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-euphoric phytocannabinoid derived from cannabis1. It improves outcomes in stroke models by reducing neuroinflammation2. CBD has also been successfully used to treat childhood epilepsy3. Evidence suggests concomitant resolution of developmental delays, including improved vocal communication4. 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 consistent 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 recovery5,6 (Fig 1 & 2). Because in other systems CBD has anti-inflammatory and antioxidant effects, these processes may be important to this vocal recovery.

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

Study Design

Day 1-6
Experimental Timeline
Day 7
Day 8-9
Day 9

Pre-microlesion injection of vehicle or 10mg/kg CBD once daily
Post-microlesion injection of vehicle or 10mg/kg CBD IM once daily
After second post-microlesion injection Isolated microlesion punch of vocal motor and learning regions

Figure 2. Two circuits controlling vocal learning and production. The anterior forebrain vocal Learning pathway (AFP) includes Area X, MAMA, 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 3. Six days pre-treatment with 1, 10 and 100 mg/kg CBD reduced the magnitude and speeds recovery of K-1 Distance (a measure of phonology) following lesion-related disruptions.

Figure 4. Pro-inflammatory gene expression of L1β, IL6, and TNFα were measured in vocal motor (HVC & RA) and learning regions (MAMA & 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).

Figure 5. 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.

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.

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 of both 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 A5 output LMAN)
• CBD treatment reduced microglial recruitment to the lesion site

References

Acknowledgments
This work was supported by GW Pharmaceuticals.