to gene silencing through hyperpolyamination of histone tails. We show here that MSK-1 is directly linked, via histone H3 phosphorylation, to the nucleosomal response at the PGC-1α promoter, and transcription via CREB phosphorylation, as evidenced by chromatin immunoprecipitation assays. Furthermore, striatal neurons from MSK-1 KO mice showed no PGC-1α upregulation. Together, these findings point to MSK-1 control of chromatin remodeling via H3 phosphorylation and CRE-mediated transcription at the PGC-1α promoter.

MSK-1 is particularly abundant in the striatum (41), and the MSK-1 deficiency observed in HD might contribute to striatal vulnerability. Indeed, we found that aging MSK-1 KO mice exhibit spontaneous striatal atrophy. Striatal atrophy is also observed in HD patients (61), as well as in PGC-1α KO mice (10) and Creb1Camkcre4Cre-/- double-mutant mice (62). However, the neuropathological features of striatal degeneration may be different in these conditions (namely HD, PGC-1α KO, CREB double mutant, MSK1-KO). In particular, PGC-1α KO mice have a spongeform striatal degeneration (13) that is not observed in HD or in the MSK-1 KO mice. This suggests that MSK-1 deficiency and subsequent PGC-1α down-regulation are part of multiple dysfunctions that result in neurodegeneration in HD. 3-NP is a mitochondrial neurotoxin that induces selective degeneration of striatal neurons and HD-like symptoms in humans, monkeys and rodents (63). As in PGC-1α KO mice (10), 3-NP-induced striatal dysfunction and cell death were precipitated by MSK-1 knock out. Together, these data provide further evidence of a link between MSK-1, mitochondrial biogenesis and striatal survival in HD.

In conclusion, we propose that MSK-1 down-regulation is an early and key event in a signaling cascade that drives PGC-1α down-regulation in HD, thus contributing to mitochondrial dysfunction and defective energy metabolism in striatal neurons. MSK-1 down-regulation causes an alteration of histone H3 phosphorylation in HD that could contribute to the dysregulation of various neuronal genes. The down-regulation of PGC-1α is likely to play a key role in this process. Strategies aimed at restoring MSK-1 function early in the disease process might provide a new therapeutic approach to HD.

**MATERIALS AND METHODS**

**In vivo experiments**

Lentiviral vector production. The GFP-MSK-1 plasmid was a generous gift from Rachel Toth (MRC Phosphorylation Unit, Dundee, UK). The cDNA-encoding MSK-1 was cloned in a self-inactivating lentiviral transfer vector containing the woodchuck hepatitis virus post-regulatory element and mouse PGK-1 as internal promoter (64).

Lentiviral vectors encoding the first 171 amino acids of mutated (82 CAG) (Exp-Htt) or normal (18 CAG) (Htt) huntingtin, and a lentiviral vector encoding MSK-1, were produced as previously described (35,65). The particle content of viral batches was determined with a p24 antigen enzyme-linked immunosorbent assay.

*Animals and lentivirus injection*. Adult male Wistar rats (Iffa-Credo) weighing about 200 g were used. The animals were housed in a controlled room temperature with a 12 h light/dark cycle and was given food and water *ad libitum*. The experiments conformed to the European Community directive 86/609/EEC on the care and use of laboratory animals.

Stereotaxic injections (David Kopf Instruments, Tujunga, CA, USA) were performed under ketamine (75 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) anesthesia. The animals were divided into three groups of 10. The first group received the vector encoding Htt alone in the left striatum and the vectors encoding both Htt and MSK-1 in the right striatum. The second group received the vector expressing Exp-Htt alone in the left striatum and the vectors encoding both Exp-Htt and MSK-1 in the right striatum. The third group received only the vector expressing MSK-1, in the left striatum.

The vectors were administered at a dose of 300 ng p24 equivalent (µL), at the following coordinates: 0.5 mm rostral to bregma, 3 mm lateral to midline and 4.5 mm ventral from the skull surface, at a rate of 0.2 µL/min via a syringe (Hamilton, Reno, NV, USA) connected to an automatic injector. The needle was left in place for 5 min before being gently withdrawn.
Tissue preparation
Four or 10 weeks after lentivirus injection, the animals were anesthetized by sodium pentobarbital overdose, perfused transcardially, post-fixed in 4% paraformaldehyde, 10% picric acid for 24 h and finally cryoprotected in 15% sucrose and 0.1 M phosphate buffer for 48 h. The brains were then excised and placed in isopentane at −25°C, before cutting 25 μm coronal sections with a sliding microtome cryostat (Leitz/Wettslar; Leica Microsystems, Nussloch, Germany). Slices through the entire striatum were collected and stored in 24-well trays as free-floating sections in TB containing 30% ethylene glycol and 30% glycerol. The trays were stored at −20°C until immunohistochemical processing.

Primary antibodies
The following primary antibodies were used: a mouse monoclonal antibody recognizing the dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32; a generous gift from Dr P. Greengard, Rockefeller University, New York, NY, USA), a rabbit anti-MSK-1 antibody (MSK-1; Sigma-Aldrich, St. Louis, MO, USA), a mouse monoclonal anti-huntingtin antibody (Euromedex, Souffelweyersheim, France), a rabbit polyclonal anti-phospho-Ser10-histone H3 antibody (Upstate Biotechnology, Billerica MA, USA), a mouse anti-neuronal nuclei (NeuN) antibody (Millipore, Billerica, MA, USA), a goat polyclonal anti-PGC-1α antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a rabbit polyclonal anti-phospho-Ser133-CREB antibody (Cell Signaling, Danvers, MA, USA).

Immunohistochemical procedures
DARPP-32, MSK-1 and Htt immunohistochemistry. For light microscopy, free-floating sections were processed as previously described (34). Briefly, the sections were incubated in phosphate buffer saline (PBS) or tris buffer saline (TBS) solution containing 10% methanol, 0.9% H2O2 and then in 0.2% triton-containing buffer. For DARPP-32 immunostaining, the sections were saturated at room temperature for 1 h in 0.1 M PBS containing 10% NGS (Vector Laboratories, Burlingame CA, USA) and then incubated with the anti-DARPP-32 antibody (mouse 1:50 000 in 5% NGS-containing PBS) overnight at 4°C. For MSK-1 and HTT immunostaining, the sections were saturated at room temperature for 1 h in 0.1 M PBS containing 3% bovine serum albumin (BSA), then incubated with the primary antibodies overnight at 4°C (rabbit MSK-1 diluted 1:2000, and mouse Htt diluted 1:200) and, after rinsing, with an anti-mouse biotinylated secondary antibody (1:200, Vector Laboratories) diluted in 1% NGS BUFFER, or with an anti-rabbit biotinylated secondary antibody (1:1000, Vector Laboratories) diluted in TBS (0.25 M Tris and 0.5 M NaCl, pH 7.5), for 2 h at room temperature. The complexes were visualized with the Vectastain ABC kit (PK-4000, Vector Laboratories), with 3,3′-diaminobenzidine tetrahydrochloride as substrate. The tissue sections were mounted, dehydrated by passing twice through ethanol to xylene and cover slipped with Eukit.

P-H3, PGC-1α, MSK-1, P-CREB, Htt and NeuN immunofluorescence. For fluorescence microscopy, free-floating sections were rinsed in TBS and incubated for 15 min with 0.2% Triton X-100 in TBS. After three rinses, the floating sections were saturated for 1 h at room temperature with 3% BSA (for P-H3, P-CREB, MSK, immunohistochemistry) or with 10% NGS (for NeuN, PGC-1α immunohistochemistry) in TBS. The sections were then rinsed three times in TBS and incubated with the primary antibody (phospho-Ser-10-histone H3 1:500, Upstate Biotechnology; phospho-Ser-133-CREB 1:750, Upstate Biotechnology; MSK, 1:2000, Sigma-Aldrich; PGC-1α, 1:500, Santa Cruz Biotechnology; or NeuN, 1:400, Millipore); overnight at 4°C in TBS. The sections were then incubated for 90 min at room temperature with the Alexa Fluor secondary antibody (Invitrogen, Carlsbad, CA, USA). For P-CREB immunohistochemistry, sections were incubated for 2 h with the secondary biotinylated antibody (anti-rabbit 1:200, Vector Laboratories) and, after three rinses, with Cy3-conjugated streptavidin (1:300, Sigma-Aldrich). All P-CREB immunohistochemistry procedures were performed with TBS containing 0.2% Triton X-100 and 1% BSA. After three rinses in TBS, tissue sections were mounted under cover slips with Vectashield (Vector Laboratories) for fluorescence microscopy.

Determination of the volume of infection
Striatal dysfunction and the volume of infection were evaluated by measuring the areas of striatal tissue exhibiting DARPP-32 depletion and Htt overexpression on 25 μm thick sections, spaced 175 μm apart, throughout the striatum, using Image J 1.40G software.

Volume was estimated as \( d^3 \times (a_1 + a_2 + a_3 \ldots) \), where \( d \) is distance between serial sections (175 μm), and \( a_1, a_2, a_3 \ldots \) are the DARPP-32-depleted or Htt-overexpressing areas of individual sections examined with a 2.5× objective.

MSK-1 KO mouse tissue preparation and histological evaluation
Nine-month-old MSK-1 KO mice and WT littermates were anesthetized rapidly with pentobarbital (30 mg/kg, i.p.; Sanofi-Aventis, Paris, France) and perfused transcardially with fixative solution containing 4% paraformaldehyde (w/v) in 0.1 M Na2HPO4/NaH2PO4 buffer, pH 7.5. The brains were post-fixed overnight in the same solution at 4°C. Sections of 30 μm thickness were cut with a vibratome (Leica) and kept at −20°C in 0.1 M phosphate buffer containing 30% ethylene glycol (v/v) and 30% glycerol (v/v). Sections were incubated for 15 min in cresyl violet and sequentially destained in ethanol 95°C, ethanol 100°C and xylene, before mounting with Eukitt.

Ventricular volumes were estimated on serial coronal tissue sections (nine sections per animal) of the rostral segment of the neostriatum to the level of third ventricle, using ImageJ software.

3-NP intoxication of MSK-1 WT and KO mice
3-nitropropionic acid (3-NP, Fluka, ref 73808) was injected intraperitoneally as previously described (66,67). 3-NP was dissolved in saline, adjusted to pH 7.4 with 1 N NaOH, and...
then filtered. 3-NP was injected twice daily for 2 days at 12 h intervals (8:00 A.M. and 8:00 P.M.) at a dose of 60 mg/kg for the first two injections and 80 mg/kg for the second two injections. 3-NP and saline (control) were injected in MSK-1 KO and WT littermate mice at 6–7 weeks of age, with five animals per group. The mice were killed 24 h after the last injection for histological processing, including cresyl violet and NeuN staining. Neuron loss was evaluated on two sections per animal, using a 10× objective.

**In vitro experiments**

**Transfection.** Transient transfection of primary striatal cell cultures with MSK-1 plasmids was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Cells in 10 cm² dishes (for RNA extraction and ChIP assays) or 2 cm² wells (for immunocytochemistry) were transfected with 4 or 0.8 µg of DNA, respectively, for 3.5 h. The cells were then rinsed with fresh culture medium and incubated at 37°C for 24 h before the experiments.

**Immunocytochemistry.** Striatal neurons were fixed in 2% paraformaldehyde in PBS for 40 min at room temperature and permeabilized with methanol/acetone (1:1 ratio) for 10 min at 4°C. After three washes with PBS, the cells were saturated for 1 h at room temperature with 10% NGS and then incubated with a goat polyclonal anti-PGC-1α (K-15) primary antibody (1:500, Santa Cruz Biotechnology, SC-5816) in PBS overnight at 4°C. The cells were rinsed with PBS and incubated for 90 min at room temperature with a donkey anti-goat Alexa Fluor 546 secondary antibody (1:500, Molecular Probes). After three washes with PBS, the cells were stained with Hoechst (1:20 000 in PBS) for 5 min at room temperature, washed thrice in PBS and mounted under cover slips with Vectashield (Vector Laboratories).

**Chromatin immunoprecipitation.** Chromatin immunoprecipitation assays were performed with the ChIP assay kit (Upstate Biotechnology, ref 17–295) as described by the manufacturer, with some modifications. Striatal neurons were cultured in 60 mm dishes. At 7 days in vitro, 1.8 × 10⁶ striatal neurons were stimulated with 100 µM glutamate and then fixed for 10 min in 1% formaldehyde at 37°C. After two washes with cold PBS containing protease inhibitors (complete EDTA-free) (Roche Diagnostics, Meylan, France), chromatin was solubilized and extracted in sodium dodecyl sulfate lysis buffer, followed by sonication. After centrifugation, the supernatant was made up to a final volume of 2 ml with ChIP dilution buffer (ChIP assay kit; Upstate Biotechnology). The supernatant was cleared at 4°C for 1 h with 45 µl of protein A or G agarose/salmon sperm DNA for polyclonal or monoclonal antibodies, respectively. After centrifugation, the following antibodies were added to the supernatant: 8 µl of mouse monoclonal anti-phosphor-Ser10-H3 (Abcam, ab14955); 10 µl of rabbit polyclonal anti-MSK-1 (H-65) (Santa Cruz Biotechnology, SC-25417) and 8 µl of rabbit polyclonal anti-CREB (48H2) (Upstate, Cell Signaling, ref 9197), and the immunoprecipitation mix was incubated at 4°C overnight, before adding 60 µl of protein A or G agarose/salmon sperm DNA at 4°C for a further hour. Immunoprecipitated DNA was eluted from the beads and cross-links were reversed for 4 h at 65°C, followed by proteinase K treatment (10 mg/ml) for 1 h at 45°C. The DNA samples were then extracted with phenol/chloroform, precipitated with ethanol and resuspended in water for PCR studies. Samples of input DNA were prepared in the same way.

The DNA thus obtained was analyzed by real-time quantitative PCR with a set of primers for the PGC-1α promoter region. The normalizing primer was H4. PCR reactions were performed in triplicate in the presence of SYBR Green (Roche Diagnostics).

**Statistical analysis**

GraphPad Prism software was used for statistical analyses. All data are expressed as mean ± SEM, and the mean significant difference between experimental groups was determined with one-way or two-way analysis of variance (ANOVA) followed by Bonferroni’s post hoc test. For comparisons between two groups, i.e. DARPP-32 depletion in Exp-Htt versus Exp-Htt + MSK-1 striata, ventricle volume and neuron loss in mice intoxicated with 3-NP, the mean significant difference was determined with Student’s unpaired t-test. All in vivo infection experiments were performed with eight animals per group, and in vitro results were obtained from three independent experiments. Significance was assumed at \( P \leq 0.05 \).

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

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**Conflict of Interest statement.** None declared.

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