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Cyclin-Dependent Kinase 5 Dysfunction Contributes to Depressive-like Behaviors in Huntington's Disease by Altering the DARPP-32 Phosphorylation Status in the Nucleus Accumbens

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ABSTRACT

BACKGROUND: Depression is the most common psychiatric condition in Huntington's disease (HD), with rates more than twice those found in the general population. At the present time, there is no established molecular evidence to use as a basis for depression treatment in HD. Indeed, in some patients, classic antidepressant drugs exacerbate chorea or anxiety. Cyclin-dependent kinase 5 (Cdk5) has been involved in processes associated with anxiety and depression. This study evaluated the involvement of Cdk5 in the development and prevalence of depressive-like behaviors in HD and aimed to validate Cdk5 as a target for depression treatment.

METHODS: We evaluated the impact of pharmacological inhibition of Cdk5 in depressive-like and anxiety-like behaviors in *Hdh*$^{+/Q111}$ knock-in mutant mice by using a battery of behavioral tests. Biochemical and morphological studies were performed to define the molecular mechanisms acting downstream of Cdk5 activation. A double huntingtin/DARPP-32 (dopamine- and cAMP-regulated phosphoprotein 32) knock-in mutant mouse was generated to analyze the role of DARPP-32 in HD depression.

RESULTS: We found that *Hdh*$^{+/Q111}$ mutant mice exhibited depressive-like, but not anxiety-like, behaviors starting at 2 months of age. Cdk5 inhibition by roscovitine infusion prevented depressive-like behavior and reduced DARPP-32 phosphorylation at Thr75 in the nucleus accumbens. *Hdh*$^{+/Q111}$ mice heterozygous for DARPP-32 Thr75Ala point mutation were resistant to depressive-like behaviors. We identified β-adducin phosphorylation as a Cdk5 downstream mechanism potentially mediating structural spine plasticity changes in the nucleus accumbens and depressive-like behavior.

CONCLUSIONS: These results point to Cdk5 in the nucleus accumbens as a critical contributor to depressive-like behaviors in HD mice by altering DARPP-32/β-adducin signaling and disrupting the dendritic spine cytoskeleton.

Keywords: β-adducin, Cdk5, DARPP-32, Dendritic spines, Depressive-like behavior, Huntington's disease

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Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the gene encoding the huntingtin protein. The disease is commonly known for the choreiform movements that develop in the later, more advanced disease stages. However, cognitive deficits and psychiatric symptoms often appear at early stages and may even precede motor disturbances. Depression is one of the most common psychiatric symptoms in HD, estimated to occur in 20% to 50% of presymptomatic HD carriers (1). Depression can aggravate chorea and cognitive deficits and increases the risk of suicide. Thus, treating these mood alterations may help to slow the disease progression and improve patients' quality of life (2). A variety of drugs that modulate neurotransmitters, mainly serotonin reuptake inhibitors, are currently available for treatment of depression in HD, but some of them may exacerbate chorea or do not produce the expected beneficial effects (3,4), stressing the need for novel therapeutic approaches. In searching for a new pharmacological target, we have focused on cyclin-dependent kinase 5 (Cdk5). Cdk5 is a serine/threonine kinase with high activity in the central nervous system and is critical for a variety of neuronal functions (5–10). In addition to its physiological actions, aberrant Cdk5 activity is a critical determinant in neuronal dysfunction associated with neurodegenerative and neuropsychiatric disorders (5). Our group has demonstrated increased Cdk5 activity in brains of HD mice associated with neuronal dysfunction and cognitive decline (11,12), which favors the search for therapeutic approaches aimed toward normalizing Cdk5 activity to ameliorate motor and cognitive deficits in HD.

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Interestingly, Cdk5 has recently been associated with anxiety and depression. Cdk5 activity and protein levels are increased in the prefrontal cortex (PFC) and hippocampus of mice with induced chronic stress and in specific subregions of the PFC in postmortem brains of patients with major depression (13). Consistently, systemic administration of Cdk5 inhibitors in rat models of depression (14) or microinjection of Cdk5 inhibitors in the dentate gyrus of rats with induced chronic mild stress (15) prevents the emergence of depressive-like behaviors. Moreover, forebrain-specific Cdk5 knockout mice exhibit reduced depressive-like behaviors (16,17), whereas Cdk5 deletion in the ventral tegmental area (VTA) and in dopaminergic neurons in the midbrain induces depressive-like phenotypes (18). Altogether, these findings shed light on the possibility of designing new therapies targeting Cdk5 activity as a new treatment to prevent or ameliorate depression in HD. In this study, we used a combination of behavioral studies, biochemistry, and molecular analysis to examine the role of Cdk5 dysregulation on depressive-like behaviors in mutant HD knock-in mice.

METHODS AND MATERIALS

A detailed description is provided in the Supplement.

Animals

We used heterozygous knock-in mutant mice for huntingtin, $Hdh^{+/Q111}$, carrying 109 CAG repeats insertion in the *Huntingtin* gene (19,20) and DARPP-32 (dopamine- and cyclic adenosine monophosphate [cAMP]-regulated phosphoprotein 32) coded by the *PPP1R2* gene with a point mutation of Thr75 to alanine ($PPP1R2^{+/T75A}$, here referred to as $D32^{+/T75A}$) (21).

Behavioral Testing

Mice were subjected to a behavioral test battery to check for several neurological phenotypes as described in the Supplement.

Pharmacological Treatment

Wild-type (WT) and $Hdh^{+/Q111}$ mutant mice 2 months of age received intracerebral infusions of roscovitine at a concentration of 20 mg/kg/day into the lateral ventricle during 2 weeks (22) (see Supplement).

Brain Monoamine Analysis

The levels of dopamine (DA) and serotonin and their metabolites, dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxy-3-indolacetic acid, were determined from brain tissue homogenates using reverse-phase high-performance liquid chromatography with electrochemical detection (see Supplement).

Western Blotting

Protein extracts from brain samples were prepared and quantified, and Western blotting was performed as previously described (12) (see Supplement).

Dendritic Spine Labeling and Confocal Analysis

Neurons from nucleus accumbens (NAc) were labeled using the Helios Gene Gun System (Bio-Rad, Hercules, CA) as previously described (23). DiI-labeled medium spiny neurons of the NAc were imaged using a Leica Confocal SP5 microscope (Leica Microsystems, Wetzlar, Germany) with a ×63 oil-immersion objective. Details for the imaging conditions are described in the Supplement.

Statistical Analysis

Details of the statistical analysis are described in the Supplement.

RESULTS

Early Development of Depressive-like, but Not Anxiety-like, Behavior in Mutant Huntingtin $Hdh^{+/Q111}$ Male Mice

Although the onset of motor symptoms define the symptomatic phase in HD, psychiatric disturbances, particularly depression, may manifest long before motor coordination deficits (24–26). To unravel the molecular mechanisms involved in these affective disturbances, we examined whether $Hdh^{+/Q111}$ mice recapitulate the human condition by showing depressive-like behaviors at early disease stages. To this aim, 2-month-old $Hdh^{+/Q111}$ mutant and $Hdh^{+/+}$ (i.e., $Hdh^{Q7/Q7}$) WT mice were subjected to a battery of behavioral tests commonly used to assess depressive-like behaviors (27,28) (Figure 1A). We found that $Hdh^{+/Q111}$ mutant mice exhibited a depressive-like phenotype that included increased latency to feed in the novelty-suppressed feeding test (NSFT), despair behavior manifested as increased immobility time in the forced swimming test (FST) and the tail suspension test (TST), and increased anhedonia demonstrated by the lack of preference for sucrose over water compared with WT mice (sucrose preference test) (SPT). Consistent with data in patients with HD, the severity of the depressive-like behavior did not increase over time, as similar findings were obtained when 8-month-old $Hdh^{+/Q111}$ mutant mice were tested and compared with age-matched WT mice (Figure 1A).

To determine whether a component of anxiety may contribute to these depressive-like symptoms, anxiety-like behaviors were evaluated using several anxiety paradigms (Figure 1B). Neither the time spent in the open arms in the elevated plus maze nor the time spent in the center in the open field or in the light compartment in the dark/light paradigm were significantly different between $Hdh^{+/Q111}$ mutant and WT mice, indicating normal anxiety behavior in mutant mice. Of relevance, no changes in the total distance traveled in the open field were observed between WT and $Hdh^{+/Q111}$ mutant mice at 2 months of age, whereas a shorter distance was found at 8 months, consistent with previous reported data (29,30) (Supplemental Figure S1). Though reduced locomotor activity could influence the results obtained in the TST or the FST, it is important to note that no worsening in depressive-like behavior is observed along the disease progression, with $Hdh^{+/Q111}$ mutant mice displaying a similar increase in immobility time at 2 and 8 months of age. Together, these results demonstrate that $Hdh^{+/Q111}$ mutant male mice at 2 months of age display depressive-like, but not anxiety-like, behavior, a phenotype that is independent of disease progression.

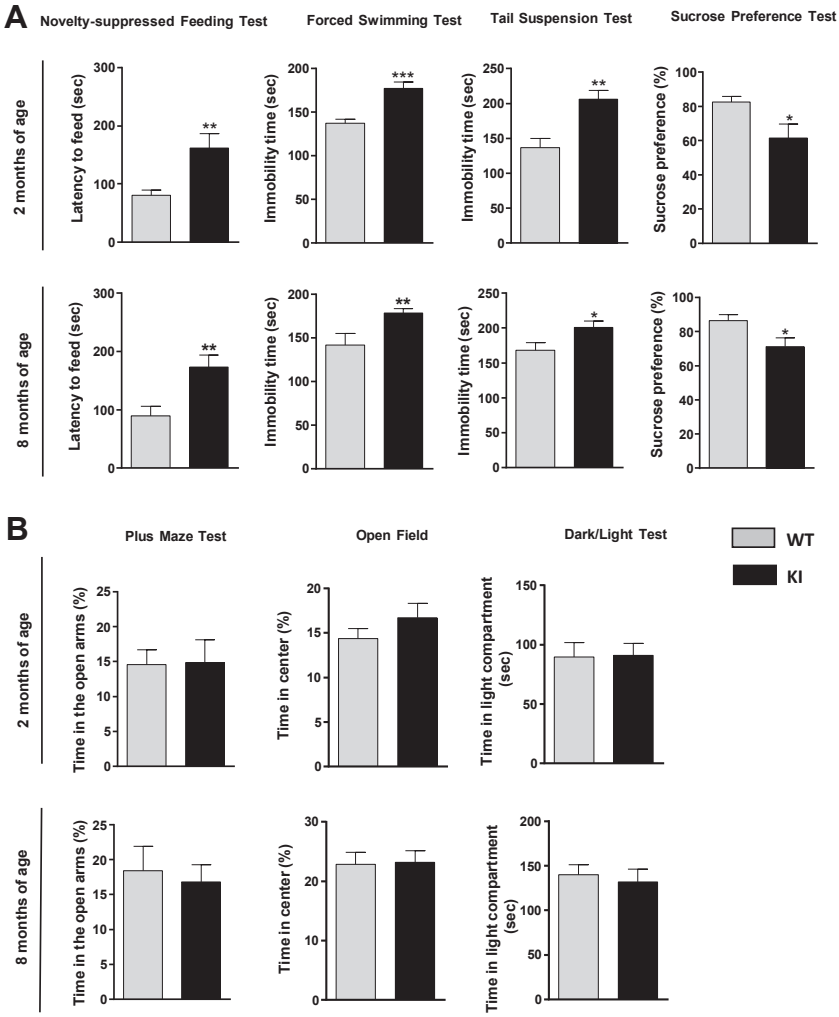

Figure 1. *Hdh*$^{+/Q111}$ mutant (KI) male mice display depressive-like phenotypes at early disease stages. Depressive-like and anxiety-like behaviors were evaluated in wild-type (WT) and KI mice at 2 and 8 months of age. **(A)** Depressive-like behavior was evaluated using the novelty-suppressed feeding test, forced swimming test, tail suspension test, and sucrose preference test. Graphs show latency to feed in the novelty-suppressed feeding test, immobility time in the forced swimming test and tail suspension test scored in seconds, and percentage of volume of sucrose intake over the total volume of fluid intake in the sucrose preference test. Data were analyzed using unpaired *t* test. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ compared with WT mice. **(B)** Anxiety-like behavior was evaluated using the elevated plus maze test, open field, and dark/light test. Graphs show percentage of time spent in the open arms in the elevated plus maze test or in the center of the open field and latency to cross into the light compartment in the dark/light test scored in seconds. Data were analyzed using unpaired *t* test. Data are mean ± SEM. All experiments evaluated $n = 8–15$ mice/genotype.

DA and Serotonin Content Is Not Altered in Mutant Huntingtin *Hdh*$^{+/Q111}$ Male Mice

Perturbations in DA and serotonin neurotransmitter systems have been implicated in anxiety and depressive behaviors (31,32). To study the contribution of these neurotransmitters in the depressive-like phenotype observed in *Hdh*$^{+/Q111}$ mutant mice, high-performance liquid chromatography analysis was used in tissue homogenates from 2-month-old mice to determine the levels of DA, serotonin, and metabolites (Figure 2). We focused on three brain regions, PFC, NAc, and hippocampus, as these regions are important for behavioral functions that may be associated with the expression of depression (33–36). Similar levels of DA and serotonin (5-hydroxytryptamine) were found between *Hdh*$^{+/Q111}$ mutant and WT mice in all analyzed regions. However, *Hdh*$^{+/Q111}$ mutant mice exhibited higher levels of the serotonin metabolite 5-hydroxyindoleacetic acid in the NAc along with a significant increase in the DA metabolite homovanillic acid in both the NAc and the PFC (Figure 2A). These data indicate no major disturbances in the synthesis of DA and serotonin in *Hdh*$^{+/Q111}$ mutant mice at the age at which depressive-like symptoms are manifested. However, a moderate but significant increase in the DA turnover (homovanillic acid/DA) and the serotonin turnover (5-hydroxytryptamine/5-hydroxyindoleacetic acid) was observed in two brain regions critically involved in depressive-like behavior manifestation (Figure 2B).

Inhibition of Cdk5 Prevents Depressive-like Behavior in Mutant Huntingtin *Hdh*$^{+/Q111}$ Male Mice

Previous findings from our group have demonstrated a significant role of Cdk5 dysfunction in motor and cognitive deficits in *Hdh*$^{+/Q111}$ mutant mice (11,12). As recent studies have shown that administration of Cdk5 inhibitors in rats produced antidepressant-like actions (14,15), we sought to address whether pharmacological inhibition of Cdk5 may prevent or alleviate the depressive-like behaviors observed in *Hdh*$^{+/Q111}$ mutant mice. The experimental design is shown in Figure 3A. Chronic inhibition of Cdk5 by intracerebroventricular microinfusion of roscovitine prevented the depressive-like behavior in 2-month-old *Hdh*$^{+/Q111}$ mutant mice. Thus, roscovitine

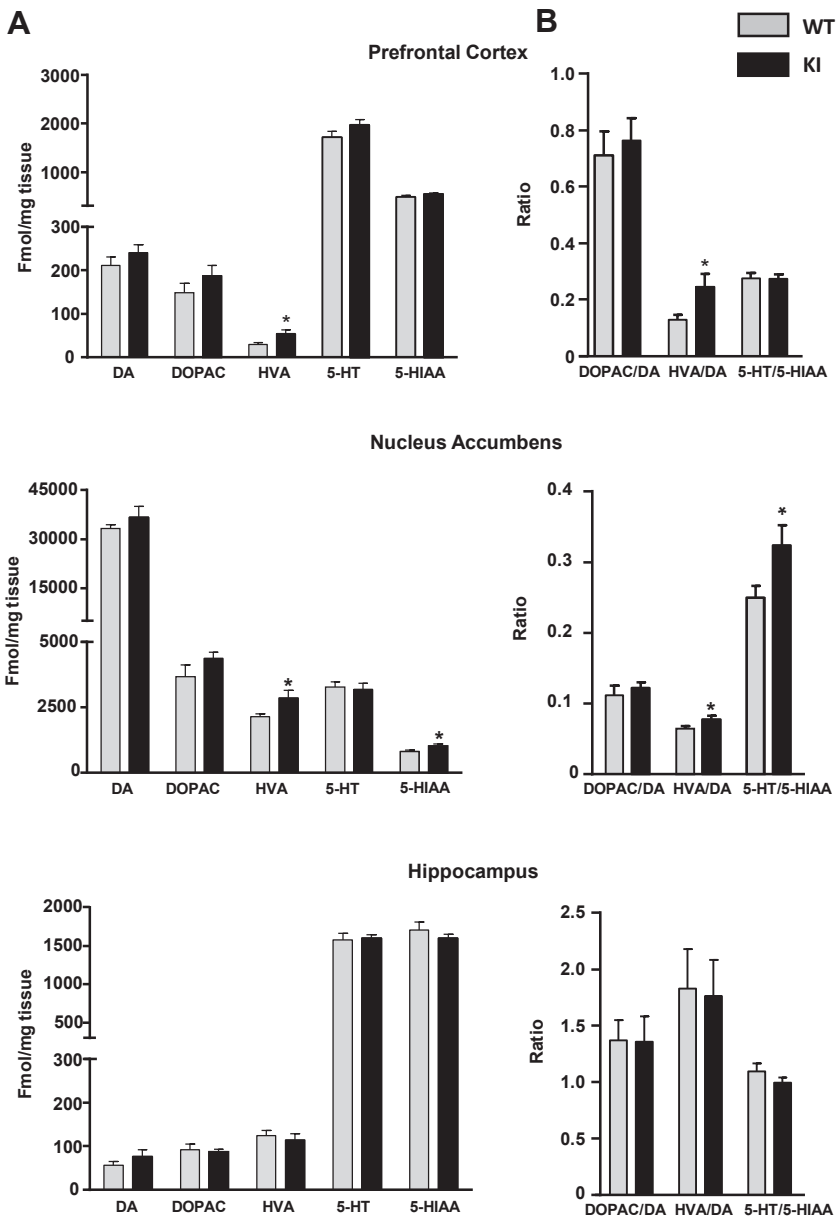

Figure 2. Dopamine (DA) and serotonin (5-hydroxytryptamine [5-HT]) metabolites are altered in the prefrontal cortex and nucleus accumbens of *Hdh*$^{+/Q111}$ mutant (KI) male mice. Graphs show **(A)** basal levels of DA, 5-HT, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) in wild-type (WT) and KI mice, represented as fmol/mg of tissue, and **(B)** dopamine and serotonin turnover represented as ratios DOPAC/DA, HVA/DA, and 5-HT/5-HIAA. Data were analyzed using unpaired *t* test. *$p < .05$ compared with WT mice. Data are mean ± SEM. All experiments evaluated $n = 6–8$ mice/genotype at 3 months of age.

treatment reversed the increase in the latency to feed in the NSFT, reversed the higher immobility time in the FST and TST, and restored the sucrose preference over water to WT levels in *Hdh*$^{+/Q111}$ mutant mice (Figure 3B). Altogether, these results demonstrate that Cdk5 is an important regulator of depressive-like behaviors in *Hdh*$^{+/Q111}$ mutant mice.

Cdk5 Inhibition Prevents Increase in DARPP-32 (Thr75) Phosphorylation in NAc of Mutant Huntingtin *Hdh*$^{+/Q111}$ Male Mice

To elucidate the biochemical mechanisms that underlie the benefits of Cdk5 inhibition in the depressive-like behavior observed in *Hdh*$^{+/Q111}$ mutant mice, we focused on two Cdk5 substrates known to participate in the dopaminergic and serotoninergic neurotransmission modulating the protein kinase A (PKA) pathway and therefore depressive-like behaviors: DARPP-32 and protein phosphatase inhibitor-1 (I1) (coded by *PPP1R1A*) (37–39). To this aim, Cdk5-mediated phosphorylation of DARPP-32 at Thr75 and I1 at Ser67 was analyzed in vehicle-infused and roscovitine-infused 2-month-old WT and *Hdh*$^{+/Q111}$ mutant mice by Western blotting (Figure 4). A specific increase in the levels of pThr75-DARPP-32 (Figure 4A) and pS67-I1 (Figure 4B) was found in the NAc of vehicle-treated *Hdh*$^{+/Q111}$ mutant mice compared with WT mice, whereas a trend toward increase was detected

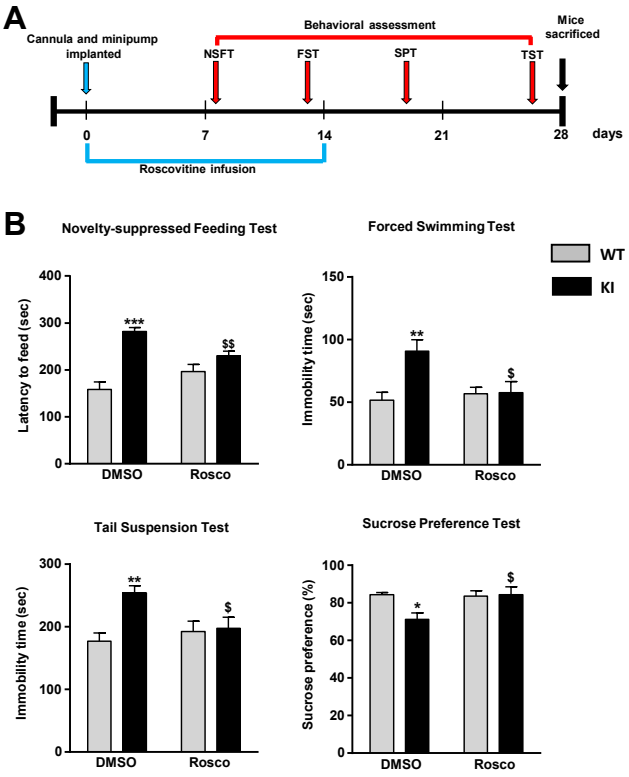

Figure 3. Pharmacological inhibition of cyclin-dependent kinase 5 prevents depressive-like behaviors in *Hdh*$^{+/Q111}$ mutant (KI) male mice. **(A)** Schematic of roscovitine (Rosco) treatment and experimental design, including novelty-suppressed feeding test (NSFT), forced swimming test (FST), tail suspension test (TST), and sucrose preference test (SPT). **(B)** Graphs show latency to feed in NSFT, immobility time in FST and TST scored in seconds, and percentage of volume of sucrose intake over the total volume of fluid intake in SPT. Data were analyzed using two-way analysis of variance with Tukey as a post hoc test. *$p < .05$, **$p < .01$, and ***$p < .001$ compared with wild-type (WT) mice; $^{\$}p < .05$ and $^{\$\$}p < .01$ compared with dimethyl sulfoxide (DMSO)–treated KI mice. Data are mean ± SEM. All experiments evaluated n = 8–10 mice/genotype/treatment. Values are represented as percentage of DMSO-treated WT mice. Mice were treated at 2 months of age, and behavioral tests were performed at 2.5 and 3 months of age.

in the PFC, both of which were abolished by roscovitine treatment. No significant differences between genotypes and treatments were observed in the dorsal striatum and hippocampus. These findings suggest that increased Cdk5 activity in *Hdh*$^{+/Q111}$ mutant mice contributes to depressive-like behaviors by increasing I1 and DARPP-32 phosphorylation mainly in the NAc, a structure whose dysfunction is well associated with depression (28,40).

Disruption of DARPP-32/β-Adducin Pathway in NAc of Mutant *Hdh*$^{+/Q111}$ Male Mice Contributes to Depressive-like Behavior by Altering Dendritic Spine Density

Remodeling of dendritic spines has been closely associated with depressive-like behaviors (41–43). Interestingly, recent findings have linked DARPP-32 function with the capacity of adducin proteins to stabilize actin filaments and therefore modulate structural plasticity (44). Thus, pThr75-DARPP-32 facilitates β-adducin Ser713 phosphorylation by inhibition of PKA and protein phosphatase 2A cascade, preventing β-adducin interaction with actin and spectrin filaments and leading to cytoskeleton destabilization in dendritic spines (44). To investigate whether this mechanism of action may influence the depressive-like behavior observed in *Hdh*$^{+/Q111}$ mutant mice, the DARPP-32/PKA/adducin signaling pathway was analyzed in the NAc of *Hdh*$^{+/Q111}$ mutant mice (Figure 5). Consistent with the reported high levels of pThr75-DARPP-32, activation of PKA measured as Thr197 phosphorylation (38,45–47) was found to be significantly decreased, whereas levels of pSer713-β-adducin increased (Figure 5A). Importantly, Cdk5 inhibition by roscovitine normalized both PKA and β-adducin phosphorylation, showing roscovitine-treated *Hdh*$^{+/Q111}$ mutant mice phosphorylation levels similar to those observed in WT mice. In view of these data and given the role of DARPP-32/β-adducin on filament cytoskeleton stabilization, we next examined the consequences of aberrant pThr75-DARPP-32 levels on dendritic spines. Vehicle-treated *Hdh*$^{+/Q111}$ mutant mice showed decreased dendritic spines on medium spiny neurons in the NAc, a reduction that was completely prevented by roscovitine treatment (Figure 5B). Altogether, these results strongly support the idea that dendritic spine loss in the NAc as a result of aberrant phosphorylation of DARPP-32/β-adducin is critically involved in the appearance of depressive-like behavior in *Hdh*$^{+/Q111}$ mutant mice.

Genetic Inhibition of pThr75 DARPP-32 Phosphorylation Reverses the Depressive-like Behavior in Mutant *Hdh*$^{+/Q111}$ Male Mice

In view of the above findings, we reasoned that if increased Cdk5 activity contributes to depressive-like behavior by increasing phosphorylation of Thr75-DARPP-32, specific reduction of this phosphorylation should prevent depressive-like states in *Hdh*$^{+/Q111}$ mutant mice. To test this hypothesis, we used a mouse line in which Thr75 was replaced in one allele by alanine to prevent phosphorylation at this site in DARPP-32 (*D32*$^{+/T75A}$). These mice were bred with *Hdh*$^{+/Q111}$ mutant mice to generate a new transgenic double mutant (DM) mouse expressing mutant Htt but partially deficient for Thr75-DARPP-32 phosphorylation (*Hdh*$^{+/Q111}$×*D32*$^{+/T75A}$) (Figure 6A), and depressive-like behavior was analyzed (Figure 6B). DM mice were indistinguishable from WT mice in all depressive-like behavioral tests performed, showing reduced latency to feed in the NSFT, decreased immobility time in the FST, increased struggle time in the TST, and higher preference for sucrose over water in the SPT compared with *Hdh*$^{+/Q111}$ mutant mice (Figure 6B). These findings suggest that phosphorylation of DARPP-32 at Thr75 mediated by Cdk5 activity is critical to modulate the depressive-like behavior in *Hdh*$^{+/Q111}$ mutant mice.

As we previously demonstrated that increased pThr75-DARPP-32 levels in the NAc of *Hdh*$^{+/Q111}$ mutant mice were associated with higher phosphorylation of β-adducin and reduction of dendritic spines (Figure 5), we next analyzed whether genetic reduction of Thr75-DARPP-32

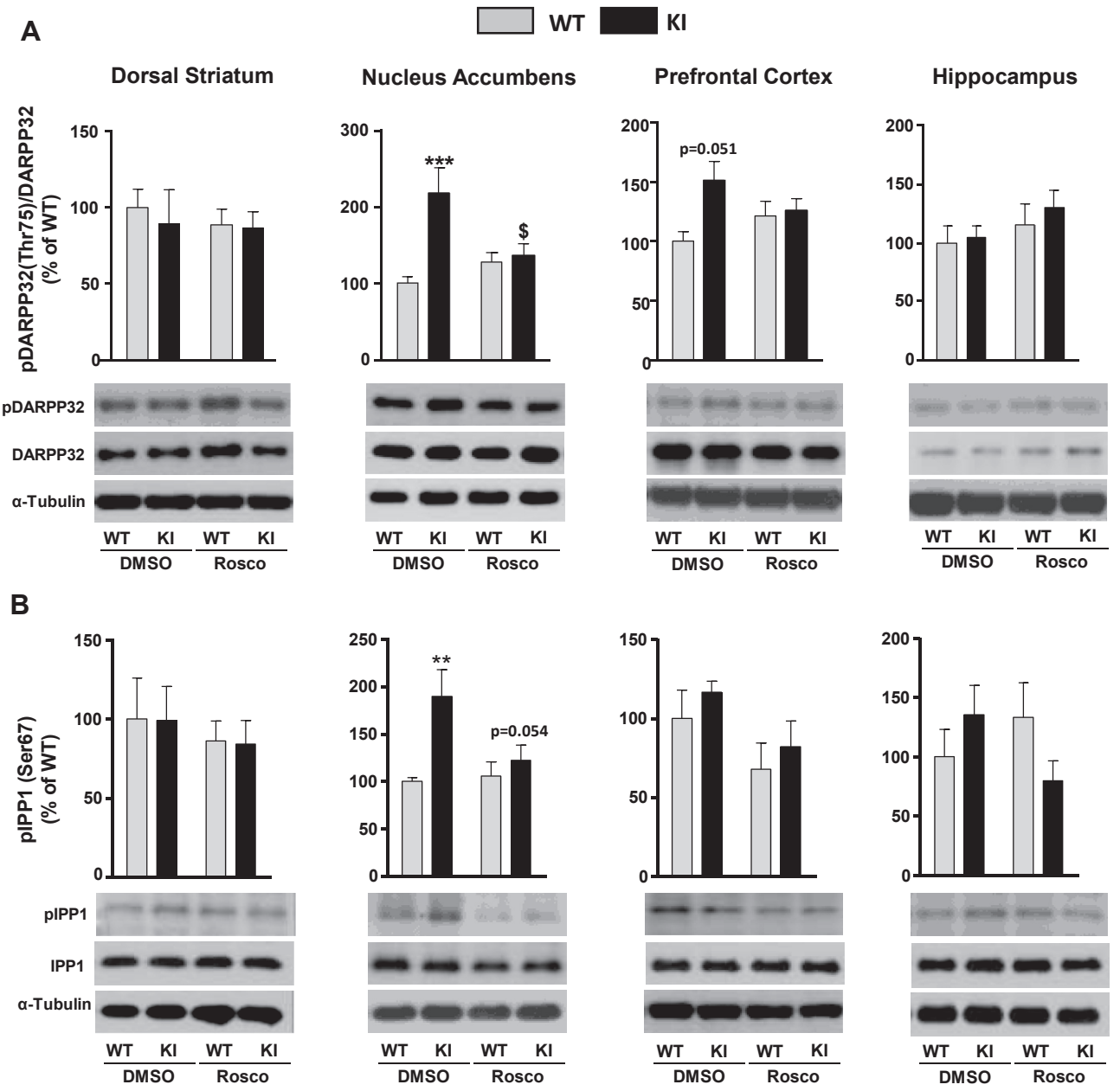

Figure 4. Pharmacological inhibition of cyclin-dependent kinase 5 prevents the increase in protein phosphatase inhibitor-1 (I1) and DARPP-32 (dopamine- and cAMP-regulated phosphoprotein 32) phosphorylation in the nucleus accumbens of *Hdh*$^{+/Q111}$ mutant (KI) male mice. Representative immunoblots showing the levels of **(A)** total DARPP-32 and phosphorylated DARPP-32 (pDARPP-32) (Thr75) and **(B)** total I1 and phosphorylated I1 (pI1) (Ser67) with α-tubulin as a loading control in the dorsal striatum, nucleus accumbens, prefrontal cortex, and hippocampus of vehicle (dimethyl sulfoxide [DMSO])- and roscovitine (Rosco)-treated 2-month-old wild-type (WT) and KI mice. Data were analyzed using two-way analysis of variance with Tukey as a post hoc test. $^{**}p < .01$, $^{***}p < .001$ compared with DMSO-treated WT mice; $^{\$}p < .05$ compared with DMSO-treated KI mice. Data are mean ± SEM. All experiments evaluated n = 6–10 mice/genotype/treatment. Values are represented as percentage of DMSO-treated WT mice. pIPP1, phosphorylated protein phosphatase inhibitor-1; IPP1, protein phosphatase inhibitor-1.

phosphorylation in *Hdh*$^{+/Q111}$ mutant mice prevented depressive-like behaviors by acting through the same pathway (Figure 7). As expected, pThr75-DARPP-32 levels were found to be significantly higher in the NAc of *Hdh*$^{+/Q111}$ mutant mice compared with WT mice, whereas a half decrease was detected in *D32*$^{+/T75A}$ mice. Importantly, in DM mice, the levels of pThr75-DARPP-32 were significantly decreased compared with *Hdh*$^{+/Q111}$ mutant mice but were similar to levels observed in *D32*$^{+/T75A}$ mice. Accordingly, phosphorylation of PKA at Thr197 was found to be markedly decreased in *Hdh*$^{+/Q111}$ mutant mice compared with all other genotypes. In contrast, no significant changes among WT,

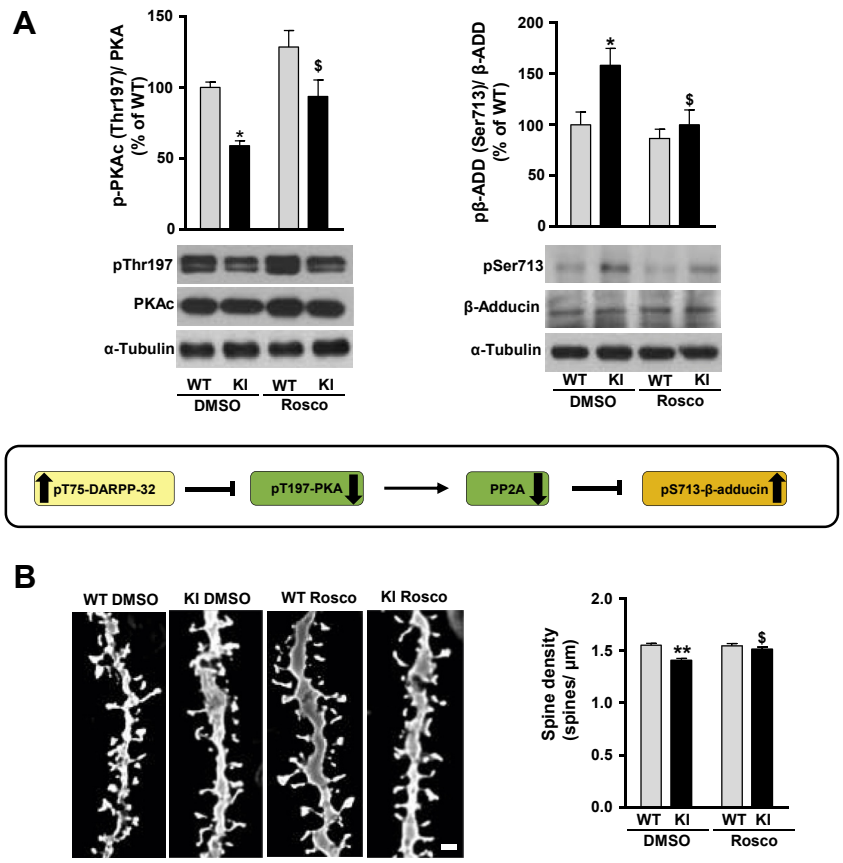

Figure 5. Pharmacological inhibition of cyclin-dependent kinase 5 decreases β-adducin phosphorylation and restores spine density of medium spiny neurons in the nucleus accumbens of *Hdh*$^{+/Q111}$ mutant (KI) male mice. **(A)** Graphs and representative immunoblots showing the levels of total protein kinase A catalytic subunit (PKAc) and phosphorylated PKAc (pPKAc) (Thr197) and total β-adducin (β-ADD) and phosphorylated β-ADD (pβ-ADD) (Ser713) with α-tubulin as loading control in the nucleus accumbens of vehicle (dimethyl sulfoxide [DMSO])- or roscovitine (Rosco)-treated 2-month-old wild-type (WT) and KI mice. Data were analyzed using two-way analysis of variance with Tukey as a post hoc test. $^{*}p < .05$ compared with DMSO-treated WT mice; $^{\$}p < .05$ compared with DMSO-treated KI mice. Data are mean ± SEM. All experiments evaluated n = 6–8 mice/genotype. Values are represented as percentage of DMSO-treated WT mice. **(B)** Quantitative analysis showing dendritic spine density per micrometer of dendritic length (right) and representative images of Golgi-impregnated dendrites of medium spiny neurons of nucleus accumbens (left) from vehicle (DMSO)- or Rosco-treated 2-month-old WT and KI mice. Scale bar = 3 μm. Data were analyzed using two-way analysis of variance with Tukey as a post hoc test. $^{**}p < .001$ compared with DMSO-treated WT mice and $^{\$}p < .05$ compared with DMSO-treated KI mice. Data are mean ± SEM. All experiments evaluated n = 4–5 mice/genotype (90–100 dendrites). Values are represented as percentage of DMSO-treated WT mice.

D32$^{+/T75A}$, and DM mice were found, though a significant increase was observed for DM mice compared with *Hdh*$^{+/Q111}$ mutant mice (Figure 7A). Next, levels of β-adducin phosphorylation at Ser713 were analyzed. *Hdh*$^{+/Q111}$ mutant mice displayed higher pS713-adducin compared with either WT or *D32*$^{+/T75A}$ and DM mice, which was consistent with the increase in pThr75-DARPP-32. Finally, dendritic spines were examined (Figure 7B). *Hdh*$^{+/Q111}$ mutant mice displayed a significant reduction in the number of spines on medium spiny neurons in the NAc compared with WT mice. Notably, reduction of pThr75-DARPP-32 completely prevented this decrease, showing similar dendritic spine density in DM mice compared with WT or *D32*$^{+/T75A}$ mice supporting the notion that aberrant pThr75-DARPP-32 in the NAc acts as a critical contributor to depressive-like behavior by altering structural synaptic plasticity.

Mutant Huntingtin *Hdh*$^{+/Q111}$ Female Mice Exhibit Early Depressive- and Anxiety-like Behaviors Along With Subtle Changes in DARPP-32/PKA/β-Adducin Pathway

Our data indicate a contribution of Cdk5 dysfunction in depressive-like behavior in *Hdh*$^{+/Q111}$ male mice by altering Thr75-DARPP-32 phosphorylation. As sex differences have been reported in HD mice (48,49), we wondered whether HD female mice also exhibited depressive- and/or anxiety-like behaviors and whether molecular changes at the DARPP-32/PKA/β-adducin pathway were also observed. Despair behavior manifested as increased immobility time in the FST and the TST was found in *Hdh*$^{+/Q111}$ female mice, whereas no changes either in the increased latency to feed in the NSFT or in the preference for sucrose in the SPT were found (Supplemental Figure S2A). Notably, and in contrast to data from *Hdh*$^{+/Q111}$ male mice, an increase in anxiety-related behaviors was evident (Supplemental Figure S2B), indicating sex differences in anxiety-like phenotypes. When molecular changes were analyzed (Supplemental Figure S3), similar levels of pThr75-DARPP-32 were found between genotypes and brain regions, though a trend tendency toward increase was observed in the NAc. Accordingly, a decrease in pThr197-PKA and an increase in pS713-β-adducin was detected in this brain region, though differences did not reach significance. Overall, these findings suggest that *Hdh*$^{+/Q111}$ female mice exhibit a specific increase in anxiety-like behaviors and a mild depressive-like phenotype associated with subtle changes in the DARPP-32/PKA/β-adducin pathway.

DISCUSSION

The present study uncovered a novel molecular mechanism underlying depressive phenotype in HD. We provide evidence that perturbation of Cdk5 function preferentially in the NAc may contribute to depressive-like phenotypes in *Hdh*$^{+/Q111}$

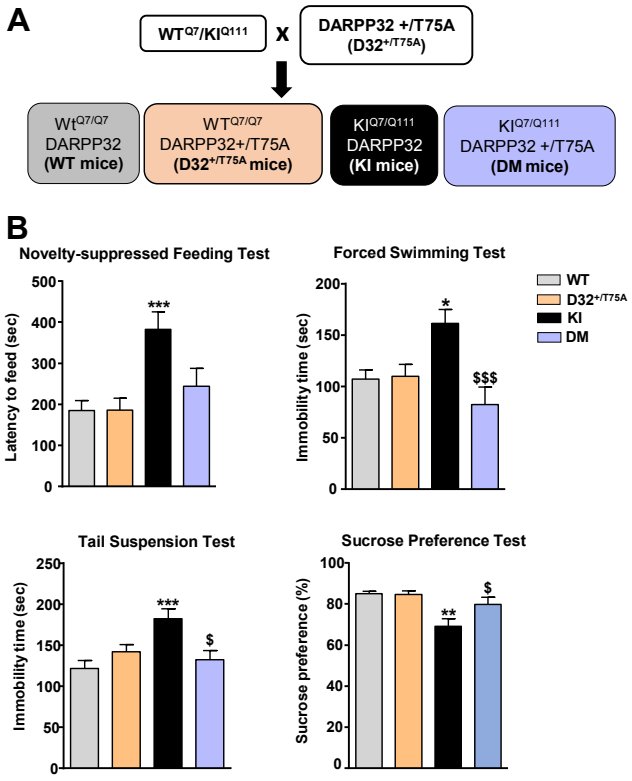

Figure 6. Reduction of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein 32) phosphorylation at the cyclin-dependent kinase 5 site (Thr75) prevents depressive-like behaviors in *Hdh*$^{+/Q111}$ mutant (KI) male mice. **(A)** Generation of new double transgenic *Hdh*$^{+/Q111}$ mutant (DM) mice in which Thr75 was replaced in one DARPP-32 allele by alanine to prevent phosphorylation at this site (D32$^{+/T75A}$). **(B)** Depressive-like behaviors were evaluated in these mice at 2 months of age by using the novelty-suppressed feeding test, forced swimming test, tail suspension test, and sucrose preference test paradigms. Graphs show latency to feed in novelty-suppressed feeding test, immobility time in forced swimming test and tail suspension test scored in seconds, and percentage of volume of sucrose intake over the total volume of fluid intake in sucrose preference test. Data were analyzed using one-way analysis of variance with Tukey as a post hoc test. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ compared with WT mice; $^{\$}p < .05$ and $^{\$\$\$}p < .001$ compared with KI mice. Data are mean ± SEM. All experiments evaluated n = 8–12 mice/genotype.

mutant mice. We identified DARPP-32 as the Cdk5 substrate that modulates the depressive-like response in *Hdh*$^{+/Q111}$ mutant mice by altering β-adducin function and the stability of the dendritic spine cytoskeleton, subsequently causing dendritic spine loss.

Using well-validated behavioral tests, we demonstrated that *Hdh*$^{+/Q111}$ mutant male mice display depressive-like, but not anxiety-like, behaviors at early disease stages, a phenotype that is not dependent on disease progression. Interestingly, *Hdh*$^{+/Q111}$ female mice exhibit mildest depressive-like behaviors, but a specific increase in anxiety, indicating subtle sex differences in moodlike phenotypes. These findings are consistent with data reported in other HD mouse models (26,27,50–54) and with data in patients with HD showing that mood disturbances are frequent not only in patients with a diagnosis of HD but also in presymptomatic HD carriers (27). A recent report using the same *Hdh*$^{+/Q111}$ mutant mice reported no evident signs of anxiety-like or depressive-like behaviors but did report motivational deficits (55). This apparent discrepancy could be due to distinct test behavioral methodologies, such as the tank dimensions in the FST or the number of testing days in the SPT.

At the molecular level, depression, anxiety, and other mood disorders have been directly associated with serotonin and DA system deficiencies (56–58). Abnormal DA and serotonin profiles have been reported in several transgenic HD mice at early disease stages before motor disturbances but in parallel with mood and cognitive alterations (59–62). In our *Hdh*$^{+/Q111}$ mutant mice, we demonstrated at 2 months of age increased serotonin turnover in the NAc along with increased DA turnover in both the NAc and the PFC, without significant alterations in either DA or serotonin levels. These data suggest that depressive-like behaviors in *Hdh*$^{+/Q111}$ mutant mice are not caused by a major deregulation of the monoaminergic system but most likely are due to altered molecular mechanisms that underlie DA signaling in the neural circuitry of depression. In searching for a potential molecular link between DA signaling and depressive-like behaviors in HD, we focused on Cdk5, a kinase essential for neuronal function that plays an important regulatory role in DA transmission through the phosphorylation of DARPP-32 (21,63–65). Notably, Cdk5 dysregulation has previously been implicated by our group in HD learning and memory deficits (12) and by others in the pathophysiology of major depression and stress (13,15,17). Interestingly, a possible molecular link between cognitive and mood disturbances has been suggested in HD. Defects in adult hippocampal neurogenesis have been described in HD mice as a contributor of memory disturbances (66–68) and have been linked to depressive-like behaviors and other psychiatric disorders (69). Reduced neurotrophic support that is due to decreased brain-derived neurotrophic factor levels or tropomyosin receptor kinase B/brain-derived neurotrophic factor signal is a well-known hallmark of HD related to increased striatal susceptibility and poor cognitive outcome (23,70–73) that has also been associated with development of depressive-like behaviors (74). However, these mechanisms are unlikely to contribute to early depressive-like symptoms in our *Hdh*$^{+/Q111}$ mutant mice, as brain-derived neurotrophic factor decrease is not detected until 6 months of age (23), and deficits in hippocampal neuron maturation of the dentate gyrus have been described in 3 month-old mice (48). In view of these findings, we postulated an involvement of Cdk5 in the early depressive-like behaviors observed in *Hdh*$^{+/Q111}$ mutant mice. Supporting this hypothesis, we found that pharmacological inhibition of Cdk5 caused antidepressant-like effects in *Hdh*$^{+/Q111}$ mutant mice in all depressive-like paradigms tested, consistent with other studies that demonstrate a negative role of Cdk5 activity on anxiety-like and depressive-like behaviors (15–18). Our findings, however, may seem in contrast to findings from Zhong *et al.* (18) showing that genetic inhibition of Cdk5, specifically in the VTA or midbrain dopaminergic neurons, induces depressive-like phenotypes in WT mice. We would like to argue that

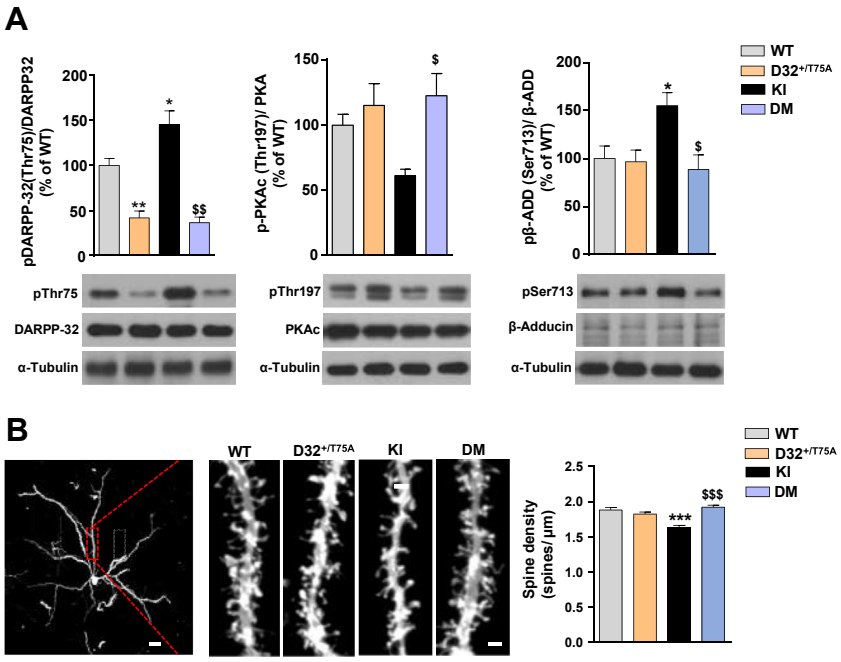

Figure 7. Genetic reduction of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein 32) phosphorylation at the cyclin-dependent kinase 5 site (Thr75) restores the DARPP-32/protein kinase A (PKA)/β-adducin (β-ADD) signaling and the structural synaptic plasticity deficits in the nucleus accumbens of *Hdh*$^{+/Q111}$ mutant (KI) male mice. **(A)** Graphs and representative immunoblots showing the levels of total DARPP-32 and phosphorylated DARPP-32 (pDARPP-32) (Thr75), total protein kinase A catalytic subunit (PKAc) and phosphorylated PKAc (pPKAc) (Thr197), and total β-ADD and phosphorylated β-ADD (pβ-ADD) (Ser713) with α-tubulin as loading control in the nucleus accumbens of wild-type (WT), D32$^{+/T75A}$, KI, and double mutant (DM) mice at 2 months of age. Data were analyzed using one-way analysis of variance with Tukey as a post hoc test. $^{*}p < .05$ and $^{**}p < .01$ compared with WT mice; $^{\$}p < .05$ and $^{\$\$}p < .01$ compared with KI mice. Data are mean ± SEM. All experiments evaluated n = 6–8 mice/genotype. Values are represented as percentage of WT. **(B)** Representative images of a DiI-labeled medium spiny neuron (scale bar = 20 µm) and dendrites of medium spiny neurons from the nucleus accumbens (scale bar = 3 µm) (left) and quantitative analysis showing dendritic spine density per micrometer of dendritic length (right) from WT, D32$^{+/T75A}$, KI, and DM mice. Data were analyzed using one-way analysis of variance with Tukey as a post hoc test. $^{***}p < .001$ compared with WT mice and $^{\$\$\$}p < .001$ compared with KI mice. Data are mean ± SEM. All experiments evaluated n = 4–5 mice/genotype (90–100 dendrites).

instead of being contradictory, these studies are complementary. Thus, while in non-HD conditions loss of Cdk5 function in the VTA could contribute to dysregulation of the mesolimbic dopaminergic system underlying depressive-like behaviors, in other pathological conditions, such as HD, inhibition of Cdk5 would not negatively impact VTA function but would prevent NAc dysfunction, ameliorating depressive-like behaviors.

Importantly, though no Cdk5 hyperactivity in the NAc of *Hdh*$^{+/Q111}$ mutant mice has been established, our data also demonstrate that Cdk5 dysfunction contributes to the emergence of depressive-like behavior in *Hdh*$^{+/Q111}$ mutant mice by boosting the phosphorylation of DARPP-32 at Thr75. This substrate of Cdk5 plays a critical role in modulating the amplitude of signal transduction events that involve cAMP-dependent protein kinase activation (38,39,63,75). Thus, following Cdk5-mediated phosphorylation of DARPP-32, DA and serotonin signaling is attenuated by inhibition of PKA and activation of PP1 (37,76). Accordingly, chronic pharmacological inhibition of Cdk5 decreased phosphorylation of DARPP-32 at Thr75, which in turn reduced inhibition of PKA, likely facilitating dopaminergic neurotransmission by means of the PKA/cAMP signaling cascade. Indeed, and consistent with these findings, it has been reported that Cdk5-dependent regulation of cAMP/PKA signaling in medium spiny neurons of the NAc can modulate behavioral responses to stress (17). Alternatively, the beneficial effects observed with roscovitine could be due to modulation of other Cdk5 substrates previously described to promote depressive-like behaviors (13,17,18,77,78). Therefore, to test the specific contribution of DARPP-32 to depressive-like behavior in HD, we generated a double mutant by crossing *Hdh*$^{+/Q111}$ mice with mice in which Thr75 was replaced in one allele by alanine to prevent DARPP-32 phosphorylation at this site. Our results demonstrate a specific requirement of DARPP-32 Thr75 for the development of depressive-like phenotypes in *Hdh*$^{+/Q111}$ mutant mice, as genetic reduction of DARPP-32 Thr75 completely abolished depressive-like symptoms in DM mice. This specific Cdk5-mediated phosphorylation of DARPP-32 has been reported to be reduced after acute or chronic administration of fluoxetine, a well-known serotonin reuptake inhibitor, whereas the effect of fluoxetine is completely suppressed in DARPP-32 knockout mice (37), which indicates that one of the most widely prescribed medications for the treatment of depression acts by reducing phosphorylated DARPP-32 Thr75. Intriguingly, tetrabenazine, an inhibitor of vesicular monoamine transporter 2 used to treat chorea in patients with HD (79), has been reported to increase the incidence of depressive mood in these patients (79,80). Although the effect on depression and suicidality in HD is controversial, it should be noted that this drug has been reported to induce Thr75 phosphorylation of DARPP-32 in NAc neurons and depressive-like symptoms in rats (81).

Structural plasticity, such as changes in spine and dendrite density and morphology, has been described in specific brain regions involved in depressive behavior (42,43,82). Recent studies have shown that Cdk5-mediated phosphorylation of DARPP-32 enhances β-adducin phosphorylation on Ser713 leading to cortical cytoskeleton destabilization in dendrites and spines (44). Consistent with these findings and the aberrant function of Cdk5 that we have described in the NAc of

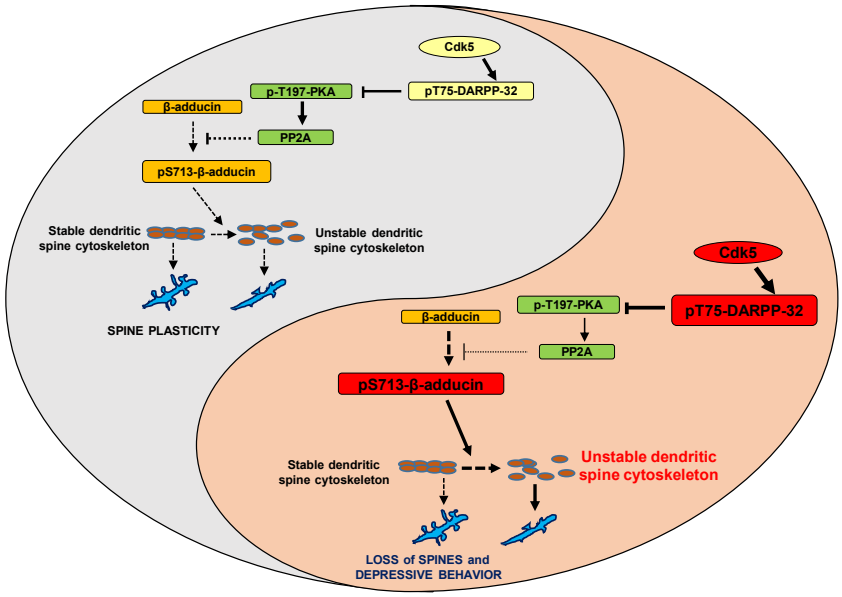

Figure 8. Proposed model of cyclin-dependent kinase 5 (Cdk5)/dopamine- and cAMP-regulated phosphoprotein 32 (DARPP-32)/β-adducin signaling involved in altered synaptic plasticity and depression in Huntington's disease. The scheme on the left shows that at physiological conditions, activation of protein kinase A (PKA) phosphorylates protein phosphatase 2A (PP2A). Activation of PP2A contributes to β-adducin dephosphorylation at Ser713, increasing its interaction with the actin/spectrin cytoskeleton resulting in dendritic stabilization. This interaction can also be modulated by Cdk5, as its substrate pT75-DARPP-32 increases phosphorylation of β-adducin at Ser713 by inhibiting PKA. The scheme on the right shows how mutant huntingtin may disrupt this signaling pathway. Increased Cdk5 activity resulting from mutant huntingtin toxicity leads to phosphorylation of DARPP-32 at Thr75 that, in turn, inhibits PKA with subsequent reduction in PP2A activity. Because of decreased PP2A activity, the phosphorylation levels of β-adducin at Ser713 increase, which prevents its interaction with the actin/spectrin cytoskeleton leading to dendritic spine destabilization that will contribute to loss of spine maintenance and synapse structural integrity.

Hdh$^{+/Q111}$ mutant mice, increased phosphorylation of β-adducin along with a significant decrease in spine density was found in this brain region in *Hdh*$^{+/Q111}$ mutant mice. This potentially deleterious effect on β-adducin and dendritic spines was fully avoided by genetic reduction of phosphorylated DARPP-32 Thr75 or by pharmacological inhibition of Cdk5, which emphasizes the role of the Cdk5/DARPP-32 pathway in NAc functional and structural disturbances leading to depressive-like symptoms in *Hdh*$^{+/Q111}$ mutant mice (Figure 8). These findings may seem contradictory to previous studies showing an increase in spine density and total dendritic length in the NAc of mice exposed to stress, a condition related to depressive-like behavior appearance (82–84). However, depressive-like behavior in mutant *Hdh*$^{+/Q111}$ mice is an intrinsic response to mutant huntingtin expression, and a distinct mechanism may be involved. Indeed, classical serotonin reuptake inhibitors available for the treatment of depression in HD may exacerbate chorea or do not produce the expected beneficial effects (3,4,85), supporting the idea that the molecular mechanisms that contribute to major depressive disorder may differ from mechanisms involved in depression in HD. In fact, a progressive decline in the NAc volume of patients with HD in premanifest stages has been demonstrated (86), whereas in human postmortem studies, reduced volume (87,88) and expression of genes involved in synaptic remodeling have been found in the NAc of depressed subjects (89).

In conclusion, this study demonstrates the importance of Cdk5 in HD in the early onset of depressive-like behaviors in mice. Further elucidation of Cdk5/DARPP-32/β-adducin action in models of depressive-like phenotypes will indicate whether this is a specific mechanism mediated by Cdk5 at the basis of depression in HD and will open new avenues toward pharmacological strategies based on specific molecular pathways to treat depression in HD.

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ARTICLE INFORMATION

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