Human Molecular Genetics, 2020, Vol. 29, No. 1 117–131

doi: 10.1093/hmg/ddz265
Advance Access Publication Date: 7 November 2019
General Article

GENERAL ARTICLE

Cerebellar contribution to the cognitive alterations in SCA1: evidence from mouse models

Melissa Asher[1], Juao-Guilherme Rosa[1], Orion Rainwater[2], Lisa Duvick[2], Michael Bennyworth[3], Ruo-Yah Lai[4], CRC-SCA [5], Sheng-Han Kuo[4] and Marija Cvetanovic[1,3,*]

[1]Department of Neuroscience, University of Minnesota, Minneapolis, MN 55455, USA, [2]Institute for Translational Neuroscience, University of Minnesota, Minneapolis, MN 55455, USA, [3]Mouse Behavior Core, University of Minnesota, Minneapolis, MN 55455, USA, [4]Department of Neurology, Columbia University, New York, NY 10032-3784, USA, and [5]Clinical Research Consortium for Spinocerebellar Ataxia (CRC-SCA)#

*To whom correspondence should be addressed at: Department of Neuroscience, University of Minnesota, 2101 6th Street SE, Minneapolis, MN 55455, USA. Tel: +612-626-4918; Email: mcvetano@umn.edu

Abstract

Spinocerebellar ataxia type 1 (SCA1) is a fatal neurodegenerative disease caused by abnormal expansion of glutamine (Q) encoding CAG repeats in the gene Ataxin-1 (ATXN1). Although motor and balance deficits are the core symptoms of SCA1, cognitive decline is also commonly observed in patients. While mutant ATXN1 is expressed throughout the brain, pathological findings reveal severe atrophy of cerebellar cortex in SCA1 patients. The cerebellum has recently been implicated in diverse cognitive functions, yet to what extent cerebellar neurodegeneration contributes to cognitive alterations in SCA1 remains poorly understood. Much of our understanding of the mechanisms underlying pathogenesis of motor symptoms in SCA1 comes from mouse models. Reasoning that mouse models could similarly offer important insights into the mechanisms of cognitive alterations in SCA1, we tested cognition in several mouse lines using Barnes maze and fear conditioning. We confirmed cognitive deficits in *Atxn1*$^{154Q/2Q}$ knock-in mice with brain-wide expression of mutant ATXN1 and in ATXN1 null mice. We found that shorter polyQ length and haploinsufficiency of ATXN1 do not cause significant cognitive deficits. Finally, *ATXN1[82Q]* transgenic mice—with cerebellum limited expression of mutant ATXN1—demonstrated milder impairment in most aspects of cognition compared to *Atxn1*$^{154Q/2Q}$ mice, supporting the concept that cognitive deficits in SCA1 arise from a combination of cerebellar and extra-cerebellar dysfunctions.

Introduction

Spinocerebellar ataxia type 1 (SCA1) is a late-onset, progressive and fatal neurodegenerative disease caused by an abnormal CAG repeat expansion in the coding region of *Ataxin-1* (*ATXN1*) gene (1,2). As CAG encodes for glutamine (Q), SCA1 belongs to the group of polyglutamine (polyQ) diseases, which also include Huntington's disease, spinobulbar muscular atrophy, SCA2, 3, 6, 7, 17 and dentatorubral-pallidolysian atrophy (DRPLA) (3,4). CAG repeat length is correlated with disease severity in SCA1, with longer CAG repeats causing earlier age of onset, faster progression and more severe symptoms (5,6). In SCA1, first clinical signs of impaired balance and motor coordination most commonly develop in the fourth decade. The progressive motor disability

†See Appendix 1 for details of the Clinical Research Consortium for Spinocerebellar Ataxia

Received: April 25, 2019. **Revised:** September 30, 2019. **Accepted:** October 23, 2019

in SCA1 patients can be so prominent as to overshadow other aspects of the clinical presentation. However, studies that examined non-motor symptoms report that some of SCA1 patients also experience cognitive deficits (7–14). Moreover, in juvenile onset SCA1 patients (60 or more CAG repeats), cognitive deficits are among the first manifestations of disease and can start as early as 4 years of age and remain an important symptom throughout the disease (15). While motor deficits in SCA1 are thought to be caused by dysfunction and loss of Purkinje neurons in the cerebellum, a brain region known for its importance in the control of movement (16,17), etiology of cognitive deficits remains less understood. Examining the underlying causes of cognitive deficits is necessary to increase our understanding of SCA1 pathogenesis and bring us closer to the future therapies that will treat all aspects of SCA1.

Mouse models have been a crucial tool for understanding the mechanisms of SCA1 pathogenesis. For example, *Atxn1* knockout (*Atxn1*$^{-/-}$) mice show little to no motor deficits, indicating that mutant ATXN1 causes ataxia by toxic gain of function rather than loss of function (18). In addition, *ATXN1[82Q]* transgenic mice (19) (in which expression of mutant ATXN1 is limited to cerebellar Purkinje neurons) develop motor deficits with a similar onset and severity to those of *Atxn1*$^{154Q/2Q}$ knock-in mice (20) (in which mutant ATXN1 is expressed throughout the brain), suggesting that mutant ATXN1 expression in the cerebellum contributes to the motor impairments in SCA1. These comprehensive mouse studies contributed to our understanding that cerebellar dysfunction and gain of mutant ATXN1 function play critical role in SCA1 motor impairments.

On the other hand, cognitive studies in SCA1 mouse models have been less comprehensive. While *Atxn1*$^{154Q/2Q}$ knock-in mice and Atxn1 null mice demonstrated cognitive deficits on Morris Water Maze (MWM), systematic analysis of cognitive performance (e.g. using the same tests on the same genetic background at the same age) is lacking (18,20).

Recent studies provided functional, anatomical and clinical evidence that cerebellum contributes to a host of non-motor functions (21–24), including cognition in many species including mice (25–28). In addition, human and mouse genetic studies implicated ATXN1 (29–31) and the length of its polyQ region (32) in cognitive functions (33,34). We thereby reasoned that systematic examination of cognitive performance in mouse models could offer important insights into the mechanisms of cognitive alterations in SCA1, as it has already done for motor impairment. To address the possible role of polyQ length, loss of ATXN1 function and cerebellar dysfunction, and to confirm previous studies, we performed cognitive testing on the same genetic background and at the same age employing two knock-in mouse models of SCA1, *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ with different polyQ length, Atxn1 null and heterozygous mice, and cerebellum-specific transgenic *ATXN1[82Q]* line.

We demonstrate that cognitive decline in SCA1 knock-in mice correlates with the polyQ length in a similar manner that is seen for motor and balance deficits in SCA1 patients and in SCA1 mouse models. Importantly, we have found poor performance on cognitive tests in *ATXN1[82Q]* mice with Purkinje neuron-restricted expression of mutant ATXN1. While *ATXN1[82Q]* mice were very impaired in developing/adopting optimal goal-reaching strategy in Barnes maze, in most other aspects of cognition, they were less impaired than *Atxn1*$^{154Q/2Q}$ mice. Together with evidence from other studies suggesting that cerebellum contributes to cognition, these results may suggest that cognitive deficits in SCA1 arise from a combination of cerebellar and extra-cerebellar changes. In our two independent cohorts, male *ATXN1[82Q]* mice showed worse cognitive and motor performance. These results may indicate that male cerebellum is more vulnerable in disease—as suggested by studies in other cerebellar mutants (35,36). However, as we have seen this possible effect of sex only in the cerebellum-specific transgenic mice, it is important to note that these findings could be an idiosyncrasy of this specific transgenic line.

In contrast to motor deficits, we find that cognitive deficits could be caused by a complete loss of ATXN1 function in homozygous (but not heterozygous) *Atxn1* knockout mice. This result confirms previous studies indicating that ATXN1 may play a role in regulating cognition (18,37) and suggests that loss of normal ATXN1 function may contribute to cognitive deficits in SCA1 patients. As partial loss of ATXN1 in Atxn1 heterozygous (*Atxn1*$^{+/-}$) mice was not sufficient to cause cognitive decline, our results may indicate safety of SCA1 gene-reduction therapies (38) with regard to cognitive performance.

Finally, we used CRC-SCA natural history study to examine whether human patients show gender differences in ataxia severity (measured by Scale for Assessment and Rating of Ataxia (SARA) score) and self-reported cognitive deficits. In contrast with the mouse results, our analysis indicated that female SCA1 and SCA3 patients demonstrate more severe ataxia and cognitive decline, respectively.

Results

Ataxin-1 knock-in mice show cognitive alterations that correlate with polyglutamine repeat length

We first examined cognitive and mood performance of two SCA1 knock-in mouse lines: *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ mice that express mutant mouse *Atxn1* with 154 and 78 CAG repeats, respectively. Because in these mice, *Atxn1* is expressed under the control of its endogenous promoter, they maintain the physiological spatio-temporal expression pattern and levels of mutant ATXN1. *Atxn1*$^{78Q/2Q}$ mice show significant rotarod deficits only at 9 months of age and no visible ataxia up to 18 months of age (39). For this reason, *Atxn1*$^{154Q/2Q}$ mice were created with a longer polyglutamine repeat expansion (20). *Atxn1*$^{154Q/2Q}$ mice demonstrate most features of SCA1, including motor deficits (as measured by rotarod performance) at 12 weeks (40) and premature death starting around 32 weeks (38). These mice also perform worse on the hidden platform version of the MWM, indicating impaired cognition (20).

We tested cognition in both *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{78Q/2Q}$ mice in parallel to determine if repeat length affects cognition as it does motor ability. To minimize potential complications due to motor deficits, we tested mice at 8 weeks, prior to the age at which *Atxn1*$^{154Q/2Q}$ mice demonstrate detectable ataxia on rotarod in our hands (12 weeks) (40). In addition, we chose the Barnes maze over the MWM as a test of spatial learning and memory as it requires walking and not swimming, making it less demanding on mice, which may be experiencing subtle coordination and movement deficits.

In Barnes maze, mice were subjected to four training trials a day for four days to learn the location of the escape box, followed by a probe trial on the fifth day where the escape box was removed to test their recall. We did not find significant difference in walking speed on Barnes maze between 8-week-old *Atxn1*$^{154Q/2Q}$ mice and their wild-type (WT) littermates (probe day WT average 0.062 m/s, *Atxn1*$^{154Q/2Q}$ average 0.052 m/s, t-test $P = 0.06203$, Supplementary Fig. 1), indicating absence of gross motor impairments at this age that could confound the

interpretation of cognitive tests. Both WT and *Atxn1*$^{154Q/2Q}$ mice seem to learn the task; i.e. they improved during training, as evidenced by decreased path length over the four training days (Fig. 1A, significant effect of day by two-way-repeated-measures(RM) ANOVA, $F_{(3,81)} = 22.37$, $P < 0.0001$). However, *Atxn1*$^{154Q/2Q}$ mice performed poorly on the probe trial compared to WT-control littermate mice (Fig. 1B, average time in goal zone WT 38.82 sec, $N = 17$, *Atxn1*$^{154Q/2Q}$ mice 26.19 sec, $N = 13$, $P = 0.0017$, two-way ANOVA with Bonferroni's *post hoc* test).

Mice can find the escape hole using distinct search strategies that reflect different levels of learning. We employed the Barnes maze unbiased strategy (BUNS) classification (41) to determine whether WT and knock-in mice used similar search strategies. BUNS describes six categories of search strategies, which are assigned a decreasing cognitive score: (1) Direct, in which the mouse takes the most efficient path directly to the goal (cognitive score = 1); (2) corrected, in which the mouse first goes toward one of the holes adjacent to the goal and makes a correction to reach the goal (cognitive score = 0.75); (3) long correction, in which the mouse first moves toward a hole far from the goal and then turns around and goes directly to the goal (cognitive score = 0.5); (4) focused search, in which the mouse scans a larger area near the goal (cognitive score = 0.5), (5) serial, in which the mouse goes around the maze checking each hole in series (cognitive score = 0.25), and (6) random, in which the mouse takes a long and inefficient path with no obvious pattern (cognitive score = 0) (Fig. 1C).

Analysis of search strategies on the four training days showed that WT mice adopted more spatial strategies (i.e. direct, corrected, long correction and focused search) with each day, but that *Atxn1*$^{154Q/2Q}$ mice relied heavily on a serial search strategy (e.g. on day 4: WT mice used 61.7% spatial strategies, 25% serial

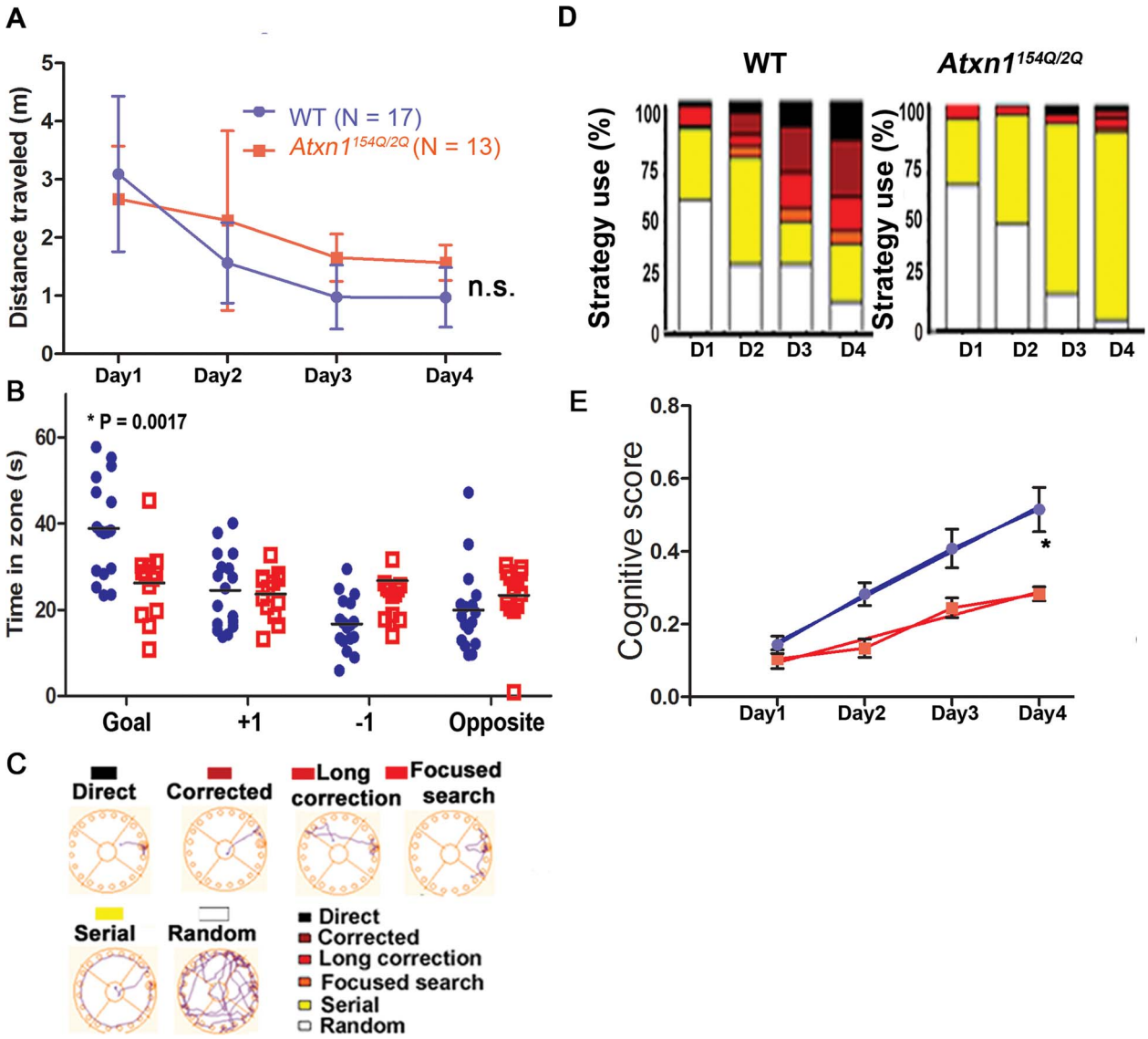

Figure 1. *Atxn1*$^{154Q/2Q}$ mice exhibit cognitive deficits on Barnes maze. *Atxn1*$^{154Q/2Q}$ mice and their WT littermates were tested on the Barnes maze. **(A)** Average path length on each of the four training days (two-way ANOVA, data are presented as mean ± SD). **(B)** Time spent in the goal quadrant during the probe trial (two-way ANOVA, data are presented as mean ± SD). **(C-D)** Barnes maze search strategies (C) and their use (D) in *ATXN1[82Q]* **(right)** mice and their WT littermate controls **(left)**. **(E)** Cognitive scores over the four training days.

and 13.3% random, $N = 17$; while *Atxn1*$^{154Q/2Q}$ mice used 9.8% spatial strategies, 84.3% serial and 5.9% random, $N = 13$; Fig. 1D).

Similarly, WT mice improved their cognitive scores at a significantly faster rate than *Atxn1*$^{154Q/2Q}$ mice (WT: $Y = 0.1239 \times X + 0.005$, *Atxn1*$^{154Q/2Q}$: $Y = 0.064 \times X + 0.029$, significantly higher slope for WT than *Atxn1*$^{154Q/2Q}$ mice, $F_{(1,116)} = 5.76$, $P = 0.0179$; Fig. 1E). This suggests a problem with acquiring spatial memories that *Atxn1*$^{154Q/2Q}$ mice compensated for by adopting a serial search, non-spatial strategy to find escape hole. Both female and male *Atxn1*$^{154Q/2Q}$ mice were significantly impaired.

Walking speed on the Barnes maze did not differ between *Atxn1*$^{78Q/2Q}$ mice and their WT littermates, indicating that as suggested by previous studies, these mice do not have crude motor deficits until 9 months (Supplementary Fig. 2A, probe day WT average 0.084 m/s, *Atxn1*$^{78Q/2Q}$ average 0.074 m/s, Student's t-test $P = 0.2529$). *Atxn1*$^{78Q/2Q}$ mice were not appreciably different from WT littermates during the Barnes maze training period, as measured by path length (Supplementary Fig. 2B, no significant effect of genotype $F_{(1,120)} = 0.56$, $P = 0.4568$, $N = 23$ WT and 15 *Atxn1*$^{78Q/2Q}$ mice), nor during recall on the probe trial (Supplementary Fig. 2C, average time in goal zone WT 45.68 sec, $N = 16$; *Atxn1*$^{78Q/2Q}$ 42.44 sec, $N = 12$, one-way ANOVA with Bonferroni's *post hoc* test $P > 0.05$). *Atxn1*$^{78Q/2Q}$ mice and their WT littermates both learned to use spatial search strategies at similar rates (Supplementary Fig. 2D and E, linear regression: WT: $Y = 0.0771 \times X + 0.21$, *Atxn1*$^{78Q/2Q}$: $Y = 0.01008 \times X + 0.12$, no significant difference between slopes of regression lines, $F_{(1,156)} = 0.73$, $P = 0.3937$, or between elevations, $F_{(1,156)} = 1.207$, $P = 0.2735$).

We used contextual fear conditioning as an additional measure of cognition. Mice received a series of five foot shocks in one context. Twenty-four h later, mice were placed in the same context (the conditioned context) and a different context (the baseline context) to measure recall. During fear conditioning, *Atxn1*$^{154Q/2Q}$ mice acquired freezing responses similarly to WT mice, with no significant effect of genotype (Fig. 2A, $F_{(1,120)} = 3.56$, $P = 0.0617$, $N = 16$ WT and 13 *Atxn1*$^{154Q/2Q}$ mice) or genotype × shock number interaction ($F_{(4,120)} = 1.36$, $P = 0.2530$) by two-way ANOVA). However, when their recall was tested 24 h later, *Atxn1*$^{154Q/2Q}$ mice did not freeze any more in the conditioned context than in the baseline context (baseline context average freezing: WT 13.87%, *Atxn1*$^{154Q/2Q}$ 13.82% freezing, $P > 0.9999$; conditioned context average freezing: WT 33%, *Atxn1*$^{154Q/2Q}$ 13.45% g, $P = 0.0005$, two-way ANOVA followed by Sidak's multiple comparisons test, effect of genotype $F(1, 24) = 6.058$, $P = 0.0214$, $N = 15$ WT and 11 *Atxn1*$^{154Q/2Q}$ mice, Fig. 2B). These results indicate that while *Atxn1*$^{154Q/2Q}$ mice are capable of acquiring fear responses, they demonstrate a profound memory deficit 24 h later.

Atxn1$^{78Q/2Q}$ mice also acquired freezing responses similarly to WT littermates (no effect of genotype by two-way ANOVA; $F_{(1,32)} = 0.63$, $P = 0.4342$, $N = 15$ WT and $N = 17$ *Atxn1*$^{78Q/2Q}$ mice, Supplementary Fig. 3A). When tested for recall 24 h later, *Atxn1*$^{78Q/2Q}$ mice froze at WT levels in both the baseline and conditioned context (Supplementary Fig. 3B, baseline context: WT average 18.74% freezing, *Atxn1*$^{78Q/2Q}$ average 21.93% freezing; conditioned context: WT average 67.15% freezing, *Atxn1*$^{78Q/2Q}$ average 60.09% freezing, one-way ANOVA with Bonferroni's *post hoc* test comparing WT and *Atxn1*$^{78Q/2Q}$, $P > 0.05$).

These results indicate that *Atxn*$^{154Q/2Q}$ mice have cognitive impairment while *Atxn1*$^{78Q/2Q}$ mice do not. This suggests that cognitive deficits, like motor deficits, are affected by CAG repeat length in *Atxn1* knock-in mice and can only be observed with longer CAG repeats.

ATXN1[82Q] mice, with Purkinje neuron-restricted expression of mutant ATXN1, perform poorly on cognitive tests

Both SCA1 patients and SCA1 mouse models demonstrate pronounced cerebellar neurodegeneration with significant loss of Purkinje neurons in the cerebellar cortex (20,42). Because the cerebellum has a well-accepted role in controlling movement, it is thought that cerebellar pathology underlies motor deficits in SCA1 patients. This was further supported by an ataxic phenotype seen in *ATXN1[82Q]* mice, a transgenic mouse model of SCA1 in which expression of mutant ATXN1[82Q] is limited to cerebellar Purkinje neurons under the control of the *Purkinje cell protein 2* (*Pcp2*) promoter. As increasing evidence supports a role for the cerebellum in cognition and mood (43–45), we examined whether cerebellar dysfunction is sufficient to cause cognitive and mood deficits in *ATXN1[82Q]* mice.

We observed no difference in walking speed on the Barnes maze between 8-week-old *ATXN1[82Q]* mice and WT

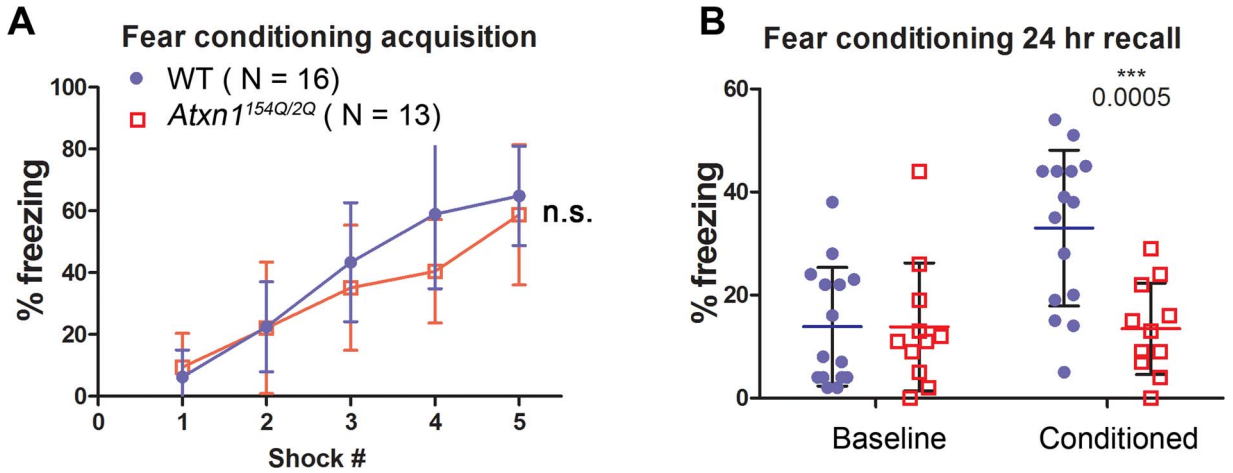

Figure 2. Fear conditioning in SCA1 knock-in mice. **(A)** Acquisition of freezing responses during context fear conditioning in WT and *Atxn1*$^{154Q/2Q}$ mice, reported as percent freezing during the 60 s interval after each shock (two-way ANOVA, data are presented as mean ± SD). **(B)** Twenty-four-h recall in a baseline context and the conditioned context for WT and *Atxn1*$^{154Q/2Q}$ mice (two-way ANOVA, data are presented as mean ± SD).

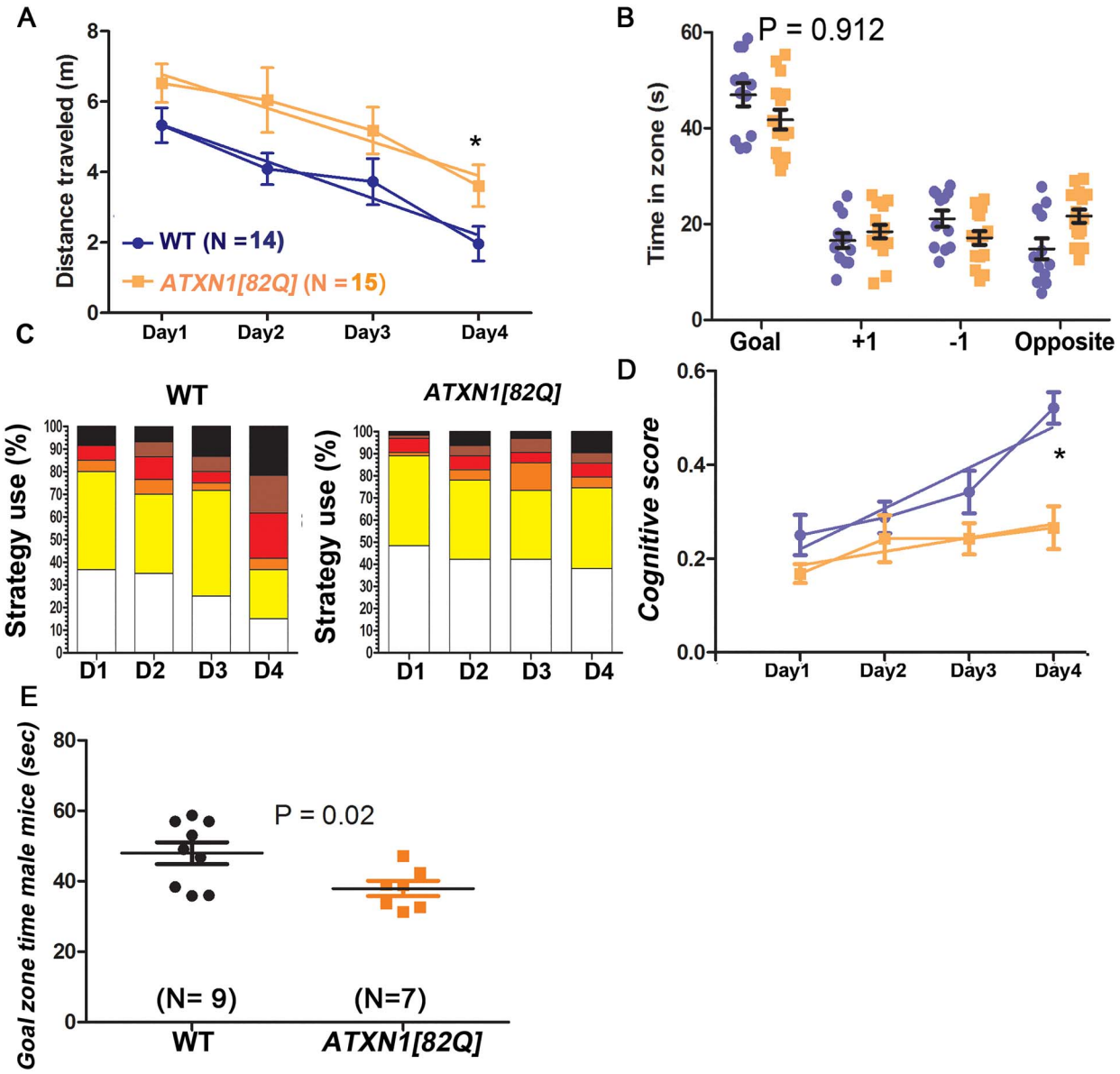

Figure 3. Cerebellum-specific *ATXN1[82Q]* transgenic mice have impaired learning and recall on Barnes maze with male mice being more affected. *ATXN1[82]* mice and their WT littermates were tested on the Barnes maze. **(A)** Average path length on each of the four training days (two-way ANOVA, data are presented as mean ± SD). **(B)** Time spent in the goal quadrant during the probe trial (two-way ANOVA, data are presented as mean ± SD). **(C)** Barnes maze search strategies in *ATXN1[82Q]* mice (**right**) and their WT littermate controls (**left**). **(D)** Cognitive scores over the four training days. **(E)** Time spent in the goal quadrant during the probe trial in male mice (data are presented as mean ± SD, Student's t-test).

littermate controls (Supplementary Fig. 4A, WT = 0.099 m/s, *ATXN1[82Q]* = 0.097 m/s, $N = 14$ and $N = 15$ respectively, $P = 0.6272$, Student's t-test), confirming that in our hands, *ATXN1[82Q]* mice do not show evidence of simple motor impairments at this age.

ATXN1[82Q] mice were impaired in learning over the four training days (measured as a decrease in path length) compared to WT littermates (significant effect of genotype [$F_{(1,29)} = 5.81$ $P = 0.0225$] two-way ANOVA, $N = 14$ WT and 15 *ATXN1[82Q]* mice, Fig. 3A).

We analyzed search strategies to better understand this learning impairment. While WT mice adopted spatial strategies, *ATXN1[82Q]* mice still predominantly used random and serial search strategies to find the escape hole even on day 4 of training (Fig. 3C, day 4: WT mice used 63.3% spatial, 21.7% serial and 15% random strategies; while *ATXN1[82Q]* mice used 25.4% spatial, 33.3% serial and 41.2% random strategies). This indicates impaired spatial learning and/or inability to develop and switch to the use of more efficient strategies. This is also evidenced by a significantly lower slope on the regression line of their cognitive score over time (Fig. 3D, WT: $Y = 0.087 \times X + 0.1333$, *ATXN1 [82Q]*: $Y = 0.0279 \times X + 0.1456$, $F_{(1,120)} = 13.587$, $P < 0.0001$).

On the probe trial, *ATXN1 [82Q]* mice as a whole were not significantly different from WT controls (WT average 46.99 sec, $N = 14$; *ATXN1 [82Q]* average 41.79 sec, $N = 15$, two-way ANOVA effect of genotype F (1,100) = 0.01, $P = 0.9125$, Fig. 3B). This may indicate that they are more impaired in developing efficient strategies to reach the escape hole, as opposed to remembering its location. We also noticed that the male *ATXN1[82Q]* mice

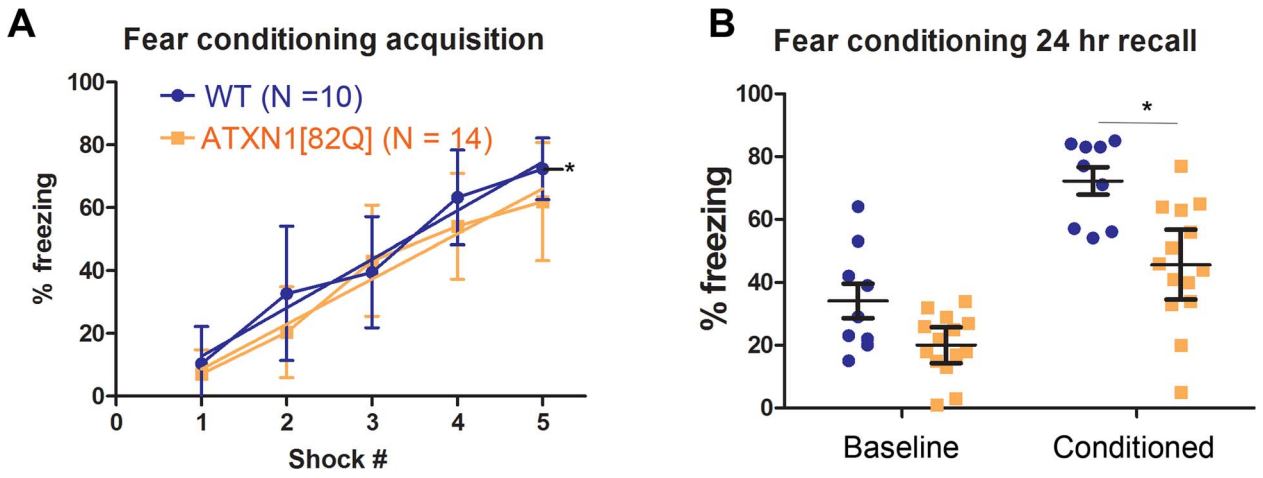

Figure 4. *ATXN1[82Q]* **mice acquire freezing response but have deficient recall 24 h later. (A)** Acquisition of freezing responses during context fear conditioning in WT and *ATXN1[82Q]* mice, reported as percent freezing during the 60 s interval after each shock (two-way ANOVA, data are presented as mean ± SD). **(B)** Freezing during 24 h recall in a baseline context and the conditioned context for WT and *ATXN1[82Q]* mice (two-way ANOVA, data are presented as mean ± SD).

performed significantly worse on the probe trial compared to their WT male littermates (Fig. 3E, WT average 47.96 s, $N = 9$; *ATXN1[82Q]* average 37.92 s, $N = 7$, Student's t-test $P = 0.0253$), while female *ATXN1[82Q]* mice were not distinguishable from female WT littermate controls (Supplementary Fig. 4B**,** WT average 41.51 s, $N = 5$; *ATXN1[82Q]* average 45.94 s, $N = 8$, Student's t-test $P = 0.3991$).

These results indicate that both cerebellum-specific transgenic *ATXN1[82Q]* mice and global knock-in $Atxn1^{154Q/2Q}$ mice perform poorly on the Barnes Maze test. However, in contrast to $Atxn1^{154Q/2Q}$ mice that were able to develop and switch to serial strategy (84.3% on day 4) over random search (5.9%), *ATXN1[82Q]* mice maintained a mix of random (41.2%) and serial search (33.3%), indicating that they might be more impaired in development and switching to more efficient strategies.

We used fear conditioning to examine associative learning in the cerebellum-specific SCA1 mice. *ATXN1[82Q]* mice acquired freezing responses to a slightly lower extent than WT controls (after the last shock WT mice showed 72.4% freezing versus 61.98% freezing in *ATXN1[82Q]* mice, $N = 10$ WT and 14 *ATXN1[82Q]* mice, significant effect of genotype: $F\ (1,110) = 4.72$, $P = 0.0319$, Fig. 4A). In the recall test 24 h later, *ATXN1[82Q]* mice froze less than their WT littermates; this effect was statistically significant in the conditioned context, but not in the control baseline context (Fig. 4B, conditioned context: WT average 72.22% freezing, *ATXN1[82Q]* average 45.64% freezing, $P < 0.001$; baseline context: WT average 34.11% freezing, *ATXN1[82Q]* average 22.0% freezing, $P > 0.05$, two-way ANOVA with Bonferroni's *post hoc* testing). As in the Barnes maze, this effect was largely driven by the male mice, while females were not statistically different from female controls (male mice: WT 70.71% versus *ATXN1[82Q]* 44.3%, $P = 0.01$, female mice: WT 57.3% versus *ATXN1[82Q]* 48%, $P = 0.0637$).

Loss of ATXN1 impairs cognition in mice

Loss of ATXN1 has been implicated in cognitive alterations in humans (29). Previous studies showed that polyglutamine expansion causes both gain and loss of ATXN1 function (46,47). Thus, we examined whether complete or partial loss of ATXN1 function affects performance on cognitive tests in homozygous and heterozygous Atxn1 knockout mice. $Atxn1^{-/-}$ mice have a global deletion of exon 8 of mouse *Atxn1* that contains the majority of the coding region (amino acids 1–616) (18). These mice were originally created on a 129/SvEv background and demonstrated cognitive deficits on MWM and in fear conditioning tests, but had very mild motor deficits (18). Since genetic background is known to influence mouse behavior and other lines that we examined were on C57B6/J background, we examined Atxn1 null mice that were backcrossed onto a C57B6/J background.

Consistent with the previous findings, we found no difference in walking speed between WT and $Atxn1^{-/-}$ mice on the Barnes maze at 8 weeks of age. We were, however, surprised to find a significant decrease in the walking speed in heterozygous $Atxn1^{+/-}$ mice (Supplementary Fig. 5A, one-way ANOVA, WT mice average 0.118 m/s, $N = 15$, $Atxn1^{+/-}$ mice average 0.0875 m/s, $N = 22$, $Atxn1^{-/-}$ mice average 0.126 m/s, $N = 11$, Bonferroni's *post hoc* testing comparing $Atxn1^{+/-}$ to WT mice $P < 0.05$ and $Atxn1^{-/-}$ to WT mice $P > 0.05$). Despite this, mice in each group improved their performance (as measured by path length) over the four training days (Fig. 5A) and we detected no significant effect of genotype on path length.

On the probe trial, we detected a significant effect of genotype on time spent in the goal zone by one-way ANOVA ($F_{(2,45)} = 11.35$, $P = 0.0001$) where $Atxn1^{-/-}$ mice—but not $Atxn1^{+/-}$ mice—performed significantly worse than their WT controls (average time in goal quadrant: WT 43.82 s, $Atxn1^{+/-}$ 47.75 s, $Atxn1^{-/-}$ 28.55 s, $P > 0.05$ for WT versus $Atxn1^{+/-}$ and $P < 0.0001$ for WT versus $Atxn1^{-/-}$, Bonferroni multiple comparisons test, Fig. 5B).

We next examined how loss of ATXN1 expression affects development of more efficient strategies. Although $Atxn1^{+/-}$ mice were indistinguishable from WT mice on the probe trial, they used more serial searches, a non-spatial strategy during training; this effect was further exacerbated in null mice (Fig. 5C, serial search strategies use: WT mice 18.33%, $Atxn1^{+/-}$ mice 36.14%, $Atxn1^{-/-}$ mice 65.9%). Thus, similar to $Atxn1^{154Q/2Q}$ mice, $Atxn1^{+/-}$ and $Atxn1^{-/-}$, mice compensated for decreased spatial learning by adopting a serial search strategy. This was also reflected in their cognitive scores (WT: $Y = 0.091 \times X + 0.098$, $Atxn1^{+/-}$: $Y = 0.098 \times X + 0.02$, $Atxn1^{-/-}$: $Y = 0.08 \times X + 0.05$, Supplementary Fig. 5B). These results suggest

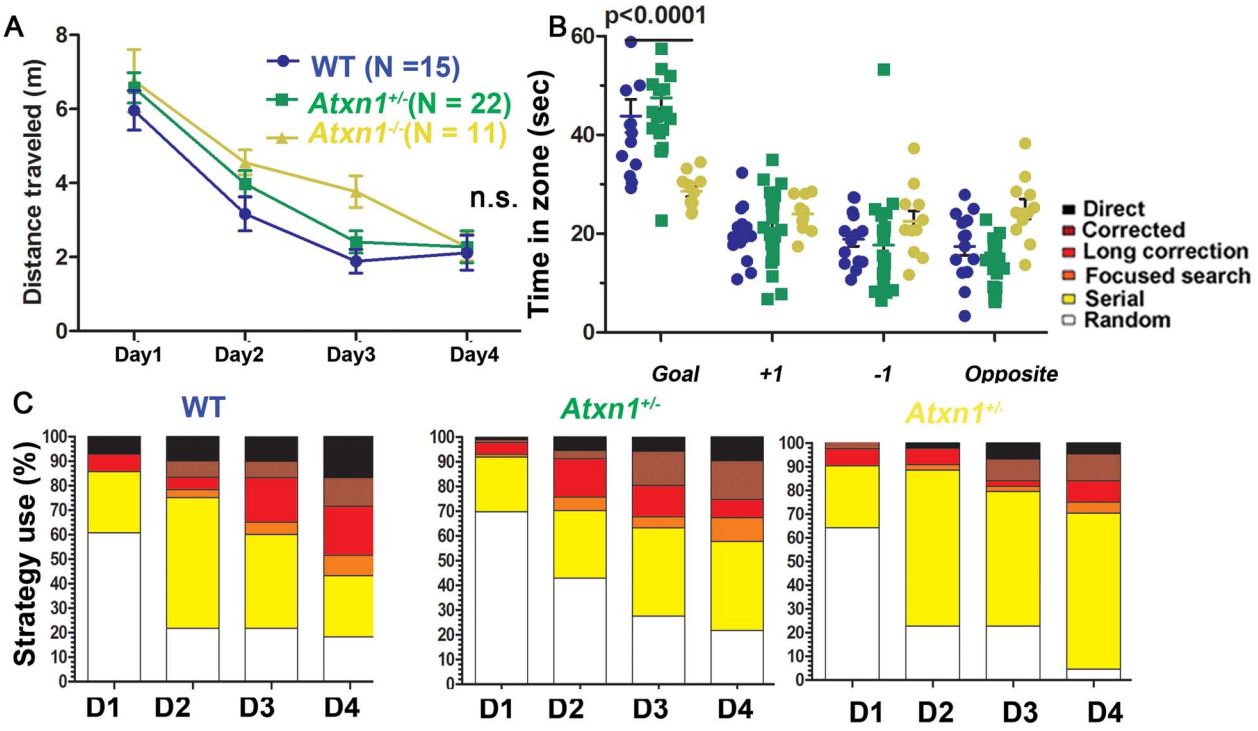

Figure 5. Barnes maze performance is impaired in homozygous (*Atxn1* $^{-/-}$) but not in heterozygous (*Atxn1* $^{+/-}$), *Atxn1* knockout mice. WT, *Atxn1*$^{+/-}$ and *Atxn1*$^{-/-}$ mice were tested on the Barnes maze. **(A)** Average path length on each of the four training days (two-way ANOVA, data are presented as mean ± SD). **(B)** Time spent in the goal quadrant during the probe trial (two-way ANOVA, data are presented as mean ± SD). **(C)** Use of Barnes maze search strategies.

that while *Atxn1*$^{+/-}$ mice may have mild motor and learning deficits, full loss of ATXN1 is required for impaired performance on the Barnes maze.

During contextual fear conditioning, all three groups were able to acquire freezing responses (Fig. 6A, significant effect of shock number by two-way ANOVA, $F_{(4,172)} = 95.85$, $P < 0.0001$, $N = 15$ WT, 21 *Atxn1*$^{+/-}$ and 10 *Atxn1*$^{-/-}$ mice). *Atxn1*$^{-/-}$ mice showed a trend toward freezing less during the last part of acquisition compared to WT littermate controls (67.7% freezing in WT mice versus 49.6% in *Atxn1*$^{-/-}$ mice after the fifth shock) that did not reach statistical significance (one-way ANOVA with Bonferroni, $P > 0.05$). When tested for recall 24 h later (Fig. 6B), *Atxn1*$^{-/-}$ mice, but not *Atxn1*$^{+/-}$ mice, froze significantly less than WT in the conditioned context (WT average 47.68%, *Atxn1*$^{+/-}$ average 46.14% freezing, *Atxn1*$^{-/-}$ average 19.83%, two-way ANOVA, significant effect of genotype $F_{(2,43)} = 6.06$, $P = 0.0002$, $N = 15$ WT, 21 *Atxn1*$^{+/-}$ and 10 *Atxn1*$^{-/-}$ mice). Neither *Atxn1*$^{-/-}$ mice nor *Atxn1*$^{+/-}$ mice differed from WT littermate controls in the baseline context (WT average 15.98% freezing, *Atxn1*$^{+/-}$ average 18.12% freezing, *Atxn1*$^{-/-}$ average 4.9% $P = 0.9758$, Sidak's multiple comparisons test). We did not detect any difference between male and female mice in these tests.

Together, these results indicate that *Atxn1*$^{-/-}$ mice have profound cognitive deficits. In contrast, while *Atxn1*$^{+/-}$ mice may have slightly slower learning, they were indistinguishable from WT littermate controls on recall tests.

Male *ATXN1[82Q]* mice demonstrate poorer motor coordination than female mice

In our cohort of *ATXN1[82Q]* transgenic mice, males showed greater cognitive deficits than females. To examine whether there is a sex effect on the ataxia phenotype in this mouse model of SCA1, we analyzed rotarod performance of a separate cohort of *ATXN1[82Q]* mice at 12 and 20 weeks.

At 12 weeks, female *ATXN1[82Q]* mice were not statistically different than male *ATXN1[82Q]* mice (Fig. 7A, average latency to fall: male WT mice 201.5 ± 14.8 s, $N = 12$, male *ATXN1[82Q]* mice, 151.9 ± 17.8 s, $N = 12$, female WT mice, 256.3 ± 29.6 s, $N = 14$, female *ATXN1[82Q]* mice 130.9 ± 19.9 s, $N = 12$,). However, at 20 weeks of age, male *ATXN1[82Q]* mice performed worse on the rotarod than females, while performance of WT male and female mice was not significantly different (Fig. 7B, average latency: male WT mice 311.5 ± 37.5 s, $N = 11$, male *ATXN1[82Q]* mice, 118.3 ± 10.5 s, $N = 7$, female WT mice 335.5 ± 29.6 s, $N = 12$, female *ATXN1[82Q]* mice 174.2 ± 16.2 s, $N = 10$).

Thus, our results indicate that male *ATXN1[82Q]* mice are more impaired than females, both in cognition and motor coordination.

Sex effects on disease symptoms in SCA patients

Sex effects have been observed in other adult onset neurological disorders, such as Parkinson's disease (48, 49) and multiple sclerosis (50, 51), but have not been extensively explored in SCAs. To examine the effects of sex on clinical phenotype of SCA patients, we studied the CRC-SCA natural history dataset, focusing on the symptoms of ataxia and cognitive impairment. We separately analyzed SCA1, 2, 3 and 6 as four independent cohorts.

We did not observe any differences between men and women in terms of baseline age, disease duration, CAG repeat number or SARA scores. We found that women with SCA3 are more likely to have cognitive impairment than men with SCA3 (Supplemental Table 1).

We performed logistic regression models to study the effects of sex in cognition in SCA patients. While we did not observe

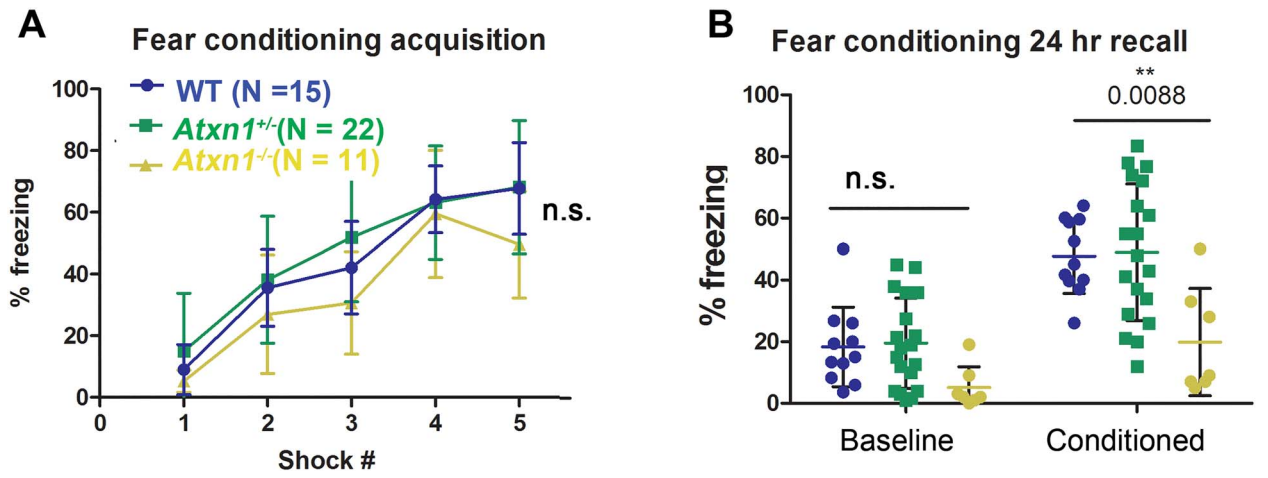

Figure 6. Fear conditioning is impaired in homozygous (*Atxn1* $^{-/-}$) but not in heterozygous (*Atxn1* $^{+/-}$) *Atxn1* knockout mice. (A) Acquisition of freezing responses during context fear conditioning in *Atxn1*$^{+/-}$ *and* *Atxn1*$^{-/-}$ mice and their WT littermates, reported as percent freezing during the 60 s interval after each shock (two-way ANOVA, data are presented as mean ± SD). **(B)** Twenty-four-h recall in a baseline context and the conditioned context (two-way ANOVA, data are presented as mean ± SD).

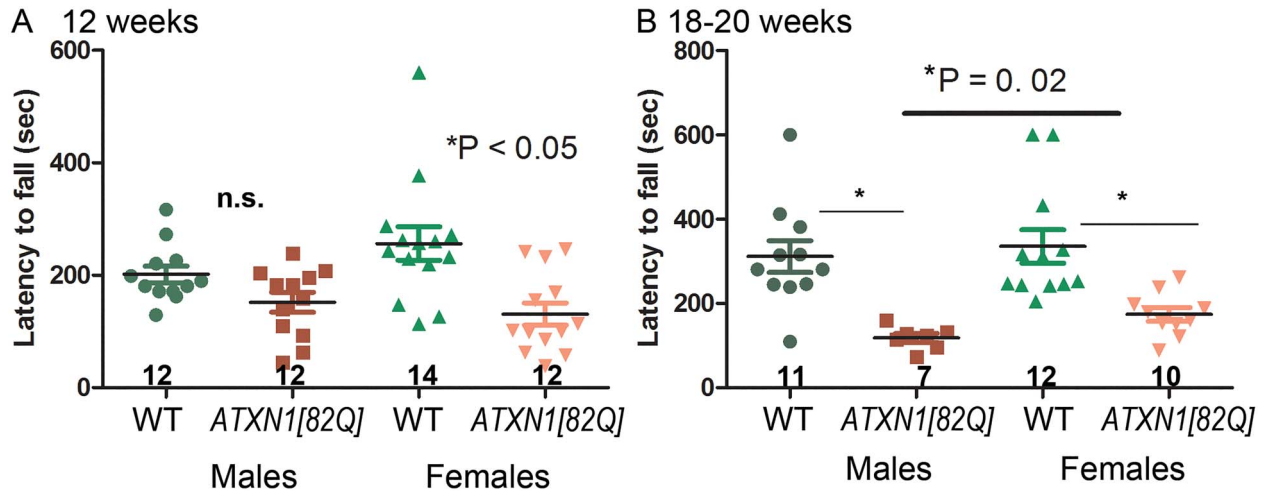

Figure 7. Motor deficits are more severe in male SCA1 transgenic mice. (A-B) *ATXN1[82Q]* mice and their WT littermates were tested on rotarod at early (A: 12 weeks) and mid-stage of disease (B: 18–20 weeks). Average latency to fall on day 4 is presented with SEM. One-way ANOVA *P*-values.

any sex effects in SCA1, 2 and 6 patients, sex played a role in cognition in SCA3 patients, after adjusting for age, disease duration and CAG repeat numbers, with women being more likely to develop cognitive impairment (Supplemental Table 2).

In contrast, we found that sex affects ataxia severity in SCA1 after adjusting for age, disease duration and CAG repeats. On average, women with SCA1 had SARA scores 3.32 points higher than men with SCA1 (Table 1)—this difference is equivalent to 2 years of SCA1 disease progression from the natural history study (52).

Discussion

We used Barnes maze and contextual fear conditioning to systematically investigate cognition in several existing mouse lines on a C57/Bl6 background starting at 8 weeks of age. SCA1 mice do not exhibit motor deficits at this age in our hands (40,53), and we chose Barnes maze (BM) to minimize the effects of the stress on the mouse performance (no food deprivation nor swimming) (54) and contextual fear conditioning as it is passive learning that can be used in mice with motor deficit. In addition, we used the automated BUNS (41) classification algorithm to analyze learning strategies in the Barnes maze, as recent studies indicated that cerebellar Purkinje cells contribute to choosing the optimal trajectory toward a goal during navigation (26,55).

We report here that male transgenic *ATXN1[82Q]* mice with Purkinje neuron restricted expression of mutant ATXN1 and *Atxn1*$^{154Q/2Q}$ SCA1 knock-in mice, which express mutant ATXN1 ubiquitously, perform poorly on Barnes maze and fear conditioning. It is important to note that *Atxn1*$^{154Q/2Q}$ mice were more impaired than *ATXN1[82Q]* mice on most measures (i.e. less time in the goal zone in Barnes maze and less % freezing in fear conditioning), suggesting that both cerebellar and extra-cerebellar dysfunctions contribute to cognitive decline in SCA1. This concept is supported by postmortem pathological analyses describing pathology in other brain regions in SCA1 patients (42,56,57). A prime extra-cerebellar suspect is the hippocampus, which is crucial to both spatial and context-dependent memory. Indeed, we and others have demonstrated that mutant ATXN1 impairs hippocampal neurogenesis and electrophysiological functions (20,58,59).

Table 1. Studying factors that might influence ataxia severity

Variables	Regression coefficients of baseline SARA[a]							
	SCA1		SCA2		SCA3		SCA6	
	B	p-value	B	p-value	B	p-value	B	p-value
Age (years)	0.47	**0.000**	0.28	**0.005**	0.41	**0.000**	0.19	**0.037**
Gender[b]	3.32	**0.048**	−1.08	0.437	−0.80	0.521	−0.64	0.670
Disease duration (years)	0.45	**0.001**	0.34	**0.001**	0.41	**0.000**	0.30	**0.001**
CAG repeat (numbers)	1.40	**0.000**	1.59	**0.000**	1.20	**0.000**	1.16	0.189

Abbreviations: SCA = Spinocerebellar ataxia; SARA = Scale for Assessment and Rating of Ataxia.

All regression coefficients and *p*-value were calculated in the linear regression model, adjusting for age of first visit, gender, disease duration and CAG repeat.
Men = 0 and women = 1.

ATXN1[82Q] mice were incapable of switching from a random search strategy to a more efficient serial search strategy. This result may provide further evidence to the suggested role of the cerebellar Purkinje cells in supporting optimal trajectories in goal-directed navigation (26,55). This would be consistent with the executive function deficits seen in patients with cerebellar injury (43) and SCAs, including SCA1 (60,61). Previous studies demonstrated bi-directional functional communication between the cerebellum and the prefrontal cortex (21,23,62) and cerebellum and hippocampus (28,62), areas involved in spatial navigation, strategy switching and problem solving (63–66). Importantly, we could not detect human *ATXN1[82Q]* mRNA in cortex nor in hippocampus of *ATXN1[82Q]* mice (Supplementary Fig. 6). Thus, we speculate that the cognitive deficits observed in these mice arise from impairments in cerebellar function that alters its interactions with other brain areas such as the prefrontal cortex or hippocampus.

Reduced freezing of transgenic *ATXN1[82Q]* mice on training and trend toward reduced freezing in basal context complicates the interpretation of cognitive deficits in contextual fear conditioning test and may hint at additional behavioral deficits, such as the expression of fear response. Intriguingly, recent studies implicated cerebellum as a part of the neural circuit underlying the expression of freezing and identified cerebellar connections with the periaqueductal gray (PAG) brain region involved in both innate and learnt fear response (67–69).

It is important, however, to note that the observed cognitive deficits in *ATXN1[82Q]* transgenic mice could also be an idiosyncrasy of this specific transgenic line (e.g. caused by overexpression or other genes affected by transgene insertion) (70). Future studies, using conditional ATXN1 knock-in mice, either expressing mutant ATXN1 only in the cerebellum or allowing for deletion of mutant ATXN1 only in the cerebellum, are needed to unequivocally establish relevance/contribution of cerebellar dysfunction to cognitive deficits in SCA1. Whole genome sequence analysis is required to identify the insertion site as well as the presence of transgene insertion/deletions in *ATXN1[82Q]* mice.

Although our cohorts were relatively small, we found worse cognitive and motor deficits in male *ATXN1[82Q]* mice in two independent cohorts. While these results may indicate that male cerebellum is more vulnerable in SCA1 transgenic mice, consistent with previous studies showing sex differences in cerebellar function (35) in WT mice and worse motor deficits in male mice in other cerebellar mutants (*reeler* and *staggerer* mutations (36,68,71), an autism-linked *Gabrb3* mutation (35), and SCA3 transgenic mice (72)), it is important to note that sex difference has not been reported previously in any of the SCA1 mice and that we did not observe it in knock-in *Atxn1*$^{154Q/2Q}$ mice. Thus, it is possible that it is a peculiar feature of this transgenic mouse line or that larger numbers of mice are needed to reliably detect changes in the female transgenic mice. Therefore, future studies will need to replicate these results in a larger cohort of transgenic mice and in knock-in mice with conditional cerebellum-selective expression/inactivation of mutant ATXN1.

Recent studies demonstrate that the cerebellum is involved in non-motor functions (23,44,45,75–79). The most convincing evidence comes from connection studies (80–82), imaging (44,83), and the effects that cerebellar dysfunctions/lesions whether inherited (79) or acquired during childhood (77) or adulthood (43,84), have on cognition, social behavior and mood. For example, the cerebellum is heavily connected to association areas of cortex with non-motor functions (85,86). Moreover, patients with lesions affecting the posterior cerebellum (43) may develop the cerebellar cognitive affective syndrome characterized by impaired executive function, poor visuospatial cognition, linguistic abnormalities and personality changes such as impulsive behavior (43,87,88). The cerebellum is activated during a wide range of cognitive tasks without a heavy motor component (83,89). Based on these and our own results, we propose that the cerebellum acts in concert with other brain areas to produce normal cognition, and that cerebellar degeneration may contribute to cognitive decline.

It is important to note that in agreement with previous studies (18,20), we found that both *Atxn1*$^{154Q/2Q}$ and *Atxn1*$^{-/-}$ mice had severe cognitive deficits. We were able to replicate these conclusions using a different background strain (C57/BL6 as opposed to FVB or mixed FVB/C57 backgrounds, respectively) and different cognitive tests (Barnes maze as opposed to MWM), suggesting that the cognitive deficits are caused by polyQ mutation or loss of ATXN1 rather than by artifacts related to genetic background (18,90,91) or behavioral testing methods.

We also investigated less extreme manipulations of ATXN1 with *Atxn1*$^{78Q/2Q}$ mice (which have a shorter polyQ expansion) and *Atxn1*$^{+/-}$ mice (which have an approximately 50% reduction in ATXN1). The finding that heterozygous knockout mice do not have the same severe cognitive deficits as homozygous knockout mice may also have important implications for SCA1 therapies. Because promising experimental gene therapies for SCA1 (16,38,92) suppress the expression of normal ATXN1 allele in addition to the mutant allele, it is crucial to understand the role of endogenous ataxin-1 to predict and avoid side effects.

Although mouse studies have suggested that mutant ATXN1 contributes to motor deficits via a toxic gain-of-function mechanism in the cerebellum, cognitive changes may be caused by loss of ATXN1 function, given that *Atxn1*$^{-/-}$ mice show severe cognitive deficits (18). This is supported by studies in human patients: chromosomal deletions including the *ATXN1* region cause severe cognitive impairments (29). In addition, ATXN1 has been identified as a risk factor for Alzheimer's disease (34,93) and schizophrenia, with reduced ATXN1 expression in the cortex of schizophrenia patients (32,94). Because both the knockout mouse and human studies investigated global loss of ATXN1 function, brain regions other than cerebellum could be contributing to impaired cognition. In addition, the similarity of learning and memory deficits in *Atxn1*$^{-/-}$ and *Atxn1*$^{154Q/2Q}$ mice suggests that cognitive deficits may be caused by a dominant negative effect of mutant ATXN1 (47). Because loss of ATXN1 function affected cognition in these studies, we investigated heterozygous Atxn1 knockout mice, which have a 50% decrease in ataxin-1 levels comparable to that induced by gene suppression therapy (38). Fortunately, the heterozygous mice had normal cognition on all of our tests, suggesting that these therapies would be well tolerated with regard to cognitive performance in WT mice.

Mutant ATXN1-induced cognitive deficits, like motor deficits, depended on CAG repeat length; global *Atxn1*$^{78Q/2Q}$ knock-in mice with a shorter polyglutamine expansion were cognitively normal. This effect of repeat length could be mediated by the neuropathological or molecular changes in cerebellum or in other brain regions. Although we cannot reproducibly detect cerebellar pathology (synaptic loss and atrophy of Purkinje cell dendrites) in *Atxn1*$^{154Q/2Q}$ mice at 8 weeks (40), when we observed cognitive deficits, there are likely more subtle early changes such as altered gene expression. However, *Atxn1*$^{78Q/2Q}$ mice exhibit no cerebellar degeneration even at 19 months of age (39). Outside of the cerebellum, Watase *et al*. demonstrated electrophysiological changes in the hippocampus of *Atxn1*$^{154Q/2Q}$ mice (albeit at a much later age of 24 weeks) (20), and we previously described a decrease in hippocampal neurogenesis in *Atxn1*$^{154Q/2Q}$ mice at earlier ages (59). Given the contribution of neurogenesis to hippocampal function, we decided to test whether there are any changes in neurogenesis in the *Atxn1*$^{78Q/2Q}$ mice. Using BrdU as a way to identify actively dividing cells, we found that there is a no significant decrease in the number of BrdU-positive cells in the dentate gyrus of *Atxn1*$^{78Q/2Q}$ mice (compared to control WT littermates; Supplementary Fig. 7), consistent with the behavioral results showing no impairment in spatial memory in these mice.

We also investigated cognitive and motor deficits in the largest SCA natural history dataset in North America. On average, we have found that 33% of patients self-report cognitive deficits. Intriguingly, women with SCA1 have more severe ataxia progression than men, while women with SCA3 have faster cognitive decline. The worse progression in female SCA patients was previously observed in the large European natural history studies (96,97). While increased number of patient and mouse studies is required for definitive conclusions regarding the possible role of sex in SCAs patients and in mouse models of SCA to be made, they caution that sex should be considered when designing preclinical and clinical studies (73,74,98,99).

Mouse models have been an essential tool in understanding SCA1 pathogenesis by implicating cerebellar pathology and gain of ATXN1 function as underlying mechanisms of ataxia. Here, we demonstrate their usefulness in studying how cerebellar dysfunction and loss of ATXN1 may contribute to cognitive decline as well.

Materials and Methods

Mice

Atxn1$^{-/-}$ mice (18) on a C57/Bl6 background were a gift from Dr Huda Zoghbi. *Atxn1*$^{154Q/2Q}$, *Atxn1*$^{78Q/2Q}$ and *ATXN1[82Q]* mice originally on FVB background were gifts from Dr Harry Orr and were backcrossed onto a C57/Bl6 background. Mice were housed in a temperature- and humidity-controlled room on a 12 h light/12 h dark cycle with access to food and water *ad libitum*. Mice were tested starting from 8 weeks of age and went through the same order of testing (Barnes maze followed by fear conditioning). All animal experiments were performed in compliance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals and approved by the University of Minnesota Institutional Animal Care and Use Committee.

Cognitive testing

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

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 × 11 cm long × 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 were kept 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, which did not enter the escape box within 3 min, were gently guided to it. Training day data are reported as a path length (distance traveled before entering the escape hole) and analyzed by two-way RM 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 BUNS classification tool as described by Illouz *et al*. (41). 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.

In one cohort of mice, WT mice did not demonstrate learning. As this indicates technical problems, we have excluded that whole cohort from the analysis.

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 foot shocks (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 $\leq$15 lasting $\geq$500 ms.). Twenty-four 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. About, 1–2 h after being placed in the condi-

tioned context, mice were placed in a second context for 3 min to measure baseline freezing. The baseline context used the same chambers but differed from the conditioned context in 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). Acquisition of freezing responses is reported as percent freezing in the 60 s period following each of the five foot shocks, analyzed by two-way RM ANOVA. Twenty-four h recall is reported as percent freezing in each context over the 3 min test period, analyzed by two-way-RM ANOVA.

Motor deficits

Motor deficits were assessed using the rotarod test as previously described in (40). Briefly, mice were placed on a rotarod apparatus (Ugo Basile), which accelerated from a speed of four rotations per minute (rpm) to 40 rpm over a 5-min period. We recorded the latency for each mouse to fall off the rotarod, to a maximum of 10 min. Mice were subjected to four trials per day for four consecutive days, with at least 10 min of rest between each trial. Performance data from day 4 were analyzed using one-way ANOVA with Bonferroni's *post hoc* test. Significance was assumed at $P < 0.05$. The experimenter was blinded to genotype for all tests.

Reverse transcription and quantitative polymerase chain reaction

Total RNA was isolated from dissected cerebella, hippocampi and cortexes (three samples each of WT and *ATXN1[82Q]* mice) using TRIzol reagent (Life Technologies) following the manufacturer's protocols. Tissue was homogenized using RNase-free disposable pellet pestles (Fisher Scientific) in a motorized chuck. RNA was then treated with DNase to remove any contaminating genomic DNA (TURBO DNA-free™ Kit #AM1907, Thermo Scientific) and reverse transcribed in independent duplicate reactions using random hexamers and 500 ng RNA in 10 µL iScript (ADV (172–5038)) reactions. cDNA was diluted 5-fold and 2 µL was used in a qPCR reaction with Light Cycler 480 Probes Master kit (Roche 04–707–494-001) on a Roche 480 Lightcycler. The target gene human Ataxin1 (*hATXN1*) primers (Forward: *AGA GAT AAG CAA CGA CCT GAA GA*, Reverse: *CCA AAA CTT CAA CGC TGA CC*) and probe FAM (465–510) (Probe #67, Universal Probe Library, Roche) and reference gene, mouse glyceraldehyde 3-phosphate dehydrogenase *mGAPDH* primers and probe Hex/Yellow555 (533–580) (05046211001, Universal Probe Library, Roche), reactions were amplified in separate wells under the following cycling conditions: 95°C for 10 s, 60°C for 10 s and for 45 cycles. C_t values were determined using the Roche's second derivative max calculation. The mRNA levels were determined with the $2^{-\Delta\Delta Ct}$ (Ct = threshold cycle) formula normalized to *mGAPDH* RNA and using *ATXN1[82Q]* mice as a reference.

Statistical analysis for animal models

Statistical tests were performed using GraphPad Prism. Data was tested for normal distribution using Kolmogorov–Smirnov and Shapiro–Wilk tests. Parametric tests were performed if normal distribution of the data was established, otherwise non-parametric tests were chosen. Data was analyzed using two-way ANOVA, one-way ANOVA followed by either Tukey's HSD or Bonferroni's *post hoc* test, or Student's t-test.

Clinical features and analysis in SCA patients

We used the National Institutes of Health-funded CRC-SCA natural history dataset (52) for this study. We focused on the symptoms of ataxia and cognitive impairment. Ataxia severity was measured using SARA by ataxia experts (101,102). SARA scores are continuous variables with a higher score indicating more severe symptoms. On the other hand, cognitive impairment is a binary variable to encode the presence or absence or cognitive impairment, which was determined by a composite examination of patients' reports, corroborated by information provided by care-givers and assessment of a neurologist during face-to-face interview. We separately analyzed SCA1, 2, 3 and 6 as four independent cohorts. Linear regression models were constructed using ataxia (SARA) or cognitive deficits as outcome variables, and age, sex and pathological CAG repeat expansions as independent variables to assess the relationship between sex and ataxia and/or cognitive decline after adjusting for age and pathological CAG repeat numbers. We also constructed logistic regression models using cognitive impairment as the binary outcome variable and age, genetic sex and pathological CAG repeat expansions as independent variables. Finally, we examined how the progression of ataxia could be influenced by genetic sex by constructing generalized estimating equation models.

BrdU administration and immunohistochemistry

BrdU (Sigma-Aldrich) was prepared as a 20 mg/mL solution in sterile saline and injected intraperitoneally into 8-week-old mice at a dose of 100 mg/kg every 12 h for three doses, and mice were sacrificed 28 days after the last injection. Brains were perfused with ice-cold PBS, fixed in 4% paraformaldehyde overnight and then incubated in 30% sucrose. For immunohistochemistry of BrdU, 40 µm thick sections were treated with 5 M HCl in 0.1% Triton X-100 for 15 min to expose BrdU binding sites. After five washes in PBS, samples were incubated overnight in anti-BrdU antibody (rat, Bio-Rad #MCA2060) followed by secondary anti-rat Alexa 488 antibody. Samples were mounted using anti-fade mounting media with DAPI (Vectashield, Vector) and analyzed using an Olympus FV1000 confocal microscope. Quantification of positive BrdU+ dentate gyrus was performed by ImageJ, which was previously described in (58). At least three mice per genotype were used for the analysis with statistics performed using the Student's t-test.

Data availability

All the data and materials will be available upon contacting the authors for requests.

Supplementary Material

Supplementary Material is available at HMG *HMG* online.

Conflict of interest statement

The authors declare that they have no conflict of interests.

Acknowledgements

We are grateful to Drs. Huda Y. Zoghbi and Harry T. Orr for providing mouse lines and to all members of Cvetanovic and Orr laboratories for suggestions.

Funding

This work was supported by the Marija Cvetanovic's start-up funds and funding from the Bob Allison Ataxia Research Center.

References

1. Banfi, S., Servadio, A., Chung, M.Y., Kwiatkowski, T.J., McCall, A.E., Duvick, L.A., Shen, Y., Roth, E.J., Orr, H.T. and Zoghbi, H.Y. (1994) Identification and characterization of the gene causing type 1 spinocerebellar ataxia. *Nature Genetics*, **7**, 513.
2. Orr, H.T., Chung, M.Y., Banfi, S., Kwiatkowski, T.J., Servadio, A., Beaudet, A.L., McCall, A.E., Duvick, L.A., Ranum, L.P. and Zoghbi, H.Y. (1993) Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type 1. *Nature Genetics*, **4**, 221.
3. Zoghbi, H.Y. and Orr, H.T. (2009) Pathogenic mechanisms of a polyglutamine-mediated neurodegenerative disease, spinocerebellar ataxia type 1. *Journal of Biological Chemistry*, **284**, 7425–7429.
4. Gusella, J.F. and MacDonald, M.E. (2000) Molecular genetics: unmasking polyglutamine triggers in neurodegenerative disease. *Nature Reviews Neuroscience*, **1**, 109.
5. Genís, D., Matilla, T., Volpini, V., Rosell, J., Dávalos, A., Ferrer, I., Molins, A. and Estivill, X. (1995) Clinical, neuropathologic, and genetic studies of a large spinocerebellar ataxia type 1 (SCA1) kindred:(CAG) n expansion and early premonitory signs and symptoms. *Neurology*, **45**, 24–30.
6. Quan, F., Janas, J. and Popovich, B.W. (1995) A novel CAG repeat configuration in the SCA1 gene: implications for the molecular diagnostics of spinocerebellar ataxia type 1. *Human Molecular Genetics*, **4**, 2411–2413.
7. Bürk, K., Globas, C., Bösch, S., Klockgether, T., Zühlke, C., Daum, I. and Dichgans, J. (2003) Cognitive deficits in spinocerebellar ataxia type 1, 2, and 3. *Journal of Neurology*, **250**, 207–211.
8. Kish, S.J., El-Awar, M., Schut, L., Leach, L., Oscar-Berman, M. and Freedman, M. (1988) Cognitive deficits in olivo-pontocerebellar atrophy: implications for the cholinergic hypothesis of Alzheimer's dementia. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, **24**, 200–206.
9. Moriarty, A., Cook, A., Hunt, H., Adams, M.E., Cipolotti, L. and Giunti, P. (2016) A longitudinal investigation into cognition and disease progression in spinocerebellar ataxia types 1, 2, 3, 6, and 7. *Orphanet Journal of Rare Diseases*, **11**, 82.
10. Illarioshkin, S.N., Slominsky, P.A., Ovchinnikov, I.V., Markova, E.D., Miklina, N.I., Klyushnikov, S.A., Shadrina, M., Vereshchagin, N.V., Limborskaya, S.A. and Ivanova-Smolenskaya, I.A. (1996) Spinocerebellar ataxia type 1 in Russia. *Journal of Neurology*, **243**, 506–510.
11. Fancellu, R., Paridi, D., Tomasello, C., Panzeri, M., Castaldo, A., Genitrini, S., Soliveri, P. and Girotti, F. (2013) Longitudinal study of cognitive and psychiatric functions in spinocerebellar ataxia types 1 and 2. *Journal of Neurology*, **260**, 3134–3143.
12. Ma, J., Wu, C., Lei, J. and Zhang, X. (2014) Cognitive impairments in patients with spinocerebellar ataxia types 1, 2 and 3 are positively correlated to the clinical severity of ataxia symptoms. *International Journal of Clinical and Experimental Medicine*, **7**, 5765.
13. Jacobi, H., Reetz, K., du Montcel, S.T., Bauer, P., Mariotti, C., Nanetti, L., Rakowicz, M., Sulek, A., Durr, A., Charles, P. and Filla, A. (2013) Biological and clinical characteristics of individuals at risk for spinocerebellar ataxia types 1, 2, 3, and 6 in the longitudinal RISCA study: analysis of baseline data. *The Lancet Neurology*, **12**, 650–658.
14. Manto, M. and Lorivel, T. (2009) Cognitive repercussions of hereditary cerebellar disorders. *Cortex*, **47**, 81–100.
15. Zoghbi, H.Y., Pollack, M.S., Lyons, L.A., Ferrell, R.E., Daiger, S.P. and Beaudet, A.L. (1988) Spinocerebellar ataxia: variable age of onset and linkage to human leukocyte antigen in a large kindred. *Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society*, **23**, 580–584.
16. Paulson, H.L., Shakkottai, V.G., Clark, H.B. and Orr, H.T. (2017) Polyglutamine spinocerebellar ataxias—from genes to potential treatments. *Nature Reviews Neuroscience*, **18**, 613.
17. Clark, H.B., Burright, E.N., Yunis, W.S., Larson, S., Wilcox, C., Hartman, B., Matilla, A., Zoghbi, H.Y. and Orr, H.T. (1997) Purkinje cell expression of a mutant allele of SCA1 in transgenic mice leads to disparate effects on motor Behaviors, followed by a progressive cerebellar dysfunction and histological alterations. *Journal of Neuroscience*, **17**, 7385–7395.
18. Matilla, A., Roberson, E.D., Banfi, S., Morales, J., Armstrong, D.L., Burright, E.N., Orr, H.T., Sweatt, J.D., Zoghbi, H.Y. and Matzuk, M.M. (1998) Mice lacking ataxin-1 display learning deficits and decreased hippocampal paired-pulse facilitation. *Journal of Neuroscience*, **18**, 5508–5516.
19. Burright, E.N., Clark, H.B., Servadio, A., Matilla, T., Feddersen, R.M., Yunis, W.S., Duvick, L.A., Zoghbi, H.Y. and Orr, H.T. (1995) SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. *Cell*, **82**, 937–948.
20. Watase, K., Weeber, E.J., Xu, B., Antalffy, B., Yuva-Paylor, L., Hashimoto, K., Kano, M., Atkinson, R., Sun, Y., Armstrong, D.L. and Sweatt, J.D. (2002) A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. *Neuron*, **34**, 905–919.
21. Schmahmann, J.D. (2019) The cerebellum and cognition. *Neuroscience Letters*, **688**, 62–75.
22. Caligiore, D., Pezzulo, G., Baldassarre, G., Bostan, A.C., Strick, P.L., Doya, K., Helmich, R.C., Dirkx, M., Houk, J., Jörntell, H. and Lago-Rodriguez, A. (2017) Consensus paper: towards a systems-level view of cerebellar function: the interplay between cerebellum, basal ganglia, and cortex. *The Cerebellum*, **16**, 203–229.
23. Strick, P.L., Dum, R.P. and Fiez, J.A. (2009) Cerebellum and nonmotor function. *Annual Review of Neuroscience*, **32**, 413–434.
24. Diedrichsen, J., King, M., Hernandez-Castillo, C., Sereno, M. and Ivry, R.B. (2019) Universal transform or multiple functionality? Understanding the contribution of the human cerebellum across task domains. *Neuron*, **102**, 918–928.
25. Badura, A., Verpeut, J.L., Metzger, J.W., Pereira, T.D., Pisano, T.J., Deverett, B., Bakshinskaya, D.E. and Wang, S.S. (2018) Normal cognitive and social development require posterior cerebellar activity. *eLife*, **7**, 1–36.
26. Babayan, B.M., Watilliaux, A., Viejo, G., Paradis, A.L., Girard, B. and Rondi-Reig, L. (2017) A hippocampo-cerebellar cen-

tred network for the learning and execution of sequence-based navigation. *Scientific Reports*, **7**, 1–16.
27. Stoodley, C.J., D'Mello, A.M., Ellegood, J., Jakkamsetti, V., Liu, P., Nebel, M.B., Gibson, J.M., Kelly, E., Meng, F., Cano, C.A. and Pascual, J.M. (2017) Altered cerebellar connectivity in autism and cerebellar-mediated rescue of autism-related behaviors in mice. *Nature Neuroscience*, **20**, 1744–1751.
28. Watson, T.C., Obiang, P., Torres-Herraez, A., Watilliaux, A., Coulon, P., Rochefort, C. and Rondi-Reig, L. (2019) Anatomical and physiological foundations of cerebello-hippocampal interaction. *eLife*, **8**, 1–28.
29. Celestino-Soper, P.B., Skinner, C., Schroer, R., Eng, P., Shenai, J., Nowaczyk, M.M., Terespolsky, D., Cushing, D., Patel, G.S., Immken, L. and Willis, A. (2012) Deletions in chromosome 6p22. 3-p24. 3, including ATXN1, are associated with developmental delay and autism spectrum disorders. *Molecular Cytogenetics*, **5**, 17.
30. Lu, H.C., Tan, Q., Rousseaux, M.W., Wang, W., Kim, J.Y., Richman, R., Wan, Y.W., Yeh, S.Y., Patel, J.M., Liu, X. and Lin, T. (2017) Disruption of the ATXN1–CIC complex causes a spectrum of neurobehavioral phenotypes in mice and humans. *Nat. Gene*, 1–13. doi: 10.1038/ng.3808.
31. Rizzi, T.S., Arias-Vasquez, A., Rommelse, N., Kuntsi, J., Anney, R., Asherson, P., Buitelaar, J., Banaschewski, T., Ebstein, R., Ruano, D. and Van der Sluis, S. (2011) The ATXN1 and TRIM31 genes are related to intelligence in an ADHD background: evidence from a large collaborative study totaling 4,963 subjects. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*, **156**, 145–157.
32. Liu, J. and Su, B. (2018) Integrated analysis supports ATXN1 as a schizophrenia risk gene. *Schizophrenia Research*, **195**, 298–305.
33. Zhang, C., Browne, A., Child, D., DiVito, J.R., Stevenson, J.A. and Tanzi, R.E. (2010) Loss of function of ATXN1 increases amyloid β-protein levels by potentiating β-secretase processing of β-amyloid precursor protein. *The Journal of Biological Chemistry*, **285**, 8515–8526.
34. Bertram, L., Lange, C., Mullin, K., Parkinson, M., Hsiao, M., Hogan, M.F., Schjeide, B.M., Hooli, B., DiVito, J., Ionita, I. and Jiang, H. (2008) Genome-wide association analysis reveals putative Alzheimer's disease susceptibility loci in addition to APOE. *American Journal of Human Genetics*, **83**, 623–632.
35. Mercer, A.A., Palarz, K.J., Tabatadze, N., Woolley, C.S. and Raman, I.M. (2016) Sex differences in cerebellar synaptic transmission and sex-specific responses to autism-linked Gabrb3 mutations in mice. *eLife*, **5**.
36. Doulazmi, M., Frédéric, F., Lemaigre-Dubreuil, Y., Hadj-Sahraoui, N., Delhaye-Bouchaud, N. and Mariani, J. (1999) Cerebellar purkinje cell loss during life span of the heterozygous Staggerer mouse (Rora+/Rorasg) is gender-related. *The Journal of Comparative Neurology*, **273**, 267–273.
37. Lu, H.C., Tan, Q., Rousseaux, M.W., Wang, W., Kim, J.Y., Richman, R., Wan, Y.W., Yeh, S.Y., Patel, J.M., Liu, X. and Lin, T. (2017) Disruption of the ATXN1–CIC complex causes a spectrum of neurobehavioral phenotypes in mice and humans. *Nature Genetics*, **49**, 527–536.
38. Friedrich, J., Kordasiewicz, H.B., O'Callaghan, B., Handler, H.P., Wagener, C., Duvick, L., Swayze, E.E., Rainwater, O., Hofstra, B., Benneyworth, M. and Nichols-Meade, T. (2018) Antisense oligonucleotide–mediated ataxin-1 reduction prolongs survival in SCA1 mice and reveals disease-associated transcriptome profiles. *JCI Insight*, **3**(21).
39. Lorenzetti, D., Watase, K., Xu, B., Matzuk, M.M., Orr, H.T. and Zoghbi, H.Y. (2000) Repeat instability and motor incoordination in mice with a targeted expanded CAG repeat in the Sca1 locus. *Human Molecular Genetics*, **9**, 779–785.
40. Cvetanovic, M., Patel, J.M., Marti, H.H., Kini, A.R. and Opal, P. (2011) Vascular endothelial growth factor ameliorates the ataxic phenotype in a mouse model of spinocerebellar ataxia type 1. *Nature Medicine*, **17**, 1445–1447.
41. Illouz, T., Madar, R., Clague, C., Griffioen, K.J., Louzoun, Y. and Okun, E. (2016) Unbiased classification of spatial strategies in the Barnes maze. *Bioinformatics*, **32**, 3314–3320.
42. Rüb, U., Bürk, K., Timmann, D., den Dunnen, W., Seidel, K., Farrag, K., Brunt, E., Heinsen, H., Egensperger, R., Bornemann, A. and Schwarzacher, S. (2012) Spinocerebellar ataxia type 1 (SCA1): new pathoanatomical and clinicopathological insights. *Neuropathology and Applied Neurobiology*, **38**, 665–680.
43. Schmahmann, J.D. and Sherman, J.C. (1998) The cerebellar cognitive affective syndrome. *Brain: A Journal of Neurology*, **121**, 561–579.
44. Leiner, H.C., Leiner, A.L. and Dow, R.S. (1989) Reappraising the cerebellum: what does the hindbrain contribute to the forebrain? *Behavioral Neuroscience*, **103**, 998–1008.
45. Guell, X., Gabrieli, J.D. and Schmahmann, J.D. (2018) Embodied cognition and the cerebellum: perspectives from the dysmetria of thought and the universal cerebellar transform theories. *Cortex*. **100**, 140–148 doi: 10.1016/j.cortex.2017.07.005.
46. Lam, Y.C., Bowman, A.B., Jafar-Nejad, P., Lim, J., Richman, R., Fryer, J.D., Hyun, E.D., Duvick, L.A., Orr, H.T., Botas, J. and Zoghbi, H.Y. (2006) ATAXIN-1 interacts with the repressor Capicua in its native complex to cause SCA1 neuropathology. *Cell*, **127**, 1335–1347.
47. Lim, J., Crespo-Barreto, J., Jafar-Nejad, P., Bowman, A.B., Richman, R., Hill, D.E., Orr, H.T. and Zoghbi, H.Y. (2008) Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. *Nature*, **452**, 713–718.
48. Lubomski, M., Rushworth, R.L., Lee, W., Bertram, K.L. and Williams, D.R. (2014) Sex differences in Parkinson's disease. *Journal of Clinical Neuroscience*, **21**, 1503–1506.
49. Gillies, G.E., Pienaar, I.S., Vohra, S. and Qamhawi, Z. (2014) Sex differences in Parkinson's disease. *Frontiers in Neuroendocrinology*, **35**, 370–384.
50. Harbo, H.F., Gold, R. and Tintoré, M. (2013) Sex and gender issues in multiple sclerosis. *Therapeutic Advances in Neurological Disorders*, **6**, 237–248.
51. Orton, S.M., Herrera, B.M., Yee, I.M., Valdar, W., Ramagopalan, S.V., Sadovnick, A.D., Ebers, G.C. and Canadian Collaborative Study Group (2006) Sex ratio of multiple sclerosis in Canada: a longitudinal study. *The Lancet Neurology*, **5**, 932–936.
52. Ashizawa, T., Figueroa, K.P., Perlman, S.L., Gomez, C.M., Wilmot, G.R., Schmahmann, J.D., Ying, S.H., Zesiewicz, T.A., Paulson, H.L., Shakkottai, V.G. and Bushara, K.O. (2013) Clinical characteristics of patients with spinocerebellar ataxias 1, 2, 3 and 6 in the US; a prospective observational study. *Orphanet Journal of Rare Diseases*, **8**, 177.
53. Ferro, A., Qu, W., Lukowicz, A., Svedberg, D., Johnson, A. and Cvetanovic, M. (2018) Inhibition of NF-κB signaling in IKKβF/F; LysM Cre mice causes motor deficits but does not alter pathogenesis of Spinocerebellar ataxia type 1. *PLoS One*, **13**, e0200013.

54. Harrison, F.E., Hosseini, A.H. and McDonald, M.P. (2009) Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. *Behavioural Brain Research*, **198**, 247–251.
55. Lefort, J.M., Vincent, J., Tallot, L., Jarlier, F., De Zeeuw, C.I., Rondi-Reig, L. and Rochefort, C. (2019) Impaired cerebellar Purkinje cell potentiation generates unstable spatial map orientation and inaccurate navigation. *Nature Communications*, **10**, 2251.
56. Seidel, K., Siswanto, S., Brunt, E.R., den Dunnen, W., Korf, H.W. and Rüb, U. (2012) Brain pathology of spinocerebellar ataxias. *Acta Neuropathologica*, **124**, 1–21.
57. Rueb, U., Schoels, L., Paulson, H., Auburger, G., Kermer, P., Jen, J.C., Seidel, K., Korf, H.W. and Deller, T. (2013) Clinical features, neurogenetics and neuropathology of the polyglutamine spinocerebellar ataxias type 1, 2, 3, 6 and 7. *Progress in Neurobiology*, **104**, 38–66.
58. Asher, M., Johnson, A., Zecevic, B., Pease, D. and Cvetanovic, M. (2016) Ataxin-1 regulates proliferation of hippocampal neural precursors. *Neuroscience*, **322**, 54–65.
59. Cvetanovic, M., Hu, Y.S. and Opal, P. (2017) Mutant Ataxin-1 inhibits neural progenitor cell proliferation in SCA1. *The. Cerebellum*, **16**, 340–347.
60. Tedesco, A.M., Chiricozzi, F.R., Clausi, S., Lupo, M., Molinari, M. and Leggio, M.G. (2018) The cerebellar cognitive profile. *Brain*. **134**: 3672–3686. doi: 10.1093/brain/awr266.
61. Schmahmann, J.D. and Sherman, J.C (1998) The cerebellar cognitive affective syndrome. *Brain*, **121**, 561–579.
62. Choe, K.Y., Sanchez, C.F., Harris, N.G., Otis, T.S. and Mathews, P.J. (2018) Optogenetic fMRI and electrophysiological identification of region-specific connectivity between the cerebellar cortex and forebrain. *NeuroImage*, **173**, 370–383.
63. Feenstra, M.G. and de Bruin, J.P. (2004) *Prefrontal Cortex: From Synaptic plasticity to Cognition: Strategy Switching and Rat Prefrontal Cortex*. Springer, Boston, MA.
64. Rich, E.L. and Shapiro, M. (2009) Rat prefrontal cortical neurons selectively code strategy switches. *Journal of Neuroscience*, **29**, 7208–7219.
65. Brown, V.J. and Bowman, E.M. (2002) Rodent models of prefrontal cortical function. *Trends in Neurosciences*, **25**, 340–343.
66. Schuck, N.W., Gaschler, R., Wenke, D., Heinzle, J., Frensch, P.A., Haynes, J.D. and Reverberi, C. (2015) Medial prefrontal cortex predicts internally driven strategy shifts. *Neuron*, **86**, 331–340.
67. Cacciola, A., Bertino, S., Basile, G.A., Di Mauro, D., Calamuneri, A., Chillemi, G., Duca, A., Bruschetta, D., Flace, P., Favaloro, A. and Calabrò, R.S. (2019) Mapping the structural connectivity between the periaqueductal gray and the cerebellum in humans. *Brain Structure and Function*, **224**, 2153–2165.
68. Koutsikou, S., Watson, T.C., Crook, J.J., Leith, J.L., Lawrenson, C.L., Apps, R. and Lumb, B.M. (2015) The periaqueductal gray orchestrates sensory and motor circuits at multiple levels of the neuraxis. *Journal of Neuroscience*, **35**, 14132–14147.
69. Watson, T.C., Cerminara, N.L., Lumb, B.M. and Apps, R. (2016) Neural correlates of fear in the periaqueductal gray. *Journal of Neuroscience*, **36**, 12707–12719.
70. Gamache, J., Benzow, K., Forster, C., Kemper, L., Hlynialuk, C., Furrow, E., Ashe, K.H. and Koob, M.D. (2019) Factors other than hTau overexpression that contribute to tauopathy-like phenotype in rTg4510 mice. *Nature Communications*. **10**, 2479. doi: 10.1038/s41467-019-10428-1.
71. Hadj-Sahraoui, N., Frédéric, F., Delhaye-Bouchaud, N. and Mariani, J. (1996) Gender effect on Purkinje cell loss in the cerebellum of the heterozygous reeler mouse. *Journal of Neurogenetics*, **11**, 45–58.
72. Silva-Fernandes, A., Duarte-Silva, S., Neves-Carvalho, A., Amorim, M., Soares-Cunha, C., Oliveira, P., Thirstrup, K., Teixeira-Castro, A. and Maciel, P. (2014) Chronic treatment with 17-DMAG improves balance and coordination in a new mouse model of Machado-Joseph disease. *Neurotherapeutics*. **11**: 433–449. doi: 10.1007/s13311-013-0255-9.
73. Wang, Z., Gerstein, M. and Snyder, M. (2014) The cerebellum, sensitive periods, and autism. *Neuron*, **83**, 518–532.
74. Dean, S.L. and McCarthy, M.M. (2008) Steroids, sex and the cerebellar cortex: implications for human disease. *The Cerebellum*, **7**(1), 38–47.
75. Appollonio, I.M., Grafman, J., Schwartz, V., Massaquoi, S. and Hallett, M. (1993) Memory in patients with cerebellar degeneration. *Neurology*, **43**(8), 1536.
76. Drepper, J., Timmann, D., Kolb, F.P. and Diener, H.C. (1999) Non-motor associative learning in patients with isolated degenerative cerebellar disease. *Brain*, **122**, 87–97.
77. Levisohn, L., Cronin-Golomb, A. and Schmahmann, J.D. (2000) Neuropsychological consequences of cerebellar tumour resection in children: cerebellar cognitive affective syndrome in a paediatric population. *Brain*, **123**(5), 1041–1050.
78. Allin, M., Matsumoto, H., Santhouse, A.M., Nosarti, C., AlAsady, M.H., Stewart, A.L., Rifkin, L. and Murray, R.M. (2001) Cognitive and motor function and the size of the cerebellum in adolescents born very pre-term. *Brain*, **124**, 60–66.
79. Tavano, A., Grasso, R., Gagliardi, C., Triulzi, F., Bresolin, N., Fabbro, F. and Borgatti, R. (2007) Disorders of cognitive and affective development in cerebellar malformations. *Brain*, **130**, 2646–2660.
80. Schmahmann, J.D. (1996) From movement to thought: anatomic substrates of the cerebellar contribution to cognitive processing. *Human Brain Mapping*, **4**(3), 174–198.
81. Coffman, K.A., Dum, R.P. and Strick, P.L. (2011) Cerebellar vermis is a target of projections from the motor areas in the cerebral cortex. *Proceedings of the National Academy of Sciences*, **108**, 16068–16073. doi: 10.1073/pnas.1107904108.
82. Middleton, F.A. and Strick, P.L. (1994) Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science*, **266**, 458–461.
83. Kim, S.G., Ugurbil, K. and Strick, P.L. (1994) Activation of a cerebellar output nucleus during cognitive processing. *Science*, **265**, 949–951.
84. Stoodley, C.J. and Schmahmann, J.D. (2010) Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex*, **46**, 831–844.
85. Shakkottai, V.G., Batla, A., Bhatia, K., Dauer, W.T., Dresel, C., Niethammer, M., Eidelberg, D., Raike, R.S., Smith, Y., Jinnah, H.A. and Hess, E.J. (2017) Current opinions and areas of consensus on the role of the cerebellum in dystonia. *The Cerebellum*. **16**:577–594. doi: 10.1007/s12311-016-0825-6.
86. Bostan, A.C., Dum, R.P. and Strick, P.L. (2010) The basal ganglia communicate with the cerebellum. *Proceedings of the National Academy of Sciences*, **107**, 8452–8456.
87. Stoodley, C.J., MacMore, J.P., Makris, N., Sherman, J.C. and Schmahmann, J.D. (2016) Location of lesion determines motor vs. cognitive consequences in patients with cerebellar stroke. *NeuroImage: Clinical*, **12**, 765–775.

88. Tedesco, A.M., Chiricozzi, F.R., Clausi, S., Lupo, M., Molinari, M. and Leggio, M.G. (2011) The cerebellar cognitive profile. *Brain*, **134**.
89. Stoodley, C.J. and Schmahmann, J.D. (2009) Functional topography in the human cerebellum: a meta-analysis of neuroimaging studies. *NeuroImage*, **44**(2), 489–501.
90. Spencer, C.M., Alekseyenko, O., Hamilton, S.M., Thomas, A.M., Serysheva, E., Yuva-Paylor, L.A. and Paylor, R. (2011) Modifying behavioral phenotypes in Fmr1KO mice: genetic background differences reveal autistic-like responses. *Autism Research*. **4**, 40–56. doi: 10.1002/aur.168.
91. Han, H.J., Allen, C.C., Buchovecky, C.M., Yetman, M.J., Born, H.A., Marin, M.A., Rodgers, S.P., Song, B.J., Lu, H.C., Justice, M.J. and Probst, F.J. (2012) Strain background influences neurotoxicity and behavioral abnormalities in mice expressing the tetracycline transactivator. *Journal of Neuroscience*, **32**, 10574–10586.
92. Keiser, M.S., Kordasiewicz, H.B. and McBride, J.L. (2015) Gene suppression strategies for dominantly inherited neurodegenerative diseases: lessons from Huntington's disease and spinocerebellar ataxia. *Human Molecular Genetics*, **25**, R53–R64.
93. Bettens, K., Brouwers, N., Van Miegroet, H., Gil, A., Engelborghs, S., De Deyn, P.P., Vandenberghe, R., Van Broeckhoven, C. and Sleegers, K. (2010) Follow-up study of susceptibility loci for Alzheimer's disease and onset age identified by genome-wide association. *Journal of Alzheimer's Disease*, **19**, 1169–1175.
94. Joo, E.J., Lee, J.H., Cannon, T.D. and Price, R.A. (1999) Possible association between schizophrenia and a CAG repeat polymorphism in the spinocerebellar ataxia type 1 (SCA1) gene on human chromosome 6p23. *Psychiatric Genetics*, **9**, 7–11.
95. Klockgether, T., Lüdtke, R., Kramer, B., Abele, M., Bürk, K., Schöls, L., Riess, O., Laccone, F., Boesch, S., Lopes-Cendes, I. and Brice, A. (1998) The natural history of degenerative ataxia: a retrospective study in 466 patients. *Brain: A Journal of Neurology*, **121**(4), 589–600.
96. Jacobi, H., Bauer, P., Giunti, P., Labrum, R., Sweeney, M.G., Charles, P., Dürr, A., Marelli, C., Globas, C., Linnemann, C. and Schöls, L. (2011) The natural history of spinocerebellar ataxia type 1, 2, 3, and 6: a 2-year follow-up study. *Neurology*, **77**, 1035–1041.
97. Hedges, V.L., Ebner, T.J., Meisel, R.L. and Mermelstein, P.G. (2012) The cerebellum as a target for estrogen action. *Frontiers in Neuroendocrinology*, **33**, 612–626.
98. Brown, C.M., Suzuki, S., Jelks, K.A. and Wise, P.M. (2009) Estradiol is a potent protective, restorative, and trophic factor after brain injury. *Seminars in Reproductive Medicine*, **27**, 240–249.
99. Lo, R.Y., Figueroa, K.P., Pulst, S.M., Perlman, S., Wilmot, G., Gomez, C., Schmahmann, J., Paulson, H., Shakkottai, V.G., Ying, S. and Zesiewicz, T. (2016) Depression and clinical progression in spinocerebellar ataxias. *Parkinsonism & Related Disorders*, **22**, 87–92.
100. Schmitz-Hübsch, T., Du Montcel, S.T., Baliko, L., Berciano, J., Boesch, S., Depondt, C., Giunti, P., Globas, C., Infante, J., Kang, J.S. and Kremer, B. (2006) Scale for the assessment and rating of ataxia: development of a new clinical scale. *Neurology*, **66**, 1717–1720.