CANNABIDIOL (CBD) EFFICACY TO IMPROVE VOCAL RECOVERY IS ASSOCIATED WITH ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY

Mark A. Tripson, Katherine N. Johnson and Ken Soderstrom

Department of Pharmacology and Toxicology, The Brody School of Medicine at East Carolina University, Greenville, NC, USA

Introduction

CBD is the main non-euphorigenic phytocannabinoids derived from cannabis[1]. It improves outcomes in stroke models by **reducing neuroinflammation[2]**. CBD has also been successfully used to treat childhood epilepsy[3]. Evidence suggests concomitant **resolution of developmental delays**, including **improved vocal communication[4,5]**. Translational to human speech, **zebra finch song** is a complex behavior learned during a sensitive period of vocal development, and therefore is a promising model to understand mechanisms responsible for **potential CBD-related improvement of delayed speech**.

Improved Vocal Recovery

Like human language, adult zebra finch song quality is maintained through continuous sensorimotor refinement involving dual circuits controlling vocal learning and production (Fig 1). Song **syntax and phonology** slowly degrade following deafening (that interferes with sensorimotor maintenance) and are rapidly, but transiently **disrupted following partial lesions** of a vocal motor cortical region called HVC (proper name). We found previously that CBD both **reduces the magnitude** of lesion-related disruptions, and **speeds vocal recovery[4] (Fig 1 & 2).** Because in other systems CBD has anti-inflammatory and antioxidative effects, these processes may be important to this vocal recovery.

Figure 1.

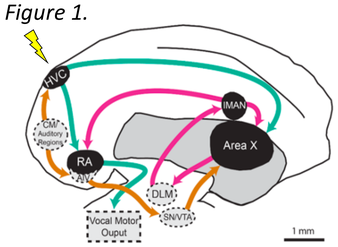

Figure 2.

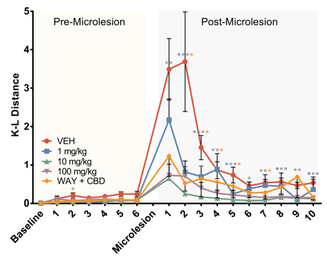

Figure 1. Dual circuits controlling vocal learning and production. The anterior forebrain vocal Learning pathway (AFP) includes Area X, lMAN, and DLM with efferent projections to RA. Vocal motor pathway includes HVC with efferent projections to RA. Stereotaxic lesions target a small area of HVC to induce vocal disruption.

Figure 2. Six days pre-treatment with 1, 10 and 100 mg/kg CBD reduced the magnitude and speeds recovery of K-L Distance (a measure of phonology) following lesion-related disruptions.

Hypothesis

- CBD anti-inflammatory activity is important to its mechanism to improve vocal recovery

Study Design

Figure 3.

Experimental Timeline

Day 1-6 → Day 7 → Day 8-9 → Day 9

Day 1-6	Day 7	Day 8-9	Day 9
Pre-microlesion injection of vehicle or 10mg/kg CBD once daily	Pre-microlesion injection of vehicle or 10mg/kg CBD followed by HVC microlesion	Post-microlesion treatment of vehicle or 10mg/kg CBD IM once daily	After second post-lesion injection: isolated biopsy punch of vocal motor and learning regions

Figure 3. Adult songbirds were given once daily injections in a volume of 50 µl IM to pectoralis based on previously published work. 10 mg/kg CBD was used because it was shown to be the lowest dose with the highest efficacy in reducing vocal deficits compared to vehicle (Fig 2). Following the second post-operative injection, brain tissue was either fixed for sectioning and IHC staining or an isolated extraction of two cortical vocal motor (HVC & RA) and two cortical-striatal learning regions (lMAN & Area X) were performed using a sterile micro punch technique for RNA extraction.

Pro-Inflammatory Gene Expression

Figure 4.

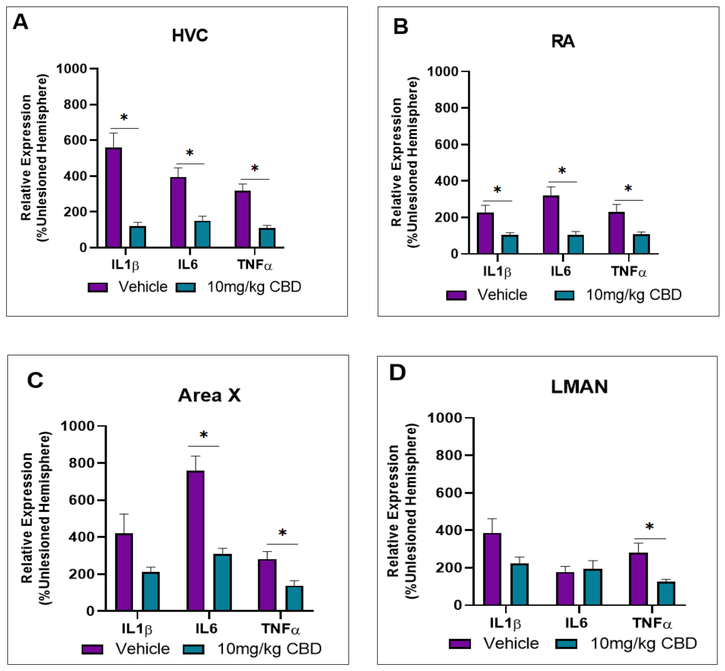

Figure 4. Pro-Inflammatory gene expression of IL1β, IL6, and TNFα were measured in vocal motor (HVC & RA) and learning regions (lMAN & Area X). There is distinct CBD efficacy within motor cortical regions HVC and RA (Fig 4A & 4B). Microlesions notably increased expression of TNFα and IL6 within Area X (Fig 4C) and TNFα within LMAN (Fig 4D) and was ameliorated in the group treated with CBD. Microlesions were given unilaterally and unlesioned hemisphere served as within subject controls. Relative expression was measured using qRT-PCR with GAPDH as an internal control. Data were analyzed by two-way ANOVA followed by Sidak's post-hoc tests ($p<0.05$ considered significant).

Anti-Inflammatory Gene Expression

Figure 5.

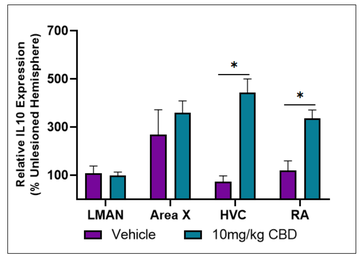

Figure 5. CBD treatments selectively increased expression of the anti-inflammatory cytokine, IL-10 within vocal motor regions HVC and RA.

Oxidative Stress Gene Expression

Figure 6.

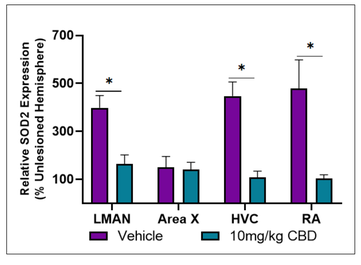

Figure 6. CBD treatments selectively decreased oxidative stress following unilateral lesion in both motor and learning circuits. Dual innervation of dopaminergic terminals from HVC and LMAN converge at RA.

Lesion-Induced Inflammatory processes

Figure 7.

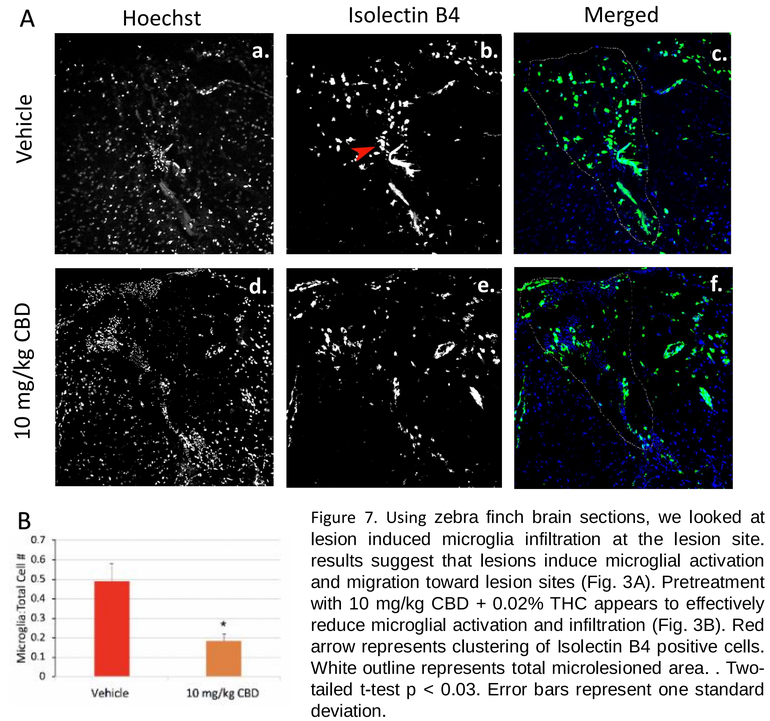

Figure 7. Using zebra finch brain sections, we looked at lesion induced microglia infiltration at the lesion site. results suggest that lesions induce microglial activation and migration toward lesion sites (Fig. 3A). Pretreatment with 10 mg/kg CBD + 0.02% THC appears to effectively reduce microglial activation and infiltration (Fig. 3B). Red arrow represents clustering of Isolectin B4 positive cells. White outline represents total microlesioned area. . Two-tailed t-test $p < 0.03$. Error bars represent one standard deviation.

Conclusions

- 10 mg/kg CBD treatment reduced the magnitude of lesion-related disruptions and sped vocal recovery
- CBD treatment significantly reduced lesion-induced pro-inflammatory cytokine expression in both the motor and learning circuits
- CBD treatment increased anti-inflammatory mediator expression within the vocal motor circuit
- CBD treatment decreased oxidative stress within the lesion target HVC and region it projects to (i.e. motor cortex (RA) and AFP output LMAN)
- CBD treatment reduced microglial recruitment to the lesion site

References

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Acknowledgments

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