

Evaluating the Effects of Deep Brain Stimulation (DBS) in Mice with
Spinocerebellar Ataxia (SCA1)

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Abstract

Spinocerebellar ataxia type 1 (SCA1) is a progressive neurodegenerative disorder that affects gait, balance, and other movements. The motor malfunctions in SCA1 are caused by cerebellar cortical degeneration, specifically altered Purkinje neuronal output. The Purkinje cells project inhibitory signals to the deep cerebellar nuclei (DCN), thus playing a vital role in motor coordination. These neurons are lost in those affected by SCA1, resulting in DCN hyperexcitability. Therefore, reducing DCN hyperexcitability could help eliminate the motor abnormalities in ataxia.

The present study investigates the effects of deep brain stimulation (DBS) in the DCN of SCA1 transgenic (B05) mice. The effects were evaluated by behavioral testing primarily gait assessment and compared B05 mice that received acute high frequency stimulations (HFS) to those without the treatment.

The results of this study demonstrate the feasibility of targeting the DCN in mice and the ability to successfully apply acute HFS. Gait assessment was carried out effectively on all mice through the DigiGait® treadmill apparatus. Furthermore, the data indicates that B05 mice subject to HFS-DBS show marginal improvement in movement control and coordination suggesting that DBS could be beneficial for patients with ataxia.

Keywords: spinocerebellar ataxia type 1 (SCA1), deep cerebellar nuclei (DCN), high frequency stimulation (HFS)

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Introduction

Spinocerebellar ataxia type 1 (SCA1) is an autosomal-dominantly inherited neurodegenerative disorder that typically shows symptoms in the third or fourth decade of life. SCA1 patients suffer from motor dysfunction, muscle atrophy, cognitive impairment and often die 10-30 years after the onset (Opal & Ashizawa, 2017). Usually, symptoms worsen over time and result in death from complications related to brainstem dysfunction (Burrigh et al., 1995). Currently, there is no effective cure or treatment for SCA1.

SCA1 is caused by the expansion of a CAG tract in exon 8 of the Ataxin-1 gene affecting the brainstem, spinocerebellar tracts, and particularly Purkinje cells of the cerebellar cortex (Burrigh et al., 1995). The CAG-repeat mutation on the Ataxin-1 gene (chromosome 6) results in the encoding of a mutated Ataxin-1 protein. The greater length of the unstable CAG-repeat (41-81 comparing to 6-39 in normal gene) results in abnormal protein aggregation. Although the specific function(s) of Ataxin-1 is still largely unknown, its abnormal aggregates cause degeneration in the nuclei of susceptible neurons, specifically cerebellar Purkinje cells (Burrigh et al., 1995).

The Purkinje cells project inhibitory signals to the deep cerebellar nuclei (DCN), thus playing a vital role in motor coordination (Grüsser-Cornehls & Bährle, 2001). An absence of the inhibitory signals to the deep cerebellar nuclei (DCN) results in hyperexcitability and thereby motor impairment (Shakkottai et al., 2004). Hence, regulating the firing frequency of the DCN neurons could ameliorate symptoms. To

study the pathogenesis of the SCA1 mutation, Burrig et al., (1995) developed transgenic mice which express the human SCA1 gene with either a normal or expanded CAG tract. The earliest onset of ataxia, at 12 weeks, was found in the B05 mouse model (Burrig et al., 1995). This model shows significant loss of Purkinje cells.

Deep brain stimulation (DBS) has been utilized to treat movement disorders such as Parkinson's Disease, dystonia, and essential tremor, but ataxia symptoms have not been frequently addressed with DBS. There is no established mechanism on how DBS works, though it is believed that DBS reduces neuronal activity inhibitory cells (Agnesi, Johnson, & Vitek, 2013).

There are currently several studies which look at the effects of electrical stimulation in rodents for other movement disorders, but there is a lack of research addressing ataxia. Subthalamic nucleus (STN) DBS is used in Parkinson's disease and shows alleviation of motor impairments, but there is little knowledge regarding its molecular mechanisms (Fischer et al., 2017). Musacchio et al., (2017) have found evidence that there is a neuroprotective effect of STN-DBS which results in the motor improvements. Iwata and Ugawa (2005) studied the effects on motor cortical excitability caused by electrical stimulation over the cerebellum. This method was applied to patients with ataxia and demonstrated an inhibitory effect that was only seen in patients with a lesion at the dentatohalamocortical pathway. This pathway received inhibitory signals from Purkinje cells of the cerebellar cortex (Iwata et al., 2005). Baker, Schuster, Cooperrider, and Machado (2010), used a rodent model of middle cerebral artery stroke to demonstrate the effects of electrical stimulation on the lateral cerebellar nucleus

(LCN). Their results showed motor recovery suggesting that direct stimulation of the LCN can yield changes in cortical excitability.

The purpose of this study was to determine the therapeutic effects of deep brain stimulation (DBS) on SCA1 by examining potential changes in motor functions as well as Purkinje cell degeneration in the B05 transgenic mouse line. We hypothesize that DBS can be used to alleviate symptoms of SCA1 by “normalizing” the neuronal firing rate in DCN.

Methods

Animals

To further investigate the pathogenesis of the SCA1 mutation, Burrig et al., (1995) generated transgenic mice expressing the human SCA1 gene with an expanded CAG tract. One of these, the transgenic mouse line B05, contains 30 copies of the transgene and a 12-week onset of ataxia, which is the model utilized in this study. B05 mice were assigned to one of three groups: a HFS- DBS (STIM), a SHAM group, and an untreated control group.

Surgery

Stereotactic DBS surgeries were performed on mice in both SHAM and STIM groups. The animals were placed in an induction chamber, and anesthetized with isoflurane supplied as a constant mix with O₂ at 3%. The animals were transferred to the stereotactic frame and monitored for complete anesthesia and normal respiration throughout the procedure. After the animals were fully anesthetized, as indicated by the lack of toe pinch reflex, they were prepared for the aseptic stereotactic implantation of stimulation electrodes into the cerebellum. The surgical site on top of the head of the

animal was thoroughly scrubbed with 70% isopropyl alcohol (3x), and 10% povidone-iodine (PVP-I) (3x) for surgery. Body temperature was maintained at 37°C with a temperature-controlled heating pad or snuggle-safe animal warmer as appropriate. Animals underwent stereotactic placement of bipolar double-cored polyurethane-insulated stainless-steel stimulating electrodes (Plastics One Products) unilaterally. Mice were placed in a stereotactic frame with the incisor bar set 3 mm above the interneural line. Electrodes were implanted in the cerebellum in the dentate nucleus at the following coordinates (from Bregma: anterior/posterior -6.5 mm, medial/lateral 2 mm, and dorsal/ventral -1.5 mm). The electrodes were stereotaxically moved to the appropriate coordinates and were secured in place using cranioplastic cement. Animals were then allowed to recover from their surgery for 24-48 hours.

DBS

The STIM group received acute HFS after the recovery period for one week at one hour every day and the SHAM group received no stimulation. The stimulus amplitude varied from 100 to 300 uA, with a pulse duration of 60us, and frequency of 300Hz.

DigiGait®

Gait assessment was performed using a DigiGait® treadmill apparatus for all mice groups pre- and post-surgery. The DigiGait apparatus consists of a clear plexiglass chamber to contain the mouse on top of a clear treadmill belt. The treadmill speed was set to 20 cm/sec and mice were video recorded while running on the belt. Video processing involved conversion of paw contacts on the treadmill to digital pawprints, which were used to determine over 40 gait indices. These indices give

outputs of stride length, stance width, ataxia coefficient, and gait symmetry. Stride length is the distance between strides of the same paw and this value should be approximately equal among the four paws. Stance width is the width between either the forelimbs or hindlimbs, and hindlimb stance width is known to be wider in the unstimulated B05 mice. Ataxia coefficient is the difference between the maximum and minimum stride lengths divided by the mean stride length. Gait symmetry is a ratio of forelimb stepping frequency to hindlimb stepping frequency, and in healthy animals, this value should be closer to 1. The untreated B05 mice were expected to have shorter stride length, wider gait width, higher ataxia coefficient and lower gait symmetry. The treated B05 mice were expected to show improvement in these gait measures.

Tissue Processing

Anatomical localization of the electrodes was confirmed using serial brain sections. Specific attention was paid to the location of the electrode tip within each hemisphere, and its relationship to adjacent structures. A cryostat microtome was used to section the brain tissue into 7 micron thick sections.

Results

Stereotactic Accuracy

Implementation of DBS electrodes into the DCN of B05 mice and application of acute HFS was successfully accomplished. Light microscopy imaging of mice brain tissue sections confirmed the correct targeting as seen in Figure 1.



Figure 1. A light microscopic image of successfully targeted DCN in B05 mice brain tissue section.

DigiGait

Recordings of the gait assessment performed using a DigiGait® treadmill apparatus for all mice groups pre- and post-surgery revealed differences in gait performance pre-stimulation and post-stimulation. Figure 2 (top) shows a B05 mouse presenting with ataxic gait symptoms during the pre-surgery run. In contrast, Figure 2 (bottom) reveals that same B05 mouse presenting with improved gait after receiving HFS after electrode implementation. Figure 3 depicts the STIM B05 mouse inside DigiGait® with an electrode implanted in the DCN.



Figure 2. B05 mouse presenting with ataxic gait pre-stimulation and improved gait post-stimulation.



Figure 3. B05 mouse inside DigiGait® with electrode implanted in DCN.

Temporospatial Parameters

DigiGait® assessment demonstrated improvement in ataxic mice treated with HFS DBS, with respect to movement control and coordination. The B05 mice show an impaired gait in which they are over-reliant on their hindlimbs. This results in the investment of excess hindlimb power which is reflected in MAX dA/dT and paw area. Paw area at peak stance is the amount of paw that is in contact with the belt at the point of maximum contact. There is a significant difference in the amount of paw area in the STIM mice compared to the paw area prior, which shows an improvement in movement control (Figure 4). Hind paw area and power generation of the hind paw during propulsion (MAX dA/dT) was returned to more wildtype values in STIM mice as seen in Figure 4. This implies that our treatment corrects the lack of control and deceleration in B05 mice.

The inability of mice to control limb power leads to the variability seen in the gait, which is measured as an ataxia coefficient. Figure 5 demonstrates improvement in ataxia coefficient. STIM mice approaching closer to the value of 1 indicate no variability and lower values indicate high variability and less control of gait. Additionally, investment of too much power into the hindlimbs is reflected in the longer time in stance and propulsion in B05 mice, which is ameliorated post DBS (Figure 6).

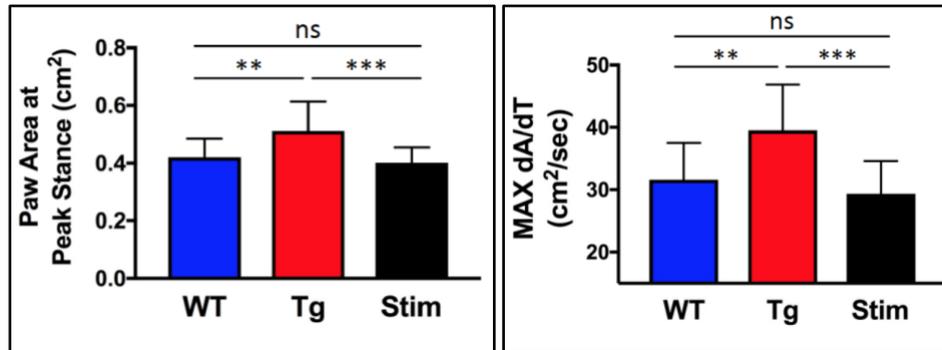


Figure 4. Measure of the amount of paw that is in contact with the belt at the point of maximum contact (Paw Area) and a measure of the power generated by the hindpaws as they push off the belt for the next step (MAX dA/dT).

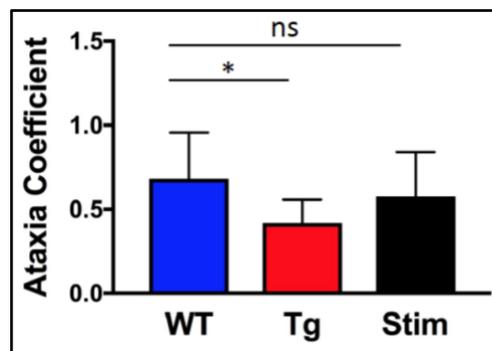


Figure 5. Ataxia coefficient calculated by $[\text{max stride length} - \text{min stride length}] / \text{mean stride length}$.

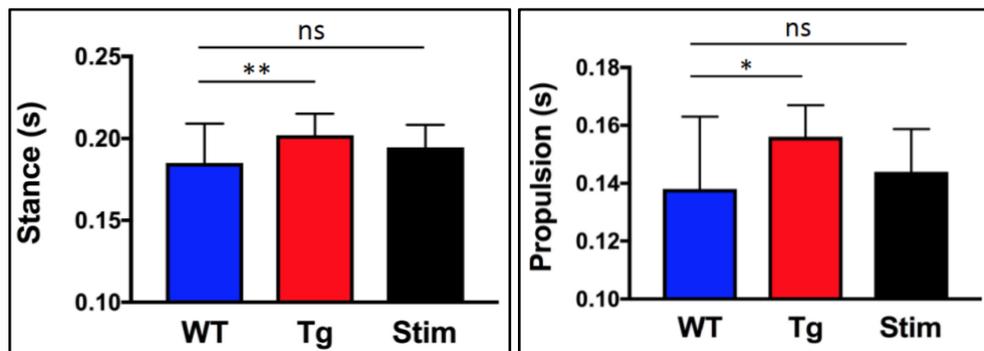


Figure 6. The amount of time that the paw spends on the belt (stance) which is further subdivided into propulsion of the hindlimbs. Changes in stance time are due to changes in the time spent during the propulsion phase.

RNA sequencing and gene expression were looked at to determine if DBS alters the splicing of mRNAs in the cerebellum of SCA1 B05 mice. The hnRNP proteins control RNA homeostasis of many genes. They contribute to several aspects of nucleic

acid metabolism including alternative splicing, mRNA stabilization, and transcriptional and translational regulation. These proteins are highly expressed in the brain and known to control the splicing of their own transcripts. A preliminary look at changes in mRNA splicing in B05 mice post DBS has shown a significant difference (Figure 7).

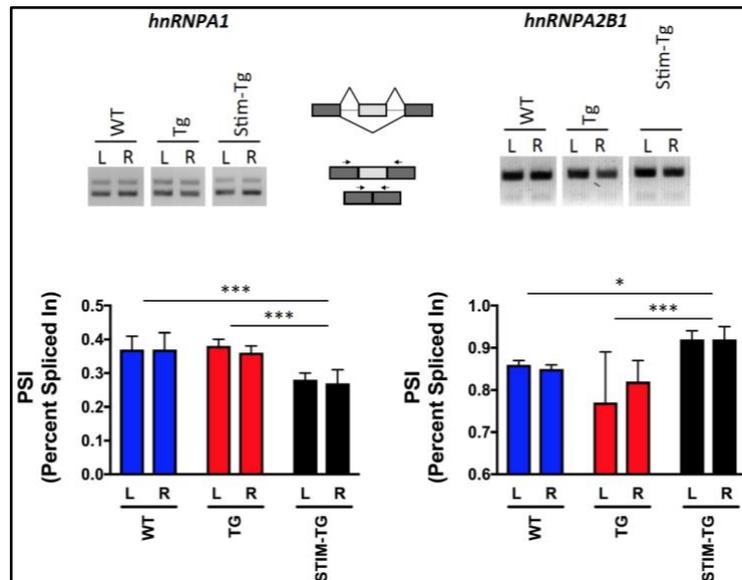


Figure 7. Alterations in mRNA splicing post DBS.

Discussion

This study is the first, to the author's knowledge, to use cerebellar DBS as a therapy for SCA1 in a preclinical model. Results from this study have shown the practicality and potential for application of DBS to ameliorate the abnormal motor functions of SCA1. The DCN in B05 mice was successfully targeted, which is essential for reliable results. In this study, we found that acute HFS via DBS resulted in the improvement of certain gait parameters in SCA1 mice including paw area, power generation of the hind paw during propulsion, ataxia coefficient, stance and propulsion.

Future studies should include other mouse models of cerebellar ataxia, such as SCA8, SCA6, as results from these may reveal more details about neurological

sequelae. The results rely on there being a significant difference between wild type vs. transgenic mice. Therefore, testing other strains of mice with cerebellar ataxia would be important to see if there is a more optimal strain as well as to determine if the DBS would be significant in other models.

More in depth investigations of RNA sequencing and gene expression levels must be considered in the future to better understand whether DBS induces changes at the transcriptome level. Further investigations with a larger mice population, a different genotype of mouse and greater data collection will be pursued to improve robustness of the dataset. Additionally, chronic stimulation paradigms will be necessary to confirm the initial observations and make this a translational therapy in humans. Exploring other possible targets in the cerebellum or other structures to stimulate is also another option to consider. Lastly, different stimulation frequencies and duration of time of administration should be explored to determine the optimal settings for DBS. This treatment idea for ataxia is novel and will require further research; however, we hope that it will become a clinically viable solution soon.

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