Changes in the expression of extracellular regulated kinase (ERK 1/2) in the R6/2 mouse model of Huntington's disease after phosphodiesterase IV inhibition

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ABSTRACT

The mitogen-activated protein kinases (MAPKs) superfamily comprises three major signaling pathways: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases or stress-activated protein kinases (JNKs/SAPKs) and the p38 family of kinases.
ERK 1/2 signaling has been implicated in a number of neurodegenerative disorders, including Huntington's disease (HD). Phosphorylation patterns of ERK 1/2 and JNK are altered in cell models of HD. In this study, we aimed at studying the correlations between ERK 1/2 and the neuronal vulnerability to HD degeneration in the R6/2 transgenic mouse model of HD. Single and double-label immunofluorescence for phospho-ERK (pERK, the activated form of ERK) and for each of the striatal neuronal markers were employed on perfusion-fixed brain sections from R6/2 and wild-type mice. Moreover, Phosphodiesterase 4 inhibition through rolipram was used to study the effects on pERK expression in the different types of striatal neurons. We completed our study with western blot analysis. Our study shows that pERK levels increase with age in the medium spiny striatal neurons and in the parvalbumin interneurons, and that rolipram counteracts such increase in pERK. Conversely, cholinergic and somatostatinergic interneurons of the striatum contain higher levels of pERK in the R6/2 mice compared to the controls. Rolipram induces an increase in pERK expression in these interneurons. Thus, our study confirms and extends the concept that the expression of phosphorylated ERK 1/2 is related to neuronal vulnerability and is implicated in the pathophysiology of cell death in HD.

Introduction

Huntington's disease (HD) is a devastating neurodegenerative disorder characterized by progressive and severe cognitive, psychiatric, and motor dysfunction (Walker, 2007). The disease is caused by expansion of a CAG repeat in exon 1 of *IT15,* which encodes for the protein huntingtin (The Huntington's disease collaborative research group, 1993). The expanded n-terminal poly-glutamine tract in mutant huntingtin is toxic (Mangiarini et al., 1996), with the length of the expansion correlating with age of onset and disease course (Andrew et al., 1993; Melone et al., 2005; Perrone and Melone, 2008).

Although huntingtin is widely expressed in the brain (Fusco et al., 1999), HD is associated with a specific pattern of neurodegeneration.

The striatal medium spiny projection neurons are most susceptible to the degeneration and loss of striatal volume is the prominent pathological finding (de la Monte et al., 1988; Vonsattel et al., 1985), while cholinergic and somatostatinergic interneurons are more resistant.

The striatum is highly innervated by Brain Derived Neurotrophic Factor (BDNF)-releasing synapses. BDNF is delivered to the striatum via activity-dependent anterograde release from excitatory corticostriatal axons (Canals et al., 2001; Zuccato and Cattaneo, 2007). BDNF is essential for striatal function and survival (Gratacòs et al., 2001; Zuccato et al., 2001). Interestingly, in HD, because mutated huntingtin loses its physiological role, a dramatic loss of BDNF occurs, contributing prominently to the striatal degeneration.

BDNF signaling is mediated primarily by its high affinity receptor, the receptor tyrosine kinase tropomyosin-related kinase B (TrkB). TrkB activation initiates three major intracellular signaling cascades: the shc/Frs2 and ras/raf-mediated activation of a MAPK phosphorylation cascade involving mitogen-activated ERK kinase (MEK) and extracellular-signal-regulated kinase (ERK 1/2), the shc/Frs2 and GAB1-mediated activation of a phoshphotidylinositol-3-kinase PI3K

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pathway involving Akt1, and the direct TrkB-mediated activation of phospholipase C gamma (PLCc) which produces inositol triphosphate (IP3) and diacylglycerol (DAG) and increases intracellular free calcium (Corominas et al., 2007; Numakawa et al., 2010).

ERK 1/2 is a strong anti-apoptotic and pro-survival mediator (Kim et al., 2009). ERK 1/2 is also involved in the phosphorylation of synapsin I (Jovanovic et al., 1996, 2008; Matsubara et al., 1996), which is implicated in the regulation of neurotransmitter release and synapse formation, and whose function is altered in HD models (Liévens et al., 2002). Moreover, ERK 1/2 downregulation is linked to neurodegenerative conditions such as ischemia (Liebelt et al., 2010) and traumatic spinal cord injury (Yu et al., 2010).

The MAP kinase/ERK 1/2 signaling pathway has been shown to play a key role in this transcriptional regulation in the striatum (Roze et al., 2008), and is activated by the binding of BDNF to its receptor TrkB, which leads to the phosphorylation of cAMP response element binding protein (CREB) (Kingsbury and Krueger, 2007).

CREB is involved in the neuronal vulnerability to HD degeneration, and neuroprotection achieved by the Phosphodiesterase IV inhibitor rolipram is mediated by increased CREB and BDNF levels in the R6/2 model of HD (DeMarch et al., 2008; Giampà et al., 2009). Neuroprotection achieved by the increase CREB and BDNF should involve the activation of ERK 1/2 pathway (Yao et al., 2009). Thus, we aimed at investigating if neuroprotection achieved by PDEIV inhibition by rolipram involved changes in the expression of pERK in R6/2 mice.

Changes in pERK expression in the R6/2 mice were described (Liévens et al., 2002). However, a precise description of possible changes in pERK expression in the different neuronal subtypes was still lacking. Thus, the analysis of ERK 1/2 expression was performed in the different neuronal subtypes of the striatum.

Materials and methods

Heterozygous transgenic R6/2 males of the CBAXC57BL/6 strain were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and maintained by backcrossing carrier males with CBAXC57BL/6_F1 females. The offspring were genotyped by polymerase chain reaction assay of DNA obtained from tail tissue. Mice were weaned and, after genotyping, treatments began at 4 weeks of age (n = 24/group) and lasted until the animals were killed at 7 and 13 weeks of age. The study groups included: R6/2 mice that were given saline by intraperitoneal injection (0.9%), R6/2 mice with rolipram given by intraperitoneal injection [dissolved in saline (1.5 mg/kg/day) and made fresh daily], rolipram-treated and saline-treated wild-type mice.

Mice were handled under the same conditions by one investigator on the same day and at the same time. Mice were identified by a randomly assigned code so that the studies were performed blind as to the genetic identity. The mice were housed five in each cage under standard conditions with *ad libitum* access to food and water. All studies were conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and approved by the Santa Lucia Foundation Animal Care and Use committee. All the histological data were collected by observers who were blinded to treatment.

Immunohistochemical studies

Tissue processing. For the histological examination, animals at 7 and 13 weeks of age were transcardially perfused under deep (chloral hydrate) anesthesia with saline solution containing 0.01 ml heparin, followed by 60 ml of 4% paraformaldehyde in saline solution. The brains were removed and postfixed overnight at +4C° and cryoprotected in 10% sucrose and 20% glycerol in 0.1 M phosphate buffer (PB) with sodium azide 0.02% for 48 h at 4C°. Brains were sectioned frozen on a sliding microtome at 40 μm thickness (Fusco et al., 2001).

Single and dual-label immunofluorescence

Primary omission controls, normal serum controls and preimmune serum controls using immunizing peptide were used to confirm the specificity of our immunohistochemical labeling (Fusco et al., 2003). Sections were incubated with a cocktail of rabbit anti-pERK (Immunological Sciences) and one of the following striatal neuronal markers: mouse anti-calbindin 28 kDa (Sigma) to evaluate the expression, at a protein level, of pERK in the striatal matrix spiny projection neurons; mouse anti-choline acetyl transferase (ChAT; Chemicon) to study the expression of pERK in cholinergic interneurons; mouse anti-nitric oxide synthase (NOS; Sigma, St-Louis, MO) to study the distribution of pERK in the somatostatin neuropeptide Y-NOS containing interneuron subset; mouse anti-parvalbumin (PARV; Chemicon Temecula CA), for the labeling of pERK into parvalbuminergic interneurons. All primary antisera were used at a 1:200 concentration (except for anti-pERK which was used at a 1:300), in 0.1 m PB containing Triton X-100, 0.3% and sodium azide, 0.02% for 72 h at 4 °C. Sections were then rinsed three times for 15 min at room temperature, and subsequently incubated with a cocktail of goat anti rabbit Cy2-conjugated secondary antibody and donkey anti-mouse Cy3-conjugated secondary antibody (both Jackson Immunoresearch, West Grove, PA, USA) for 2 h at room temperature. Tissue was mounted on gelatin-coated slides, coverslipped with GEL-MOUNT and examined under an epi-illumination fluorescence microscope (Zeiss Axioskop 2), and a CLSM (Zeiss LSM 700) was used to acquire all the images.

Striatal tissue protein extraction—Western blotting

Western blot analysis of phosphorylation state of ERK 1/2 after rolipram inhibition of PDE4 was performed. The animals (n = 4 per group) were deeply anesthetized and killed by decapitation at the age of 7 and 13 weeks (wild-type and R6/2). The brain was quickly removed and the striatum was dissected out and homogenized in lysis buffer containing 1% Triton X-100, 10 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA, 5 mM EDTA, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 1 mM β-glycerolphosphate, phosphatase inhibitors (1 mM Na3VO4, and 10 mM NaF) and protease inhibitor mixture (Sigma-Aldrich). Samples were left for 30 min on ice and then centrifuged at 1200 ×g for 20 min at 4 °C, the supernatants were collected and stored at −80 °C. The protein concentration was determined using the Bradford colorimetric assay (Bio-Rad, Milano, Italy).

Thirty μg of the extracted proteins were resolved by 10% SDS-PAGE gels and transferred to PVDF membranes (Millipore, Billerica, USA). Nonspecific sites were blocked by 0.1% Tween 20 and 5% milk powder in TBS for 1 h at room temperature. Membranes were then incubated overnight at 4 °C with the specific anti-pERK rabbit antibody (1:1000, Immunological Science, Italy) in TBS/1% Tween-20 with 1% of milk powder. After primary antibody incubation, the membranes were incubated for 1 h at room temperature with the appropriate horseradish peroxidase-conjugated secondary antibody (1:5000, Immunological Science) and the reaction was visualized by using an enhanced chemiluminescent detection system (Millipore, Bilerica, MA). Incubation with a rabbit total ERK 1/2 (1:1000; Cell Signaling, Beverly, MA) was performed to obtain loading controls. Densitometric quantification of the immunoblots was performed using Image J software.

Statistical analysis

To obtain an indirect measure of the amount of pERK immunostaining in spiny projection neurons, and interneurons, image analysis of calbindin positive, cholinergic, somatostatinergic, parvalbuminergic interneurons was performed by means of image J software. Briefly, the background was removed and the image was converted to greyscale. The double labeled neurons were identified through the use

of the function "region of interest (ROI) manager" of the program. The intensity of fluorescent pERK immunolabeling was calculated in each of three separate fields (one dorsolateral, one central and one medial, each 1 mm in diameter) on each hemisphere in each of three rostro-caudally spaced sections in each of saline, rolipram-treated R6/2 mice and wild-type littermates.

The data obtained by image analysis of pERK immunolabeling in all subtypes of striatal neurons were expressed as mean +/− SD. Differences in the time course experiments were analyzed by two-way ANOVA followed by Tukey's posthoc analysis, and the significance level was set to P<0.05.

For Western blot analysis, the density of pERK protein band was measured using a Kodak Image Station and the ratio between pERK/total ERK was calculated for each group. Statistical comparisons between groups were made by ANOVA including the factors of genotype, age and treatment as warranted. All posthoc analysis used HSD Tukey test and the significance level was set to P<0.05.

Results

Immunohistochemistry

The expression of pERK in the medium spiny neurons increases with age and is decreased by rolipram

The protein expression of pERK was studied in the immunohisto-chemically labeled striatal medium spiny projection neurons of R6/2 mice and wild-type littermates. The analysis was performed at 8 and 13 weeks of age, when R6/2 are fully symptomatic and close to the end stage. pERK levels were lower in the spiny neurons of R6/2 at 8 weeks compared to wild-types. At the second time point of 13 weeks, pERK levels increased both in the wild-types and in the R6/2 mice (F(1,1198) = 20.22; P<0.000. At this time point, pERK levels were comparable between R6/2 and their wild-type littermates (P<0.9). (Figs. 1, 5). Moreover, the intensity of pERK immunolabeling increased significantly in both wild-types and R6/2 at 13 weeks,
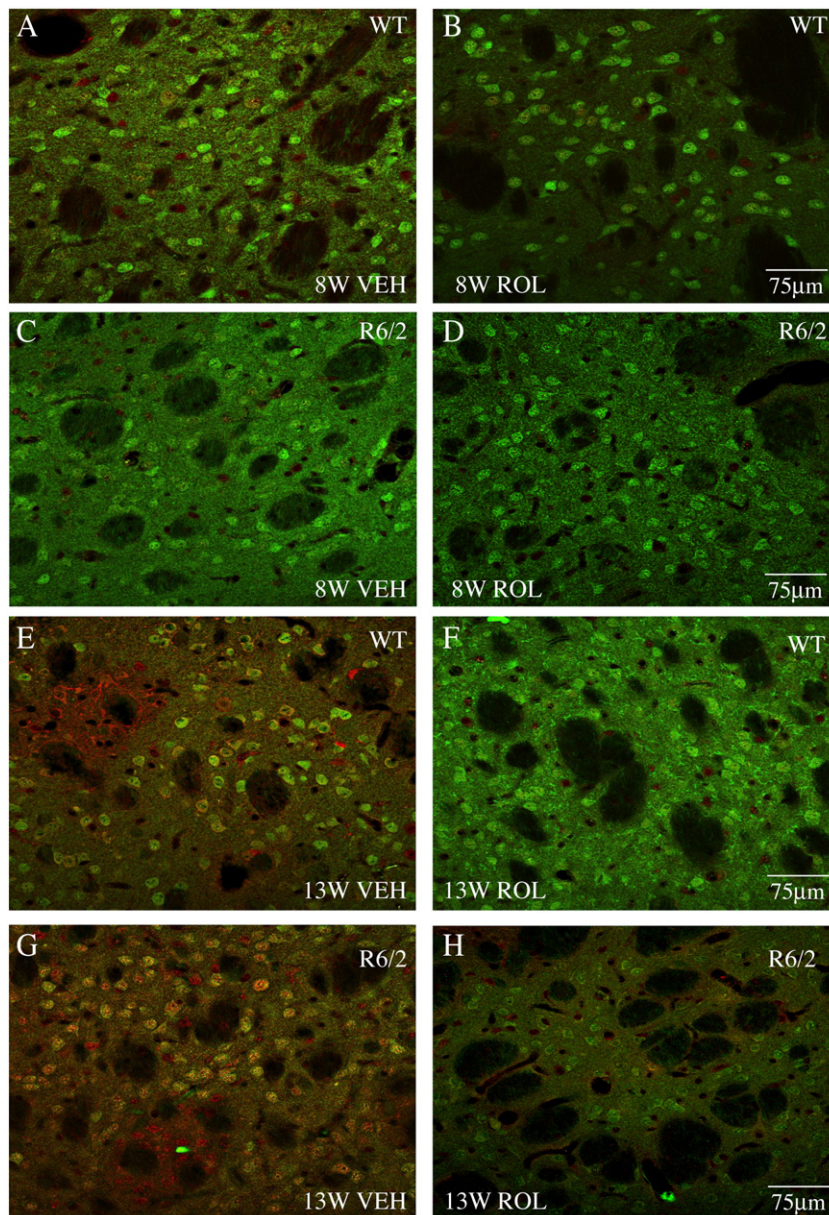

Fig. 1. Confocal laser scanning microscopy (CLSM) images of double-label immunofluorescence for pERK and projections neurons marker calbindin (CALB). (A–C–E–G) pERK immu-nolabeling (visualized in red-cy3 immunofluorescence) and CALB immunolabeling (visualized in green-cy2 immunofluorescence) in wild-type and R6/2 HD mice striatum at 8 and 13 weeks of age treated with vehicle. In (B–D–F–H) pERK and CALB immunolabeling in Wt and R6/2 HD mice striatum at 8–13 weeks of age treated with Rolipram.

although there were no differences between genotypes. As shown in Fig. 5A, the administration of PDE4 inhibitor rolipram decreased dramatically pERK protein expression both in the wild-type and in the R6/2 animals. Thus, we observed that pERK levels in the spiny projection neurons were comparable between wild-type and R6/2 mice, and that rolipram had a negative effect on pERK expression in those neurons, regardless of the genotype (F(1,1198) = 349.20; P<0.000).

The expression of pERK in cholinergic neurons decreases with age in the R6/2 mice and is increased by rolipram

In the cholinergic interneurons of the striatum, which are the neuronal subpopulation most resistant to HD degeneration, pERK levels were higher in the R6/2 than in the wild-type mice at 8 weeks. This was different from what we observed in the projection neurons, where pERK levels were comparable between wild-types and R6/2. Interestingly, pERK decreased at 13 weeks in the R6/2, whereas they remained unchanged in the wild-type mice. We also observed that PDE4 inhibition with rolipram induced an increase in pERK levels at 13 weeks both in the R6/2 and in the wild-type, F(1,102) = 16.99 P<0.0001. The increase observed at 13 weeks with rolipram treatment was statistically significant, however the levels of pERK expression did not reach the ones observed at 8 weeks in either group (Figs. 2, 5B).

Expression of pERK is decreased by rolipram in the parvalbuminergic interneurons in the R6/2 mice

The parvalbuminergic interneurons of the striatum are, among the interneurons, the most vulnerable subtype to HD degeneration. The protein expression of pERK was comparable between R6/2 and corresponding wild-type mice at 8 weeks. At 13 weeks rolipram induced a decrease in pERK compared to R6/2 treated with vehicle F(1,259) = 44.56 P<0.0000 (Figs. 3, 5C).

The expression of pERK is increased by rolipram in the somatostatinergic interneurons in the R6/2 mice

Unlike the spiny projection parvalbuminergic neurons, the somatostatin neuropeptide Y-NOS-containing interneurons are relatively spared in HD. In (nNOS)-positive neurons, as we have observed in large aspiny cholinergic neurons pERK levels were higher in the R6/2 than in the wild-types both at 8 weeks and at 13 weeks. Selective rolipram PDE4 inhibition induced, also in this subset of neurons, an increase in pERK levels at both time points F(1,164) = 49.37 P<0.0000 (Figs. 4 and 5).

Western blotting on striatal tissue confirms the increased pERK expression observed in the medium spiny neurons

This Western blot study was conducted on striatal tissue *in toto*. The majority of striatal neurons being represented by projection neurons (over 90–95%), our results arguably reflect the amount of pERK protein in the medium spiny neurons. Our quantitative analysis on the Western blotting data, showed that, at early stage of HD (8 weeks of age), the phosphorylation levels of ERK 1/2 were significantly lower in the R6/2 mice compared to the wild-type animals, regardless of the treatment. In agreement with the data collected with the immunofluorescence analysis, the intensity of activated ERK 1/2 was significantly higher in both R6/2 and wild-type mice at 13 weeks of age. An increase in pERK between the 8 weeks and the 13 weeks time point was observed in both R6/2 and wild-types. Rolipram treatment counteracted this effect with a significant decrease that was most evident in the R6/2 at 13 weeks F(1, 30) = 16.56; P<0.0000 (Figs. 6A, B).

Discussion

In this study, we showed the expression of pERK in the different neuronal populations of the striatum of the normal mouse and of
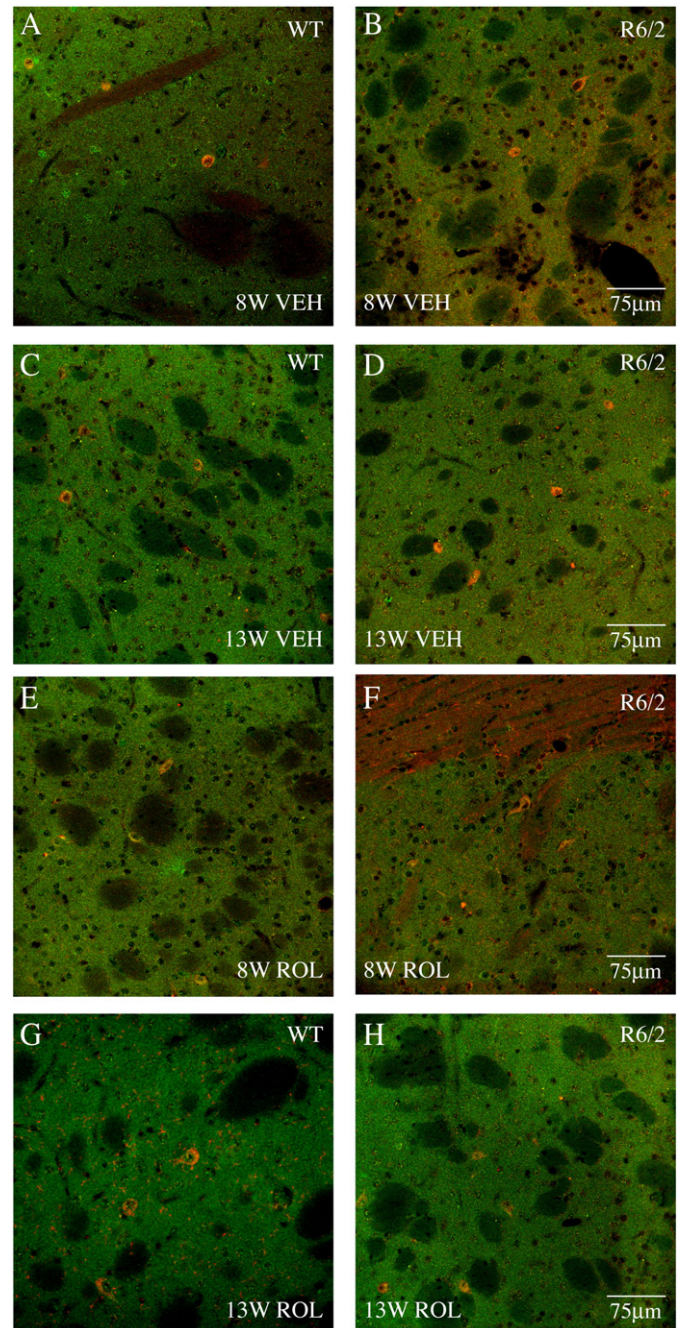

Fig. 2. Confocal laser scanning microscopy (CLSM) images of double-label immunofluorescence for pERK and cholinergic striatal interneurons (ChAT). (A–D) pERK immunolabeling (visualized in green-cy2 immunofluorescence) and CHAT immunolabeling (visualized in red-cy3 immunofluorescence) in wild-type and R6/2 HD mice striatum at 8–13 weeks of age treated with vehicle. (E–H) pERK and ChAT immunolabeling in wild-type and R6/2 HD mice striatum at 8–13 weeks of age treated with rolipram.

the R6/2 mouse model of HD. Moreover, we observed changes in the expression of pERK protein after the inhibition of phosphodiesterase 4, which was shown to be neuroprotective in both the rat and the mouse model of HD (DeMarch et al., 2007, 2008; Giampà et al., 2009, 2010).

Neuronal degeneration does not afflict all types of striatal neurons in the same fashion. In fact, while GABAergic spiny projection neurons degenerate massively (Albin et al., 1990a,b, 1992; Reiner et al., 1988), interneurons display different levels of resistance to HD. In
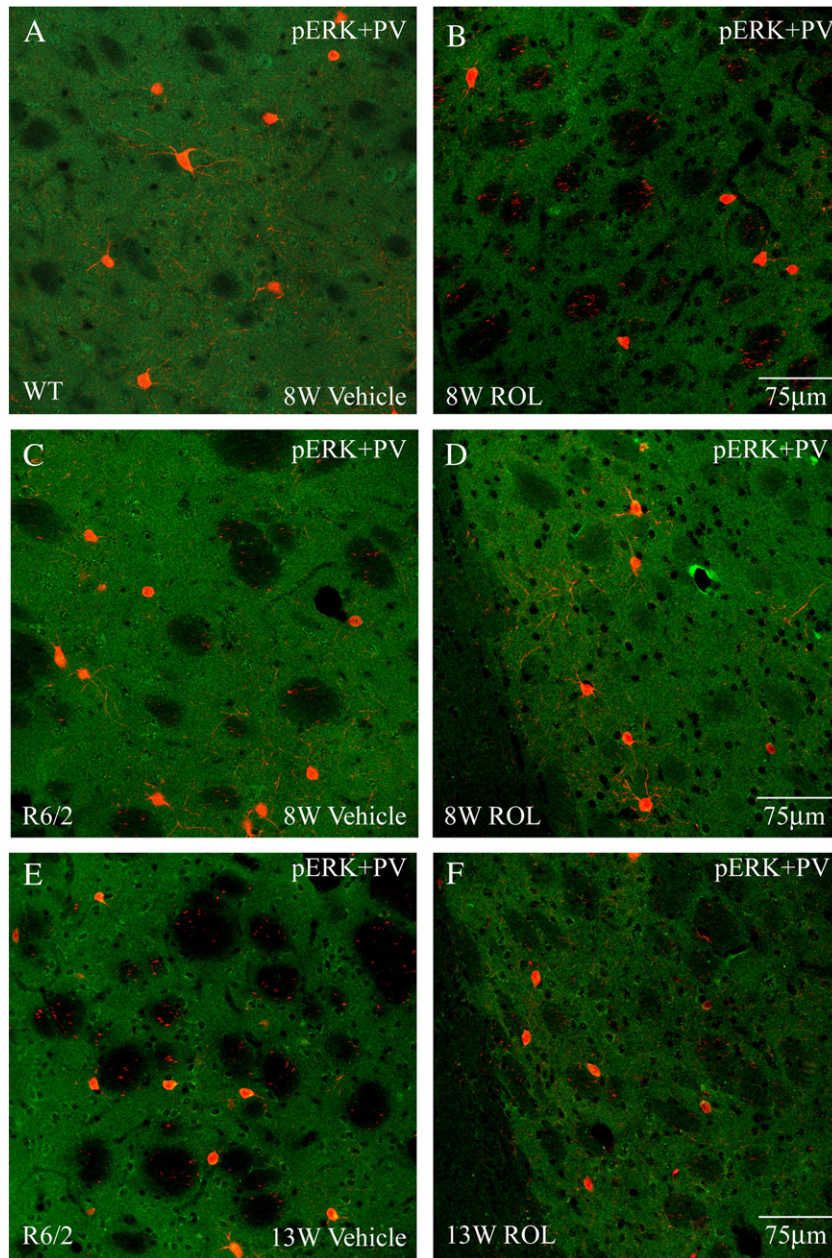
Fig. 3. Confocal laser scanning microscopy (CLSM) images of double-label immunofluorescence for pERK and parvalbuminergic striatal interneurons (PV). (A–C–E) pERK immuno-labeling (visualized in green-cy2 immunofluorescence) and PV immunolabeling (visualized in red-cy3 immunofluorescence) in wild-type and R6/2 HD mice striatum at 8–13 weeks of age treated with vehicle. (B–D–F) pERK and PV immunolabeling in wild-type and R6/2 HD mice striatum at 8–13 weeks of age treated with rolipram.

particular, parvalbuminergic interneurons degenerate (Vonsattel and DiFiglia, 1998), whereas cholinergic, somatostatinergic, and calretinin interneurons survive in HD (Albin et al., 1990a; Cicchetti and Parent, 1996; Ferrante et al., 1985, 1986, 1987). Such diverse vulnerability was also observed in the quinolinic acid rat model of HD (Figueredo-Cardenas et al., 1998).

For the first time, we describe that pERK protein is expressed differently in the striatal neuronal subsets, both in the wild-type and in the R6/2 mice. Indeed, we observed that projection neurons as well as parvalbuminergic interneurons, which are most vulnerable to HD degeneration, contain pERK levels that tend to increase with age (in the wild-type animals) and with the progression of the disease (in the R6/2 mice).

Conversely, the subsets of neurons that are more resistant to HD degeneration, such as the somatostatin-NOS-NPY (Figueredo-Cardenas et al., 1997) and the cholinergic interneurons (Meade et al., 2000) showed that pERK decreased with age (in the wild-type) and progression of the disease (in the R6/2).

Studies focusing on the intrinsic characteristics of the different striatal neurons in models of HD have tried to shed light on what makes certain neurons more vulnerable, and certain neurons more resistant, to HD degeneration (Chen et al., 1998; Figueredo-Cardenas et al., 1997; Fusco et al., 1999; Sun et al., 2002a,b).

In a previous study, our group had shown that neurons that are more resistant to HD degeneration, namely, the striatal cholinergic interneurons, contain higher amounts of BDNF, which is essential for striatal survival and is greatly decreased in HD, compared to the more vulnerable medium spiny neurons (Fusco et al., 2003).

Moreover, striatal cholinergic interneurons are more enriched with the CREB in its phosphorylated form (Giampà et al., 2006). CREB is
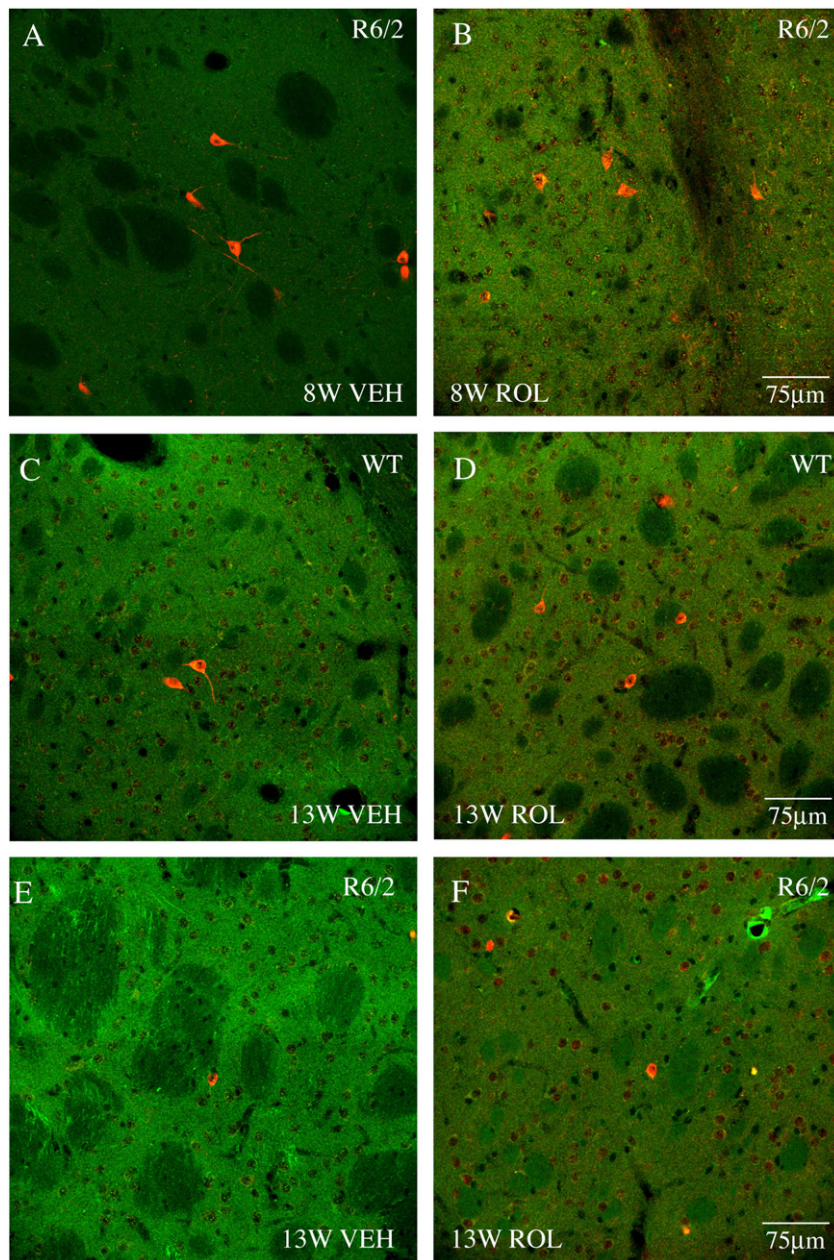
Fig. 4. Confocal laser scanning microscopy (CLSM) images of double-label immunofluorescence for pERK and somatostatinergic striatal interneurons (NOS). (A–C–E) pERK immunolabeling (visualized in green-cy2 immunofluorescence) and NOS immunolabeling (visualized in red-cy3 immunofluorescence) in wild-type and R6/2 HD mice striatum at 8–13 weeks of age treated with vehicle. (B–D–F) pERK and NOS immunolabeling in rolipram-treated wild-type and R6/2 HD mice striatum at 8–13 weeks of age.

required for the survival of adult CNS neurons (Mantamadiotis et al., 2002), and mediates nuclear calcium-regulated gene transcription following neuronal cell membrane depolarization (Hardingham et al., 1998). Moreover, a neuroprotective role of CREB was clearly demonstrated in a hypoxic–ischemic brain damage model of programmed cell death (Walton et al., 1996, 1999). In particular, inhibition of cAMP response element (CRE)-mediated gene transcription has been hypothesized to contribute to HD pathology (Jiang et al., 2003; Kazantsev et al., 1999; Nucifora et al., 2001; Shimohata et al., 2000; Steffan et al., 2000, 2001).

Of note, several different agents can induce the phosphorylation of both ERK 1/2 and CREB, as the two factors seem to be interlinked in one or more pathways. In fact, in the striatum, glutamate-induced phosphorylation of CREB appears to be mediated via activation of ERK 1/2 (Sgambato et al., 1998; Vanhoutte et al., 1999). Moreover,

there is evidence indicating that the functioning of cAMP pathway is inextricably linked with that of the extracellular signal-regulated kinase (ERK 1/2/mitogen-activated protein kinase (MAPK) pathway (Baillie et al., 2000).

Our results show, however, that the beneficial effect of rolipram acts differently in the different cell types of the striatum. Indeed, rolipram showed to decrease pERK levels in the neurons that are more vulnerable to HD degeneration, whereas it induced an upregulation of ERK 1/2 in the more resistant neurons, such as the cholinergic and the SS/NOS/NPY interneurons. In particular, the effect of rolipram was not detectable at 8 weeks, when pERK levels were comparable between groups. Rolipram effect was, in fact, more evident at 13 weeks of age in the R6/2 mice, showing that the compound was able to maintain the levels of pERK necessary for cell functions.
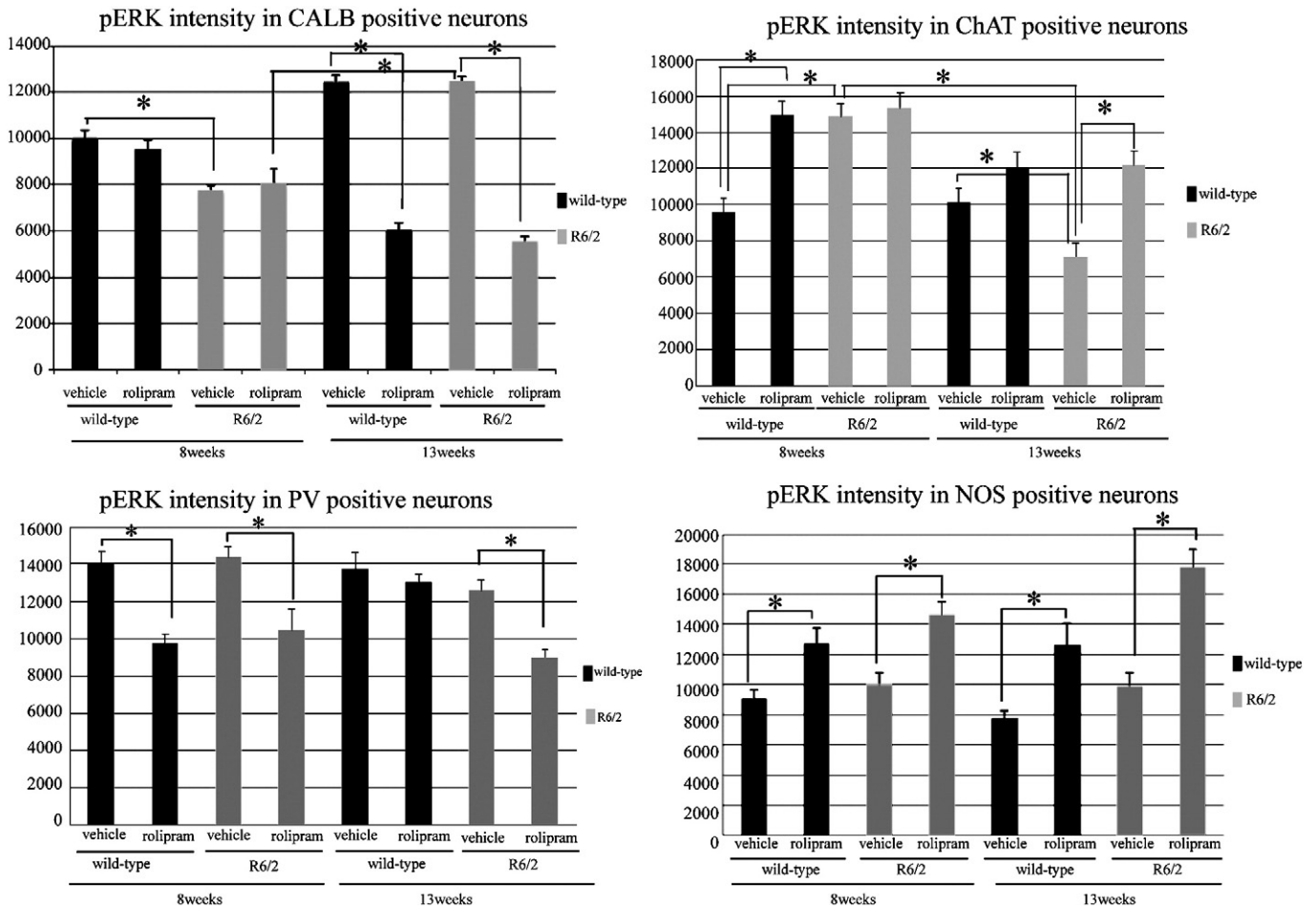
Fig. 5. Graph summarizing the different levels of pERK (expressed in arbitrary units) in the CALB immunoreactive projection neurons (A) and in the interneurons subtypes (B–D) of striata following rolipram and vehicle treatment and sacrificed at the different time points. Please note that the levels of pERK decrease dramatically at 13 weeks of age after rolipram treatment.

Our results were corroborated by the western blot study, which showed, in whole striatal samples, changes in pERK that were comparable to the ones observed in the immunofluorescence analysis.

In a previous study, an increase in pERK was observed in R6/2 mice at 12 weeks of age (Liévens et al., 2002). Our data expand these findings and describe how rolipram-induced PDE4 inhibition decreases such as high levels of pERK in the medium spiny neurons, which account for the largest part of striatal neuronal population.

The observation that changes in ERK 1/2 phosphorylation did not parallel the changes in CREB described previously (Giampà et al., 2006) seems to be conflicting with the concept that CREB and ERK 1/2 pathways are interlinked, particularly because rolipram induces an increase in CREB both in the mouse and rat models of HD (DeMarch et al., 2007, 2008; Giampà et al., 2009). Indeed, the inhibition of another kind of PDE, namely, PDE10, induces the activation of CREB and also of ERK 1/2 (Kleiman et al., 2011). Thus, one could expect that the
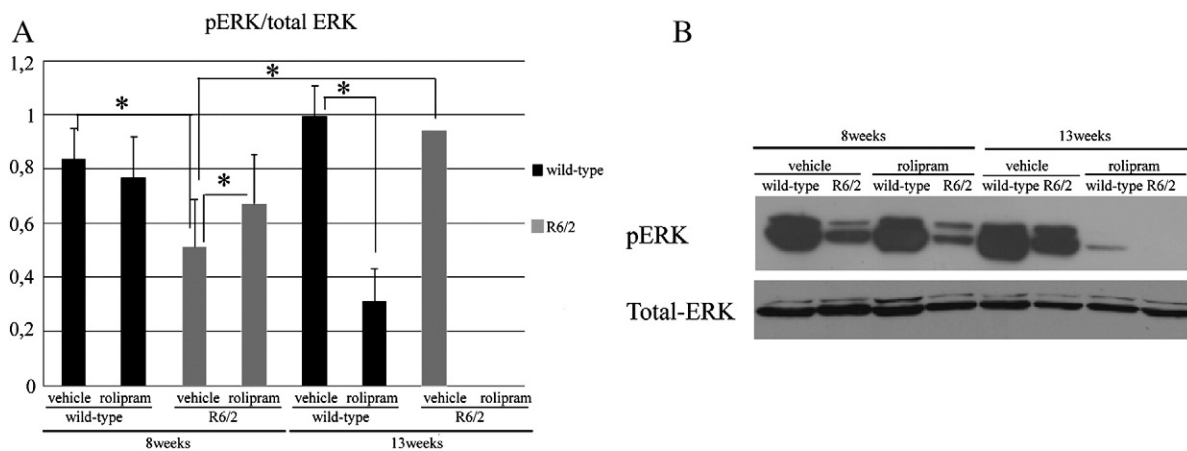

Fig. 6. Regulation of ERK 1/2 activation after rolipram treatment in wild-type and R6/2 mice. A–B: pERK levels were analyzed by Western blotting of protein extracts obtained from the striatum of WT and R6/2 mice at different time points (8 and 13 weeks).

neuroprotective effect of rolipram induced an increase in pERK because of the increased pCREB that was observed previously.

This, however, seems to be oversimplified when taking into account the complexity of the cross-talk between cAMP-PKA and ERK 1/2 signaling and that ERK 1/2 can play different, and even opposite, roles in the cell.

The role of ERK 1/2 in a variety of cellular functions including cell death depends on the cell type as well as on the signal that triggers cell death (Bioessays, 2003; Colucci-D'Amato et al., 2003; Stanciu et al., 2000). Indeed, ERK 1/2 facilitates apoptosis in several paradigms (Aoki et al., 2011; Bhat and Zhang, 1999; Fukunaga and Miyamoto, 1998) including MPP+-mediated apoptosis in SH-SY5Y neuroblastoma cells (Gómez-Santos et al., 2002). Yet MAPK/ERK 1/2 is associated with survival (Hetman et al., 1999) and is upregulated in the penumbra of the ischemic lesion in the focal cerebral ischemia model (Ferrer et al., 2003).

Furthermore, a bulk of data shows that the duration of ERK 1/2 activation, caused by different stimuli in a given cell type, determines its subcellular compartmentalization and/or trafficking. The latter, in turn, dictates whether ERK 1/2-expressing cells would enter a program of cell death or survival (Chu et al., 2004; Colucci-D'Amato et al., 2003; Stanciu and DeFranco, 2002).

In neurons, the high number of different stimuli, such as neurotrophins, neurotransmitters, growth factors, depolarization, leading to ERK 1/2 activation, renders ERK 1/2 an essential signal cross-roads within the cell. Thus, the beneficial effect of rolipram on a number of different types of neurons could result from its fine tuning of ERK 1/2 phosphorylation. In this context, it is worth noting that also the cAMP-PKA pathway, affected by rolipram treatment, can exert either an inhibitory or a facilitating stimulation on MAPK-ERK 1/2 signaling, according to the cell types (Frödin et al., 1994; Sevetson et al., 1993).

In addition, to add complexity to the PKA-ERK 1/2 cross-talk, it is worth mentioning that phosphorylated ERK 1/22 is able to directly inhibit, a commonly expressed isoenzyme of PDE4, PDE4D3, thus increasing cAMP levels (Hoffmann et al., 1999).

In light of this, we did not correlate the upregulation of CREB observed after the administration of the PDE4 inhibitor rolipram (Giampà et al., 2009) to the changes in the expression of ERK 1/2 described in the present study. However, we can speculate that PDE4 inhibition can be beneficial by lowering pERK in those cells where an excessive amount is expressed (such as the medium spiny neurons), and at the same time by increasing pERK in those neurons that have lower levels such, as the cholinergic and SS/NOS/NPY interneurons, so that the anti-apoptotic effect can take place.

The situation that we have uncovered here offers the opportunity for the rolipram-induced increase in CREB to lead to either an increase or a decrease in ERK 1/2 phosphorylation. This could be due to the possibility of different ERK 1/2 pathways than can be activated according to the neuronal subtype and its characteristic vulnerability to HD degeneration.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.nbd.2012.01.011.

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