HHS Public Access

Author manuscript

Neurobiol Dis. Author manuscript; available in PMC 2023 March 01.

Published in final edited form as:

Neurobiol Dis. 2023 March ; 178: 106023. doi:10.1016/j.nbd.2023.106023.

BDNF is altered in a brain-region specific manner and rescues deficits in Spinocerebellar Ataxia Type 1

Juao-Guilherme Rosa[a], **Katherine Hamel**[a], **Alyssa Soles**[a], **Carrie Sheeler**[a], **Ella Borgenheimer**[a], **Stephen Gilliat**[a], **Kaelin Sbrocco**[a], **Ferris Ghanoum**[a], **Hillary P. Handler**[b,c], **Colleen Forster**[d], **Orion Rainwater**[c], **Marija Cvetanovic**[a,b,*]

[a]Department of Neuroscience, University of Minnesota, 2101 6th Street SE, Minneapolis, MN 55455, United States of America

[b]Institute for Translational Neuroscience, University of Minnesota, 2101 6th Street SE, Minneapolis, MN 55455, United States of America

[c]Department of Lab Medicine and Pathology, United States of America

[d]BLS Histology, IHC Laboratory, United States of America

Abstract

Spinocerebellar ataxia type 1 (SCA1) is an adult-onset, dominantly inherited neurodegenerative disease caused by the expanded polyQ tract in the protein ATAXIN1 (ATXN1) and characterized by progressive motor and cognitive impairments. There are no disease-modifying treatments or cures for SCA1. Brain-derived neurotrophic factor (BDNF) plays important role in cerebellar physiology and has shown therapeutic potential for cerebellar pathology in the transgenic mouse model of SCA1, *ATXN1[82Q]* line that overexpress mutant ATXN1 under a cerebellar Purkinje-cell-specific promoter. Here we demonstrate decreased expression of brain derived neurotrophic factor (BDNF) in the cerebellum and medulla of patients with SCA1. Early stages of disease seem most amenable to therapy. Thus, we next quantified *Bdnf* expression in $Atxn1^{154Q/2Q}$ mice, a knock-in mouse model of SCA1, during the early symptomatic disease stage in four

*Corresponding author at: Department of Neuroscience, University of Minnesota, 2101 6th Street SE, Minneapolis, MN 55455, United States of America. mcvetano@umn.edu (M. Cvetanovic).

Author contributions

M.C. conceptualized the study. J-G.R., C.S., K.H., A.S., H. P. H., and S.G. performed experiments. J-G.R., M.C., F.G., H. P. H, A. S., and C.S. analyzed data. M.C., and J-G.R., wrote the manuscript with input from H.T.O., A. K., C.S., K.H., A.S., and S.G. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Animal experimentation was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota and was conducted in accordance with the National Institutes of Health's (NIH) Principles of Laboratory Animal Care (86–23, revised 1985), and the American Physiological Society's Guiding Principles in the Use of Animals (National Research Council (US), 2011).

Consent for publication

Not applicable.

Availability of supporting data

Data are available on request from the authors.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbd.2023.106023.

clinically relevant brain regions: cerebellum, medulla, hippocampus and motor cortex. We found that during the early stages of disease, *Bdnf* mRNA expression is reduced in the hippocampus and cerebellum, while it is increased in the cortex and brainstem. Importantly, we observed that pharmacological delivery of recombinant BDNF improved motor and cognitive performance, and mitigated pathology in the cerebellum and hippocampus of $Atxn1^{154Q/2Q}$ mice. Our findings demonstrate brain-region specific deficiency of BDNF in SCA1 and show that reversal of low BDNF levels offers the potential for meaningful treatment of motor and cognitive deficits in SCA1.

Keywords

SCA1; BDNF; Neurodegeneration; Brain region specific; Motor deficits; Cognition

1. Background

Spinocerebellar ataxia type-1 (SCA1) is an inherited neurodegenerative disorder caused by 39 or more pure CAG repeats in the *ATAXIN-1* (*ATXN1*) gene (Zoghbi and Orr, 2009). Patients with SCA1 suffer from progressive deficits in motor coordination (i.e. ataxia), and cognition (Klinke et al., 2010; Zoghbi et al., 1988; Koeppen, 2005) that are likely reflective of pathology in the cerebellum, brainstem, cortex and hippocampus (Rüb et al., 2012; Seidel et al., 2012a; Diallo et al., 2019; Moriarty et al., 2016; Jacobi et al., 2013; Koscik et al., 2020; Seidel et al., 2012b; Rüb et al., 2013). There are currently no disease-modifying treatments or cure available for SCA1 patients (Friedrich et al., 2018a), indicating a need for novel therapies.

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, is highly expressed in the brain and important for both cognitive and motor aspects of brain function (Sendtner, 2005). BDNF plays key roles in both development and maintenance of brain function by inducing survival signaling and neuroplasticity (Miranda et al., 2019). BDNF expression levels vary across human brain regions, being highest in the cerebellum (Devlin et al., 2021), implicating BDNF's importance for cerebellar function. BDNF null mice exhibit reduced dendritic arborization of cerebellar Purkinje cells, loss of synapses, impaired short- and long-term synaptic plasticity, and defective coordination and balance, all supporting the critical roles of BDNF in cerebellar structure and function (Ernfors and Lee, 1994; Carter et al., 2002). In addition, BDNF regulates hippocampal neurogenesis and cognition (Rossi et al., 2006; Liu and Nusslock, 2018). Brain disorders, including neurodegenerative and psychiatric diseases, are usually associated with a reduced BDNF levels in the brain (Polyakova et al., 2015; Fumagalli et al., 2006; Cattaneo et al., 2016). We have previously demonstrated that BDNF is decreased in the cerebellum of transgenic mouse model of SCA1 *ATXN1[82Q]* mice that overexpress mutant ATXN1 under a cerebellar Purkinje-cell-specific promoter (Sheeler et al., 2021). Moreover, we have found that BDNF treatment ameliorates cerebellar pathology and motor deficits in *ATXN1[82Q]* mice. While these results were promising, several important questions remained. First, is BDNF expression altered in the cerebella of patients with SCA1? To answer this, we

quantified BDNF expression in cerebellar tissue donated by SCA1 affected patients and matched controls.

Next, ATXN1 is expressed in brain cells such as neurons, astrocytes, and microglia [http://www.brainrnaseq.org/, accessed on December 2022] throughout the brain including the medulla, hippocampus, and motor cortex [https://www.proteinatlas.org/, accessed on December 2022]. These are all regions affected in SCA1 that likely contribute to clinical symptoms of SCA1. We asked whether BDNF expression is altered in these regions as well. Additionally, several studies indicated that early stages of neurodegeneration present the ideal therapeutic window (Friedrich et al., 2018b; Zu et al., 2004a). Therefore, we also aimed to determine how BDNF expression is altered during early disease stages in these different brain regions and how BDNF expression changes with disease progression. To address these questions, we quantified *Bdnf* expression in four different clinically relevant brain regions -cerebellum, hippocampus, brain stem and motor cortex - using SCA1 knock-in $Atxn1^{154Q/2Q}$ mice, where *Atxn1* gene is expressed throughout the brain (Watase et al., 2002; Asher et al., 2020). Recent studies indicate BDNF as an effective treatment for motor or cognitive deficits in brain disorders affecting different brain regions, including Huntington's disease, Alzheimer's disease, Parkinson's disease, and SCA6 (Zuccato and Cattaneo, 2007; Nagahara et al., 2009; Cook et al., 2021; Howells et al., 2000). Therefore, we investigated whether BDNF can rescue both motor and cognitive deficits in SCA1 knock-in mice. As we recently demonstrated that astrocytes and microglia exhibit brain-region specific alterations in SCA1 (Rosa et al., 2022), we investigated the effects of BDNF on neurons, astrocytes and microglia in cerebellum, medulla, hippocampus and motor cortex of wild-type and SCA1 mice (Watase et al., 2002; Suh et al., 2019; Cvetanovic et al., 2016).

2. Methods

2.1. Mice

The creation of the $Atxn1^{154Q/2Q}$ mice was previously described (Watase et al., 2002). Because repeat length in trinucleotide repeat expansions is unstable and prone to further expansion (Gatchel and Zoghbi, 2005), we routinely perform genetic sequencing of our mouse lines. We found that the number of repeats has recently expanded in our colony from 154 CAG to 166 CAG. Based on previous studies, this increase in CAG repeat number is expected to increase severity of disease and lower age of symptom onset. An equal number of male and female $Atxn1^{154Q/2Q}$ and wild-type (WT) mice were randomly allocated to BDNF or control artificial cerebrospinal fluid (aCSF) groups ($N = 10–12$).

We surgically implanted ALZET pumps (Alzet Model 1004) into 7-week-old mice in a subcutaneous pocket in their back. A delivery cannula was placed into the right lateral ventricle using stereotaxic surgery (A/P, 1.1 mm; M/L, 0.5 mm; D/V, −2.5 mm from Bregma) as previously described (Seidel et al., 2012a). ALZET pumps delivered BDNF or aCSF at a steady flow rate for 4 weeks following implantation (20 μg of human recombinant BDNF (R&D Systems Cat. 248-BD-250/CF) in 100 μl per micropump, resulting in a delivery rate of 0.71 μg/day) (Sheeler et al., 2021).

In all experiments, investigators were blinded to the genotype/treatment. While we started with the same number of animals in each group, the final number of animals per condition varied depending on the success of surgery, including survival from surgery and correct placement of the cannula. Five mice died: two female WT mice treated with aCSF and BDNF, and one female and two male *Atxn1*$^{154Q/2Q}$ mice treated with BDNF. All mice underwent postmortem examination, and this revealed the cannula was placed correctly and not obstructed in any of the mice.

Animal experimentation was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota, and was conducted in accordance with the National Institutes of Health's (NIH) Principles of Laboratory Animal Care (86–23, revised 1985), and the American Physiological Society's Guiding Principles in the Use of Animals (National Research Council (US), 2011).

2.2. Cognitive testing

Mice were subjected to contextual fear conditioning prior to BDNF delivery (6 weeks of age). Mice were tested via Barnes maze 3 weeks after BDNF delivery started (10 weeks of age). Both cognitive tests were administered as previously done and are described below (Asher et al., 2020).

Sample sizes in the behavioral tests were determined using power analysis and prior experience with these tests, or previous reports using similar methodology. Experimenters were blinded to genotype and treatment during all tests.

2.2.1. Barnes maze—The maze was a white circular platform 91 cm in diameter with 20 5-cm circular holes spaced evenly around the edge, raised approximately 92 cm above the floor. One of the holes led to a 5 cm wide x 11 cm long x 5 cm deep opaque box (the "escape box") and the other 19 were covered. The testing room had visual cues on the walls to serve as landmarks, and all objects in the room remained in the same places for every trial. The position of each mouse was tracked using AnyMaze software. Mice were exposed to the maze for four 3-min trials per day during four consecutive training days (intertrial interval of approximately 15 min). Mice that did not enter the escape box within 3 min were gently guided to it. Training day data is reported as a path length (distance traveled before entering the escape hole), and analyzed by two-way repeated measures ANOVA. A probe test was conducted 24 h after the last training session. For the probe test, the escape hole was covered, and each mouse was allowed to explore the maze freely for 90 s. The time spent in each quadrant of the maze was recorded, and the amount of time spent in the goal quadrant (the quadrant centered on the goal hole) was analyzed by one-way ANOVA.

Search strategies on the training days were automatically classified and assigned cognitive scores using the Barnes maze unbiased strategy (BUNS) classification tool as described by Illouz et al. (Illouz et al., 2016). In order to compare learning rates between groups, cognitive scores for each mouse on each of the 16 training trials were plotted in GraphPad Prism 7.0. Linear regression was performed for each group and the slopes and elevations of the lines were compared using Prism's Analysis function.

2.2.2. Contextual fear conditioning—Conditioning took place in chambers with a floor consisting of stainless-steel rods through which shocks were delivered (Med Associates #ENV-008-FPU-M). On day 1, mice were placed in the chambers for a 10-min period during which they received five shocks to the foot (0.70 mA, 2-s duration). Freezing during the 60 s after each shock was quantified automatically using VideoFreeze software (freezing was defined as a motion index ≤15 lasting ≥500 ms). 24 h after the initial conditioning, mice were returned to the same chambers with the shock generators turned off and freezing behavior was monitored for 3 min. 1–2 h after being placed in the conditioned context, mice were placed in a second context [the same chambers but different floor texture (smooth plastic versus metal rods), shape (curved plastic wall versus square metal wall), and odor (0.5% vanilla extract versus 33% Simple Green)] for 3 min to measure baseline freezing. Acquisition of freezing responses is reported as percent freezing in the 60-s period following each of the 5 shocks to the foot, analyzed by two-way repeated measures ANOVA. 24-h recall is reported as percent freezing in each context over the 3-min test period, analyzed by two-way repeated measures ANOVA.

2.3. Rotarod analysis

Mice were tested on rotarod (#47600; Ugo Basile) to evaluate motor deficits as described previously prior to BDNF delivery (6 weeks of age) and 4 weeks after the BDNF delivery started (11 weeks of age). Rotarod paradigm consisted of four trials per day over four days with acceleration from 5 to 40 rotations per minute (rpm) over minutes 0 to 5, followed by 40 rpm constant speed from 5 to 10 min. Latency to fall was recorded (Ferro et al., 2018).

2.4. 5-bromo-2′-deoxyuridine (BrdU) Administration

An intraperitoneal injection of BrdU (Biolegend) was administered following the final rotarod trial at a dosage of 100 mg/kg every 12 h for three doses. Mice were sacrificed 72 h after the last injection as previously described (Asher et al., 2016a).

2.5. Immunofluorescent (IF) staining

IF was performed on a minimum of six different floating 45-μm-thick brain slices from each mouse (six technical replicates per mouse per region or antibody of interest). Confocal images were acquired using a confocal microscope (Olympus FV1000) using a 20× oil objective. *Z*-stacks were taken consisting of twenty non-overlapping 1-μm-thick slices of each stained brain slice per brain region (i.e., six z-stacks per mouse, each taken from a different brain slice). The laser power and detector gain were standardized and fixed between mice within a surgery cohort, and all images for mice within a cohort were acquired in a single imaging session to allow for quantitative comparison.

We used primary antibodies against Purkinje cell marker calbindin (mouse, Sigma-Aldrich, C9848), bromodeoxyuridine (BrdU) (rat, BioRad, MCA2060), and vesicular glutamate transporter 2 (VGLUT2) (guinea pig, Millipore, AB2251-I) as previously described (Kim et al., 2018; Asher et al., 2016b). Quantitative analysis was performed using ImageJ (NIH) as described previously. To quantify relative intensity of staining for calbindin, we measured the average signal intensity in the region of interest and normalized it to that of the WT mouse of that cohort. The density of new neuronal cells was determined by normalizing

the number of BrdU+ cells to the dentate gyrus area. To quantify atrophy of the cerebellar molecular layer, we took six measurements per image of the distance from the base of the Purkinje soma to the end of their dendrites, the average being the molecular layer width for that image. Recession of climbing fibers was quantified by normalizing the width of VGLUT2 staining to the width of the molecular layer.

We also performed IF on 6-μm-thick slices from paraffin embedded cerebellum samples from human SCA1 patients and normal healthy controls provided to us by Dr. Arnulf H. Koeppen, Albany, NY (Table 1). Confocal images were acquired using a confocal microscope (Olympus FV1000) using a 40× oil objective. *Z*-stacks were taken consisting of five 1-μm-thick slices of each stained brain slice. The laser power and detector gain were standardized and fixed between samples. Human tissue was stained with BDNF antibody (rabbit, Abcam). The percent area of staining for BDNF was quantified by creating a mask of BDNF staining and recording the fraction of the region of interest covered by staining. To quantify BDNF+ puncta, we utilized the ImageJ analyze particles function that recorded the count and total area of particles between the size of 0.05 μm^2 and 5.0 μm^2 and a circularity between 0.5 and 1.0. We normalized particle counts and total particle area to the total area of the region of interest in order to calculate BDNF+ puncta density and BDNF+ puncta percent area, respectively.

2.6. Enzyme-linked immunosorbent assay (ELISA)

Subcortical tissue was dissected on ice and the weight of the tissue for each mouse was measured, then frozen. Proteins were extracted from frozen tissue using Tris-Triton Lysis Buffer [150 nM sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS (sodium dodecyl sulfate), and 50 mM Tris (pH 8.0)] as has been described previously (Mellesmoen et al., 2019). Following lysate preparation, total BDNF was quantified in duplicates using the Total BDNF Quantikine ELISA kit (Biotechne-R&D Systems).

2.7. RNA extraction, sequencing, and analyses

Cerebellum, medulla, cerebral cortex, and hippocampal tissue was isolated from 26-week-old wild-type and $Atxn1^{154Q/2Q}$ mice and stored in RNAlater solution (Thermo Fisher Scientific). Total RNA was isolated using TRIzol reagent (Thermo Fisher Scientific) following the manufacturer's protocols. Tissue was homogenized using RNase-Free disposable pellet pestles in a motorized chuck. Purified RNA was sent to the University of Minnesota Genomics Center for quality control, including quantification using fluorimetry via RiboGreen assay kit (Thermo Fisher Scientific) and RNA integrity was assessed via capillary 34 electrophoresis using an Agilent BioAnalyzer 2100 to generate an RNA integrity number (RIN). RIN values for submitted RNA were above 8.0 for all samples except one medulla sample (RIN = 6.8). All submitted RNA samples had >1 μg total mass. Library creation was completed using oligo-dT purification of polyadenylated RNA, which was reverse transcribed to create cDNA. cDNA was fragmented, blunt ended, and ligated to barcode adaptors. Libraries were size selected to 320 bp ± 5% to produce average inserts of approximately 200 bp, and size distribution was validated using capillary electrophoresis and quantified using fluorimetry (PicoGreen, Thermo Fisher Scientific) and qPCR. Libraries were then normalized, pooled, and sequenced on an S4 flow cell by an Illumina NovaSeq

6000 using a 150-nucleotide, paired-end read strategy. The resulting FASTQ files were trimmed, aligned to the mouse reference genome (GRCm38), sorted, and counted using the Bulk RNAseq Analysis Pipeline from the Minnesota Supercomputing Institute's Collection of Hierarchical UMII/RIS Pipelines (v0.2.0) (Baller et al., 2019). Genes <300 bp are too small to be accurately captured in standard RNAseq library preparations, so they were discarded from all downstream analyses.

Differential gene expression analysis was performed using the edgeR package (McCarthy et al., 2012; Robinson et al., 2009) (v3.30.3) in R (R Foundation for Statistical Computing v3.6.1). All four brain regions were analyzed independently. Genes with fewer than 10 counts across all samples in each region were excluded. Genes with FDR values less than or equal to 0.05 were considered significant.

2.8. Reverse transcription and quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from dissected mouse cerebella, brainstem, cortex, and hippocampus using TRIzol (Life Technologies), and RT-qPCR was performed as described previously (Moriarty et al., 2016). We used IDT Primetime primers. Relative mRNA levels were calculated using 18S RNA as a control and wild-type mice as a reference using $2^{-\Delta\Delta\text{-Ct}}$ as previously described.

2.9. Western blotting

Cerebellum and hippocampus tissues were collected from wild-type and *Atxn1*$^{154Q/2Q}$ mice at 10 weeks of age. Protein lysates were made using RIPA lysis buffer (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1% sodium deoxycholate, 1% NP-40, 0.2% SDS, phosphatase (Sigma) and protease inhibitors cocktail (Roche)). Protein concentrations were measured using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific). Samples from each brain region containing 40 μg total protein was boiled in Laemmli loading buffer and run on a 4%–20% or 12% BioRad precast gel. Protein was transferred to a nitrocellulose membrane using the BioRad Trans-Blot Turbo system. Blots were blocked overnight at 4 °C in 5% milk PBST (phosphate-buffered saline, 0.1% Tween 20). Blot sections were probed overnight at 4 °C 1:1000 with the BDNF (Abcam: 108319) or TrkB (Abcam: 18987) antibodies diluted in 5% milk PBST. Blots were washed 3 times with PBST and then placed in 5% milk PBST plus 1:2500 rabbit specific horseradish peroxidase (HRP) antibodies (GE Healthcare) at 4 °C overnight. Blots were washed 3 times with PBST followed by Super Signal West Dura (Thermo. Fisher Scientific) detection reagents and imaged on an ImageQuant LAS 4000. The blots were stripped with Restore Western Blot Stripping Buffer (Thermo Fisher Scientific) for 15 min at room temperature, washed 3 times with PBST, and blocked overnight at 4 °C in 5% milk PBST. These blots were then probed overnight at 4 °C 1:10000 with α-Tubulin antibody (Millipore Sigma), washed 3 times with PBST, placed in 5% milk PBST plus 1:2500 mouse specific HRP antibodies (GE Healthcare) at room temperature for 4 h, and visualized via the same method as described above.

2.10. Statistics

Wherever possible, sample sizes were calculated using power analyses based on the standard deviations from our previous studies, significance level of 5%, and power of 90%.

Statistical tests were performed with GraphPad Prism 7.0. Data was analyzed using two-way ANOVA (to assess the impact of genotype and treatment), unpaired Student's *t*-test (to test statistical significance of differences between groups of interest wherein only one variable was different (treatment or genotype), or one-way ANOVA followed by the post-hoc test. Outliers were determined using GraphPad PRISM's Robust regression and Outlier removal (ROUT) with a Q = 1% for non-biased selection.

2.11. Study approval

Animal experimentation was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Minnesota and was conducted in accordance with the National Institutes of Health's (NIH) Principles of Laboratory Animal Care (86–23, revised 1985), and the American Physiological Society's Guiding Principles in the Use of Animals (National Research Council (US), 2011).

3. Results

3.1. Cerebellar BDNF levels are reduced in patients with SCA1

To determine whether BDNF is altered in cerebella from patients with SCA1, we stained cerebellar slices of patients with SCA1 and their sex- and age-matched healthy controls (Table 1) for BDNF protein. We then quantified density and the area occupied by the BDNF labeled puncta in the cerebellar molecular layer of patients with SCA1 (Andreska et al., 2014) (Fig. 1A). We found a significant decrease in BDNF area (~ 50% decrease, 7.68 in controls and 3.4 in patients with SCA1), as well as in BDNF puncta density and the area of BDNF labeled puncta. Another brain region significantly affected in SCA1 is brain stem. We thus investigated whether BDNF expression is also altered in the medulla of patients with SCA1. We found a smaller but significant reduction in BDNF area (~ 20%, 10.2 in controls, 8.05 in patients with SCA1) in the medulla of patients with SCA1 (Fig. 1B). These results indicate that BDNF protein is reduced in the cerebellum and medulla of patients with SCA1.

3.2. Spatial differences in BDNF expression during early disease in Atxn1$^{154Q/2Q}$ mice

While BDNF is decreased in the cerebellum and medulla of patients with SCA1 at the terminal stage of disease, early disease stages are most amenable to effective therapies. We next investigated whether BDNF expression is altered during this promising therapeutic window. As patient's tissues at these early stages are not available, we used SCA1 knock-in mouse model *Atxn1*$^{154Q/2Q}$ line that express mutant *Atxn1* under endogenous promoter. Moreover, as the endogenous promoter drives expression of mutant *Atxn1* throughout the brain, SCA1 pathology was detected in the cerebellum, brainstem, cortex, and hippocampus of *Atxn1*$^{154Q/2Q}$ mice (Banfi et al., 1996). We first investigated how are *Bdnf* mRNA levels altered in these four different clinically relevant brain regions at 12 weeks to represent the early symptomatic disease stage in *Atxn1*$^{154Q/2Q}$ mice without neuronal loss (Friedrich et al., 2018b; Zu et al., 2004b). Using quantitative RT-PCR, we found significant *Bdnf* mRNA reduction in the cerebellum and hippocampus of *Atxn1*$^{154Q/2Q}$ mice. In contrast, the cortex and medulla of *Atxn1*$^{154Q/2Q}$ mice exhibited a significant increase in the *Bdnf* mRNA expression (Fig. 2). To determine how *Bdnf* expression changes with disease progression we next examined *Bdnf* mRNA expression at 26 weeks during late disease stages using

RNA sequencing. Intriguingly, we found that *Bdnf* is significantly decreased in cortex and hippocampus, trending toward decrease in the cerebellum and is not significantly different from wild-type controls in medulla at 26 weeks (Supplementary Fig. 1). These results suggest spatial-temporal differences in *Bdnf* expression in SCA1 mice that may indicate region specific roles of BDNF during different stages of SCA1.

3.3. Pharmacological delivery of BDNF after onset of symptoms mitigates motor and cognitive deficits in Atxn1$^{154(1/2(1}$ mice

To test the therapeutic effects of BDNF in SCA1, we initiated BDNF treatment with Alzet pumps delivering recombinant BDNF into the lateral ventricle, after the onset of disease symptoms, at 7 weeks of age (Fig. 3A) (Sheeler et al., 2021). Prior to treatment we assessed SCA1-like phenotypes at this early stage of disease using motor (rotarod) and cognitive (contextual fear conditioning) tests. We also measured their weight to determine whether they already exhibit failure-to-gain weight phenotype. At 6 weeks of age, *Atxn1*$^{154Q/2Q}$ mice had significantly reduced latency to fall on rotarod and decreased freezing in fear conditioning, indicating significant motor and cognitive deficits at this age. Mice also had a lower weight relative to WT controls (Supplementary Fig. 2). Using western blotting we also quantified protein expression of BDNF and its receptor tyrosine kinase receptor B (TrkB) at 10 weeks (Jin, 2020; Rose et al., 2003). We have found a trending reduction in BDNF precursor pre-proBDNF (preBDNF) and mature-BDNF (mBDNF) protein in the hippocampus (~ 50% of wild-type levels) and a slight reduction in preBDNF protein (~ 20%) in the cerebellum (Supplementary Fig. 3A–B). We could not detect reliably strong mature BDNF bands in cerebellar lysates. Both full length (flTrkB) and truncated TrkB (tTrkB) proteins were decreased in the hippocampus and cerebellum, but significance was reached only for tTrkB in the cerebellum and full length TrkB in the hippocampus (Supplementary Fig. 3C–D). These results suggest that at the time of treatment there is a trending reduction in BDNF signaling in cerebellum and hippocampus.

Mice underwent surgery in which a cannula was implanted into the right lateral ventricle and an ALZET pump was placed subcutaneously to deliver recombinant BDNF or aCSF for four weeks. BDNF delivery was confirmed using ELISA at the end of the experiment at 13 weeks of age (Supplementary Fig. 4).

We first examined the effects of BDNF on SCA1-like phenotypes including motor and cognitive deficits. Four weeks of BDNF delivery significantly increased the latency to fall during rotarod test of *Atxn1*$^{154Q/2Q}$ mice, indicating beneficial effect of BDNF on motor deficits in this SCA1 knock-in mouse model (Fig. 3B). Cognitive deficits were examined via Barnes maze. BDNF delivery reduced the latency of *Atxn1*$^{154Q/2Q}$ mice to enter the escape hole during the training phase of the Barnes maze (Fig. 3C), suggesting that BDNF has a beneficial effect on learning. However, it is possible that trending BDNF-induced improvement in the speed of SCA1 mice could contribute to this (Supplementary Fig. 5). To further examine how BDNF affects ability to learn in SCA1 mice, we used the automated Barnes maze unbiased strategy analysis tool (BUNS) (Illouz et al., 2016). Mice can solve the Barnes maze using spatial strategies (direct, corrected, long correction and focused search indicated by black to red colors in Fig. 3), non-spatial search strategy (serial, going

around the maze until they find escape hole, indicated by yellow color), or by randomly exploring the maze (using no strategy at all, indicated by white color) (Supplementary Fig. 6A). *Atxn1*$^{154Q/2Q}$ mice preferentially use random and serial strategies instead of spatial strategies to find the escape hole (6.25% compared to 26% spatial strategy use by wild-type mice) (Ernfors and Lee, 1994) (Fig. 3C). Importantly, we found that delivery of BDNF significantly improved strategy development *Atxn1*$^{154Q/2Q}$ mice (from 6.25% use of spatial search strategies, including direct, corrected, long corrected and focused search, in *Atxn1*^{154Q}; aCSF mice to 18% use of spatial strategies in *Atxn1*^{154Q}; BDNF treated mice, Fig. 3C). Each strategy is assigned a cognitive score, and linear regression analysis of the rate of cognitive score change per training day also demonstrated that BDNF significantly improved strategy development in *Atxn1*$^{154Q/2Q}$ mice (Fig. 3D). These results suggest that BDNF has a mitigating effect on the lack of strategy development in *Atxn1*$^{154Q/2Q}$ mice.

Despite previous studies linking BDNF and body weight (Pelleymounte et al., 1995), BDNF delivery had no effect on the failure to gain weight phenotype in *Atxn1*$^{154Q/2Q}$ mice (Supplementary Fig. 6B).

3.4. BDNF ameliorates cerebellar and hippocampal pathology in Atxn1$^{154Q/2Q}$ mice

It is reasonable to propose that during the early disease stage, decreased expression of *Bdnf* may contribute to SCA1 pathology in the cerebellum and hippocampus. Therefore, we next examined whether delivery of recombinant BDNF mitigates SCA1 pathology in the cerebellum and hippocampus.

To test whether BDNF ameliorates cerebellar Purkinje cell pathology, we stained cerebellar slices with calbindin - a marker of Purkinje cells - and vesicular glutamate transporter 2 (VGLUT2) - a marker of climbing fiber synapses on Purkinje cells (Supplementary Fig. 7A). Cerebellar SCA1 pathology is commonly quantified by a decrease in the width of the molecular layer (ML) in the cerebellar cortex, thought to indicate dendritic atrophy of Purkinje cells, reduced calbindin expression and reduced extension of VGLUT2 terminals on Purkinje cell dendrites (VGLUT2/ML) (Qu et al., 2017). As expected control aCSF-treated *Atxn1*$^{154Q/2Q}$ mice had significantly reduced width of molecular layer compared to a CSF; WT mice (Fig. 4A). Moreover, BDNF; *Atxn1*$^{154Q/2Q}$ had significantly larger molecular layer thickness compared to aCSF; *Atxn1*$^{154Q/2Q}$ mice indicating that BDNF ameliorates PC atrophy. Similarly, while aCSF; *Atxn1*$^{154Q/2Q}$ had significantly reduced calbindin intensity and reduced VGLUT2/ML ratio, indicating synaptic loss, we found no significant difference in these measures between BDNF; *Atxn1*$^{154Q/2Q}$ and BDNF; WT mice (Fig. 4B and, Supplementary Fig. 7B). These results indicate that BDNF mitigates PC atrophy, expression of calbindin and loss of VGLUT2 synapses in the cerebella of SCA1 mice.

To examine the effects of BDNF on cerebellar astrocytes and microglia, we stained cerebellar slices with glial fibrillary acidic protein (GFAP), a marker of astrocytes that is increased in gliosis, and microglial marker intracellular calcium binding protein (Iba1). We have quantified intensity of GFAP staining and area occupied by GFAP to quantify morphological changes (hypertrophy or atrophy), as well as density and area of Iba1 positive microglia. We found no significant effects of BDNF on GFAP intensity, GFAP

area (Supplementary Fig. 8A), and in Iba1 density and area microglia (Supplementary Fig. 9A) in either wild-type or SCA1 mice.

To determine the effects of BDNF on SCA1 hippocampal pathology, we first quantified proliferation of cells in dentate gyrus as we previously found that this measure of hippocampal neurogenesis is reduced in *Atxn1*$^{154Q/2Q}$ mice (Asher et al., 2016a; Cvetanovic et al., 2017). We injected BDNF and aCSF treated SCA1 and wild-type mice with BrdU to label proliferating cells, and quantified BrdU incorporation in dentate gyrus using immunohistochemistry (Fig. 4C). As we have shown previously, aCSF; *Atxn1*$^{154Q/2Q}$ mice had significantly decreased number of BrdU labeled cells, indicating decreased proliferation. BDNF treatment rescued proliferation in the dentate gyrus of *Atxn1*$^{154Q/2Q}$ mice and caused a trending increase in proliferation in wild-type mice (Fig. 4D).

Neuronal loss in CA2 region of hippocampus was previously described during late disease stages in *Atxn1*$^{154Q/2Q}$ mice. By measuring the thickness of the molecular layers, we did not find any changes in either CA2 or CA3 regions in aCSF or BDNF-treated *Atxn1*$^{154Q/2Q}$ mice (Supplementary Fig. 10A).

We found a significant increase in the GFAP intensity in the hippocampus of BDNF treated wild-type mice and trending increases in GFAP intensity and area in BDNF treated wild-type and *Atxn1*$^{154Q/2Q}$ mice (Supplementary Fig. 8B). We investigated further how BDNF affects astrocytes by quantifying expression of key astrocytic genes in the hippocampus. Astrocytes play key roles in regulating neuronal functions, including homeostasis of glutamate, ions, and water. We have recently demonstrated decreased expression of core homeostatic genes regulating extracellular levels of glutamate (*Slc1a2*), potassium (*Kcnj10*), astrocyte communication (*Gja1*) and water (*Aqp4*) in the hippocampus of *Atxn1*$^{154Q/2Q}$ mice (Rosa et al., 2021). Therefore, we used quantitative RT-PCR to examine whether BDNF modulates expression of these genes in the hippocampus of *Atxn1*$^{154Q/2Q}$ mice. We found that BDNF significantly increased the expression of *Aqp4*, *Slc1a2*, *Gja1*, and *Kcnj10* in the wild-type and in *Atxn1*$^{154Q/2Q}$ mice (Fig. 4E). To discriminate whether this increase in astrocytic gene expression was due to increase in the astrocyte number or increased expression per astrocytes we quantified density of astrocytes in the dentate gyrus. We did not observe any change in astrocyte density with BDNF treatment in either wild-type or *Atxn1*$^{154Q/2Q}$ mice (Supplementary Fig. 11), indicating that BDNF affects gene expression in the hippocampal astrocytes.

Investigating microglia we found trending increases in density and percent area of Iba1+ microglia with BDNF treatment in the hippocampus of both wild-type and SCA1 mice (Supplementary Fig. 9B).

We also examined how BDNF affects neurons, astrocytes and microglia in medulla and cortex (regions where *Bdnf* mRNA expression is increased in SCA1 mice) but we have found few significant differences in our measurements. Of note, density of NeuN+ neurons was not altered in either of these regions in SCA1 mice at this stage of disease (Supplementary Fig. 10B and C). Intriguingly, BDNF itself significantly reduced GFAP intensity in the medulla of wild-type mice (Supplementary Figs. 8 C–D, 9C–D).

Finally, we examined how exogenous BDNF affected endogenous *Bdnf* mRNA expression in wild-type and SCA1 mice. The only statistically significant effect of BDNF treatment (by two-way ANOVA) was on *Bdnf* mRNA levels in the hippocampus (Supplementary Fig. 12).

4. Discussion

Here, we report reduced BDNF expression in the cerebellum and medulla of patients with SCA1 and in the hippocampus and cerebellum of early disease stage knock-in $Atxn1^{154Q/2Q}$ mice. We demonstrate that pharmacological delivery of BDNF improves motor and cognitive performance, and ameliorates pathology in the cerebellum and hippocampus of $Atxn1^{154Q/2Q}$ mice.

These results build on our previous studies showing decreased BDNF in the cerebellum of *ATXN1[82Q]* mice, Purkinje cell specific transgenic SCA1 line. First, we demonstrated that BDNF protein is decreased in cerebellum of patients with SCA1 compared to unaffected age-and sex-matched controls. Moreover, brainstem is another brain region affected in SCA1, and we have found decreased BDNF protein in the medulla of patients with SCA1. While these results indicate that BDNF levels are decreased in patients with SCA1, they are limited in representing the terminal stage of disease stage. To investigate whether BDNF is decreased early in disease and whether BDNF delivery has a therapeutic potential, we turned to an SCA1 knock-in mouse model expressing mutant ATXN1 throughout the brain, $Atxn1^{154Q/2Q}$ mice. These mice are characterized by motor and cognitive deficits, and failure to gain weight. In addition to cerebellar pathology, previous studies found a reduction in hippocampal neurogenesis, neuronal loss in CA2 area, and changes in gene expression in medulla and cortex.

We found that during early symptomatic stages of disease BDNF expression was altered in a region-specific manner in these mice. For instance, we found reduced *Bdnf* mRNA expression in the cerebellum and hippocampus, while *Bdnf* mRNA expression was increased in the medulla and cortex of 12-week-old $Atxn1^{154Q/2Q}$ mice. We propose that regional specificity of *Bdnf* mRNA expression changes may indicate brain region specific contribution of BDNF to the early SCA1 pathology. Moreover, protein expression of pre- and mature BDNF and its receptor TrkB (full length and truncated) was decreased in cerebellum and hippocampus, providing further evidence of reduced BDNF signaling in these brain regions early in SCA1. At the late disease stage, we found significantly reduced expression of *Bdnf* mRNA in cerebellum and in the hippocampus of $Atxn1^{154Q/2Q}$ mice compared to WT littermate controls. This is reminiscent of Alzheimer's disease (AD) study demonstrating that BDNF expression varies over the course of the disease and correlates with the severity of dementia (Tapia-Arancibia et al., 2008). In this AD study BDNF was significantly increased during early stages whereas at more severe stages of AD, it was decreased.

We hypothesized that reduced BDNF expression may contribute to SCA1 pathology in the cerebellum and hippocampus. To test this, we used pharmacological approach to deliver recombinant BDNF or aCSF for four weeks with ALZET pumps. We found that BDNF treatment significantly improved motor and cognitive performance of BDNF; $Atxn1^{154Q/2Q}$

mice compared to aCSF-treated *Atxn1*$^{154Q/2Q}$ controls. These behavioral improvements were accompanied by ameliorated cerebellar and hippocampal pathology in BDNF treated *Atxn1*$^{154Q/2Q}$ mice. These results support the therapeutic potential of BDNF in SCA1 during early stages of SCA1.

It is important to note that BDNF did not rescue all SCA1 phenotypes. BDNF was previously implicated in regulating weight, with genetic deletion of *Bdnf* causing animals to exhibit energy balance dysregulation and severe obesity (Rios, 2013). As SCA1 mice exhibit failure to gain weight phenotype during early stages with disease, we were cautious that one possible negative side effect of BDNF could be further weight loss. However, we had not detected any effect of BDNF on the failure to gain weight phenotype seen in SCA1 mice. As this effect of BDNF is thought to be exerted via hypothalamus, the regulatory center for feeding and weight control, this result may indicate that BDNF expression is not altered in the hypothalamus of SCA1 mice. Alternatively, failure to gain phenotype may be driven by peripheral regions in which BDNF is not altered at this disease stage. Our study is limited as it cannot distinguish whether BDNF exerts its beneficial effects in SCA1 mice by directly affecting neurons, hippocampal stem cells, or astrocytes, and whether similar effects will be seen in human cellular counterparts. Future studies using human neurons, stem cells, and astrocytes derived from induced pluripotent cells (iPSCs) donated by patients with SCA1 will allow us to interrogate direct effects of BDNF on each human cell type (Sheeler et al., 2020).

In conclusion, our findings demonstrate an important role for BDNF in SCA1 pathogenesis and suggest that reversal of low BDNF levels offers the potential for meaningful treatment in patients with SCA1. BDNF plays a key role in a healthy brain function, and loss of BDNF can contribute to pathology in neurodegenerative diseases and depression (Miranda et al., 2019). BDNF expression is decreased in aging, and in patients and in mouse models has been described in other polyQ neurodegenerative diseases such as Huntington's disease (HD) (Zuccato and Cattaneo, 2007; Zuccato et al., 2001) and SCA6 (Cook et al., 2021; Takahashi et al., 2012). Decreased BDNF has also been shown to correlate with behavioral impairments in aging, Alzheimer's disease (AD), and Parkinson's disease (PD) (Moriarty et al., 2016; Jacobi et al., 2013) (Koscik et al., 2020; Seidel et al., 2012b; Rüb et al., 2013). Despite differences in brain region and neuronal vulnerability in these diseases, BDNF treatment has been shown to be beneficial in preclinical studies (Friedrich et al., 2018a; Sendtner, 2005; Miranda et al., 2019; Devlin et al., 2021; Ernfors and Lee, 1994; Sheeler et al., 2021; Mellesmoen et al., 2019), indicating that BDNF has a great therapeutic potential with a broad disease target (Nagahara et al., 2009; Gharami et al., 2008; Xie et al., 2010; Canals et al., 2004; Tsukahara et al., 1995). The first human clinical trials for BDNF in Alzheimer's disease (AD) and mild cognitive impairment (MCI) started in 2021 and novel nanoparticle approaches were developed to non-invasively deliver BDNF to the brain (Vitaliano et al., 2022). Our results build upon this work and support the translational potential of BDNF for SCA1.

In addition, our findings advance understanding of SCA1 neurodegeneration in several ways. First, they suggest regional differences in SCA1 pathogenesis, with decreased BDNF being associated with early SCA1 pathology in cerebellum and hippocampus. Second, our results

implicate that BDNF increases the expression of astrocytic homeostatic genes key for the neuronal function in hippocampus. As astrocyte density is not altered by BDNF this result may implicate that some of the beneficial effects of BDNF may be contributed by astrocytes. Third, our work provides clues to develop biomarkers to monitor disease progression (such as BDNF itself). BDNF decrease can be measured in blood, and has been suggested as a candidate biomarker of other pathological conditions (Lima Giacobbo et al., 2019). Since BDNF is widely implicated in brain pathology, our results could prove relevant to other ataxias and other neurodegenerative diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We are very grateful to Dr. Arnulf H. Koeppen, Albany, NY for sharing cerebellar and medullar samples from patients with SCA1 and for the thoughtful feedback. We are very grateful to Dr. Harry Orr, University of Minnesota, MN for sharing SCA1 mice and for the thoughtful feedback. We acknowledge all the members of the Orr and Cvetanovic laboratories for thoughtful discussions and feedback on the study. Work in this study was aided by the Mouse Behavioral Core and Histology and IHC Laboratory at the University of Minnesota.

Full financial disclosure for the previous 12 months

MC, SG, FG, and KS were supported by National Institute of Health NINDS awards (R01 NS197387 and R01 NS109077 to M.C.), National Ataxia Foundation, Wallin Neuroscience Foundation, and Pilot grant in Addiction. CS was supported by the UM-MnDRIVE Graduate Assistantship Award and KH was supported by the UM Doctoral Dissertation Fellowship.

Data availability

Data will be made available on request.

Abbreviations:

SCA1	Spinocerebellar Ataxia Type 1
BDNF	Brain Derived Neurotrophic Factor
polyQ	polyglutamine
ASO	antisense oligonucleotides
GFAP	Glial Fibrillary Acidic Protein
iPSCs	induced pluripotent stem cells
HD	Huntington’s disease
AD	Alzheimer’s disease
PD	Parkinson’s disease

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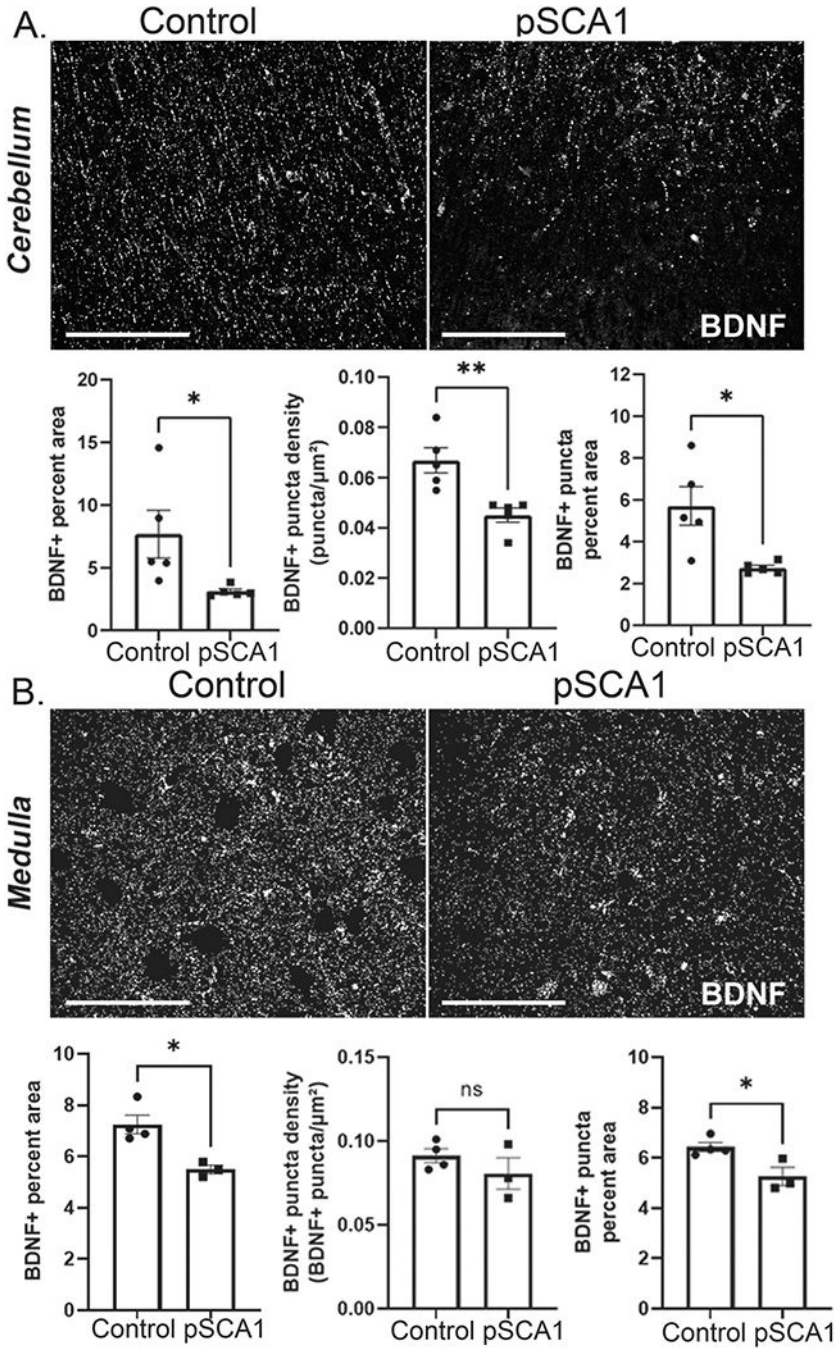

Fig. 1.

BDNF expression is decreased in the cerebellum of patients with SCA1.

Slices from the cerebellar cortex (A) or medulla (B) of the patients with SCA1 (pSCA1) and healthy age- and sex- matched controls were stained with BDNF. Confocal images were used to quantify BDNF% area, puncta density and puncta area. Data is presented as mean ± SEM with average values for each independent experimental sample represented by a dot. * $p < 0.05$, ** $p < 0.01$ Student's *t*-test. Scale bar = 100 μm.

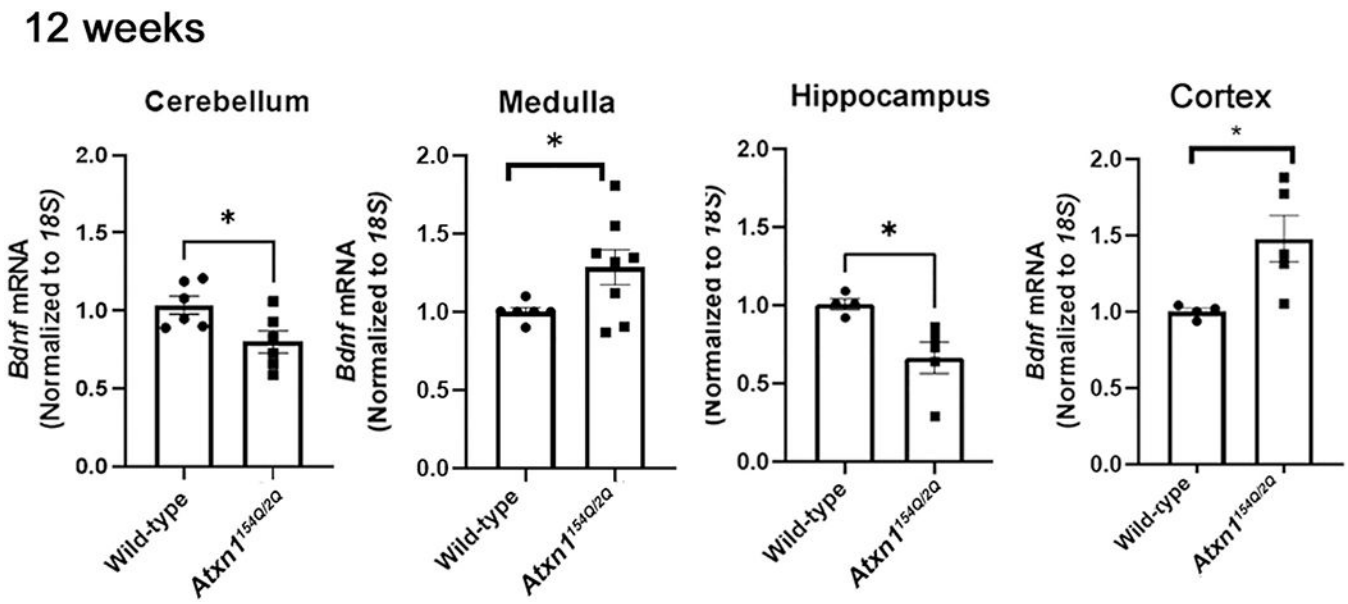

Fig. 2.

BDNF expression is altered in a brain region specific manner in *Atxn1*$^{154Q/2Q}$ mice.

The cerebellum, medulla, cortex and hippocampus were dissected from 12 weeks old *Atxn1*$^{154Q/2Q}$ mice and their wild-type littermate controls (N = 4–8), mRNA was extracted and RTqPCR was used to evaluate expression of *Bdnf* mRNA. Data is presented as mean ± SEM with average values for each mouse represented by a dot. * $p < 0.05$ Welch's t-test.

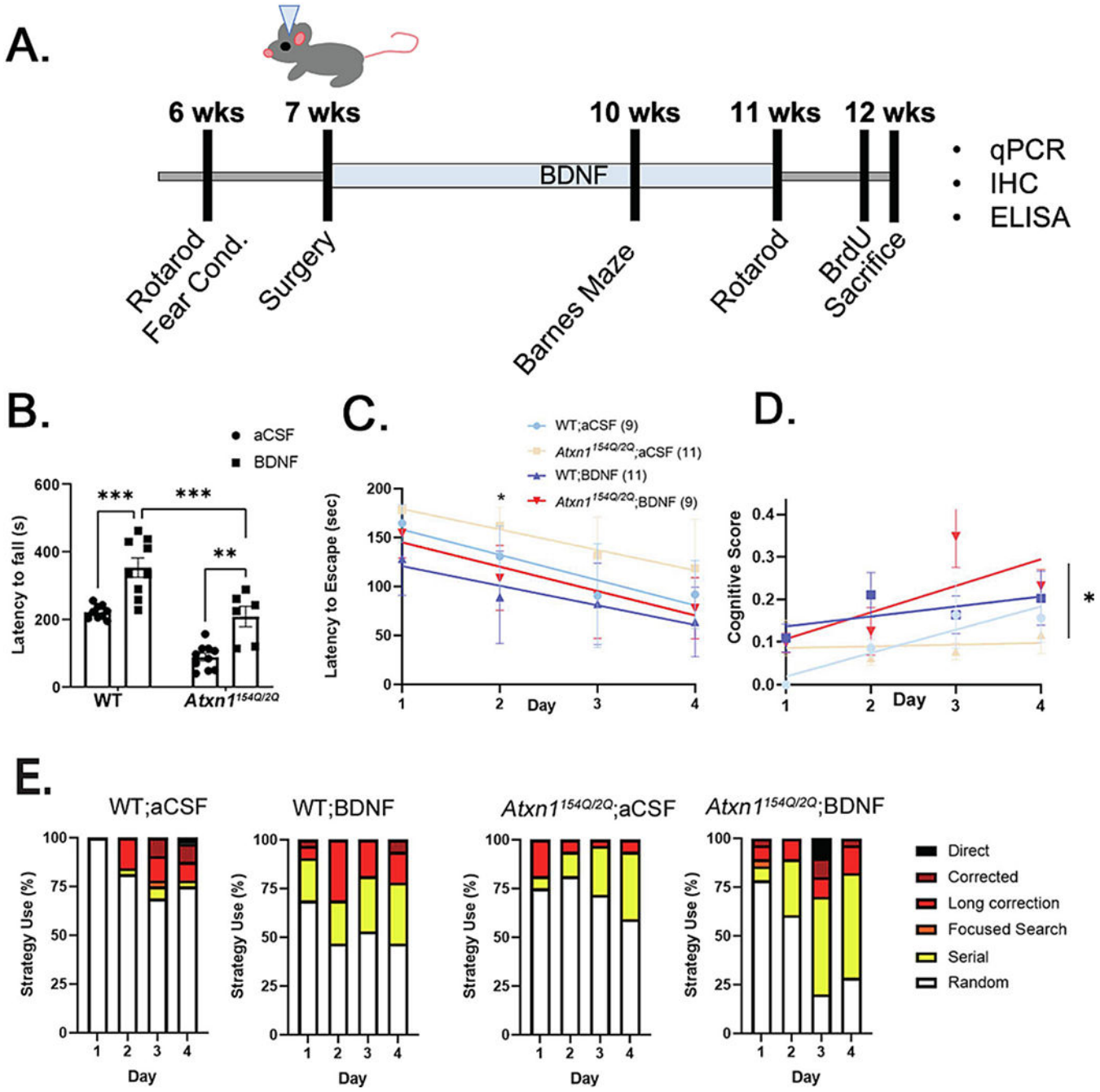

Fig. 3.

BDNF treatment ameliorates motor and cognitive deficits in *Atxn1*$^{154Q/2Q}$ mice. A. Schematics of the experiment.

Stereotaxic surgery was used to implant a cannula into a lateral ventricle of 7- week-old *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls (N = 20 each). Each cannula was connected to the subcutaneous osmotic ALZET pump continuously delivering BDNF or aCSF (N = 10) for four weeks. At 11 and 10 weeks rotarod and Barnes maze were used to evaluate motor and cognitive performance. B. Latency to fall on rotarod assay. C. Latency

to escape during four training days on Barnes maze. D. BUNS cognitive score across testing days. E. Strategy development and use during training days of Barnes maze. Data is presented as mean ± SEM with average values for each independent experimental sample represented by a dot. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. Two-way ANOVA with multiple comparisons.

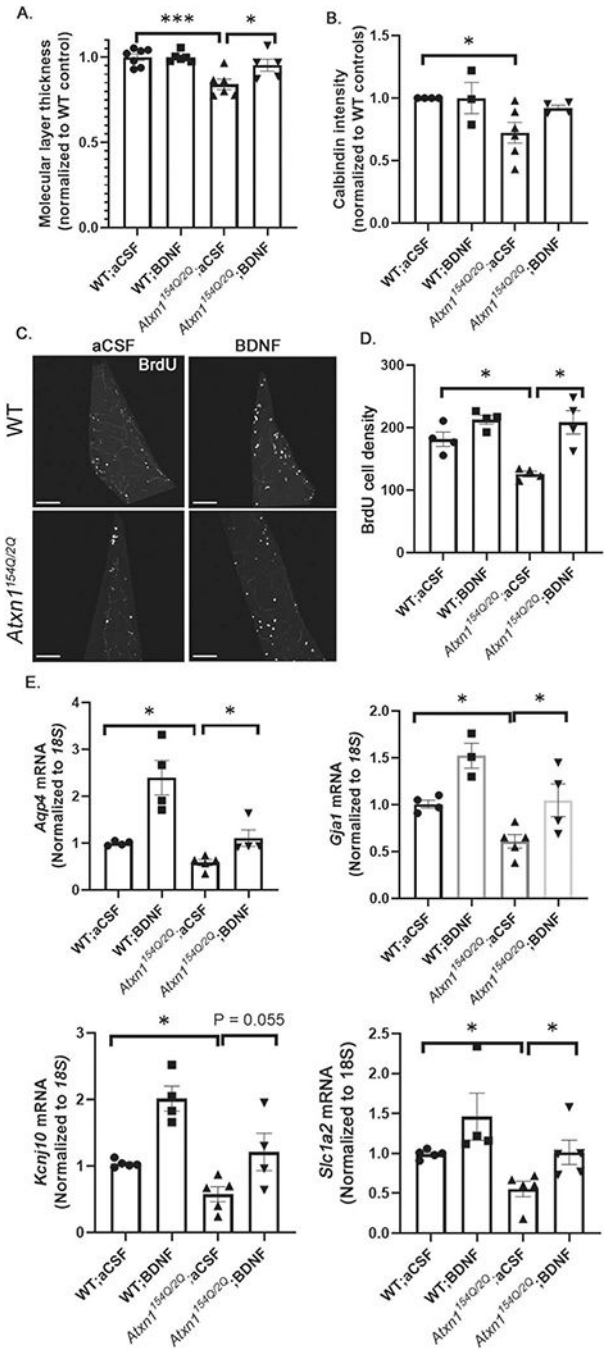

Fig. 4.

BDNF treatment ameliorates cerebellar and hippocampal pathology in SCA1 mice. Purkinje cell dendritic atrophy (molecular layer thickness, A) and calbindin expression (B) were assessed in the molecular layer of the cerebellum at 12 weeks. At 11 weeks mice were injected with BrdU to label proliferating cells in the hippocampal dentate gyrus. Slices were stained with BrdU antibody (C) and confocal images were used to quantify density of proliferating (BrdU labeled) cells (D). Scale bar = 100 μm. E. mRNA was extracted from the hippocampus and RTqPCR was used to evaluate expression of astrocyte specific genes.

Data is presented as mean ± SEM with average values for each independent experimental sample represented by a dot. * $p < 0.05$, ***$p < 0.005$, Two-way ANOVA with multiple comparisons.

Table 1

Tissue samples from patients with SCA1 and controls

Region	Identifier	Sex	Age	Repeats
Cerebellum	Control 1	M	70	Unknown
Cerebellum	Control 2	M	63	Unknown
Cerebellum	Control 3	F	63	Unknown
Cerebellum	Control 4	F	73	Unknown
Cerebellum	Control 5	F	69	Unknown
Cerebellum	SCA1	M	64	45
Cerebellum	SCA1	M	65	42
Cerebellum	SCA1	F	63	41
Cerebellum	SCA1	F	81	38
Cerebellum	SCA1	F	66	46
Medulla	Control 1	M	55	Unknown
Medulla	Control 2	M	59	Unknown
Medulla	Control 3	F	63	Unknown
Medulla	Control 4	F	69	Unknown
Medulla	SCA1	M	59	47
Medulla	SCA1	F	66	46
Medulla	SCA1	F	63	41