Improved Locomotor Recovery in a Rat Model of Spinal Cord Injury by Non-Invasive BioLuminescent-OptoGenetic (BL-OG) Stimulation

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INTRODUCTION

Despite ever improving treatment methods, there still is no way to effectively restore function after spinal cord injury. A promising approach is to re-engage spinal cord neurons caudal to the injury site. A potential method to achieve this is optogenetics. Traditionally, optogenetic stimulation allows activation of neurons using an external light source. However, the invasiveness and need for an implanted optical fiber severely limits the viability of this strategy for treating spinal cord injuries.

In this study, a light-producing luciferase (sbGLuc, a variant of *Gaussia* luciferase) was used as an internal light source fused to a highly light-sensitive blue-shifted channelrhodopsin, CheRiff, to generate a luminescent opsin (luminopsin, LMO). Cells expressing the LMO are activated through bioluminescence following an intraperitoneal injection of the luciferin coelenterazine (CTZ). When activated, the light sensitive opsin opens, and the flow of cations results in the excitation of the neuron (BioLuminescent OptoGenetics, BL-OG). Previous work from this lab has found that BL-OG activation of neurons caudal to the lesion site improved locomotor function. By employing an opsin with increase light sensitivity, the current work specifically aims to analyze the efficacy of intraperitoneal injection of CTZ as opposed to the more invasive lateral ventricle cannula method.

METHODOLOGY

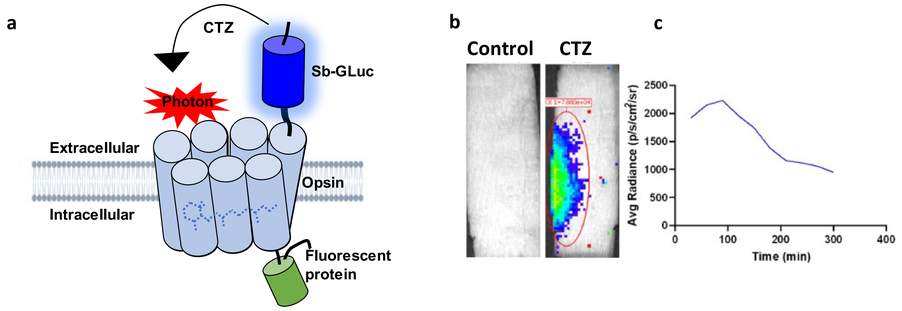


Figure 1: Schematic of the genetically engineered LMO and peak bioluminescence emission between 60 and 120 minutes in a CheRiff-expressing rat (B: IVIS image).

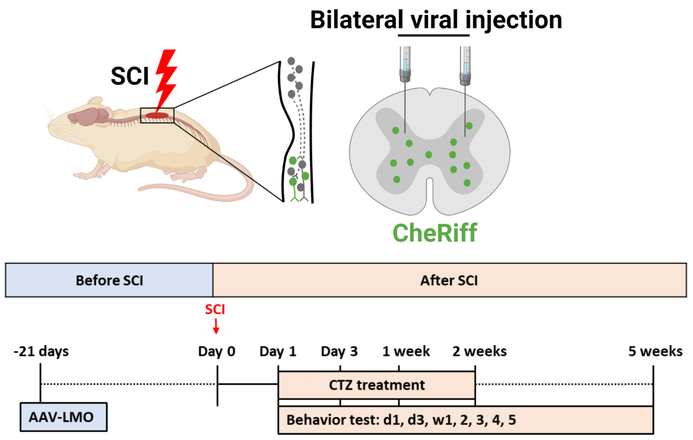


Figure 2: Schematic of the experimental model and timeline of experimental procedures with the first surgery for virus injection three weeks prior to contusion spinal cord injury.

RESULTS: CheRiff-LMO expressing rats undergoing neuronal stimulation showed improved locomotor function after SCI

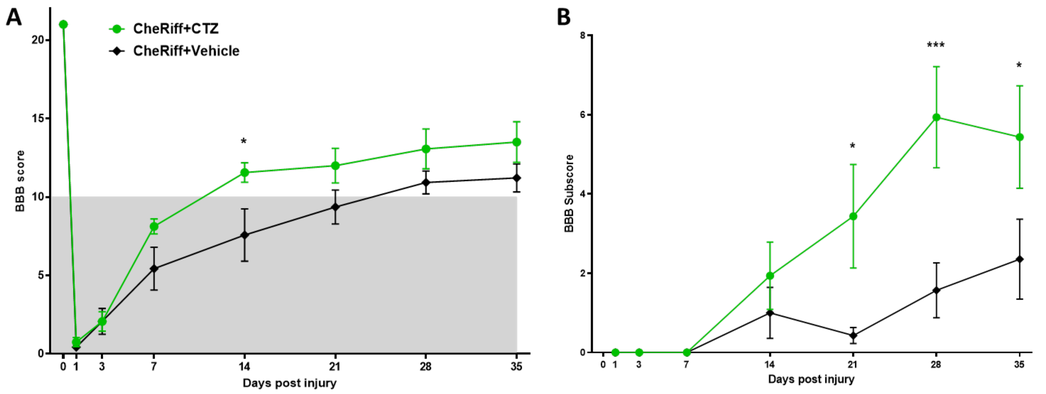
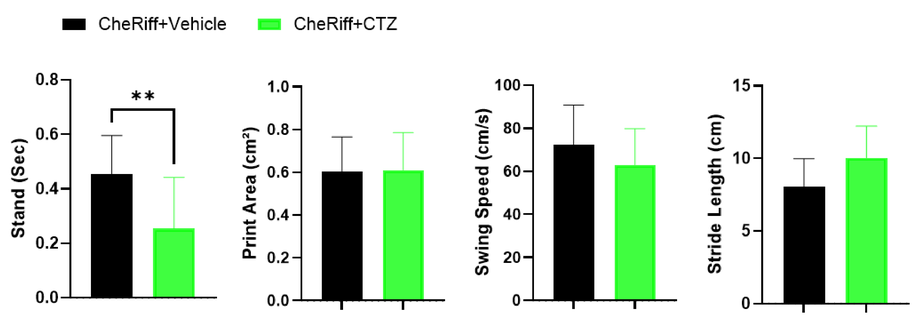


Figure 3: Rats expressing the CheRiff-containing LMO and treated with CTZ showed improved locomotor function after SCI. BBB locomotor rating score (A) and subscore (B) was performed to assess functional recovery of rats' hind limbs on Day 1, Day 3, Week 1, Week 2, Week 3, Week 4, and Week 5 following spinal cord injury. CheRiff+Vehicle (n=7) and CheRiff+CTZ (n=8). CheRiff+CTZ vs. CheRiff+Vehicle *p < 0.05, ***p < 0.001. Error bars ± standard error of mean (SEM).

RESULTS: CatWalk automated gait analysis detected difference in stand between vehicle and CTZ-treated rats

Figure 4: Stand, print area, swing speed, and stride length of vehicle or CTZ treated spinal cord injured rats. All animals expressed the LMO-CheRiff. Rats that received CTZ, i.e. neuronal stimulation, showed a significant decrease in stand, indicative of improved locomotion, but no improvement in the other parameters analyzed. CheRiff+Vehicle (n=7) and CheRiff+CTZ (n=8). CheRiff+CTZ vs. CheRiff+Vehicle **p < 0.001. Error bars ± standard error of mean (SEM).



RESULTS: Experimental and control groups were comparable regarding viral transduction and severity of contusion injury

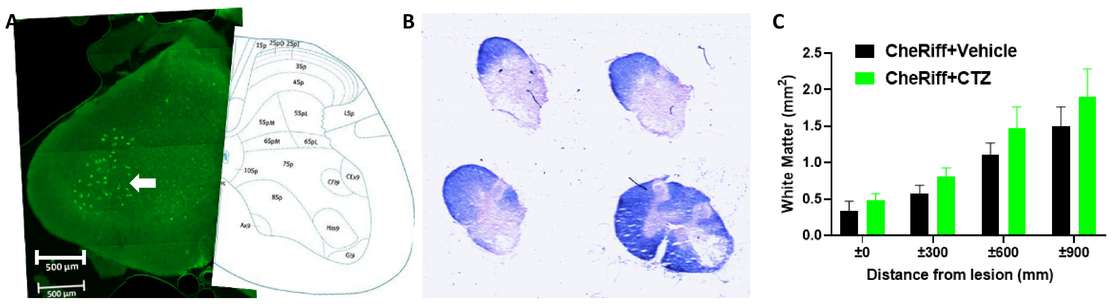


Figure 5: Expression of AAV-hSyn-CheRiff-LMO in the lumbar spinal cord (A), eriochrome cyanine staining (B; white matter is blue) and quantification of spared white matter from the lesion site outward (C).

CONCLUSION AND FUTURE DIRECTIONS

Peripheral application of the luciferase substrate efficiently activates sbGLuc-CheRiff, resulting in significant improvement of locomotor function and gait (stand). Future experiments will be targeted at using further optimized luciferases with ever more light emission and more light sensitive opsins in combination with luciferins that cross the blood brain barrier more efficiently. We anticipate that these steps will further improve locomotor function after SCI using BL-OG with high efficiency.

ACKNOWLEDGMENTS

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