Bushart Dave (Orcid ID: 0000-0002-3852-127X)

Shakkottai Vikram (Orcid ID: 0000-0003-2742-6469)

Author Manuscript

A chlorzoxazone-baclofen combination improves cerebellar impairment in spinocerebellar ataxia type 1

David D. Bushart PhD[1,2], Haoran Huang BS[1], Luke J. Man BS[1], Logan M. Morrison BS[1,3], Vikram G. Shakkottai MD, PhD[1,4,*]

[1]Department of Neurology, University of Michigan, Ann Arbor, MI 48109

[2]Ohio State University College of Medicine, Columbus, OH 43210 (current location)

[3]Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI 48109

[4]Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI 48109

*Corresponding Author:

Vikram G. Shakkottai

4009 BSRB

109 Zina Pitcher Place

Ann Arbor, MI 48109

(734) 615-6891

vikramsh@med.umich.edu

Word count: 3940 words

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/mds.28355

This article is protected by copyright. All rights reserved.

Author Manuscript

Running title: Chlorzoxazone-baclofen improves cerebellar function in SCA1

Key words: Ataxia, SCA1, chlorzoxazone, baclofen, neuronal dysfunction

Financial disclosure/conflict of interest: US Patent Application Number: 16/182965, titled: Therapeutic Combination for Treatment of Cerebellar Ataxia. Inventors: Vikram Shakkottai, David Bushart. Assignee: The Regents of the University of Michigan

Funding sources: NIH R01 NS085054 (VGS)

Abstract

Background: A combination of central muscle relaxants, chlorzoxazone and baclofen (chlorzoxazone-baclofen), has been proposed for treatment of cerebellar symptoms in human spinocerebellar ataxia (SCA). However, central muscle relaxants can worsen balance. The optimal dose for target engagement without toxicity remains unknown.

Objectives: Using the genetically precise *Atxn1*$^{154Q/2Q}$ model of SCA1, we determine the role of cerebellar dysfunction in motor impairment. We also aim to identify appropriate concentrations of chlorzoxazone-baclofen needed for target engagement without toxicity to plan for human clinical trials.

Methods: We use patch clamp electrophysiology in acute cerebellar slices and immunostaining to identify the specific ion channels targeted by chlorzoxazone-baclofen. Behavioral assays for coordination and grip strength are used to determine specificity of chlorzoxazone-baclofen for improving cerebellar dysfunction without off-target effects in *Atxn1*$^{154Q/2Q}$ mice.

Results: We identify irregular Purkinje neuron firing in association with reduced expression of the ion channels *Kcnma1* and *Cacna1g* in *Atxn1*$^{154Q/2Q}$ mice. Using *in vitro* electrophysiology in brain slices, we identify concentrations of chlorzoxazone-baclofen that improve Purkinje neuron

This article is protected by copyright. All rights reserved.

spike regularity without reducing firing frequency. At a disease stage in *Atxn1*$^{154Q/2Q}$ mice when motor impairment is due to cerebellar dysfunction, orally administered chlorzoxazone-baclofen improves motor performance without affecting muscle strength.

Conclusion: We identify a tight relationship between baclofen-chlorzoxazone concentrations needed to engage target, and levels above which cerebellar function will be compromised. We propose to use this information for a novel clinical trial design, using sequential dose escalation within each subject, to identify dose levels that are likely to improve ataxia symptoms while minimizing toxicity.

Author Manuscript

Introduction

The CAG triplet repeat-associated spinocerebellar ataxias (SCA) are a group of neurodegenerative diseases that result in progressive loss of motor function and early death [1]. Distinct genetic mutations underlie each of the CAG triplet repeat-associated SCAs; nevertheless, recent work illustrates that shared underlying mechanisms of disease may contribute to neuronal dysfunction across these disorders [2-5]. SCA1, one of the most common and well-studied of the SCAs, is caused by a glutamine-encoding CAG triplet expansion in the *ATXN1* gene. Cerebellar Purkinje neurons are prominently affected in SCA1 and are believed to underlie motor impairment, while dysfunction of brainstem nuclei and other non-cerebellar structures correlates more closely with early death [6-8]. However, the relative contribution of these structures to motor impairment at various stages of disease remains unclear.

This article is protected by copyright. All rights reserved.

Author Manuscript

Recent work demonstrates that Purkinje neuron dysfunction is a central cause of motor impairment in rodent models of SCA. In murine models, abnormalities in Purkinje neuron membrane excitability often manifest as irregular, slow, or absent spiking. Interestingly, specific ion-channels have been associated with firing dysfunction across several of these models [2-5], suggesting shared mechanisms of disease and potentially overlapping therapeutic targets. These channels include the large conductance calcium-activated potassium (BK) channel, along with calcium sources that can activate BK function [5]. In the transgenic ATXN1[82Q] mouse model of SCA1, targeting reduced BK channel function with the FDA-approved potassium channel activating compounds chlorzoxazone and baclofen (chlorzoxazone-baclofen) can restore Purkinje neuron spiking and improve motor dysfunction [9]. Due to Purkinje neuron-specific expression of the ATXN1[82Q] transgene in this model of SCA1 [10], the ability of chlorzoxazone-baclofen to improve cerebellar symptoms without worsening non-cerebellar symptoms is not currently known.

The benefit of chlorzoxazone-baclofen on Purkinje neuron spiking has previously been assessed at a single concentration in the transgenic ATXN1[82Q] model of SCA1 [9]. In these mice, Purkinje neurons undergo depolarization block due to profound loss of BK channel current early in disease, causing a majority of cells to cease firing at the onset of symptoms [3]. Since ATXN1[82Q] transgene expression is restricted to Purkinje neurons in this model, it is unclear whether chlorzoxazone-baclofen may have off-target effects outside of the cerebellum. It is therefore imperative to identify the concentration ranges of chlorzoxazone-baclofen that can optimally improve Purkinje neuron function while minimizing extra-cerebellar toxicity. This will

This article is protected by copyright. All rights reserved.

enable the design of future clinical trials to assess the safety and efficacy of chlorzoxazone-baclofen in human SCA.

In the present work, we use a genetically precise knock-in mouse model of SCA1 to explore appropriate concentrations and target engagement of chlorzoxazone-baclofen. Our studies demonstrate that BK channels are the likely calcium-activated potassium channel target in SCA1. Importantly, chlorzoxazone-baclofen co-treatment improves motor function in SCA1 mice at an age when the motor phenotype is not confounded by changes in grip strength. In addition, chlorzoxazone-baclofen show no signs of toxicity in SCA1 mice and do not worsen deficits in grip strength at a later disease timepoint when motor weakness is present. These data indicate that chlorzoxazone-baclofen treatment specifically targets cerebellar dysfunction in SCA1, and supports the further development of chlorzoxazone-baclofen as a treatment for cerebellar symptoms in SCA1.

Methods

Animals

All animal studies were reviewed and approved by the University of Michigan Institutional Animal Care and Use Committee and were conducted in accordance with the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals. *Atxn1*$^{154Q/2Q}$ mice, which express an expanded CAG triplet repeat in the endogenous *Atxn1* locus [11], were maintained on a C57BL/6 background. Heterozygous *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate

Author Manuscript

This article is protected by copyright. All rights reserved.

controls were used for all studies. All studies were performed at 14 weeks of age or 20 weeks of age. Sexes were balanced for all animal studies.

Supplemental Methods

Please see the Supplemental Methods section of this manuscript for additional information.

Results

Purkinje neuron dysfunction is associated with motor impairment preceding neurodegeneration in *Atxn1*$^{154Q/2Q}$ mice

Purkinje neuron dysfunction is an early feature of disease in many models of spinocerebellar ataxia (SCA) [2-4, 12-17]. Changes in Purkinje neuron firing are directly relevant to disease, as improving Purkinje neuron firing also improves motor dysfunction in murine SCA models [4, 9, 13, 15-17]. Abnormalities in Purkinje neuron firing are particularly relevant in SCA1, as pharmacologic and genetic strategies to improve Purkinje neuron intrinsic excitability improves not just motor dysfunction but also degeneration in murine models of disease [3, 17, 18]. Prior studies in SCA1 have focused primarily on the transgenic ATXN1[82Q] model of SCA1, where polyglutamine expanded ATXN1 expression is restricted to Purkinje neurons. While the ATXN1[82Q] model of SCA1 accurately models cerebellar features of disease, the relevance of cerebellar dysfunction to the overall motor phenotype is unknown in the more genetically precise *Atxn1*$^{154Q/2Q}$ model of SCA1, in which a polyglutamine-expanded *Atxn1* transgene is knocked into the endogenous *Atxn1* locus, and therefore widely expressed in the nervous system and elsewhere. In this model of SCA1 with a hyperexpanded polyglutamine repeat

Author Manuscript

This article is protected by copyright. All rights reserved.

motor dysfunction and premature death have been attributed, at least in part to spinal cord and brainstem motor neuron degeneration [7, 11]. We therefore sought to identify whether cerebellar dysfunction is present, and relevant to the motor phenotype in *Atxn1*$^{154Q/2Q}$ mice.

We examined Purkinje neuron membrane excitability in *Atxn1*$^{154Q/2Q}$ mice at 14 weeks of age, an age at which motor impairment is prominent [11]. No evidence of neurodegeneration is present in *Atxn1*$^{154Q/2Q}$ mice even at 20 weeks of age (Supplementary Figure 1A-B). When compared to Purkinje neurons from wild-type littermate controls, *Atxn1*$^{154Q/2Q}$ Purkinje neurons display irregular spiking (Figure 1A-C) with no change in firing frequency (Figure 1D). Since Purkinje neuron firing differs between the anterior and posterior cerebellum in SCA murine models [4, 5], we independently analyzed neurons from the anterior cerebellum (lobules IV-V) and nodular zone (lobule X) and found a similar increase in spike irregularity with no change in firing frequency across both cerebellar regions. Additionally, since alterations in Purkinje neuron physiology may reflect changes in either intrinsic pacemaking or synaptic activity, we performed recordings in *Atxn1*$^{154Q/2Q}$ and wild-type control Purkinje neurons before and after the addition of synaptic inhibitors (6,7-dinitroquinoxaline-2,3-dione [DNQX], 10 µM; picrotoxin, 50 µM). No significant difference in either spike frequency (Supplementary Figure 1C) or regularity (Supplementary Figure 1D) was noted after the addition of synaptic inhibitors, suggesting that altered Purkinje neuron spiking in *Atxn1*$^{154Q/2Q}$ mice is intrinsically driven. When neurons were held at -80 mV and injected with depolarizing current, we found that *Atxn1*$^{154Q/2Q}$ neurons require a significantly lower amplitude of injected current to elicit depolarization block than wild-type littermate control neurons (Figure 1E). Importantly, these alterations in spiking were

Author Manuscript

This article is protected by copyright. All rights reserved.

accompanied by no significant change in input resistance between *Atxn1*$^{154Q/2Q}$ and wild-type littermate control neurons (Figure 1F).

Since irregular Purkinje neuron spiking is associated with deficits in the afterhyperpolarization (AHP) in various mouse models of ataxia [2-4, 19], we examined differences in spike waveform between *Atxn1*$^{154Q/2Q}$ Purkinje neurons and wild-type littermate controls. We found that the AHP in *Atxn1*$^{154Q/2Q}$ mice was depolarized when compared to wild-type littermate control cells (Figure 1G-I). No other significant changes in spike waveform were noted in *Atxn1*$^{154Q/2Q}$ Purkinje neurons when compared to wild-type littermate controls (Supplementary Table 1). Together, these data indicate that a specific reduction in AHP amplitude accompanies irregular spiking and neuronal hyperexcitability in *Atxn1*$^{154Q/2Q}$ mice at a stage of disease with prominent motor impairment.

Changes in ion channel expression or function accompany changes in Purkinje neuron spiking in mouse models of SCA [12]. The observed irregular spiking and reduced AHP amplitude observed in *Atxn1*$^{154Q/2Q}$ Purkinje neurons is consistent with calcium-activated potassium (K_{Ca}) channel dysfunction or voltage-gated calcium (Ca_V) channel dysfunction [3, 15, 19-21]. We examined previously published microarray data to identify potential ion channel transcript dysregulation in *Atxn1*$^{154Q/2Q}$ and identified reduced transcripts for *Kcnma1, Cacna1g, Trpc3* and *Itpr1* [5, 22]. Reduced *Kcnma1* and *Cacna1g* transcripts were observed at 14 weeks of age in *Atxn1*$^{154Q/2Q}$ mice when compared to wild-type littermate controls [5]. We therefore sought to quantify protein expression of large conductance K_{Ca} (BK) channels (encoded by *Kcnma1*) and

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

putative calcium sources that may activate BK, including $Ca_V3.1$ (*Cacna1g*) [4], $Ca_V2.1$ (*Cacna1a*) [23], and the IP3 receptor (*Itpr1*) [24] in *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls at 14 weeks of age. In both the anterior cerebellum (Figure 2A) and nodular zone (Figure 2B), fluorescence intensity of BK and $Ca_V3.1$ (Figure 2C) was reduced in the cerebellar molecular layer of *Atxn1*$^{154Q/2Q}$ mice compared to wild-type littermate controls, while the IP3 receptor and $Ca_V2.1$ (Figure 2C) showed no change in expression. Together, these data suggest that reduced cerebellar expression of BK and $Ca_V3.1$ underlies the altered physiology observed in *Atxn1*$^{154Q/2Q}$ mice.

A combination of chlorzoxazone and baclofen improves *Atxn1*$^{154Q/2Q}$ Purkinje neuron firing

The altered Purkinje neuron spiking observed in symptomatic *Atxn1*$^{154Q/2Q}$ mice is similar to neuronal dysfunction seen in another mouse model of SCA1, the ATXN1[82Q] transgenic model [3]. More substantial loss of BK and Cav3.1 channel expression in ATXN1[82Q] mice results in a more prominent electrophysiologic phenotype, in which neurons undergo a complete loss of spiking [3]. This nevertheless represents a continuum with the irregular spiking observed in *Atxn1*$^{154Q/2Q}$ Purkinje neurons [5] (Figure 1). The shared underlying dysfunction of BK channels and reduced AHP amplitude suggests that BK channels may be an important target in SCA1.

In transgenic ATXN1[82Q] mice, combined treatment with chlorzoxazone and baclofen targets potassium channel dysfunction to improve Purkinje neuron spiking and motor impairment at both early- and mid-symptomatic ages [9]. Chlorzoxazone is a canonical K_{Ca} channel activator

This article is protected by copyright. All rights reserved.

Author Manuscript

that can target BK channels [25]. Baclofen, a $GABA_B$ receptor agonist, is a known activator of subthreshold-activated potassium channels in Purkinje neurons [26]. Subthreshold-activated potassium channels can compensate for loss of the AHP and facilitate repetitive spiking in Purkinje neurons [2]. In ATXN1[82Q] mice, a combination of chlorzoxazone and baclofen (chlorzoxazone-baclofen) was necessary to achieve adequate K_{Ca} channel activation and restoration of function [9]. We wished to determine the necessity of a combination of these agents to improve the more modestly impaired firing in *Atxn1*$^{154Q/2Q}$ mice. Since SCA1-KI mice exhibit a gradation of Purkinje neuron firing abnormalities, this allows for investigation of dose-dependence of chlorzoxazone-baclofen to correct irregular spiking. It is important to determine the optimal concentrations of chlorzoxazone-baclofen as excessive activation of K^+ channels results in reduction in Purkinje neuron firing frequency, which is independently correlated with motor dysfunction (reviewed in [27]).

The effect of different doses of chlorzoxazone and baclofen were assessed in acute cerebellar slices from 14-week *Atxn1*$^{154Q/2Q}$ mice by perfusing varying doses of either individual compounds or combinations of chlorzoxazone-baclofen. A high combined dose of chlorzoxazone (50 µM) and baclofen (2 µM) was able to improve spike regularity in *Atxn1*$^{154Q/2Q}$ Purkinje neurons, as indicated by a reduction in the coefficient of variation (CV) of the interspike interval (ISI) (Figure 3A). An intermediate combined dose of chlorzoxazone (10 µM) and baclofen (400 nM) similarly reduced the CV of the ISI in *Atxn1*$^{154Q/2Q}$ neurons (Figure 3A), while a lower combined dose of chlorzoxazone (5 µM) and baclofen (100 nM) had no effect on CV of the ISI (Figure 3A). Firing frequency was examined in these same recordings. Firing frequency

This article is protected by copyright. All rights reserved.

Author Manuscript

was greatly suppressed by the high combined dose of chlorzoxazone (50 µM) and baclofen (2 µM), while no effect on firing frequency was observed at the intermediate combined dose of chlorzoxazone (10 µM) and baclofen (400 nM) (Figure 3B). A small reduction in firing frequency was observed in the low combined dose of chlorzoxazone (5 µM) and baclofen (100 nM) (Figure 3B). Interestingly, when treated with the intermediate dose of chlorzoxazone alone (10 µM; Figure 3C) or baclofen alone (400 nM; Figure 3D), the CV of the ISI was not changed from baseline. This is contrast to perfusion of higher dose chlorzoxazone alone (50 µM) or baclofen alone (2 µM), which both suppress firing frequency (Supplementary Figure 2A-B) while also reducing the CV of the ISI (Supplementary Figure 2C-D). Taken together, these data suggest that chlorzoxazone-baclofen improves Purkinje neuron spiking in a dose-responsive manner in *Atxn1*$^{154Q/2Q}$ mice, and that the intermediate dose of a combination of chlorzoxazone (10 µM) and baclofen (400 nM) has the optimal effect of improving Purkinje neuron spiking irregularity without affecting firing frequency in acute cerebellar slices. Additionally, these data clearly indicate the necessity of a combination of chlorzoxazone and baclofen to achieve the effect of improving spike regularity while not affecting firing frequency.

Chlorzoxazone and baclofen specifically target cerebellar dysfunction in SCA1

Atxn1$^{154Q/2Q}$ mice have a hyperexpanded polyglutamine repeat. In human SCA1, large repeat expansions are associated with brainstem predominant disease and premature death in the second decade of life [28-30]. It is important to know whether cerebellar dysfunction contributes to motor impairment in *Atxn1*$^{154Q/2Q}$ mice and accurately serves as a model for testing therapy for cerebellar dysfunction, the major source of disability and quality of life in human SCA1.

This article is protected by copyright. All rights reserved.

Atxn1$^{154Q/2Q}$ mice are described to have motor neuron involvement [7], which may confound interpretation of the rotarod task in mice. We therefore evaluated grip strength, a measure of motor neuron function, using the hanging wire task. At 14 weeks of age, no impairments in grip strength were noted in *Atxn1*$^{154Q/2Q}$ mice when compared to wild-type littermate controls (Figure 4A). *Atxn1*$^{154Q/2Q}$ mice were administered chlorzoxazone-baclofen in drinking water at a dose expected to achieve the intermediate concentrations (see Figure 4 of [9]) of chlorzoxazone (1-10 µM) and baclofen (100 nM-1 µM) in the brain. Importantly, chlorzoxazone-baclofen did not affect grip strength of 14 week-old *Atxn1*$^{154Q/2Q}$ mice (Figure 4A). The rotarod assay is commonly used to test balance and gait coordination, and reflects balance impairment when not confounded by impaired muscle strength [31]. At 14 weeks, *Atxn1*$^{154Q/2Q}$ mice display significantly impaired motor function on the rotarod assay when compared to wild-type littermate controls (Figure 4B). The absence of grip-strength impairment in the *Atxn1*$^{154Q/2Q}$ mice provides more confidence that the rotarod impairment at this stage of disease is due to cerebellar dysfunction. Treatment with chlorzoxazone-baclofen significantly improved motor performance in 14 week-old *Atxn1*$^{154Q/2Q}$ mice (Figure 4B).

We next assessed grip strength in the same cohorts of chlorzoxazone-baclofen treated *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls at 20 weeks of age. Since there were no significant changes in grip strength due to treatment condition within each genotype (Figure 4C), we examined changes in grip strength when pooled by genotype at this later stage of disease. *Atxn1*$^{154Q/2Q}$ mice display a significant impairment in grip strength compared to wild-type littermate controls (Wild-type: 104.7 ± 5.112 seconds; *Atxn1*$^{154Q/2Q}$: 79.90 ± 7.158 seconds; p =

Author Manuscript

This article is protected by copyright. All rights reserved.

0.0065, Student's t-test; data presented as mean ± standard error of the mean). Reassuringly, chlorzoxazone-baclofen, which are FDA approved skeletal muscle relaxants, did not worsen the grip strength deficits in *Atxn1*$^{154Q/2Q}$ mice (Figure 4C). At this stage of disease, it would be expected that rotarod performance of *Atxn1*$^{154Q/2Q}$ mice would be further impaired, referable to the observed grip strength deficits. Consistent with this hypothesis, 20-week *Atxn1*$^{154Q/2Q}$ mice displayed significantly impaired motor performance on the rotarod compared to wild-type littermate controls to a larger degree than was observed at 14 weeks of age. Chlorzoxazone-baclofen did not rescue the rotarod impairment in *Atxn1*$^{154Q/2Q}$ mice at this stage of disease (Figure 4D), but, importantly, also did not worsen motor performance in 20 week-old *Atxn1*$^{154Q/2Q}$ mice, consistent with the lack of effect of chlorzoxazone-baclofen on grip strength at this age.

Since chlorzoxazone-baclofen is being considered for clinical use in patients with ataxia [9], we evaluated for potential toxicity of long-term use of this combination in *Atxn1*$^{154Q/2Q}$ mice. As reported previously, *Atxn1*$^{154Q/2Q}$ mice fail to gain weight compared to wild-type littermate controls, which is evident in both male and female mice at both 14 (Figure 5A-B, Supplementary Figure 3A) and 20 (Figure 5C-D, Supplementary Figure 3B) weeks of age [11]. Chlorzoxazone-baclofen did not accelerate this failure to gain weight. A slightly lower baseline weight was observed in chlorzoxazone-baclofen treated wild-type mice (Figure 5A), which was also noted in these same mice at 20 weeks of age (Figure 5C). However, this difference in weight was not accelerated in chlorzoxazone-baclofen treated female wild-type mice when compared to vehicle treated controls at the two recorded timepoints (Figure 5E), suggesting that these mice still gain weight normally and are not experiencing toxicity from chlorzoxazone-baclofen treatment.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

Together, these behavioral studies indicate that at 14 weeks, cerebellar dysfunction contributes meaningfully to motor impairment in *Atxn1*$^{154Q/2Q}$ mice and can be ameliorated by a treatment strategy targeting cerebellar dysfunction. At a later disease stage, *Atxn1*$^{154Q/2Q}$ mice display a motor phenotype that is driven by impairments of muscle strength. These findings reinforce the idea that chlorzoxazone-baclofen specifically targets motor impairment due to cerebellar dysfunction in SCA1.

Discussion

Chlorzoxazone-baclofen has been proposed for the treatment of cerebellar motor dysfunction in SCA [9]. However, the optimal dose of chlorzoxazone-baclofen for target engagement without off-target toxicity is not known. In the present study, we identify a dose range of chlorzoxazone-baclofen that improves Purkinje neuron spike regularity in the *Atxn1*$^{154Q/2Q}$ model of SCA1 without suppressing firing frequency. Importantly, chlorzoxazone-baclofen improves cerebellar motor dysfunction in *Atxn1*$^{154Q/2Q}$ mice at an age when motor impairment is cerebellar in origin, and does not negatively affect grip strength. At a later stage of disease, when motor impairment is worsened by impaired grip strength, chlorzoxazone-baclofen does not cause further impairment in motor function. Taken together, these studies suggest that chlorzoxazone-baclofen can be dosed to properly engage target in the cerebellum while simultaneously minimizing off-target toxicity. Human clinical trials of chlorzoxazone-baclofen will determine whether this drug combination may be effective to also treat cerebellar motor impairment in human SCA.

This article is protected by copyright. All rights reserved.

Author Manuscript

In the transgenic ATXN1[82Q] mouse model of SCA1, chlorzoxazone-baclofen improves cerebellar Purkinje neuron physiology and motor impairment by compensating for reduced potassium channel transcripts and function [9]. In the present study, chlorzoxazone-baclofen similarly improves motor function in *Atxn1*$^{154Q/2Q}$ mice at an age when motor dysfunction is cerebellar in origin. The less severe degree of potassium channel transcript reduction in *Atxn1*$^{154Q/2Q}$ mice compared to ATXN1[82Q] mice [5] explains the less severe Purkinje neuron firing abnormalities in *Atxn1*$^{154Q/2Q}$ mice. Despite a longer polyglutamine repeat in *Atxn1*$^{154Q/2Q}$ mice, the less severe cerebellar phenotype is likely due to more modest endogenous levels of mutant Atxn1 expression in *Atxn1*$^{154Q/2Q}$ mice as compared to the overexpression of mutant ATXN1 in ATXN1[82Q] Purkinje neurons [10, 11]. In ATXN1[82Q] mice, the benefit of chlorzoxazone-baclofen persists at later stage disease, despite prominent Purkinje neuron dendritic degeneration seen at this disease stage [9]. Chlorzoxazone-baclofen was, however, unable to sustain improved motor function in *Atxn1*$^{154Q/2Q}$ mice in later stage disease. This discrepancy may be explained by the genetic differences in these two models. The transgenic ATXN1[82Q] model of SCA1 overexpresses mutant *ATXN1* specifically in cerebellar Purkinje neurons under control of the *Pcp2* promoter [10]. Conversely, *Atxn1*$^{154Q/2Q}$ mice express glutamine-expanded *Atxn1* at its endogenous locus, resulting in widespread expression throughout the central nervous system [11]. As a result, motor impairment in ATXN1[82Q] mice is cerebellar in origin, even in late stage disease, and largely reflects Purkinje neuron dysfunction. Spinal motor neuron involvement in *Atxn1*$^{154Q/2Q}$ mice likely contributes to motor impairment in this model of SCA1 [7, 11]. The current study suggests that motor dysfunction in mid-stage disease in *Atxn1*$^{154Q/2Q}$ mice is driven by cerebellar dysfunction, as evidenced by the

This article is protected by copyright. All rights reserved.

improvement in motor function with chlorzoxazone-baclofen, whereas later stage motor impairment is a result of motor neuron dysfunction that is not improved by chlorzoxazone-baclofen. In this regard, *Atxn1*$^{154Q/2Q}$ mice likely recapitulate features of human SCA1, where morbidity is primarily cerebellar in origin in earlier stages of disease [32, 33], whereas end stage disease is due to cranial motor neuron dysfunction [34]. Chlorzoxazone-baclofen is likely therefore to have the maximal clinical benefit at stages of disease where motor dysfunction is cerebellar in origin. A combination of gene-specific disease-modifying therapy in combination with symptomatic therapy is likely to be most effective in improving quality of life in patients with ataxia.

Chlorzoxazone and baclofen are used clinically as muscle relaxants and are not recommended for simultaneous dosing in patients due to concerns about tolerability [35]. A major concern of using central muscle relaxants in SCA is the potential to worsen motor impairment [36]. However, the present data suggest that chlorzoxazone-baclofen can engage target in cerebellar Purkinje neurons while avoiding toxicity related to impaired muscle strength. An optimal effect of combined chlorzoxazone and baclofen treatment would be to improve the regularity of spiking in SCA1 Purkinje neurons without slowing firing frequency, as both irregular spiking and slow Purkinje neuron firing are associated with motor impairment in mouse models of ataxia [4, 12, 14-16, 19, 37]. In the current study, we identify a tight relationship between drug concentrations needed to engage target, and levels above which cerebellar function will be compromised. In planning for a human clinical trial with this drug combination to improve cerebellar ataxia, it will be important to identify the specific orally administered dose of chlorzoxazone-baclofen that

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

achieves appropriate brain levels. We propose that a scheme of sequential dose escalation within each clinical trial-subject would be the most effective way to identify doses of chlorzoxazone-baclofen that engage target optimally without compromising cerebellar function, by allowing for monitoring of plasma and potentially cerebrospinal fluid levels of chlorzoxazone-baclofen along with effects on cerebellar motor function. In the current study, a high cerebellar concentration of chlorzoxazone-baclofen (50 µM and 2 µM, respectively) impairs cerebellar function, while a low concentration of chlorzoxazone-baclofen (5 µM and 0.2 µM, respectively) fails to engage target. An intermediate concentration of chlorzoxazone-baclofen (10 µM and 0.4 µM, respectively) at the site of action in the cerebellum is associated with optimal improvements in cerebellar physiology. Brain concentrations of baclofen are ~5 fold lower than plasma concentrations in both humans and mice [38]. Our studies in mice suggest that brain and plasma concentrations of chlorzoxazone reach near equilibrium in mice following chronic administration [9]. We therefore propose, based on the *in vitro* data obtained in the current study, that a plasma concentration range between 8-40 µM chlorzoxazone and 1-4 µM baclofen will be associated with improvement in cerebellar function in human SCA. It is likely that the relationship between administered drug dose, plasma/CSF concentrations of chlorzoxazone-baclofen, and changes in cerebellar function with each drug dose will need to be closely monitored for individual trial participants in a potential efficacy study.

Interestingly, the ion channels that underlie altered Purkinje neuron physiology in *Atxn1*$^{154Q/2Q}$ mice are also implicated in neuronal dysfunction in other mouse models of SCA. These channels, BK (encoded by *Kcnma1*) and $Ca_V3.1$ (encoded by *Cacna1g*), show reduced

This article is protected by copyright. All rights reserved.

Author Manuscript

expression in another mouse model of SCA1 [5], several mouse models of SCA2 [5], and a mouse model of SCA7 [4]. BK channels rely upon intracellular calcium for activation, and recent evidence suggests that $Ca_V3.1$ may be an important calcium source for BK [4, 5]. Despite SCA1, SCA2, and SCA7 resulting from polyglutamine expansions in distinct genes, dysfunction of a BK channel functional module appears to contribute to altered Purkinje neuron spiking and cerebellar motor impairment in all of these disorders [5]. As illustrated in the present study, and in the ATXN1[82Q] model of SCA1 [9], chlorzoxazone-baclofen improves Purkinje neuron physiology through engagement of potassium channel targets. Therefore, it is possible that chlorzoxazone-baclofen may have relevance for treating motor impairment beyond SCA1. Notably, chlorzoxazone treatment has also been shown to improve aberrant Purkinje neuron spiking and motor performance in other murine models of cerebellar ataxia [37, 39-41]. A combination of chlorzoxazone-baclofen has not, however, been tested in rodent models of polyglutamine ataxia other than SCA1, although a related genetic strategy of viral BK channel replacement improves Purkinje neuron physiology in SCA7 [4]. It is tempting to speculate that a shared mechanism of dysfunction exists across multiple etiologies of SCA, and that a treatment strategy such as chlorzoxazone-baclofen may therefore have wider relevance for targeting cerebellar motor impairment.

Author Roles

1. Research project: A. Conception, B. Organization, C. Execution; 2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique; 3. Manuscript Preparation: A. Writing of the first draft, B. Review and Critique

This article is protected by copyright. All rights reserved.

DDB 1A, 1B, 1C, 2A, 2B, 3A, 3B

HH 1B, 1C

LJM 1C

LMM 1C, 2B, 3B

VGS 1A, 1B, 2C, 3A, 3B

Financial Disclosure

VGS receives royalties from UpToDate Inc.

All other authors have no financial conflicts of interest to disclose.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

References

1. Durr, A., *Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond.* Lancet Neurol, 2010. **9**(9): p. 885-94.
2. Dell'Orco, J.M., S.M. Pulst, and V.G. Shakkottai, *Potassium channel dysfunction underlies Purkinje neuron spiking abnormalities in spinocerebellar ataxia type 2.* Human Molecular Genetics, 2017. **26**(20): p. 3935-3945.
3. Dell'Orco, J.M., et al., *Neuronal Atrophy Early in Degenerative Ataxia Is a Compensatory Mechanism to Regulate Membrane Excitability.* Journal of Neuroscience, 2015. **35**(32): p. 11292-11307.
4. Stoyas, C.A., et al., *Nicotinamide Pathway-Dependent Sirt1 Activation Restores Calcium Homeostasis to Achieve Neuroprotection in Spinocerebellar Ataxia Type 7.* Neuron, 2020. **105**(4): p. 630-+.
5. Chopra, R., et al., *Altered Capicua expression drives regional Purkinje neuron vulnerability through ion channel gene dysregulation in spinocerebellar ataxia type 1.* Hum Mol Genet, 2020.
6. Genis, D., et al., *Clinical, neuropathologic, and genetic studies of a large spinocerebellar ataxia type 1 (SCA1) kindred: (CAG)n expansion and early premonitory signs and symptoms.* Neurology, 1995. **45**(1): p. 24-30.
7. Orengo, J.P., et al., *Motor neuron degeneration correlates with respiratory dysfunction in SCA1.* Dis Model Mech, 2018. **11**(2).
8. Rub, U., et al., *Spinocerebellar ataxia type 1 (SCA1): new pathoanatomical and clinico-pathological insights.* Neuropathol Appl Neurobiol, 2012. **38**(7): p. 665-80.

This article is protected by copyright. All rights reserved.

Author Manuscript

9. Bushart, D.D., et al., *Targeting potassium channels to treat cerebellar ataxia.* Ann Clin Transl Neurol, 2018. **5**(3): p. 297-314.

10. Burright, E.N., et al., *SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat.* Cell, 1995. **82**(6): p. 937-48.

11. Watase, K., et al., *A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration.* Neuron, 2002. **34**(6): p. 905-19.

12. Bushart, D.D. and V.G. Shakkottai, *Ion channel dysfunction in cerebellar ataxia.* Neurosci Lett, 2019. **688**: p. 41-48.

13. Shakkottai, V.G., et al., *Early changes in cerebellar physiology accompany motor dysfunction in the polyglutamine disease spinocerebellar ataxia type 3.* J Neurosci, 2011. **31**(36): p. 13002-14.

14. Hansen, S.T., et al., *Changes in Purkinje cell firing and gene expression precede behavioral pathology in a mouse model of SCA2.* Human Molecular Genetics, 2013. **22**(2): p. 271-283.

15. Jayabal, S., et al., *4-aminopyridine reverses ataxia and cerebellar firing deficiency in a mouse model of spinocerebellar ataxia type 6.* Sci Rep, 2016. **6**: p. 29489.

16. Kasumu, A.W., et al., *Selective positive modulator of calcium-activated potassium channels exerts beneficial effects in a mouse model of spinocerebellar ataxia type 2.* Chem Biol, 2012. **19**(10): p. 1340-53.

This article is protected by copyright. All rights reserved.

Author Manuscript

17. Hourez, R., et al., *Aminopyridines correct early dysfunction and delay neurodegeneration in a mouse model of spinocerebellar ataxia type 1.* J Neurosci, 2011. **31**(33): p. 11795-807.

18. Chopra, R., et al., *Protein kinase C activity is a protective modifier of Purkinje neuron degeneration in cerebellar ataxia.* Hum Mol Genet, 2018. **27**(8): p. 1396-1410.

19. Walter, J.T., et al., *Decreases in the precision of Purkinje cell pacemaking cause cerebellar dysfunction and ataxia.* Nat Neurosci, 2006. **9**(3): p. 389-97.

20. Ly, R., et al., *T-type channel blockade impairs long-term potentiation at the parallel fiber-Purkinje cell synapse and cerebellar learning.* Proc Natl Acad Sci U S A, 2013. **110**(50): p. 20302-7.

21. Sausbier, M., et al., *Cerebellar ataxia and Purkinje cell dysfunction caused by Ca2+-activated K+ channel deficiency.* Proc Natl Acad Sci U S A, 2004. **101**(25): p. 9474-8.

22. Gatchel, J.R., et al., *The insulin-like growth factor pathway is altered in spinocerebellar ataxia type 1 and type 7.* Proc Natl Acad Sci U S A, 2008. **105**(4): p. 1291-6.

23. Womack, M.D., C. Chevez, and K. Khodakhah, *Calcium-activated potassium channels are selectively coupled to P/Q-type calcium channels in cerebellar Purkinje neurons.* J Neurosci, 2004. **24**(40): p. 8818-22.

24. Zhao, G., et al., *Type 1 IP3 receptors activate BKCa channels via local molecular coupling in arterial smooth muscle cells.* J Gen Physiol, 2010. **136**(3): p. 283-91.

25. Liu, Y.C., Y.K. Lo, and S.N. Wu, *Stimulatory effects of chlorzoxazone, a centrally acting muscle relaxant, on large conductance calcium-activated potassium channels in pituitary GH3 cells.* Brain Res, 2003. **959**(1): p. 86-97.

This article is protected by copyright. All rights reserved.

Author Manuscript

26. Tabata, T., et al., *GABAergic activation of an inwardly rectifying K+ current in mouse cerebellar Purkinje cells.* J Physiol, 2005. **563**(Pt 2): p. 443-57.
27. Chopra, R. and V.G. Shakkottai, *Translating cerebellar Purkinje neuron physiology to progress in dominantly inherited ataxia.* Future Neurol, 2014. **9**(2): p. 187-196.
28. Zoghbi, H.Y., et al., *Spinocerebellar ataxia: variable age of onset and linkage to human leukocyte antigen in a large kindred.* Ann Neurol, 1988. **23**(6): p. 580-4.
29. Ranum, L.P., et al., *Molecular and clinical correlations in spinocerebellar ataxia type I: evidence for familial effects on the age at onset.* Am J Hum Genet, 1994. **55**(2): p. 244-52.
30. Haines, J.L., et al., *Spinocerebellar ataxia in a large kindred: age at onset, reproduction, and genetic linkage studies.* Neurology, 1984. **34**(12): p. 1542-8.
31. Crawley, J.N., *What's wrong with my mouse? : behavioral phenotyping of transgenic and knockout mice*. 2000, New York: Wiley-Liss. xiii, 329 p.
32. Ashizawa, T., et al., *Clinical characteristics of patients with spinocerebellar ataxias 1, 2, 3 and 6 in the US; a prospective observational study.* Orphanet J Rare Dis, 2013. **8**: p. 177.
33. Schmitz-Hubsch, T., et al., *Responsiveness of different rating instruments in spinocerebellar ataxia patients.* Neurology, 2010. **74**(8): p. 678-84.
34. Diallo, A., et al., *Survival in patients with spinocerebellar ataxia types 1, 2, 3, and 6 (EUROSCA): a longitudinal cohort study.* Lancet Neurol, 2018. **17**(4): p. 327-334.

This article is protected by copyright. All rights reserved.

Author Manuscript

35. Expert, P.b.t.A.G.S.B.C.U., *American Geriatrics Society 2019 Updated AGS Beers Criteria(R) for Potentially Inappropriate Medication Use in Older Adults.* J Am Geriatr Soc, 2019. **67**(4): p. 674-694.

36. Lexi-Comp Inc., *Lexicomp online*.

37. Egorova, P.A., et al., *In vivo analysis of cerebellar Purkinje cell activity in SCA2 transgenic mouse model.* J Neurophysiol, 2016. **115**(6): p. 2840-51.

38. Knutsson, E., U. Lindblom, and A. Martensson, *Plasma and cerebrospinal fluid levels of baclofen (Lioresal) at optimal therapeutic responses in spastic paresis.* J Neurol Sci, 1974. **23**(3): p. 473-84.

39. Alvina, K. and K. Khodakhah, *KCa channels as therapeutic targets in episodic ataxia type-2.* J Neurosci, 2010. **30**(21): p. 7249-57.

40. Gao, Z., et al., *Cerebellar ataxia by enhanced Ca(V)2.1 currents is alleviated by Ca2+-dependent K+-channel activators in Cacna1a(S218L) mutant mice.* J Neurosci, 2012. **32**(44): p. 15533-46.

41. Egorova, P.A., A.V. Gavrilova, and I.B. Bezprozvanny, *Ataxic Symptoms in Huntington's Disease Transgenic Mouse Model Are Alleviated by Chlorzoxazone.* Front Neurosci, 2020. **14**: p. 279.

42. Ankri, L., Y. Yarom, and M.Y. Uusisaari, *Slice it hot: acute adult brain slicing in physiological temperature.* J Vis Exp, 2014(92): p. e52068.

This article is protected by copyright. All rights reserved.

Author Manuscript

Figure Legends

Figure 1. Alterations in Purkinje neuron physiology in *Atxn1*$^{154Q/2Q}$ mice.

This article is protected by copyright. All rights reserved.

Author Manuscript

Representative spontaneous Purkinje neuron spiking is shown for 14 week-old (A) wild-type and (B) *Atxn1*$^{154Q/2Q}$ mice. (C) Regularity of Purkinje neuron spiking, as indicated by the coefficient of variation of the interspike interval, in wild-type and *Atxn1*$^{154Q/2Q}$ mice. (D) Firing frequency in wild-type and *Atxn1*$^{154Q/2Q}$ Purkinje neurons. (E) Threshold of injected current required to elicit depolarization block in wild-type and *Atxn1*$^{154Q/2Q}$ mice. (F) Input resistance in wild-type and *Atxn1*$^{154Q/2Q}$ Purkinje neurons. (G) Minimum spike amplitude, indicative of the fast afterhyperpolarization (AHP) in wild-type and *Atxn1*$^{154Q/2Q}$ Purkinje neurons. Quantification of the AHP and interspike interval of wild-type and *Atxn1*$^{154Q/2Q}$ Purkinje neurons in the (H) anterior cerebellar lobules and (I) posterior cerebellar lobules. * denotes $p<0.05$; ** denotes $p<0.01$, *** denotes $p<0.001$; ns denotes $p>0.05$. Two-tailed Student's t-test (C-G); two-way repeated measures ANOVA with Holm-Sidak correction for multiple comparisons (H-I).

Figure 2. Reduced expression of BK and $Ca_V3.1$ channels in *Atxn1*$^{154Q/2Q}$ cerebellum.
(A) Ion channel expression was measured in the anterior cerebellum (lobule 5) molecular layer of 14 week-old *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls. (B) Ion channel expression was measured in the nodular zone (lobule 10) molecular layer of 14 week-old *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls. (C) Representative 40x confocal microscopy images from the anterior cerebellum are shown for (C) BK, $Ca_V3.1$, IP3R, and $Ca_V2.1$ in *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls. ** denotes $p<0.01$; *** denotes $p<0.001$; ns denotes $p>0.05$. Two-tailed Student's t-test (F), with Holm-Sidak correction for multiple comparisons used in (A-B).

This article is protected by copyright. All rights reserved.

Author Manuscript

Figure 3. Chlorzoxazone-baclofen improves *Atxn1*$^{154Q/2Q}$ Purkinje neuron physiology in a dose-responsive manner.

Acute cerebellar slices were prepared from 14 week-old *Atxn1*$^{154Q/2Q}$ mice for all recordings. (A) Regularity of Purkinje neuron spiking was recorded by measuring the coefficient of variation (CV) of the interspike interval (ISI). CV of the ISI was recorded at baseline and normalized to 1.0, and was then recorded after perfusion of differing concentrations of chlorzoxazone and baclofen into the bath. Dotted line represents normalized CV of the ISI from age-matched wild-type littermate controls. (B) Firing frequency was recorded at baseline and normalized to 1.0, and was then recorded after perfusion of differing concentrations of chlorzoxazone and baclofen into the bath. Dotted line represents normalized baseline firing frequency from age-matched wild-type littermate controls. (C) Similar to (A), CV of the ISI was recorded at baseline and after perfusion of an intermediate dose of chlorzoxazone (10 μM). Dotted line represents normalized CV of the ISI from age-matched wild-type littermate controls. (D) Similar to (C), CV of the ISI was recorded at baseline and after perfusion an intermediate dose of baclofen (400 nM). Dotted line represents normalized CV of the ISI from age-matched wild-type littermate controls. * denotes $p<0.05$; ** denotes $p<0.01$; *** denotes $p<0.001$; ns denotes $p>0.05$. Paired Student's t-test.

Figure 4. Chlorzoxazone-baclofen improves cerebellar motor impairment in *Atxn1*$^{154Q/2Q}$ mice.

Atxn1$^{154Q/2Q}$ mice and wild-type littermate controls were treated with chlorzoxazone-baclofen in water bottles starting at 13 weeks of age. (A) Grip strength was measured in *Atxn1*$^{154Q/2Q}$ mice

This article is protected by copyright. All rights reserved.

and wild-type littermate controls at 14 weeks of age using a hanging wire task. (B) The time to fall off of a rotarod at constant speed was recorded for *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls at 14 weeks of age. (C) Similar to (A), at 20 weeks of age. (D) Similar to (B), at 20 weeks of age. * denotes $p<0.05$; ** denotes $p<0.01$; *** denotes $p<0.001$; ns denotes $p>0.05$. Two-way repeated measures ANOVA with Holm-Sidak correction for multiple comparisons (A, C); one-way ANOVA with Holm-Sidak correction for multiple comparisons (B, D).

Figure 5. Body weight is preserved in *Atxn1*$^{154Q/2Q}$ mice treated with chlorzoxazone-baclofen.

Body weight was measured in 14 week-old (A) male and (B) female *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls after one week of water bottle treatment of chlorzoxazone-baclofen. Similar to (A-B), body weight was measured in 20 week-old (C) male and (D) female *Atxn1*$^{154Q/2Q}$ mice and wild-type littermate controls after 7 weeks of continuous chlorzoxazone-baclofen water bottle treatment. (E) Percent change in body weight from 14 to 20 weeks of age was calculated for wild-type female mice treated with either vehicle drinking water or water containing chlorzoxazone-baclofen. *** denotes $p<0.001$; ns denotes $p>0.05$. One-way ANOVA with Holm-Sidak correction for multiple comparisons (A-D); Two-tailed Student's t-test (E).

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

COPYRIGHT TRANSFER AGREEMENT

Date: 9/21/2020

Contributor name: Luke Joseph-Karfei Man

Contributor address: 31065 Lucerne Dr Franklin Michigan 48025

Manuscript number: MDS-20-1225

Re: Manuscript entitled: A chlorzoxazone-baclofen combination improves cerebellar impairment in spinocerebellar ataxia type1 (the "Contribution")

for publication in: Movement Disorders (the "Journal")

Published by Wiley on behalf of The International Parkinson and Movement Disorder Society (the "Owner")

Dear Contributor(s):

Thank you for submitting your Contribution for publication. In order to expedite the editing and publishing process and enable the Owner to disseminate your Contribution to the fullest extent, we need to have this Copyright Transfer Agreement executed. If the Contribution is not accepted for publication, or if the Contribution is subsequently rejected, this Agreement shall be null and void. **Publication cannot proceed without a signed copy of this Agreement.**

A. COPYRIGHT

The Contributor assigns to the Owner, during the full term of copyright and any extensions or renewals, all copyright in and to the Contribution, and all rights therein, including but not limited to the right to reproduce, publish, republish, transmit, sell, transfer, distribute, and otherwise use the Contribution in whole or in part in electronic and print editions of the Journal and in derivative works throughout the world, in all languages and in all media of expression now known or later developed, and to license or permit others to do so.

B. RETAINED RIGHTS

Notwithstanding the above, the Contributor or, if applicable, the Contributor's employer, retains all proprietary rights other than copyright, such as patent rights, in any process, procedure or article of manufacture described in the Contribution. This reservation of rights does not affect or limit the rights assigned to Owner in Section A.

C. PERMITTED USES BY CONTRIBUTOR

1. License. The Owner grants to Contributor a non-exclusive, non-transferable and limited license to reproduce and distribute copies of the print or electronic "preprints" of the unpublished Contribution, in the original form submitted to the Journal prior to the peer review process, solely to colleagues within the Contributor's nonprofit organization or educational institution. The Contributor shall make no more than 100 printed copies of the preprints in any calendar year. Such preprints may be posted as electronic files on the Contributor's own personal website, on the Contributor's internal intranet at Contributor's nonprofit organization or educational institution, or on a secure external website at the Contributor's nonprofit organization or educational institution, provided that access is limited to employees and/or students at Contributor's non-profit organization or educational institution. Contributor shall not charge a fee for any

This article is protected by copyright. All rights reserved.

Author Manuscript

preprints, and Contributor's use under this Section C shall not be for any commercial purpose, or for any systematic external distribution (e.g., posting on a listserve, public website, database connected to a public access server, or automated delivery system). The license grant in this Section does not apply to for-profit corporations, and any proposed use outside of the scope of this Section C must be pre-approved in writing by the Owner. The rights granted to Contributor under this Section C do not include reproduction, distribution or any other use of rating scales, videos or other audiovisual materials associated with the Contribution.

2. Required Citation. Prior to publication, the Contributor must provide full credit and acknowledgement of the Journal in all preprints in the following format: This is a preprint of an article accepted for publication in [Journal Title], Copyright © [year] The International Parkinson and Movement Disorder Society. After publication, the Contributor must provide a citation to the Journal in all preprints in the following format: This is a preprint of an article that was published in [Journal title]: (Title of Article, Contributor, Journal Title and Volume/ Issue, Copyright © [year] The International Parkinson and Movement Disorder Society). An electronic link must be provided to the Journal's website, located at http://www.interscience.Wiley.com. The Contributor agrees not to update the preprint or replace it with the published version of the Contribution.

3. Accepted Version. Re-use of the accepted and peer-reviewed (but not the final typeset published) version of the Contribution (the "Accepted Version") is not permitted under this Agreement. There are separate arrangements with certain funding agencies governing reuse of the Accepted Version. Additional terms apply if the Contributor receives or received funding from these agencies. The details of those relationships, and other offerings allowing open web use, are set forth at the following website: http://www.wiley.com/go/funderstatement.

4. Additional Terms for Certain Funders. Certain funders, including the NIH, members of the Research Councils UK (RCUK) and Wellcome Trust require deposit of the Accepted Version in a public repository after an embargo period. Details of funding arrangements are set out at the following website: http://www.wiley.com/go/funderstatement. Additional terms may be applicable. Please contact the production editor for the journal at MDSprod@wiley.com if you have additional funding requirements.

If any Contributor receiving funds from applicable sources does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in a public repository after the following applicable embargo period has expired, subject to further conditions imposed by the RCUK:

a. 12 months from first publication online of the final published version of the Contribution for research funded by members of the Research Councils UK (RCUK) other than The Economic and Social Research Council (ESRC) and the Arts and Humanities Research Council (AHRC); or
b. 24 months from first publication online of the final published version of the Contribution for research funded by ESRC or AHRC.

5. Additional Terms for Certain Institutions. Wiley has arrangements with certain educational institutions to permit the deposit of the Accepted Version in the institutional repository after an embargo period. Details of such arrangements are set out at the following website: http://olabout.wiley.com/WileyCDA/Section/id-406074.html . Additional terms may be applicable.

If any Contributor affiliated with these applicable educational institutions does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in the educational institution's repository after the following applicable embargo period has expired. See the following website for details: http://olabout.wiley.com/WileyCDA/Section/id-817011.html.

This article is protected by copyright. All rights reserved.

Author Manuscript

D. CONTRIBUTIONS OWNED BY EMPLOYER

If the Contribution was written by the Contributor in the course of the Contributor's employment (as a "work-made-for-hire" in the course of employment), the Contribution is owned by the company/institution which must execute this Agreement (in addition to the Contributor's signature). In such case, the company/institution hereby assigns to the Owner, during the full term of copyright, all copyright in and to the Contribution for the full term of copyright throughout the world as specified in Section A above.

E. GOVERNMENT CONTRACTS

In the case of a Contribution prepared under U.S. Government contract or grant, the U.S. Government may reproduce, without charge, all or portions of the Contribution and may authorize others to do so, for official U.S. Government purposes only, if the U.S. Government contract or grant so requires. (U.S. Government, U.K. Government, and other government employees: see notes at end.)

F. CONTRIBUTOR'S REPRESENTATIONS

The Contributor represents that the Contribution is the Contributor's original work, all individuals identified as Contributors actually contributed to the Contribution, and all individuals who contributed are included. The Contribution is submitted only to this Journal and has not been published before. (If excerpts from copyrighted works owned by third parties are included, the Contributor will obtain written permission from the copyright owners for all uses as set forth in the Journal's Instructions for Contributors, and show credit to the sources in the Contribution.) The Contributor also warrants that the Contribution contains no libelous or unlawful statements, does not infringe upon the rights (including without limitation the copyright, patent or trademark rights) or the privacy of others, or contain material or instructions that might cause harm or injury. Upon request, Contributor will provide the data or will cooperating fully in obtaining and providing the data on which the Contribution is based for examination by the editors or their assignees.

G. FINANCIAL DISCLOSURES

The Contributor certifies that his/her financial and material support for this research and work, regardless of date, is clearly identified on Exhibit A to this Agreement. The Contributor has also identified on Exhibit A, all other support unrelated to this research, covering the past year from the date of submission (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields).

H. VIDEO AND PHOTOGRAPHY CONSENT

In the event that the Contribution includes, discloses or incorporates any content (including, without limitation, any video clip or photograph) which identifies any individual patient(s) ("patient identifiable content"), the Contributor obtained from such patient(s) written consent to such inclusion, disclosure or incorporation and that this consent fully complies with all legal requirements, including without limitation, all of the requirements of the laws of the jurisdiction(s) to which the patient(s) and the patient(s)' physician are subject, including the United States Health Insurance Portability and Accountability Act of 1996 ("HIPAA") if applicable. The Contributor hereby certifies that, if the patient consent form is in a language other than English, such consent form meets all of the requirements set forth in the Instructions to Authors. In addition, the Contributor hereby confirms that he/she obtained from patient(s) written consent to use the patient identifiable content in both print and online (i.e., internet/web-based) publication formats. The Contributor further certifies that the person executing any such patient consent form, to the best of his/her knowledge, had legal capacity under applicable law to execute the form on behalf of the patient.

This article is protected by copyright. All rights reserved.

Author Manuscript

I. ACKNOWLEDGEMENTS

The Contributor should obtain written permission from all individuals named in the acknowledgement since readers may infer their endorsement of data and conclusions. The Contributor certifies that all individuals named in the acknowledgement section have provided written permission to be named.

J. MISCELLANEOUS

This Agreement may be amended or modified only in a writing executed by both parties. The waiver or failure of any party to exercise any rights under this Agreement shall not be deemed a waiver or other limitation of any other right or any future right. This Agreement shall inure to the benefit of, and shall be binding upon, the parties, their respective successors and permitted assigns. This Agreement may be executed in two (2) or more counterparts, each of which shall be an original and all of which taken together shall constitute one and the same agreement. Executed copies of this Agreement may be delivered by facsimile transmission, pdf/email or other comparable electronic means. If for any reason any provision of this Agreement shall be deemed by a court of competent jurisdiction to be legally invalid or unenforceable, the validity, legality and enforceability of the remainder of this Agreement shall not be affected and such provision shall be deemed modified to the minimum extent necessary to make such provision consistent with applicable law and, in its modified form, such provision shall then be enforceable and enforced. The parties agree to do such further acts and to execute and deliver such additional agreements and instruments from time to time as either may at any time reasonably request in order to assure and confirm unto such requesting party the rights, powers and remedies conferred in the Agreement. This Agreement, including any exhibits attached hereto, contains the entire agreement and understanding of the parties with respect to the subject matter hereof, and supersedes all prior agreements, negotiations, representations and proposals, written and oral, relating thereto.

All Contributors must sign below. Contributors must check one box except that NIH grantees should check both Contributor-owned work and the NIH grantee box. If your Contribution was written during the course of employment, your employer must also sign where indicated.

Please send your original completed and signed forms by fax or email a scanned copy to the Journal production editor. For production editor contact details please visit the Journal's online author guidelines. **Do not send in hard copies of these forms.**

[X] Contributor-owned work

Luke Man — Contributor's signature — Date: 9/21/2020

Luke Man Research Assistant
Type or print name and title

Co-Contributor's signature — Date

Type or print name and title

This article is protected by copyright. All rights reserved.

[_____] Company/Institution-owned Work (made-for-hire in the Course of employment)

_____ Company or Institution (Employer-for-Hire) _____ Date

_____ Authorized signature of Employer _____ Date

_____ Contributor's signature _____ Date

_____ Type or print name and title

ATTACH ADDITIONAL SIGNATURE PAGES AS NECESSARY

[_____] U.S. Government work

Note to U.S. Government Employees

A contribution prepared by a U.S. federal government employee as part of the employee's official duties, or which is an official U.S. Government publication, is called a "U.S. Government work", and is in the public domain in the United States. In such case, Paragraph A.1 will not apply but the Contributor must type his/her name (in the Contributor's signature line) above. Contributor acknowledges that the Contribution will be published in the United States and other countries. If the Contribution was not prepared as part of the employee's duties or is not an official U.S. Government publication, it is not a U.S. Government work.

[_____] **U.K. Government work (Crown Copyright)**

Note to U.K. Government Employees

The rights in a contribution prepared by an employee of a UK government department, agency or other Crown body as part of his/her official duties, or which is an official government publication, belong to the Crown. Contributors must ensure they comply with departmental regulations and submit the appropriate authorisation to publish. If your status as a government employee legally prevents you from signing this Agreement, please contact the Journal production editor.

[_____] **Other**

Including Other Government work or Non-Governmental Organisation work

Note to Non-U.S., Non-U.K. Government Employees or Non-Governmental Organisation Employees

If your status as a government or non-governmental organisation employee legally prevents you from signing this Agreement, please contact the Journal production editor.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

Exhibit A

Financial Disclosure

The Contributor has received financial and material support for this research and work regardless of date from the following sources:

Name: _____

Address: _____

Type of support: _____

This material will be printed with the published article.

In the past year from the date of submission, the Contributor has also received the following support unrelated to this research (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields):

Name: _____

Address: _____

Type of support: _____

This material will be posted on the journal website and may be printed at the Editors' discretion.

ATTACH ADDITIONAL INFORMATION AS NECESSARY

This article is protected by copyright. All rights reserved.

Author Manuscript

COPYRIGHT TRANSFER AGREEMENT

Date: 09/12/20

Contributor name: Logan M. Morrison

Contributor address: Department of Neurology, University of Michigan, 109 Zina Pitcher Place, Ann Arbor, MI 48109

Manuscript number: MDS-20-1225

Re: Manuscript entitled: "A chlorzoxazone-baclofen combination improves cerebellar impairment in spinocerebellar ataxia type 1" (the "Contribution")

for publication in: Movement Disorders (the "Journal")

Published by Wiley on behalf of The International Parkinson and Movement Disorder Society (the "Owner")

Dear Contributor(s):

Thank you for submitting your Contribution for publication. In order to expedite the editing and publishing process and enable the Owner to disseminate your Contribution to the fullest extent, we need to have this Copyright Transfer Agreement executed. If the Contribution is not accepted for publication, or if the Contribution is subsequently rejected, this Agreement shall be null and void. **Publication cannot proceed without a signed copy of this Agreement.**

A. COPYRIGHT

The Contributor assigns to the Owner, during the full term of copyright and any extensions or renewals, all copyright in and to the Contribution, and all rights therein, including but not limited to the right to reproduce, publish, republish, transmit, sell, transfer, distribute, and otherwise use the Contribution in whole or in part in electronic and print editions of the Journal and in derivative works throughout the world, in all languages and in all media of expression now known or later developed, and to license or permit others to do so.

B. RETAINED RIGHTS

Notwithstanding the above, the Contributor or, if applicable, the Contributor's employer, retains all proprietary rights other than copyright, such as patent rights, in any process, procedure or article of manufacture described in the Contribution. This reservation of rights does not affect or limit the rights assigned to Owner in Section A.

C. PERMITTED USES BY CONTRIBUTOR

1. License. The Owner grants to Contributor a non-exclusive, non-transferable and limited license to reproduce and distribute copies of the print or electronic "preprints" of the unpublished Contribution, in the original form submitted to the Journal prior to the peer review process, solely to colleagues within the Contributor's nonprofit organization or educational institution. The Contributor shall make no more than 100 printed copies of the preprints in any calendar year. Such preprints may be posted as electronic files on the Contributor's own personal website, on the Contributor's internal intranet at Contributor's nonprofit organization or educational institution, or on a secure external website at the Contributor's nonprofit organization or educational institution, provided that access is limited to employees and/or students at Contributor's non-profit organization or educational institution. Contributor shall not charge a fee for any

This article is protected by copyright. All rights reserved.

Author Manuscript

preprints, and Contributor's use under this Section C shall not be for any commercial purpose, or for any systematic external distribution (e.g., posting on a listserve, public website, database connected to a public access server, or automated delivery system). The license grant in this Section does not apply to for-profit corporations, and any proposed use outside of the scope of this Section C must be pre-approved in writing by the Owner. The rights granted to Contributor under this Section C do not include reproduction, distribution or any other use of rating scales, videos or other audiovisual materials associated with the Contribution.

2. Required Citation. Prior to publication, the Contributor must provide full credit and acknowledgement of the Journal in all preprints in the following format: This is a preprint of an article accepted for publication in [Journal Title], Copyright © [year] The International Parkinson and Movement Disorder Society. After publication, the Contributor must provide a citation to the Journal in all preprints in the following format: This is a preprint of an article that was published in [Journal title]: (Title of Article, Contributor, Journal Title and Volume/ Issue, Copyright © [year] The International Parkinson and Movement Disorder Society). An electronic link must be provided to the Journal's website, located at http://www.interscience.Wiley.com. The Contributor agrees not to update the preprint or replace it with the published version of the Contribution.

3. Accepted Version. Re-use of the accepted and peer-reviewed (but not the final typeset published) version of the Contribution (the "Accepted Version") is not permitted under this Agreement. There are separate arrangements with certain funding agencies governing reuse of the Accepted Version. Additional terms apply if the Contributor receives or received funding from these agencies. The details of those relationships, and other offerings allowing open web use, are set forth at the following website: http://www.wiley.com/go/ funderstatement.

4. Additional Terms for Certain Funders. Certain funders, including the NIH, members of the Research Councils UK (RCUK) and Wellcome Trust require deposit of the Accepted Version in a public repository after an embargo period. Details of funding arrangements are set out at the following website: http://www.wiley.com/go/funderstatement. Additional terms may be applicable. Please contact the production editor for the journal at MDSprod@wiley.com if you have additional funding requirements.

If any Contributor receiving funds from applicable sources does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in a public repository after the following applicable embargo period has expired, subject to further conditions imposed by the RCUK:

a. 12 months from first publication online of the final published version of the Contribution for research funded by members of the Research Councils UK (RCUK) other than The Economic and Social Research Council (ESRC) and the Arts and Humanities Research Council (AHRC); or
b. 24 months from first publication online of the final published version of the Contribution for research funded by ESRC or AHRC.

5. Additional Terms for Certain Institutions. Wiley has arrangements with certain educational institutions to permit the deposit of the Accepted Version in the institutional repository after an embargo period. Details of such arrangements are set out at the following website: http://olabout.wiley.com/WileyCDA/Section/id-406074.html . Additional terms may be applicable.

If any Contributor affiliated with these applicable educational institutions does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in the educational institution's repository after the following applicable embargo period has expired. See the following website for details: http://olabout.wiley.com/WileyCDA/Section/id-817011.html.

This article is protected by copyright. All rights reserved.

Author Manuscript

D. CONTRIBUTIONS OWNED BY EMPLOYER

If the Contribution was written by the Contributor in the course of the Contributor's employment (as a "work-made-for-hire" in the course of employment), the Contribution is owned by the company/institution which must execute this Agreement (in addition to the Contributor's signature). In such case, the company/institution hereby assigns to the Owner, during the full term of copyright, all copyright in and to the Contribution for the full term of copyright throughout the world as specified in Section A above.

E. GOVERNMENT CONTRACTS

In the case of a Contribution prepared under U.S. Government contract or grant, the U.S. Government may reproduce, without charge, all or portions of the Contribution and may authorize others to do so, for official U.S. Government purposes only, if the U.S. Government contract or grant so requires. (U.S. Government, U.K. Government, and other government employees: see notes at end.)

F. CONTRIBUTOR'S REPRESENTATIONS

The Contributor represents that the Contribution is the Contributor's original work, all individuals identified as Contributors actually contributed to the Contribution, and all individuals who contributed are included. The Contribution is submitted only to this Journal and has not been published before. (If excerpts from copyrighted works owned by third parties are included, the Contributor will obtain written permission from the copyright owners for all uses as set forth in the Journal's Instructions for Contributors, and show credit to the sources in the Contribution.) The Contributor also warrants that the Contribution contains no libelous or unlawful statements, does not infringe upon the rights (including without limitation the copyright, patent or trademark rights) or the privacy of others, or contain material or instructions that might cause harm or injury. Upon request, Contributor will provide the data or will cooperating fully in obtaining and providing the data on which the Contribution is based for examination by the editors or their assignees.

G. FINANCIAL DISCLOSURES

The Contributor certifies that his/her financial and material support for this research and work, regardless of date, is clearly identified on Exhibit A to this Agreement. The Contributor has also identified on Exhibit A, all other support unrelated to this research, covering the past year from the date of submission (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields).

H. VIDEO AND PHOTOGRAPHY CONSENT

In the event that the Contribution includes, discloses or incorporates any content (including, without limitation, any video clip or photograph) which identifies any individual patient(s) ("patient identifiable content"), the Contributor obtained from such patient(s) written consent to such inclusion, disclosure or incorporation and that this consent fully complies with all legal requirements, including without limitation, all of the requirements of the laws of the jurisdiction(s) to which the patient(s) and the patient(s)' physician are subject, including the United States Health Insurance Portability and Accountability Act of 1996 ("HIPAA") if applicable. The Contributor hereby certifies that, if the patient consent form is in a language other than English, such consent form meets all of the requirements set forth in the Instructions to Authors. In addition, the Contributor hereby confirms that he/she obtained from patient(s) written consent to use the patient identifiable content in both print and online (i.e., internet/web-based) publication formats. The Contributor further certifies that the person executing any such patient consent form, to the best of his/her knowledge, had legal capacity under applicable law to execute the form on behalf of the patient.

This article is protected by copyright. All rights reserved.

Author Manuscript

I. ACKNOWLEDGEMENTS

The Contributor should obtain written permission from all individuals named in the acknowledgement since readers may infer their endorsement of data and conclusions. The Contributor certifies that all individuals named in the acknowledgement section have provided written permission to be named.

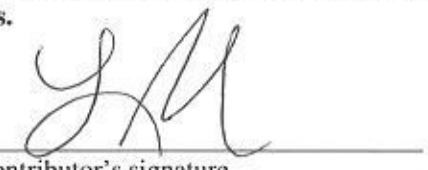
J. MISCELLANEOUS

This Agreement may be amended or modified only in a writing executed by both parties. The waiver or failure of any party to exercise any rights under this Agreement shall not be deemed a waiver or other limitation of any other right or any future right. This Agreement shall inure to the benefit of, and shall be binding upon, the parties, their respective successors and permitted assigns. This Agreement may be executed in two (2) or more counterparts, each of which shall be an original and all of which taken together shall constitute one and the same agreement. Executed copies of this Agreement may be delivered by facsimile transmission, pdf/email or other comparable electronic means. If for any reason any provision of this Agreement shall be deemed by a court of competent jurisdiction to be legally invalid or unenforceable, the validity, legality and enforceability of the remainder of this Agreement shall not be affected and such provision shall be deemed modified to the minimum extent necessary to make such provision consistent with applicable law and, in its modified form, such provision shall then be enforceable and enforced. The parties agree to do such further acts and to execute and deliver such additional agreements and instruments from time to time as either may at any time reasonably request in order to assure and confirm unto such requesting party the rights, powers and remedies conferred in the Agreement. This Agreement, including any exhibits attached hereto, contains the entire agreement and understanding of the parties with respect to the subject matter hereof, and supersedes all prior agreements, negotiations, representations and proposals, written and oral, relating thereto.

All Contributors must sign below. Contributors must check one box except that NIH grantees should check both Contributor-owned work and the NIH grantee box. If your Contribution was written during the course of employment, your employer must also sign where indicated.

Please send your original completed and signed forms by fax or email a scanned copy to the Journal production editor. For production editor contact details please visit the Journal's online author guidelines. **Do not send in hard copies of these forms.**

[√] Contributor-owned work



Contributor's signature

09/18/20

Date

Logan M. Morrison, B.S.

Type or print name and title

Co-Contributor's signature

Date

Type or print name and title

This article is protected by copyright. All rights reserved.

[_____] Company/Institution-owned Work (made-for-hire in the Course of employment)

_____ Company or Institution (Employer-for-Hire) _____ Date

_____ Authorized signature of Employer _____ Date

_____ Contributor's signature _____ Date

_____ Type or print name and title

ATTACH ADDITIONAL SIGNATURE PAGES AS NECESSARY

[_____] **U.S. Government work**

Note to U.S. Government Employees

A contribution prepared by a U.S. federal government employee as part of the employee's official duties, or which is an official U.S. Government publication, is called a "U.S. Government work", and is in the public domain in the United States. In such case, Paragraph A.1 will not apply but the Contributor must type his/her name (in the Contributor's signature line) above. Contributor acknowledges that the Contribution will be published in the United States and other countries. If the Contribution was not prepared as part of the employee's duties or is not an official U.S. Government publication, it is not a U.S. Government work.

[_____] **U.K. Government work (Crown Copyright)**

Note to U.K. Government Employees

The rights in a contribution prepared by an employee of a UK government department, agency or other Crown body as part of his/her official duties, or which is an official government publication, belong to the Crown. Contributors must ensure they comply with departmental regulations and submit the appropriate authorisation to publish. If your status as a government employee legally prevents you from signing this Agreement, please contact the Journal production editor.

[_____] **Other**

Including Other Government work or Non-Governmental Organisation work

Note to Non-U.S., Non-U.K. Government Employees or Non-Governmental Organisation Employees

If your status as a government or non-governmental organisation employee legally prevents you from signing this Agreement, please contact the Journal production editor.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

Exhibit A

Financial Disclosure

The Contributor has received financial and material support for this research and work regardless of date from the following sources:

Name: _____

Address: _____

Type of support: _____

This material will be printed with the published article.

In the past year from the date of submission, the Contributor has also received the following support unrelated to this research (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields):

Name: Rackham Graduate Student Research Grant (Rackham Graduate School)

Address: 915 E Washington St, Ann Arbor, MI 48109

Type of support: Grant

This material will be posted on the journal website and may be printed at the Editors' discretion.

ATTACH ADDITIONAL INFORMATION AS NECESSARY

This article is protected by copyright. All rights reserved.

COPYRIGHT TRANSFER AGREEMENT

Date: 9-18-2020

Contributor name: Haoran Huang

Contributor address: Room 5418, 109 Zina Pitcher, Ann Arbor, MI, 48105

Manuscript number: #MDS-20-1225

Re: Manuscript entitled: A chlorzoxazone-baclofen combination improves cerebellar impairment in spinocerebellar ataxia type 1 (the "Contribution")

for publication in: Movement Disorders (the "Journal")

Published by Wiley on behalf of The International Parkinson and Movement Disorder Society (the "Owner")

Dear Contributor(s):

Thank you for submitting your Contribution for publication. In order to expedite the editing and publishing process and enable the Owner to disseminate your Contribution to the fullest extent, we need to have this Copyright Transfer Agreement executed. If the Contribution is not accepted for publication, or if the Contribution is subsequently rejected, this Agreement shall be null and void. **Publication cannot proceed without a signed copy of this Agreement.**

A. COPYRIGHT

The Contributor assigns to the Owner, during the full term of copyright and any extensions or renewals, all copyright in and to the Contribution, and all rights therein, including but not limited to the right to reproduce, publish, republish, transmit, sell, transfer, distribute, and otherwise use the Contribution in whole or in part in electronic and print editions of the Journal and in derivative works throughout the world, in all languages and in all media of expression now known or later developed, and to license or permit others to do so.

B. RETAINED RIGHTS

Notwithstanding the above, the Contributor or, if applicable, the Contributor's employer, retains all proprietary rights other than copyright, such as patent rights, in any process, procedure or article of manufacture described in the Contribution. This reservation of rights does not affect or limit the rights assigned to Owner in Section A.

C. PERMITTED USES BY CONTRIBUTOR

1. License. The Owner grants to Contributor a non-exclusive, non-transferable and limited license to reproduce and distribute copies of the print or electronic "preprints" of the unpublished Contribution, in the original form submitted to the Journal prior to the peer review process, solely to colleagues within the Contributor's nonprofit organization or educational institution. The Contributor shall make no more than 100 printed copies of the preprints in any calendar year. Such preprints may be posted as electronic files on the Contributor's own personal website, on the Contributor's internal intranet at Contributor's nonprofit organization or educational institution, or on a secure external website at the Contributor's nonprofit organization or educational institution, provided that access is limited to employees and/or students at Contributor's non-profit organization or educational institution. Contributor shall not charge a fee for any

Author Manuscript

This article is protected by copyright. All rights reserved.

D. CONTRIBUTIONS OWNED BY EMPLOYER

If the Contribution was written by the Contributor in the course of the Contributor's employment (as a "work-made-for-hire" in the course of employment), the Contribution is owned by the company/institution which must execute this Agreement (in addition to the Contributor's signature). In such case, the company/institution hereby assigns to the Owner, during the full term of copyright, all copyright in and to the Contribution for the full term of copyright throughout the world as specified in Section A above.

E. GOVERNMENT CONTRACTS

In the case of a Contribution prepared under U.S. Government contract or grant, the U.S. Government may reproduce, without charge, all or portions of the Contribution and may authorize others to do so, for official U.S. Government purposes only, if the U.S. Government contract or grant so requires. (U.S. Government, U.K. Government, and other government employees: see notes at end.)

F. CONTRIBUTOR'S REPRESENTATIONS

The Contributor represents that the Contribution is the Contributor's original work, all individuals identified as Contributors actually contributed to the Contribution, and all individuals who contributed are included. The Contribution is submitted only to this Journal and has not been published before. (If excerpts from copyrighted works owned by third parties are included, the Contributor will obtain written permission from the copyright owners for all uses as set forth in the Journal's Instructions for Contributors, and show credit to the sources in the Contribution.) The Contributor also warrants that the Contribution contains no libelous or unlawful statements, does not infringe upon the rights (including without limitation the copyright, patent or trademark rights) or the privacy of others, or contain material or instructions that might cause harm or injury. Upon request, Contributor will provide the data or will cooperating fully in obtaining and providing the data on which the Contribution is based for examination by the editors or their assignees.

Author Manuscript

G. FINANCIAL DISCLOSURES

The Contributor certifies that his/her financial and material support for this research and work, regardless of date, is clearly identified on Exhibit A to this Agreement. The Contributor has also identified on Exhibit A, all other support unrelated to this research, covering the past year from the date of submission (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields).

H. VIDEO AND PHOTOGRAPHY CONSENT

In the event that the Contribution includes, discloses or incorporates any content (including, without limitation, any video clip or photograph) which identifies any individual patient(s) ("patient identifiable content"), the Contributor obtained from such patient(s) written consent to such inclusion, disclosure or incorporation and that this consent fully complies with all legal requirements, including without limitation, all of the requirements of the laws of the jurisdiction(s) to which the patient(s) and the patient(s)' physician are subject, including the United States Health Insurance Portability and Accountability Act of 1996 ("HIPAA") if applicable. The Contributor hereby certifies that, if the patient consent form is in a language other than English, such consent form meets all of the requirements set forth in the Instructions to Authors. In addition, the Contributor hereby confirms that he/she obtained from patient(s) written consent to use the patient identifiable content in both print and online (i.e., internet/web-based) publication formats. The Contributor further certifies that the person executing any such patient consent form, to the best of his/her knowledge, had legal capacity under applicable law to execute the form on behalf of the patient.

This article is protected by copyright. All rights reserved.

preprints, and Contributor's use under this Section C shall not be for any commercial purpose, or for any systematic external distribution (e.g., posting on a listserve, public website, database connected to a public access server, or automated delivery system). The license grant in this Section does not apply to for-profit corporations, and any proposed use outside of the scope of this Section C must be pre-approved in writing by the Owner. The rights granted to Contributor under this Section C do not include reproduction, distribution or any other use of rating scales, videos or other audiovisual materials associated with the Contribution.

2. Required Citation. Prior to publication, the Contributor must provide full credit and acknowledgement of the Journal in all preprints in the following format: This is a preprint of an article accepted for publication in [Journal Title], Copyright © [year] The International Parkinson and Movement Disorder Society. After publication, the Contributor must provide a citation to the Journal in all preprints in the following format: This is a preprint of an article that was published in [Journal title]: (Title of Article, Contributor, Journal Title and Volume/ Issue, Copyright © [year] The International Parkinson and Movement Disorder Society). An electronic link must be provided to the Journal's website, located at http://www.interscience.Wiley.com. The Contributor agrees not to update the preprint or replace it with the published version of the Contribution.

3. Accepted Version. Re-use of the accepted and peer-reviewed (but not the final typeset published) version of the Contribution (the "Accepted Version") is not permitted under this Agreement. There are separate arrangements with certain funding agencies governing reuse of the Accepted Version. Additional terms apply if the Contributor receives or received funding from these agencies. The details of those relationships, and other offerings allowing open web use, are set forth at the following website: http://www.wiley.com/go/funderstatement.

4. Additional Terms for Certain Funders. Certain funders, including the NIH, members of the Research Councils UK (RCUK) and Wellcome Trust require deposit of the Accepted Version in a public repository after an embargo period. Details of funding arrangements are set out at the following website: http://www.wiley.com/go/funderstatement. Additional terms may be applicable. Please contact the production editor for the journal at MDSprod@wiley.com if you have additional funding requirements.

If any Contributor receiving funds from applicable sources does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in a public repository after the following applicable embargo period has expired, subject to further conditions imposed by the RCUK:

a. 12 months from first publication online of the final published version of the Contribution for research funded by members of the Research Councils UK (RCUK) other than The Economic and Social Research Council (ESRC) and the Arts and Humanities Research Council (AHRC); or
b. 24 months from first publication online of the final published version of the Contribution for research funded by ESRC or AHRC.

5. Additional Terms for Certain Institutions. Wiley has arrangements with certain educational institutions to permit the deposit of the Accepted Version in the institutional repository after an embargo period. Details of such arrangements are set out at the following website: http://olabout.wiley.com/WileyCDA/Section/id-406074.html . Additional terms may be applicable.

If any Contributor affiliated with these applicable educational institutions does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in the educational institution's repository after the following applicable embargo period has expired. See the following website for details: http://olabout.wiley.com/WileyCDA/Section/id-817011.html.

Author Manuscript

This article is protected by copyright. All rights reserved.

I. ACKNOWLEDGEMENTS

The Contributor should obtain written permission from all individuals named in the acknowledgement since readers may infer their endorsement of data and conclusions. The Contributor certifies that all individuals named in the acknowledgement section have provided written permission to be named.

J. MISCELLANEOUS

This Agreement may be amended or modified only in a writing executed by both parties. The waiver or failure of any party to exercise any rights under this Agreement shall not be deemed a waiver or other limitation of any other right or any future right. This Agreement shall inure to the benefit of, and shall be binding upon, the parties, their respective successors and permitted assigns. This Agreement may be executed in two (2) or more counterparts, each of which shall be an original and all of which taken together shall constitute one and the same agreement. Executed copies of this Agreement may be delivered by facsimile transmission, pdf/email or other comparable electronic means. If for any reason any provision of this Agreement shall be deemed by a court of competent jurisdiction to be legally invalid or unenforceable, the validity, legality and enforceability of the remainder of this Agreement shall not be affected and such provision shall be deemed modified to the minimum extent necessary to make such provision consistent with applicable law and, in its modified form, such provision shall then be enforceable and enforced. The parties agree to do such further acts and to execute and deliver such additional agreements and instruments from time to time as either may at any time reasonably request in order to assure and confirm unto such requesting party the rights, powers and remedies conferred in the Agreement. This Agreement, including any exhibits attached hereto, contains the entire agreement and understanding of the parties with respect to the subject matter hereof, and supersedes all prior agreements, negotiations, representations and proposals, written and oral, relating thereto.

All Contributors must sign below. Contributors must check one box except that NIH grantees should check both Contributor-owned work and the NIH grantee box. If your Contribution was written during the course of employment, your employer must also sign where indicated.

Please send your original completed and signed forms by fax or email a scanned copy to the Journal production editor. For production editor contact details please visit the Journal's online author guidelines. **Do not send in hard copies of these forms.**

[✓] Contributor-owned work

黄昊然
Contributor's signature

9-18-20
Date

Haoran Huang
Type or print name and title

Co-Contributor's signature

Date

Type or print name and title

Author Manuscript

This article is protected by copyright. All rights reserved.

[_____] Company/Institution-owned Work (made-for-hire in the Course of employment)

_____ _____
Company or Institution (Employer-for-Hire) Date

_____ _____
Authorized signature of Employer Date

_____ _____
Contributor's signature Date

_____
Type or print name and title

ATTACH ADDITIONAL SIGNATURE PAGES AS NECESSARY

[_____] **U.S. Government work**

Note to U.S. Government Employees

A contribution prepared by a U.S. federal government employee as part of the employee's official duties, or which is an official U.S. Government publication, is called a "U.S. Government work", and is in the public domain in the United States. In such case, Paragraph A.1 will not apply but the Contributor must type his/her name (in the Contributor's signature line) above. Contributor acknowledges that the Contribution will be published in the United States and other countries. If the Contribution was not prepared as part of the employee's duties or is not an official U.S. Government publication, it is not a U.S. Government work.

[_____] **U.K. Government work (Crown Copyright)**

Note to U.K. Government Employees

The rights in a contribution prepared by an employee of a UK government department, agency or other Crown body as part of his/her official duties, or which is an official government publication, belong to the Crown. Contributors must ensure they comply with departmental regulations and submit the appropriate authorisation to publish. If your status as a government employee legally prevents you from signing this Agreement, please contact the Journal production editor.

[_____] **Other**

Including Other Government work or Non-Governmental Organisation work

Note to Non-U.S., Non-U.K. Government Employees or Non-Governmental Organisation Employees

If your status as a government or non-governmental organisation employee legally prevents you from signing this Agreement, please contact the Journal production editor.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

COPYRIGHT TRANSFER AGREEMENT

Date: 9/21/2020

Contributor name: David Bushart

Contributor address: 1005 W. 5th Ave. #221, Columbus OH 43212

Manuscript number: MDS-20-1225

Re: Manuscript entitled: Acharzoxagone-baclofen combination improves cerebellar impairment in spinocerebellar ataxia type 1. (the "Contribution")

for publication in: Movement Disorders (the "Journal")

Published by Wiley on behalf of The International Parkinson and Movement Disorder Society (the "Owner")

Dear Contributor(s):

Thank you for submitting your Contribution for publication. In order to expedite the editing and publishing process and enable the Owner to disseminate your Contribution to the fullest extent, we need to have this Copyright Transfer Agreement executed. If the Contribution is not accepted for publication, or if the Contribution is subsequently rejected, this Agreement shall be null and void. **Publication cannot proceed without a signed copy of this Agreement.**

A. COPYRIGHT

The Contributor assigns to the Owner, during the full term of copyright and any extensions or renewals, all copyright in and to the Contribution, and all rights therein, including but not limited to the right to reproduce, publish, republish, transmit, sell, transfer, distribute, and otherwise use the Contribution in whole or in part in electronic and print editions of the Journal and in derivative works throughout the world, in all languages and in all media of expression now known or later developed, and to license or permit others to do so.

B. RETAINED RIGHTS

Notwithstanding the above, the Contributor or, if applicable, the Contributor's employer, retains all proprietary rights other than copyright, such as patent rights, in any process, procedure or article of manufacture described in the Contribution. This reservation of rights does not affect or limit the rights assigned to Owner in Section A.

C. PERMITTED USES BY CONTRIBUTOR

1. License. The Owner grants to Contributor a non-exclusive, non-transferable and limited license to reproduce and distribute copies of the print or electronic "preprints" of the unpublished Contribution, in the original form submitted to the Journal prior to the peer review process, solely to colleagues within the Contributor's nonprofit organization or educational institution. The Contributor shall make no more than 100 printed copies of the preprints in any calendar year. Such preprints may be posted as electronic files on the Contributor's own personal website, on the Contributor's internal intranet at Contributor's nonprofit organization or educational institution, or on a secure external website at the Contributor's nonprofit organization or educational institution, provided that access is limited to employees and/or students at Contributor's non-profit organization or educational institution. Contributor shall not charge a fee for any

This article is protected by copyright. All rights reserved.

Scanned with CamScanner

Author Manuscript

preprints, and Contributor's use under this Section C shall not be for any commercial purpose, or for any systematic external distribution (e.g., posting on a listserve, public website, database connected to a public access server, or automated delivery system). The license grant in this Section does not apply to for-profit corporations, and any proposed use outside of the scope of this Section C must be pre-approved in writing by the Owner. The rights granted to Contributor under this Section C do not include reproduction, distribution or any other use of rating scales, videos or other audiovisual materials associated with the Contribution.

2. Required Citation. Prior to publication, the Contributor must provide full credit and acknowledgement of the Journal in all preprints in the following format: This is a preprint of an article accepted for publication in [Journal Title], Copyright © [year] The International Parkinson and Movement Disorder Society. After publication, the Contributor must provide a citation to the Journal in all preprints in the following format: This is a preprint of an article that was published in [Journal title]: (Title of Article, Contributor, Journal Title and Volume/ Issue, Copyright © [year] The International Parkinson and Movement Disorder Society). An electronic link must be provided to the Journal's website, located at http://www.interscience.Wiley.com. The Contributor agrees not to update the preprint or replace it with the published version of the Contribution.

3. Accepted Version. Re-use of the accepted and peer-reviewed (but not the final typeset published) version of the Contribution (the "Accepted Version") is not permitted under this Agreement. There are separate arrangements with certain funding agencies governing reuse of the Accepted Version. Additional terms apply if the Contributor receives or received funding from these agencies. The details of those relationships, and other offerings allowing open web use, are set forth at the following website: http://www.wiley.com/go/ funderstatement.

4. Additional Terms for Certain Funders. Certain funders, including the NIH, members of the Research Councils UK (RCUK) and Wellcome Trust require deposit of the Accepted Version in a public repository after an embargo period. Details of funding arrangements are set out at the following website: http://www.wiley.com/go/funderstatement. Additional terms may be applicable. Please contact the production editor for the journal at MDSprod@wiley.com if you have additional funding requirements.

If any Contributor receiving funds from applicable sources does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in a public repository after the following applicable embargo period has expired, subject to further conditions imposed by the RCUK:

a. 12 months from first publication online of the final published version of the Contribution for research funded by members of the Research Councils UK (RCUK) other than The Economic and Social Research Council (ESRC) and the Arts and Humanities Research Council (AHRC); or
b. 24 months from first publication online of the final published version of the Contribution for research funded by ESRC or AHRC.

5. Additional Terms for Certain Institutions. Wiley has arrangements with certain educational institutions to permit the deposit of the Accepted Version in the institutional repository after an embargo period. Details of such arrangements are set out at the following website: http://olabout.wiley.com/WileyCDA/Section/id-406074.html . Additional terms may be applicable.

If any Contributor affiliated with these applicable educational institutions does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in the educational institution's repository after the following applicable embargo period has expired. See the following website for details: http://olabout.wiley.com/WileyCDA/Section/id-817011.html.

This article is protected by copyright. All rights reserved.

Scanned with CamScanner

Author Manuscript

D. CONTRIBUTIONS OWNED BY EMPLOYER

If the Contribution was written by the Contributor in the course of the Contributor's employment (as a "work-made-for-hire" in the course of employment), the Contribution is owned by the company/institution which must execute this Agreement (in addition to the Contributor's signature). In such case, the company/institution hereby assigns to the Owner, during the full term of copyright, all copyright in and to the Contribution for the full term of copyright throughout the world as specified in Section A above.

E. GOVERNMENT CONTRACTS

In the case of a Contribution prepared under U.S. Government contract or grant, the U.S. Government may reproduce, without charge, all or portions of the Contribution and may authorize others to do so, for official U.S. Government purposes only, if the U.S. Government contract or grant so requires. (U.S. Government, U.K. Government, and other government employees: see notes at end.)

F. CONTRIBUTOR'S REPRESENTATIONS

The Contributor represents that the Contribution is the Contributor's original work, all individuals identified as Contributors actually contributed to the Contribution, and all individuals who contributed are included. The Contribution is submitted only to this Journal and has not been published before. (If excerpts from copyrighted works owned by third parties are included, the Contributor will obtain written permission from the copyright owners for all uses as set forth in the Journal's Instructions for Contributors, and show credit to the sources in the Contribution.) The Contributor also warrants that the Contribution contains no libelous or unlawful statements, does not infringe upon the rights (including without limitation the copyright, patent or trademark rights) or the privacy of others, or contain material or instructions that might cause harm or injury. Upon request, Contributor will provide the data or will cooperating fully in obtaining and providing the data on which the Contribution is based for examination by the editors or their assignees.

G. FINANCIAL DISCLOSURES

The Contributor certifies that his/her financial and material support for this research and work, regardless of date, is clearly identified on Exhibit A to this Agreement. The Contributor has also identified on Exhibit A, all other support unrelated to this research, covering the past year from the date of submission (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields).

H. VIDEO AND PHOTOGRAPHY CONSENT

In the event that the Contribution includes, discloses or incorporates any content (including, without limitation, any video clip or photograph) which identifies any individual patient(s) ("patient identifiable content"), the Contributor obtained from such patient(s) written consent to such inclusion, disclosure or incorporation and that this consent fully complies with all legal requirements, including without limitation, all of the requirements of the laws of the jurisdiction(s) to which the patient(s) and the patient(s)' physician are subject, including the United States Health Insurance Portability and Accountability Act of 1996 ("HIPAA") if applicable. The Contributor hereby certifies that, if the patient consent form is in a language other than English, such consent form meets all of the requirements set forth in the Instructions to Authors. In addition, the Contributor hereby confirms that he/she obtained from patient(s) written consent to use the patient identifiable content in both print and online (i.e., internet/web-based) publication formats. The Contributor further certifies that the person executing any such patient consent form, to the best of his/her knowledge, had legal capacity under applicable law to execute the form on behalf of the patient.

This article is protected by copyright. All rights reserved.

Scanned with CamScanner

Author Manuscript

I. ACKNOWLEDGEMENTS

The Contributor should obtain written permission from all individuals named in the acknowledgement since readers may infer their endorsement of data and conclusions. The Contributor certifies that all individuals named in the acknowledgement section have provided written permission to be named.

J. MISCELLANEOUS

This Agreement may be amended or modified only in a writing executed by both parties. The waiver or failure of any party to exercise any rights under this Agreement shall not be deemed a waiver or other limitation of any other right or any future right. This Agreement shall inure to the benefit of, and shall be binding upon, the parties, their respective successors and permitted assigns. This Agreement may be executed in two (2) or more counterparts, each of which shall be an original and all of which taken together shall constitute one and the same agreement. Executed copies of this Agreement may be delivered by facsimile transmission, pdf/email or other comparable electronic means. If for any reason any provision of this Agreement shall be deemed by a court of competent jurisdiction to be legally invalid or unenforceable, the validity, legality and enforceability of the remainder of this Agreement shall not be affected and such provision shall be deemed modified to the minimum extent necessary to make such provision consistent with applicable law and, in its modified form, such provision shall then be enforceable and enforced. The parties agree to do such further acts and to execute and deliver such additional agreements and instruments from time to time as either may at any time reasonably request in order to assure and confirm unto such requesting party the rights, powers and remedies conferred in the Agreement. This Agreement, including any exhibits attached hereto, contains the entire agreement and understanding of the parties with respect to the subject matter hereof, and supersedes all prior agreements, negotiations, representations and proposals, written and oral, relating thereto.

All Contributors must sign below. Contributors must check one box except that NIH grantees should check both Contributor-owned work and the NIH grantee box. If your Contribution was written during the course of employment, your employer must also sign where indicated.

Please send your original completed and signed forms by fax or email a scanned copy to the Journal production editor. For production editor contact details please visit the Journal's online author guidelines. **Do not send in hard copies of these forms.**

[X] Contributor-owned work

DDB
Contributor's signature

9-21-20
Date

David D. Bushart
Type or print name and title

Co-Contributor's signature

Date

Type or print name and title

This article is protected by copyright. All rights reserved.

Scanned with CamScanner

[_____] Company/Institution-owned Work (made-for-hire in the Course of employment)

_____ Company or Institution (Employer-for-Hire) _____ Date

_____ Authorized signature of Employer _____ Date

_____ Contributor's signature _____ Date

_____ Type or print name and title

ATTACH ADDITIONAL SIGNATURE PAGES AS NECESSARY

[_____] U.S. Government work

Note to U.S. Government Employees

A contribution prepared by a U.S. federal government employee as part of the employee's official duties, or which is an official U.S. Government publication, is called a "U.S. Government work", and is in the public domain in the United States. In such case, Paragraph A.1 will not apply but the Contributor must type his/her name (in the Contributor's signature line) above. Contributor acknowledges that the Contribution will be published in the United States and other countries. If the Contribution was not prepared as part of the employee's duties or is not an official U.S. Government publication, it is not a U.S. Government work.

[_____] U.K. Government work (Crown Copyright)

Note to U.K. Government Employees

The rights in a contribution prepared by an employee of a UK government department, agency or other Crown body as part of his/her official duties, or which is an official government publication, belong to the Crown. Contributors must ensure they comply with departmental regulations and submit the appropriate authorisation to publish. If your status as a government employee legally prevents you from signing this Agreement, please contact the Journal production editor.

[_____] Other

Including Other Government work or Non-Governmental Organisation work

Note to Non-U.S., Non-U.K. Government Employees or Non-Governmental Organisation Employees

If your status as a government or non-governmental organisation employee legally prevents you from signing this Agreement, please contact the Journal production editor.

Author Manuscript

This article is protected by copyright. All rights reserved.

Scanned with CamScanner

Author Manuscript

Exhibit A

Financial Disclosure

The Contributor has received financial and material support for this research and work regardless of date from the following sources:

Name: _____

Address: _____

Type of support: _____

This material will be printed with the published article.

In the past year from the date of submission, the Contributor has also received the following support unrelated to this research (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields):

Name: _____

Address: _____

Type of support: _____

This material will be posted on the journal website and may be printed at the Editors' discretion.

ATTACH ADDITIONAL INFORMATION AS NECESSARY

This article is protected by copyright. All rights reserved.

Scanned with CamScanner

Author Manuscript

COPYRIGHT TRANSFER AGREEMENT

Date: 9/19/2020

Contributor name: Vikram Shakkottai

Contributor address: 4009 BSRB 109 Zina Pitcher PL
Ann Arbor MI 48109

Manuscript number: MDS-20-1225

Re: Manuscript entitled: A chlorzoxazone–baclofen combination improves cerebellar impairment in spinocerebellar ataxia type 1 (the "Contribution")

for publication in: Movement Disorders (the "Journal")

Published by Wiley on behalf of The International Parkinson and Movement Disorder Society (the "Owner")

Dear Contributor(s):

Thank you for submitting your Contribution for publication. In order to expedite the editing and publishing process and enable the Owner to disseminate your Contribution to the fullest extent, we need to have this Copyright Transfer Agreement executed. If the Contribution is not accepted for publication, or if the Contribution is subsequently rejected, this Agreement shall be null and void. **Publication cannot proceed without a signed copy of this Agreement.**

A. COPYRIGHT

The Contributor assigns to the Owner, during the full term of copyright and any extensions or renewals, all copyright in and to the Contribution, and all rights therein, including but not limited to the right to reproduce, publish, republish, transmit, sell, transfer, distribute, and otherwise use the Contribution in whole or in part in electronic and print editions of the Journal and in derivative works throughout the world, in all languages and in all media of expression now known or later developed, and to license or permit others to do so.

B. RETAINED RIGHTS

Notwithstanding the above, the Contributor or, if applicable, the Contributor's employer, retains all proprietary rights other than copyright, such as patent rights, in any process, procedure or article of manufacture described in the Contribution. This reservation of rights does not affect or limit the rights assigned to Owner in Section A.

C. PERMITTED USES BY CONTRIBUTOR

1. License. The Owner grants to Contributor a non-exclusive, non-transferable and limited license to reproduce and distribute copies of the print or electronic "preprints" of the unpublished Contribution, in the original form submitted to the Journal prior to the peer review process, solely to colleagues within the Contributor's nonprofit organization or educational institution. The Contributor shall make no more than 100 printed copies of the preprints in any calendar year. Such preprints may be posted as electronic files on the Contributor's own personal website, on the Contributor's internal intranet at Contributor's nonprofit organization or educational institution, or on a secure external website at the Contributor's nonprofit organization or educational institution, provided that access is limited to employees and/or students at Contributor's non-profit organization or educational institution. Contributor shall not charge a fee for any

This article is protected by copyright. All rights reserved.

Author Manuscript

preprints, and Contributor's use under this Section C shall not be for any commercial purpose, or for any systematic external distribution (e.g., posting on a listserve, public website, database connected to a public access server, or automated delivery system). The license grant in this Section does not apply to for-profit corporations, and any proposed use outside of the scope of this Section C must be pre-approved in writing by the Owner. The rights granted to Contributor under this Section C do not include reproduction, distribution or any other use of rating scales, videos or other audiovisual materials associated with the Contribution.

2. Required Citation. Prior to publication, the Contributor must provide full credit and acknowledgement of the Journal in all preprints in the following format: This is a preprint of an article accepted for publication in [Journal Title], Copyright © [year] The International Parkinson and Movement Disorder Society. After publication, the Contributor must provide a citation to the Journal in all preprints in the following format: This is a preprint of an article that was published in [Journal title]: (Title of Article, Contributor, Journal Title and Volume/ Issue, Copyright © [year] The International Parkinson and Movement Disorder Society). An electronic link must be provided to the Journal's website, located at http://www.interscience.Wiley.com. The Contributor agrees not to update the preprint or replace it with the published version of the Contribution.

3. Accepted Version. Re-use of the accepted and peer-reviewed (but not the final typeset published) version of the Contribution (the "Accepted Version") is not permitted under this Agreement. There are separate arrangements with certain funding agencies governing reuse of the Accepted Version. Additional terms apply if the Contributor receives or received funding from these agencies. The details of those relationships, and other offerings allowing open web use, are set forth at the following website: http://www.wiley.com/go/funderstatement.

4. Additional Terms for Certain Funders. Certain funders, including the NIH, members of the Research Councils UK (RCUK) and Wellcome Trust require deposit of the Accepted Version in a public repository after an embargo period. Details of funding arrangements are set out at the following website: http://www.wiley.com/go/funderstatement. Additional terms may be applicable. Please contact the production editor for the journal at MDSprod@wiley.com if you have additional funding requirements.

If any Contributor receiving funds from applicable sources does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in a public repository after the following applicable embargo period has expired, subject to further conditions imposed by the RCUK:

a. 12 months from first publication online of the final published version of the Contribution for research funded by members of the Research Councils UK (RCUK) other than The Economic and Social Research Council (ESRC) and the Arts and Humanities Research Council (AHRC); or
b. 24 months from first publication online of the final published version of the Contribution for research funded by ESRC or AHRC.

5. Additional Terms for Certain Institutions. Wiley has arrangements with certain educational institutions to permit the deposit of the Accepted Version in the institutional repository after an embargo period. Details of such arrangements are set out at the following website: http://olabout.wiley.com/WileyCDA/Section/id-406074.html . Additional terms may be applicable.

If any Contributor affiliated with these applicable educational institutions does not choose the Owner's OnlineOpen option, the Contributor will be allowed to self-archive by depositing the Accepted Version in the educational institution's repository after the following applicable embargo period has expired. See the following website for details: http://olabout.wiley.com/WileyCDA/Section/id-817011.html.

This article is protected by copyright. All rights reserved.

Author Manuscript

D. CONTRIBUTIONS OWNED BY EMPLOYER

If the Contribution was written by the Contributor in the course of the Contributor's employment (as a "work-made-for-hire" in the course of employment), the Contribution is owned by the company/institution which must execute this Agreement (in addition to the Contributor's signature). In such case, the company/institution hereby assigns to the Owner, during the full term of copyright, all copyright in and to the Contribution for the full term of copyright throughout the world as specified in Section A above.

E. GOVERNMENT CONTRACTS

In the case of a Contribution prepared under U.S. Government contract or grant, the U.S. Government may reproduce, without charge, all or portions of the Contribution and may authorize others to do so, for official U.S. Government purposes only, if the U.S. Government contract or grant so requires. (U.S. Government, U.K. Government, and other government employees: see notes at end.)

F. CONTRIBUTOR'S REPRESENTATIONS

The Contributor represents that the Contribution is the Contributor's original work, all individuals identified as Contributors actually contributed to the Contribution, and all individuals who contributed are included. The Contribution is submitted only to this Journal and has not been published before. (If excerpts from copyrighted works owned by third parties are included, the Contributor will obtain written permission from the copyright owners for all uses as set forth in the Journal's Instructions for Contributors, and show credit to the sources in the Contribution.) The Contributor also warrants that the Contribution contains no libelous or unlawful statements, does not infringe upon the rights (including without limitation the copyright, patent or trademark rights) or the privacy of others, or contain material or instructions that might cause harm or injury. Upon request, Contributor will provide the data or will cooperating fully in obtaining and providing the data on which the Contribution is based for examination by the editors or their assignees.

G. FINANCIAL DISCLOSURES

The Contributor certifies that his/her financial and material support for this research and work, regardless of date, is clearly identified on Exhibit A to this Agreement. The Contributor has also identified on Exhibit A, all other support unrelated to this research, covering the past year from the date of submission (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields).

H. VIDEO AND PHOTOGRAPHY CONSENT

In the event that the Contribution includes, discloses or incorporates any content (including, without limitation, any video clip or photograph) which identifies any individual patient(s) ("patient identifiable content"), the Contributor obtained from such patient(s) written consent to such inclusion, disclosure or incorporation and that this consent fully complies with all legal requirements, including without limitation, all of the requirements of the laws of the jurisdiction(s) to which the patient(s) and the patient(s)' physician are subject, including the United States Health Insurance Portability and Accountability Act of 1996 ("HIPAA") if applicable. The Contributor hereby certifies that, if the patient consent form is in a language other than English, such consent form meets all of the requirements set forth in the Instructions to Authors. In addition, the Contributor hereby confirms that he/she obtained from patient(s) written consent to use the patient identifiable content in both print and online (i.e., internet/web-based) publication formats. The Contributor further certifies that the person executing any such patient consent form, to the best of his/her knowledge, had legal capacity under applicable law to execute the form on behalf of the patient.

This article is protected by copyright. All rights reserved.

Author Manuscript

I. ACKNOWLEDGEMENTS

The Contributor should obtain written permission from all individuals named in the acknowledgement since readers may infer their endorsement of data and conclusions. The Contributor certifies that all individuals named in the acknowledgement section have provided written permission to be named.

J. MISCELLANEOUS

This Agreement may be amended or modified only in a writing executed by both parties. The waiver or failure of any party to exercise any rights under this Agreement shall not be deemed a waiver or other limitation of any other right or any future right. This Agreement shall inure to the benefit of, and shall be binding upon, the parties, their respective successors and permitted assigns. This Agreement may be executed in two (2) or more counterparts, each of which shall be an original and all of which taken together shall constitute one and the same agreement. Executed copies of this Agreement may be delivered by facsimile transmission, pdf/email or other comparable electronic means. If for any reason any provision of this Agreement shall be deemed by a court of competent jurisdiction to be legally invalid or unenforceable, the validity, legality and enforceability of the remainder of this Agreement shall not be affected and such provision shall be deemed modified to the minimum extent necessary to make such provision consistent with applicable law and, in its modified form, such provision shall then be enforceable and enforced. The parties agree to do such further acts and to execute and deliver such additional agreements and instruments from time to time as either may at any time reasonably request in order to assure and confirm unto such requesting party the rights, powers and remedies conferred in the Agreement. This Agreement, including any exhibits attached hereto, contains the entire agreement and understanding of the parties with respect to the subject matter hereof, and supersedes all prior agreements, negotiations, representations and proposals, written and oral, relating thereto.

All Contributors must sign below. Contributors must check one box except that NIH grantees should check both Contributor-owned work and the NIH grantee box. If your Contribution was written during the course of employment, your employer must also sign where indicated.

Please send your original completed and signed forms by fax or email a scanned copy to the Journal production editor. For production editor contact details please visit the Journal's online author guidelines. **Do not send in hard copies of these forms.**

[✓] Contributor-owned work

V.G Nik

Contributor's signature

9-19-2020

Date

Vikram Shakkottai

Type or print name and title

Associate Professor of Neurology

Co-Contributor's signature

Date

Type or print name and title

This article is protected by copyright. All rights reserved.

[_____] Company/Institution-owned Work (made-for-hire in the Course of employment)

_____ Company or Institution (Employer-for-Hire) _____ Date

_____ Authorized signature of Employer _____ Date

_____ Contributor's signature _____ Date

_____ Type or print name and title

ATTACH ADDITIONAL SIGNATURE PAGES AS NECESSARY

[_____] **U.S. Government work**

Note to U.S. Government Employees

A contribution prepared by a U.S. federal government employee as part of the employee's official duties, or which is an official U.S. Government publication, is called a "U.S. Government work", and is in the public domain in the United States. In such case, Paragraph A.1 will not apply but the Contributor must type his/her name (in the Contributor's signature line) above. Contributor acknowledges that the Contribution will be published in the United States and other countries. If the Contribution was not prepared as part of the employee's duties or is not an official U.S. Government publication, it is not a U.S. Government work.

[_____] **U.K. Government work (Crown Copyright)**

Note to U.K. Government Employees

The rights in a contribution prepared by an employee of a UK government department, agency or other Crown body as part of his/her official duties, or which is an official government publication, belong to the Crown. Contributors must ensure they comply with departmental regulations and submit the appropriate authorisation to publish. If your status as a government employee legally prevents you from signing this Agreement, please contact the Journal production editor.

[_____] **Other**

Including Other Government work or Non-Governmental Organisation work

Note to Non-U.S., Non-U.K. Government Employees or Non-Governmental Organisation Employees

If your status as a government or non-governmental organisation employee legally prevents you from signing this Agreement, please contact the Journal production editor.

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

Exhibit A

Financial Disclosure

The Contributor has received financial and material support for this research and work regardless of date from the following sources:

Name: NIH

Address: Bethesda, MD

Type of support: Research Grant R01 NS085054

This material will be printed with the published article.

In the past year from the date of submission, the Contributor has also received the following support unrelated to this research (e.g., grants, advisory boards, employment, consultancies, contracts, honoraria, royalties, expert testimony, partnerships, or stock ownership in medically-related fields):

Name: Up to Date Inc

Address: Wolters-Kluwer

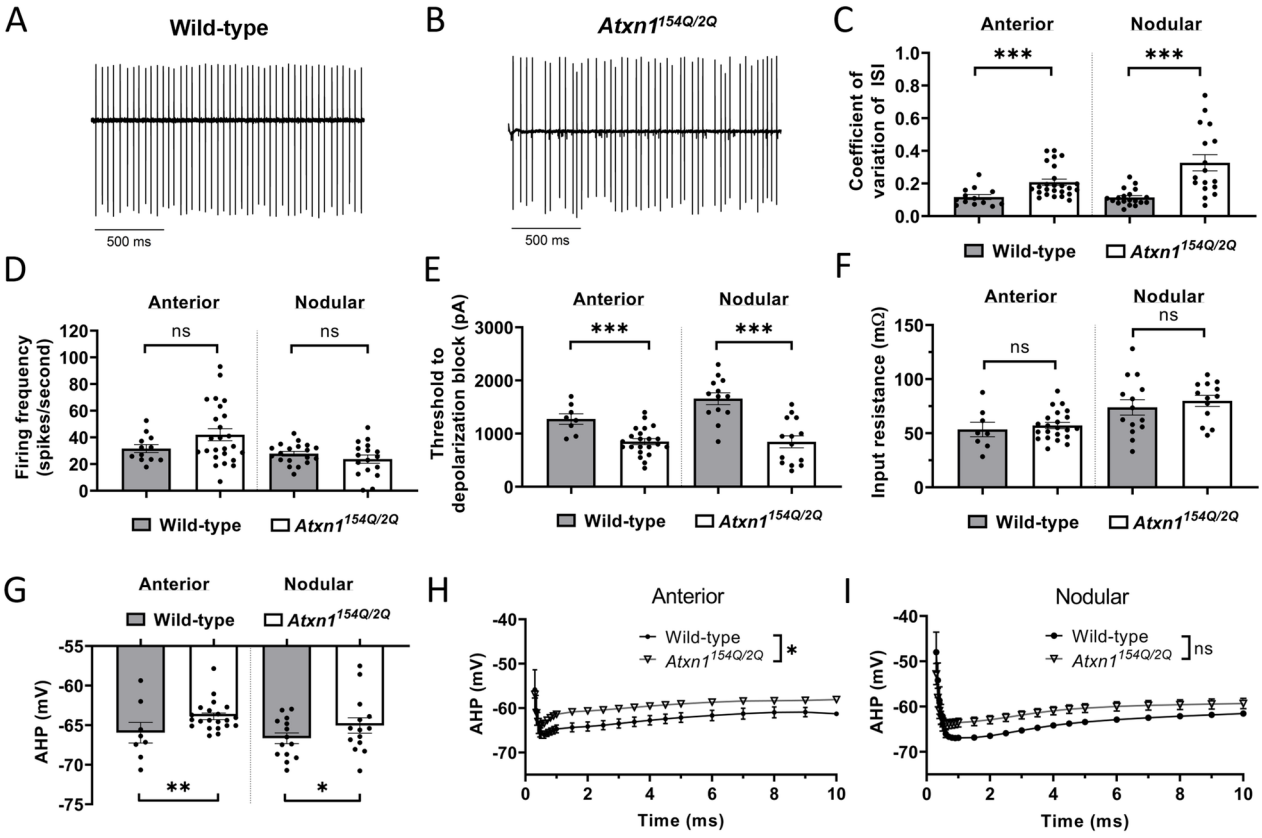
Type of support: Royalty payment for educational material on management of cerebellar ataxia

This material will be posted on the journal website and may be printed at the Editors' discretion.

ATTACH ADDITIONAL INFORMATION AS NECESSARY

This article is protected by copyright. All rights reserved.

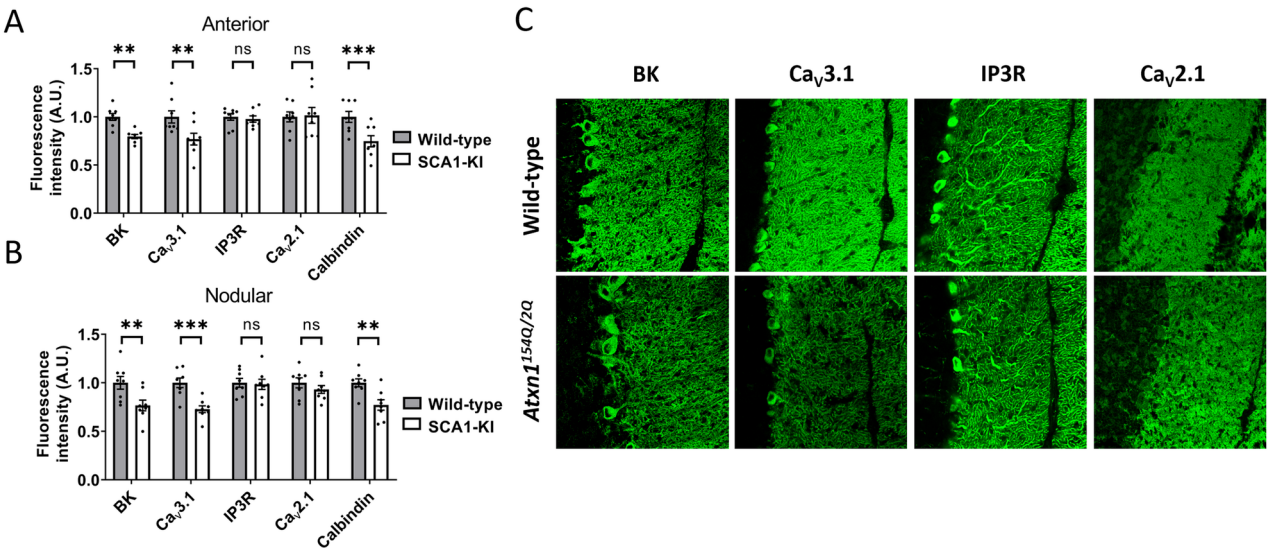
Author Manuscript



MDS_28355_Figure 1.tif

This article is protected by copyright. All rights reserved.

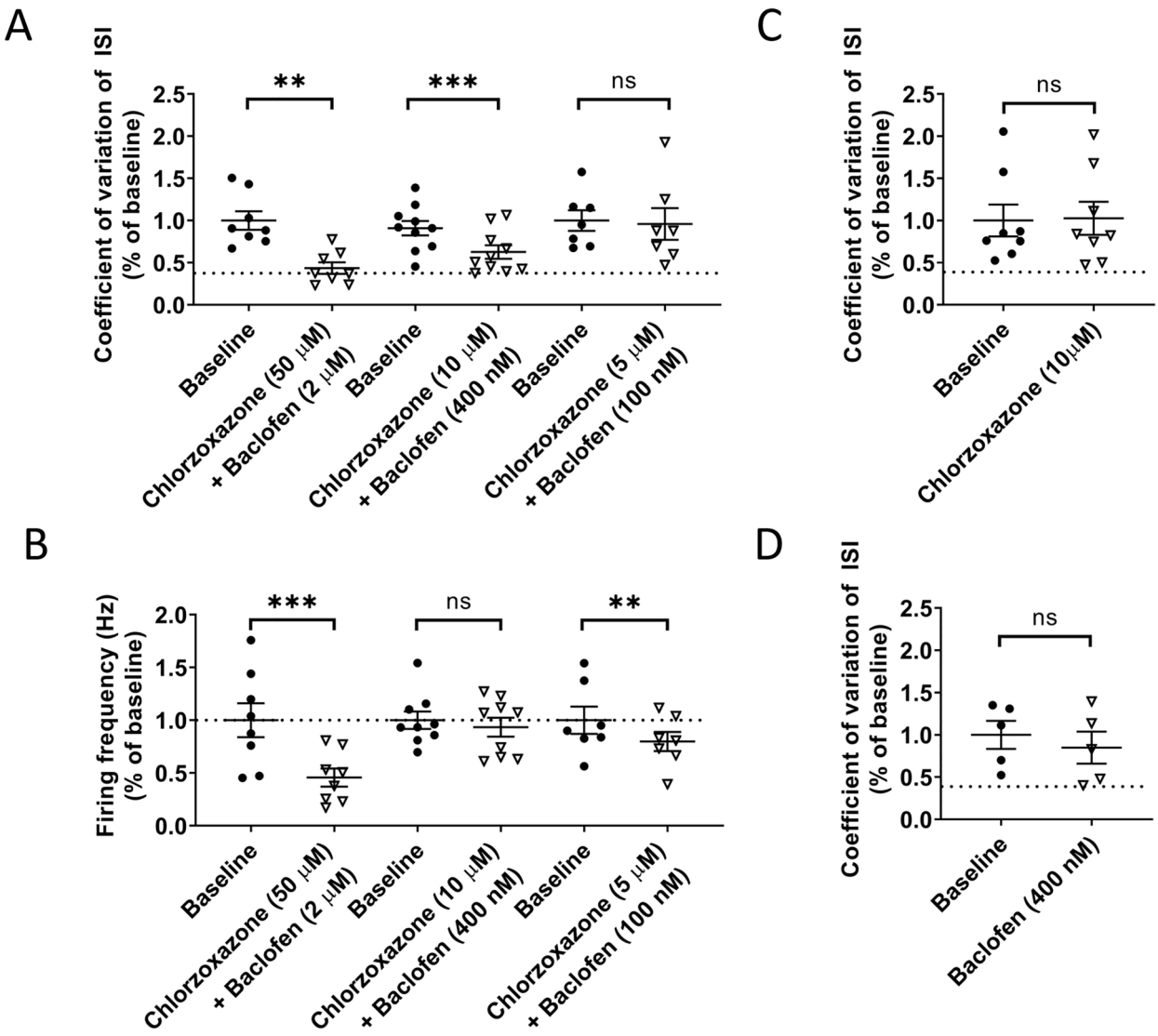
Author Manuscript



MDS_28355_Figure 2.tif

This article is protected by copyright. All rights reserved.

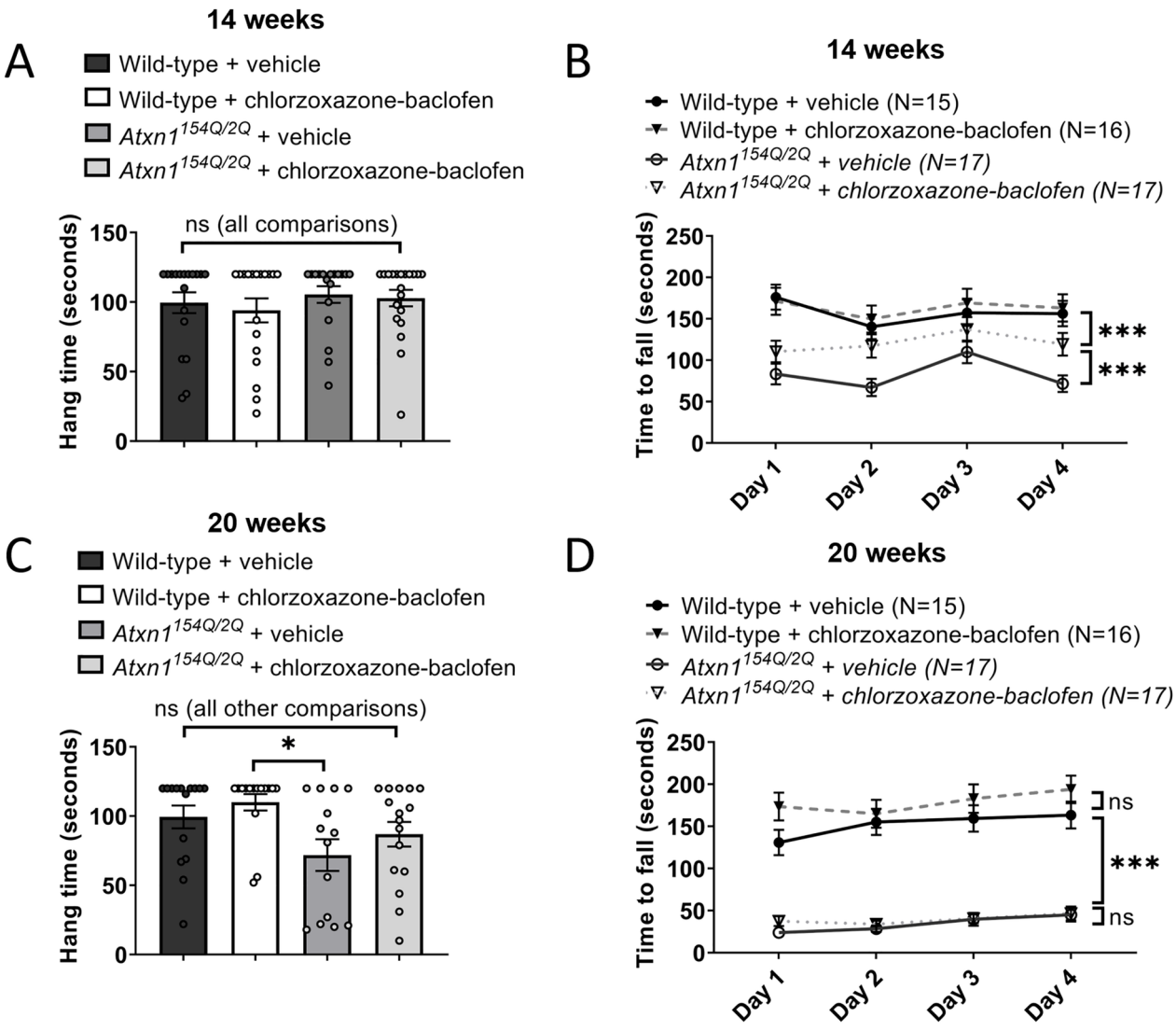
Author Manuscript



MDS_28355_Figure 3.tif

This article is protected by copyright. All rights reserved.

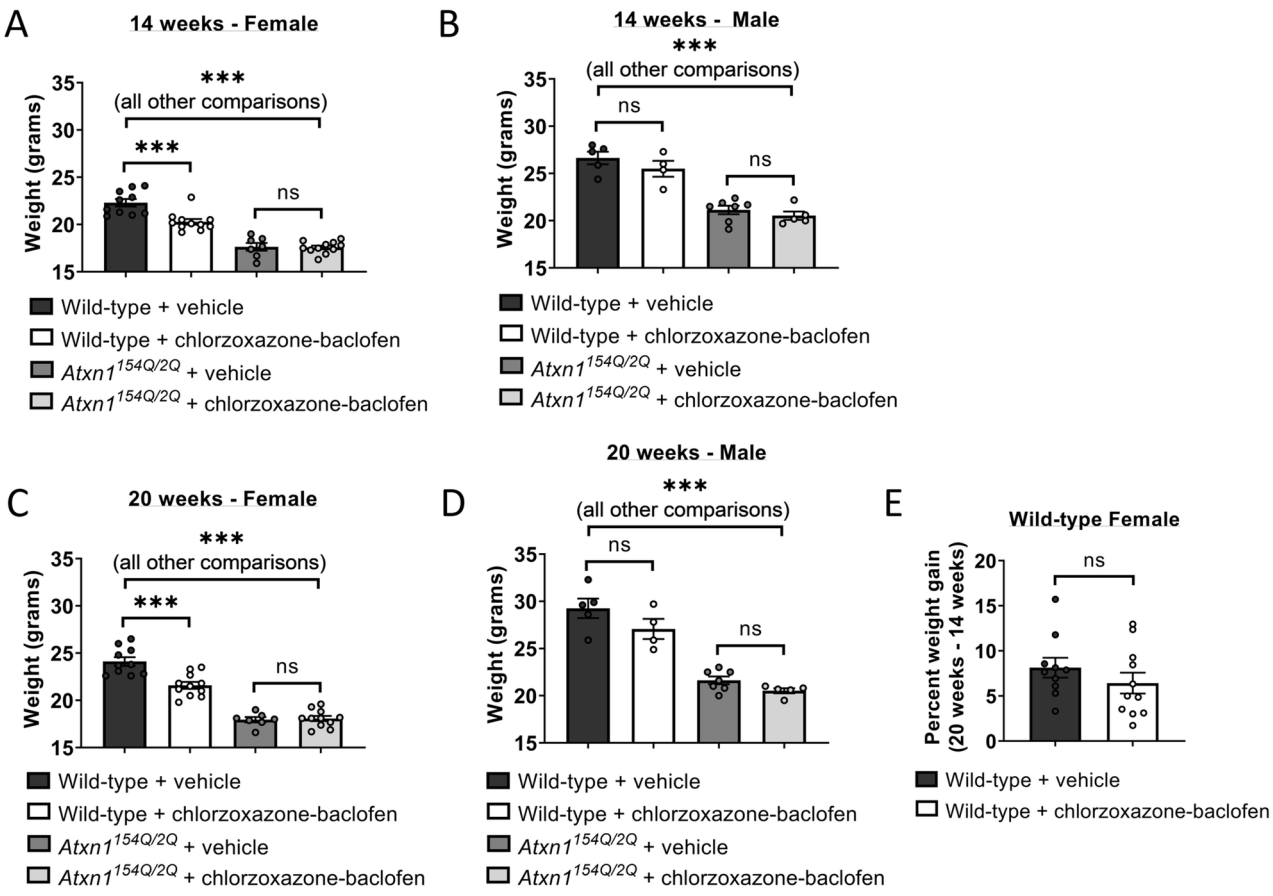
Author Manuscript



MDS_28355_Figure 4.tif

This article is protected by copyright. All rights reserved.

Author Manuscript



MDS_28355_Figure 5.tif

This article is protected by copyright. All rights reserved.