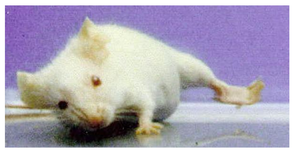

Optical Imaging of Cerebellar Dysfunction in SCA1 Mice

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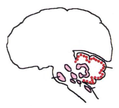
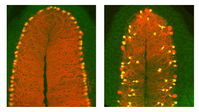
ABSTRACT

Spinocerebellar ataxia type 1(SCA1) is an inherited cerebellar neurodegenerative disorder caused by a polyglutamine tract expansion in the ataxin-1 protein. Recent studies have suggested that the mutation in ataxin-1 down-regulates mGluR1 signaling proteins. We investigated the cerebellar functioning with optical imaging and found a deficit in mGluR1 response and a diffuse climbing fiber activation of Purkinje cells. Loss of parasagittal organization is consistent with the loss of mGluR1. The SCA1 mice also exhibited the loss of off-beam inhibition. The loss of the off-beam inhibition is believed to be a sign of cerebellar dysfunction. Early in the recovery of the tet-regulatable SCA1 mice, the climbing fiber activation of Purkinje has the normal parasagittal organization, but the off-beam inhibition is still absent.





INTRODUCTION

Spinocerebellar ataxia type 1 (SCA1) is an autosomal dominant neurodegenerative disorder characterized by slurred speech, loss of limb coordination, and gait abnormalities[1]. SCA1 affected individuals experience a progressive loss of motor control that eventually leads to death 15 years after onset. SCA1 is caused by a toxic gain-of-function expansion in the polyglutamine tract of the ataxin-1 protein. The underlying neuropathology is the degeneration of the Purkinje cells and neurons of select midbrain nuclei. A successful SCA1 model has been generated by expressing mutant ataxin-1 driven by the Pcp2 promoter[2]. These mice recapitulate the hallmarks of the human SCA1 disease with progressive loss of Purkinje cell arborization, heterotopic Purkinje cell somata, and impaired motor skills. A tet-regulatable pcp2 driven mouse model of SCA1 has been recently made. These mice demonstrated the ability of the brain to reverse aspects of SCA1 pathology.

Recent studies have implicated transcriptional regulation and RNA processing in SCA1[3,4]. Microarray analysis of SCA1 mice indicate a reduction in genes involved in mGluR1 signaling[5]. The mRNA of the genes of the proteins colored gray in the schematic below have reduced mRNA levels in SCA1 mice. The mGluR1 protein levels have been confirmed to be reduced[6].

Figure 1: Microarray analysis revealed the down-regulation of genes involved in mGlur1 signaling[5]. Gray shapes indicate genes identified as down-regulated in SCA1 mice.

We hypothesized that the down-regulation of mGluR1 signaling proteins would lead to synaptic dysfunction, and this dysfunction underlies the neuronal dysfunction seen in SCA1. We sought to characterize synaptic plasticity in SCA1 transgenic mice using cerebellar optical imaging *in vivo.*

METHODS

The Ebner laboratory pioneered *in vivo* optical imaging of cerebellar activity[7,8,9]. We provided tet-regulatable SCA1 transgenic mice for optical imaging in conjunction with afferent stimulation. SCA1 transgenic mice were generated on a FVB background. FVB mice were used as control (wild-type) mice. Parallel fiber stimulation (100usec pulses at 50-200μA, 10-100 Hz for 10 sec) was delivered superficially to cerebellar surface via a tungsten electrode (1-3 MΩ). Climbing fiber stimulation was achieved by injecting current (100usec pulses at 100-200μA, 10 Hz for 10 sec) into the contralateral inferior olive with a tungsten electrode (1-3 MΩ). We provided moderately affected, 12 week old SCA1 transgenic mice, and mice that had the transgene turned off for 4 weeks after 12 weeks on (12on/4off); these mice are in the early stages of recovery.

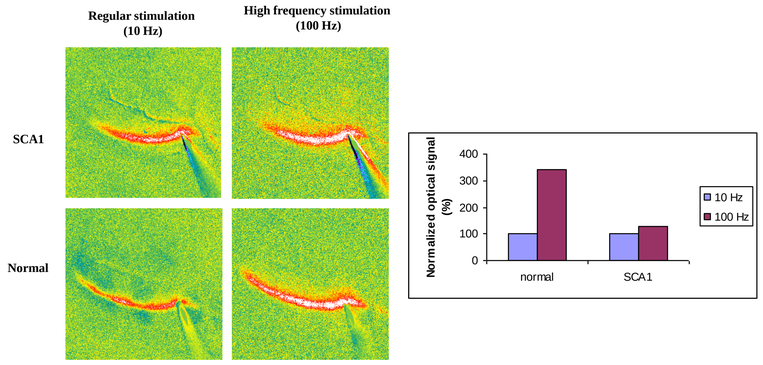


Figure 2: SCA1 mouse Purkinje cells were less reactive to an mGluR1 stimulation protocol. Purkinje cell activation with 10 Hz stimulation via the parallel Fibers does not have a major mGluR1 component. With high frequency stimulation of 100 Hz, the beam response increases 3-fold to reflect the additional stimulation of the Purkinje cell through mGluR1 pathway in the control mice[10]. High frequency (100 Hz) does not elicit an augmented optical beam response in SCA1 transgenic mice. SCA1 transgenic mice have a deficit in mGluR1 stimulation of the Purkinje cells

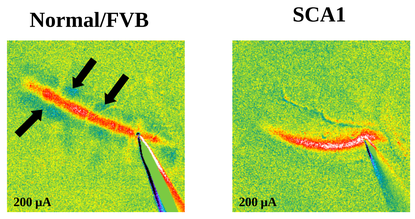


Figure 3: Parallel fiber stimulation produces an off-beam inhibitory response. Purkinje cell activity levels drop below baseline levels adjacent to a beam of parallel fiber stimulated Purkinje cells (See arrows). This drop in activity is referred to as off-beam inhibition. Off-beam inhibition is absent in SCA1 transgenic mice. Off-beam inhibition was absent in another ataxic transgenic mouse line. The loss of the off-beam inhibition is probably indicative of a dysfunctional cerebellar network since it is seen in other ataxic mice and not a primary mechanism of SCA1 dysfunction.

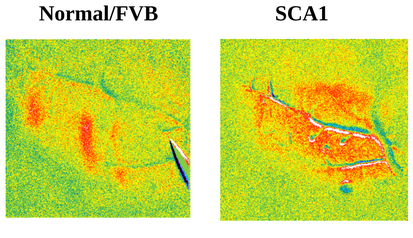


Figure 4: Climbing fiber (CF) stimulations results in diffuse activation of the Purkinje cells in SCA1 transgenic mice. CF projections are arranged into condense parasagittal stripes in the mature cerebellum of normal mice[7]. As a result, stimulation of the CFs will result in stimulation of Purkinje cells in parasagittal bands. CF stimulation in the SCA1 mice results in a diffuse activation of Purkinje cells with no distinct parasagittal bands. This diffuse activation suggest the absent of the CF parasagittal banding pattern.

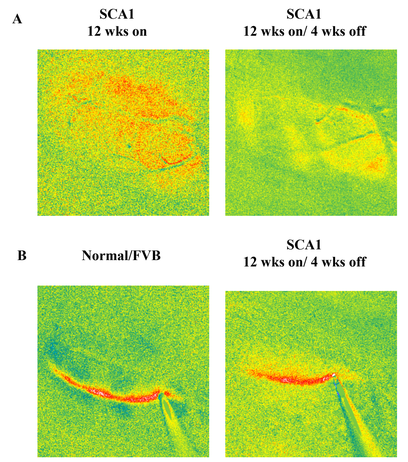


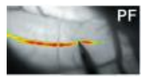
Figure 5: SCA1 transgenic mice in the early stages of recovery have normal pattern of Purkinje cell activation with CF stimulation, but off-beam inhibition was absent. The SCA1 transgene was left on for 12 weeks and turned off for 4 weeks (12on/4off). 12on/4off SCA1 transgenic mice are in early stages of recovery and do not yet have any significant recovery in Rotarod performance, a behavior test of cerebellar function, or in cell pathology. These mice do have parasagittal band pattern of Purkinje cell activation when the contra lateral inferior olive was stimulated (A). This recovery is to prior the recovery of the behavioral phenotype assessed by rotarod and to the reappearance of off-beam inhibition. Off-beam inhibition is still absence in 12on/4off SCA1 transgenic mice (B).

CONCLUSIONS

High frequency parallel fiber stimulation does not elicit an augmented optical beam in the SCA1 transgenic mice. This finding demonstrates that a previous found reduction of mGluR1 protein levels translates to a detectable deficit in mGluR activity.

SCA1 transgenic mice do not display off-beam inhibition. This off-beam inhibition has been attributed to inhibitory stellate neurons. The loss of the off-beam inhibition is hypothesized to reflect a dysfunction cerebellar neural network. The presence of the off-beam inhibition in SCA1 transgenic may be useful tool in measuring cerebellar function in future studies.

CF stimulation results in a diffuse pattern of Purkinje cell activation. The parasaggital compartmentalization of Purkinje cell activation returns prior to behavioral and cell pathology recovery indicating the CF abnormality may be related to the primary disease process. mGluR1 knockout mice also have CF abnormalities with the loss of the parasagittal banding pattern[11]. Thus, the diffuse pattern of Purkinje cell activation in SCA1 transgenic mice is consistent with an mGluR1 deficit in the SCA1 transgenic mice.



FUTURE STUDIES

1. Investigate whether SCA1 mice have altered synaptic plasticity. 12-on/1-off SCA1 transgenic mice presumably have no CF abnormality that would prevent us from the performing the synaptic plasticity experiments.
2. Investigate whether the diffuse pattern of CF activation is result of the loss of the developmental period of synaptic refinement or the induction of extra synaptic projections after normal development (Use 4on/8off SCA1 mice).
3. Investigate whether Purkinje cells receive input from multiple CF . mGluR1 knockout mice retain multiple CF innervations per Purkinje cell into adulthood[11,12].

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