Increased endogenous expression of PSD95/Dlg4 by an artificial transcription factor ameliorates cognitive and motor deficits in the Huntington's disease mouse model R6/2

Abstract: P2825

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Abstract

Huntington's disease (HD) is an incurable hereditary disorder caused by an expansion of CAG repeats in exon 1 of the Huntingtin gene. HD is characterized by motor dysfunction and cognitive decline. The pathophysiology of HD begins in cortico-striatal circuits and later spreads to other brain regions, notably the hippocampus. At the cellular level, structural changes in synapses have been observed prior to neuronal degeneration, significantly disrupting the formation and maintenance of neuronal circuits. One of the proteins associated with the physiopathology of HD is postsynaptic density protein 95 (PSD95, encoded by Dlg4), a key synaptic plasticity protein. The loss of PSD95 could explain, at least in part, cognitive and motor impairments. To restore endogenous PSD95/Dlg4 expression, our laboratory developed an epigenetic editing strategy where a zinc finger DNA-binding domain targeting the PSD95/Dlg4 gene promoter was fused to the transactivation domain VP64 (Bustos et al., Brain 2017). This artificial transcription factor (ATF), termed PSD95-6ZF-VP64, induced local epigenetic reprogramming (i.e., histone H3 acetylation) in N2a cells, increased PSD95/Dlg4 expression in hippocampal tissue, and rescued memory deficits in Alzheimer's disease mouse model. Here, we aimed to assess the therapeutic potential of the ATF PSD95-6ZF-VP64 in R6/2 mice, a HD model that recapitulates key pathological and clinical symptoms within a short time frame.

Results

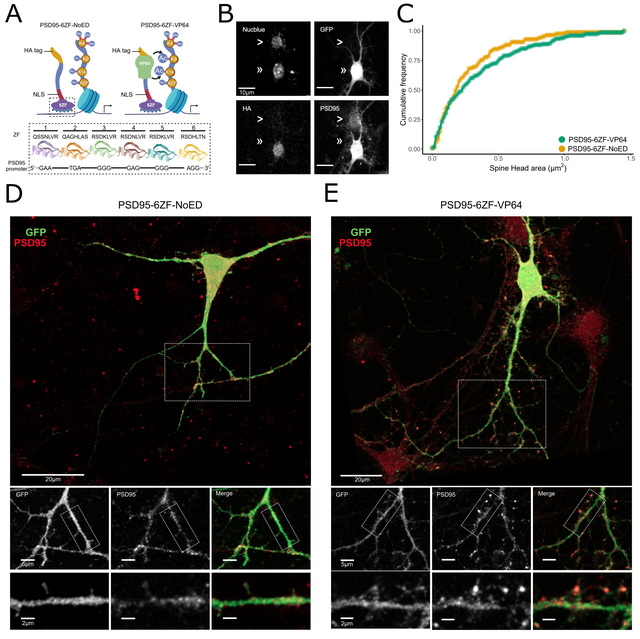

Figure 1: PSD95-6ZF-VP64 induces morphological changes in neuronal cell cultures.
(A) Diagram of PSD95-6ZF constructs: both include a zinc finger (ZF), nuclear localization sequence (NLS), and HA tag, with PSD95-6ZF-VP64 carrying the VP64 activator. (B) Representative images show infected neurons (double arrow) distinguished by GFP fluorescence and HA expression. (C) Quantification of spine head areas shows a significant increase in larger spine heads in PSD95-6ZF-VP64-treated neurons compared to controls (p<0.0005). (D-E) Immunolabeling reveals enhanced dendritic complexity and PSD95 clustering in PSD95-6ZF-VP64 neurons versus PSD95-6ZF-NoED-treated cells. Insets highlight dendritic details, showing increased PSD95 puncta density and

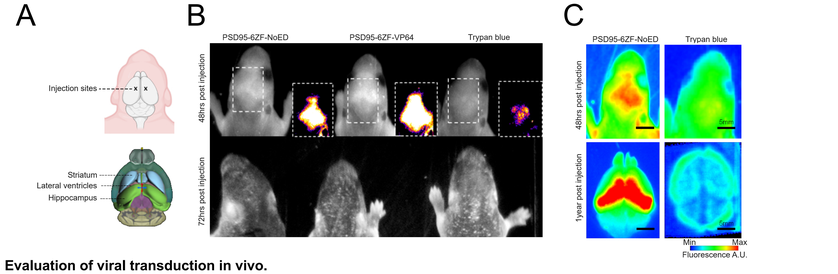

Figure 2: Evaluation of viral transduction in vivo.
(A) Injection sites in the striatum, lateral ventricles, and hippocampus are illustrated. (B) GFP fluorescence was monitored 48 and 72 hours post-injection in mice treated with PSD95-6ZF-NoED, PSD95-6ZF-VP64, or trypan blue (control). Robust GFP signals were observed in PSD95-6ZF-treated groups, while no fluorescence was detected in trypan blue controls. (C) Heatmaps display persistent GFP fluorescence in hippocampal regions one year post-injection with PSD95-6ZF constructs, confirming long-term viral expression. Fluorescence intensity is scaled relative to background.

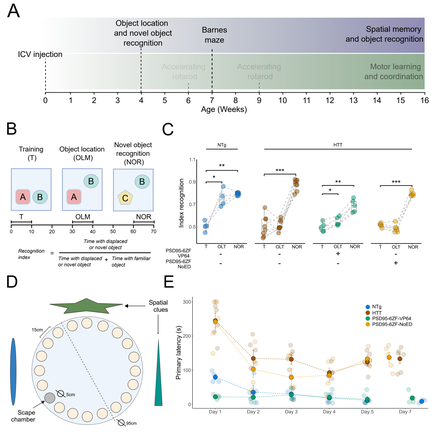

Figure 4: Behavioral assessment of PSD95-6ZF-VP64 in R6/2 mice.
(A) Timeline of the behavioral test battery, including object location memory (OLM), novel object recognition (NOR), Barnes maze, and accelerating rotarod tests. (B) Schematic of OLM and NOR tests to measure spatial and object recognition. (C) Recognition index reveals improved performance in OLM, but not NOR, for PSD95-6ZF-VP64-treated R6/2 mice compared to PSD95-6ZF-NoED and untreated groups (p<0.05, *p<0.01, **p<0.001). (D) Schematic of the Barnes maze test setup with spatial cues. (E) Primary latency in the Barnes maze demonstrates significantly improved spatial learning in

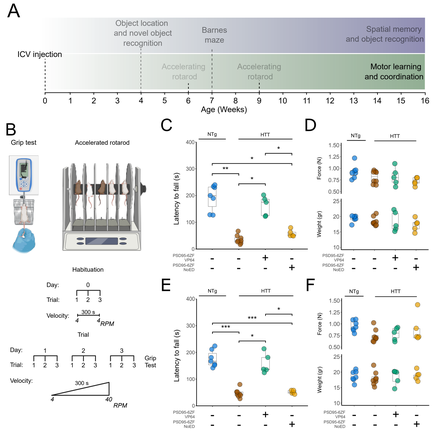

Figure 5: Motor coordination and strength in PSD95-6ZF-VP64-treated R6/2 mice.
(A) Timeline of motor learning and coordination tests, including accelerating rotarod and grip strength. (B) Diagrams of the grip test and rotarod setup. (C, E) Latency to fall on the rotarod significantly improved in PSD95-6ZF-VP64-treated R6/2 mice compared to PSD95-6ZF-NoED and untreated groups, nearing NTg levels (p<0.05, *p<0.01, **p<0.001). (D, F) Grip strength, measured as force and weight, was unaffected by PSD95-6ZF treatment in all groups, indicating specific improvements in motor coordination rather than raw strength

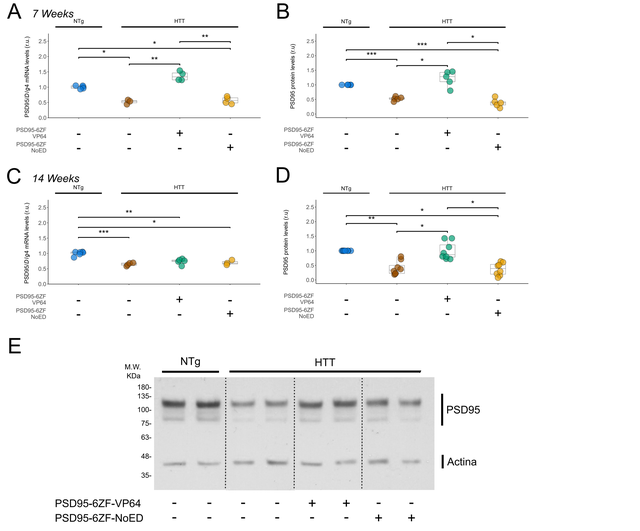

Figure 3: Restoration of PSD95 expression in R6/2 mice.
PSD95/Dlg4 mRNA and protein levels were measured in the hippocampus of 7-week-old (A, B) and 14-week-old (C, D) R6/2 mice. PSD95-6ZF-VP64 treatment significantly increased mRNA and protein levels compared to PSD95-6ZF-NoED and untreated groups, restoring them to NTg control levels. Statistical analysis revealed significant differences (p<0.05, *p<0.01, **p<0.001). (E) Western blot images confirm increased PSD95 protein in PSD95-6ZF-VP64-treated mice, with actin used as a loading control.

Conclusion

Consistent with previous studies, we found that 7- and 14-week-old R6/2 mice exhibited reduced hippocampal PSD95/Dlg4 mRNA and protein levels. Concomitant with the loss of hippocampal PSD95/Dlg4, a battery of behavioral assays revealed motor and cognitive impairments in 4- to 9-week-old R6/2 mice. In terms of cognitive deficits, R6/2 mice demonstrated impairments in object location memory (OLM) and the Barnes maze, whereas performance in novel object recognition (NOR) remained intact. Regarding motor function, R6/2 mice displayed reduced performance on the accelerated rotarod, while grip strength remained unaffected. Intracerebroventricular injections of neonatal R6/2 mice with AAV9-PSD95-6ZF-VP64 elevated hippocampal PSD95/Dlg4 mRNA and protein levels to those observed in control non-transgenic mice. Moreover, transduction with this ATF efficiently ameliorated the hippocampal-dependent deficits in spatial learning and memory (as assessed by OLM and the Barnes maze) as well as motor coordination and motor skill learning (measured by accelerated rotarod). Conclusively, this work validates PSD95 as a key player in the prodromal phase of the HD pathology and establishes this ATF as an attractive therapeutic tool for treating the disease's early phase.

References

Bustos FJ, Ampuero E, Jury N, et al. Epigenetic editing of the Dlg4/PSD95 gene improves cognition in aged and Alzheimer's disease mice. Brain. 2017;140(12):3252-3268. doi:10.1093/brain/awx272

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