

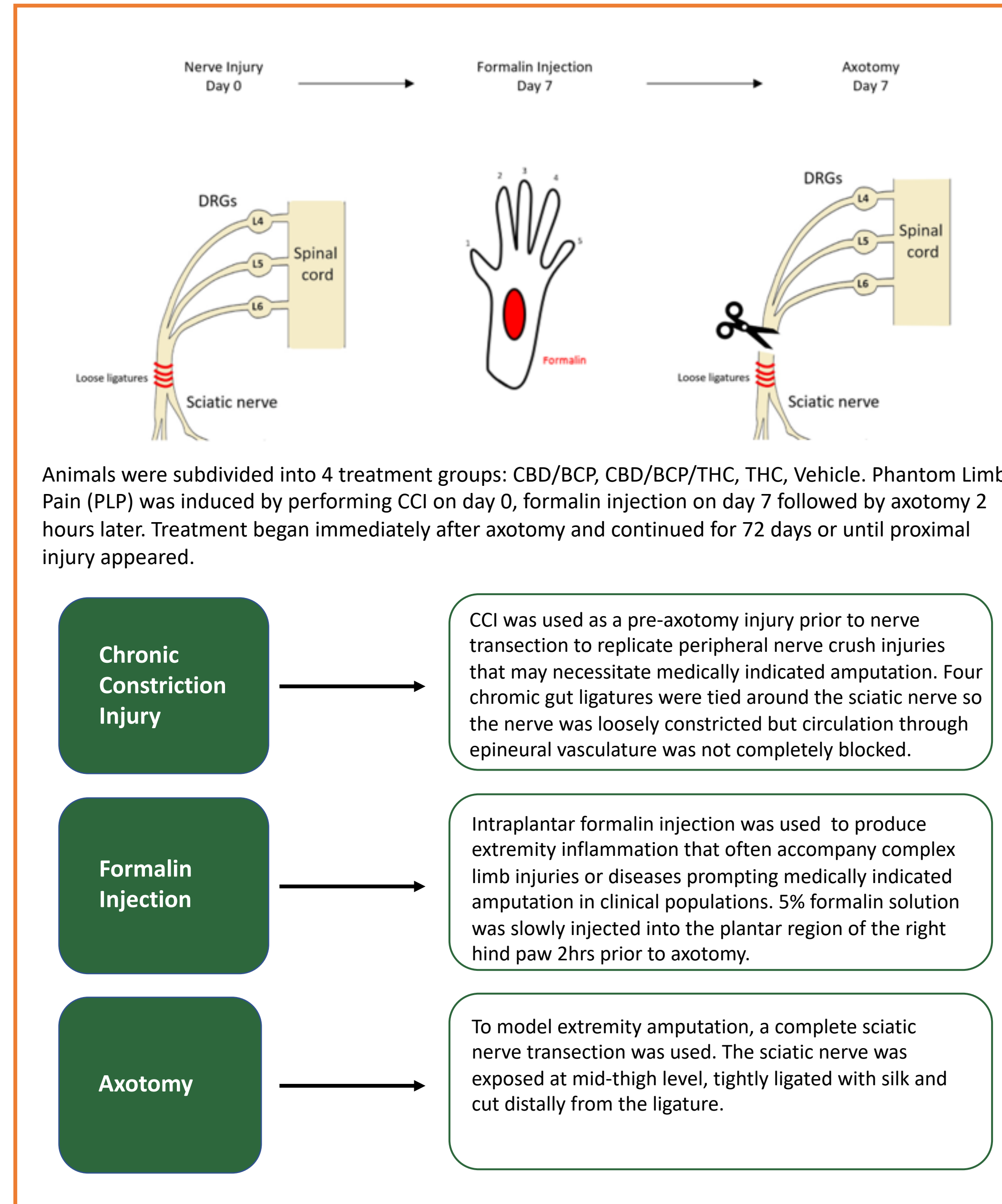
Introduction

Phantom Limb Pain (PLP) often results from medically required limb amputation and becomes difficult to manage due to underlying inflammation and other neuropathologies, affecting up to 85% of amputees. Medical marijuana is often used for pain relief and may be beneficial for chronic pain syndromes like PLP due to the wealth of cannabinoid (CB) compounds acting via distinct or synergistic mechanisms. The goal of this study was to evaluate the analgesic potential of delta-9-tetrahydrocannabinol (THC), Cannabidiol (CBD), β -caryophyllene (BCP) and their combination in preventing or reversing PLP-like behavior.

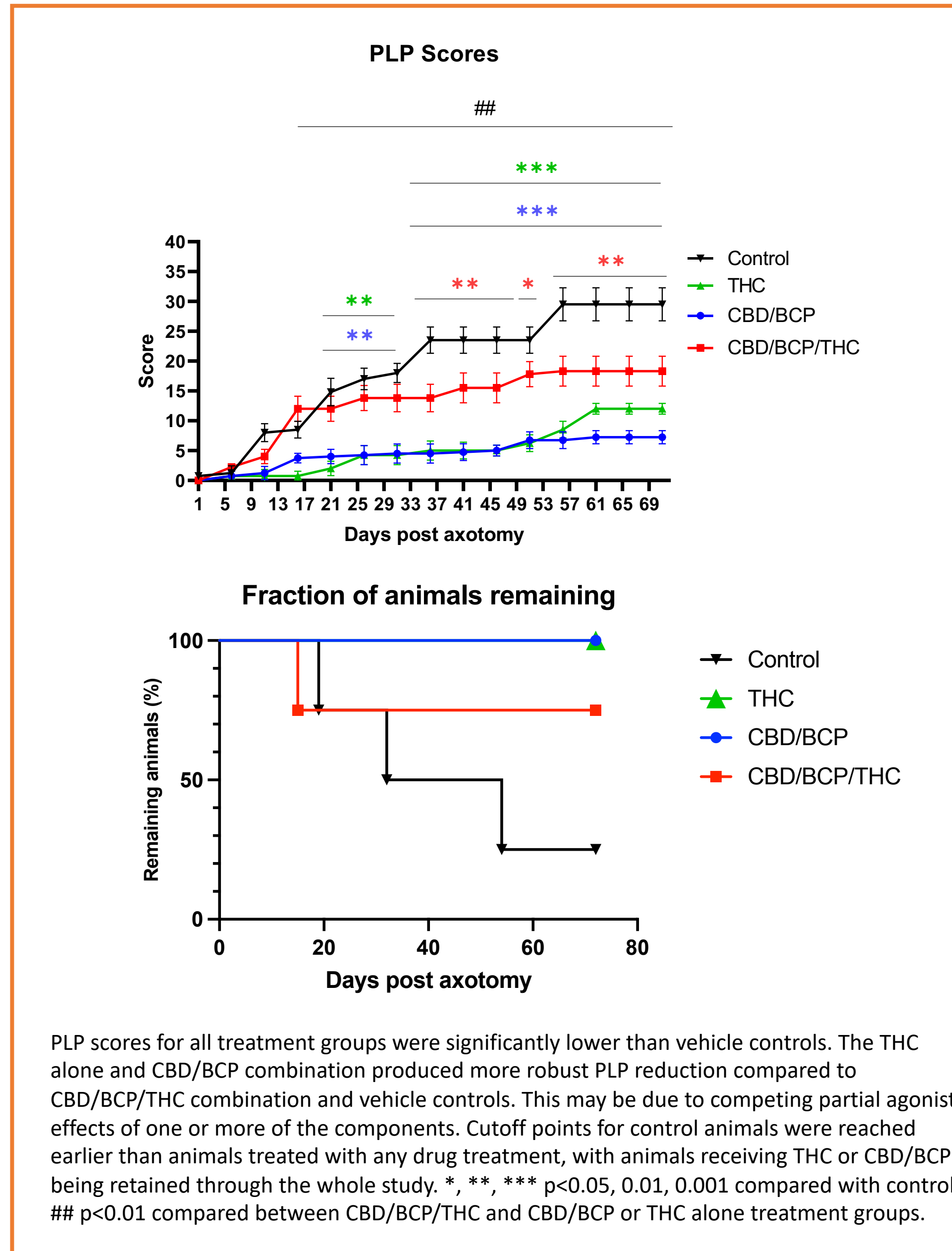
Methods

Animals: Male rats Sprague Dawley, 140g. **Surgeries:** All surgeries under anesthesia, rats allowed to fully recover before returned to the home cages. **Formalin injections:** 50 μ l of 5% formalin was injected into lateral side of the plantar hind paw. **Chronic constriction injury (CCI):** Sciatic nerve was exposed and 4 chronic gut loose ligatures placed around the nerve (Bennett, 1996). **Axotomy:** Sciatic nerve was exposed, tightly ligated with silk and transected below the ligature. **Drug treatment:** Animals were assigned to 1 of 4 treatment groups and received treatments twice daily. CBD/BCP (2.0 mg/kg:16 mg/kg), CBD/BCP/THC (2.0 mg/kg:16 mg/kg:0.04 mg/kg), THC (0.04 mg/kg). **Behavior:** Animals were observed and scored daily. **Scoring scale:** 1 point (p) for nail biting (Level I, max 5p), 3p for injury of the distal digit (Level II, starting at 5p, 20p max), 5p for injury of proximal digit (Level III, starting at 20p, cut off 25p). Animals are sacrificed when proximal injury appears, with the day of termination recorded, or by 72 days post-axotomy. Final scores were retained until the end of the experiment. Graph Pad and Sigma Stat software used for behavioral evaluations. **Tissue processing:** Perfusion with 4% paraformaldehyde, tissue cryoprotected with 30% sucrose and cryosectioned. **ELISA:** Animals euthanized by CO₂, fresh spinal tissue removed, snap frozen in dry ice and stored at -80C. Protein concentration in the homogenized tissue determined by BCA (Thermo Scientific) and processed for ELISA according to manufacturer's protocol (Abcam, RayBiotech).

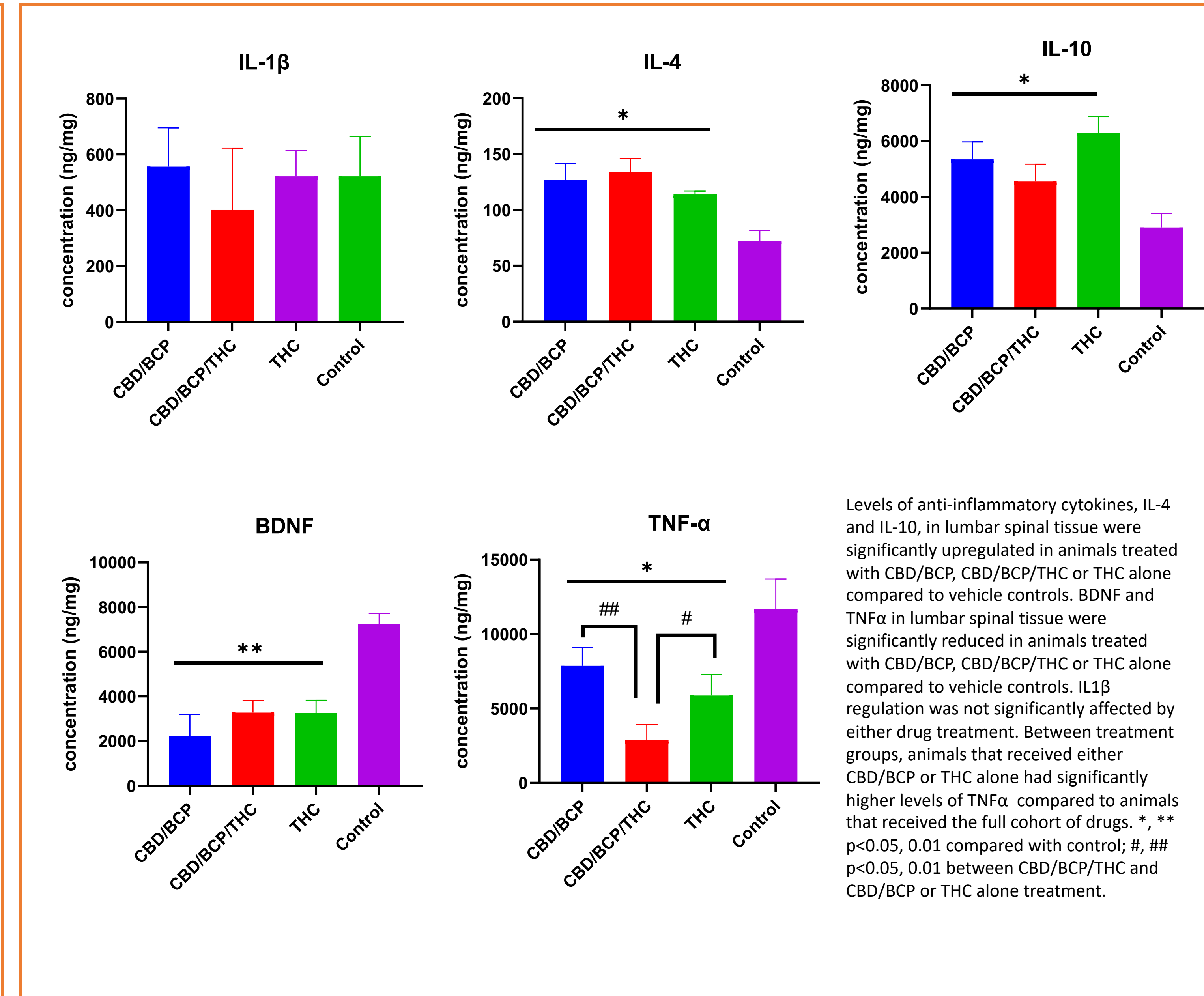
PLP Design



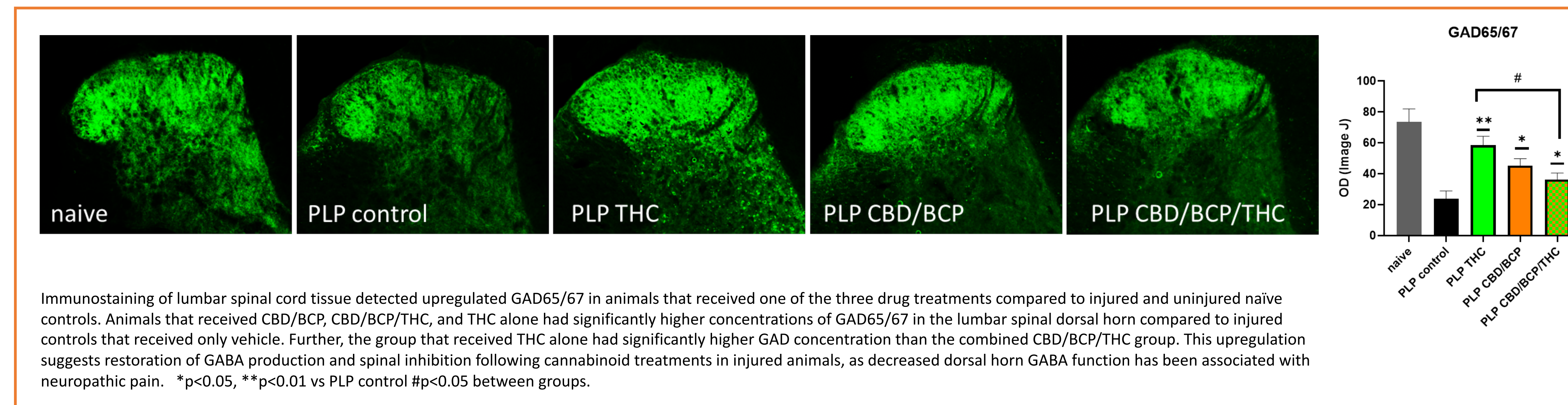
Behavior



ELISA



Immunohistochemistry



Results

- Administration of all the tested cannabis combinations showed attenuation in the severity and onset of PLP-like behaviors compared to the vehicle controls
- Comparison between experimental groups showed that animals treated with either THC alone or CBD:BCP combination displayed lower autotomy scores compared to animals receiving all three together (THC:CBD:BCP).
- All cannabinoid treatments produced increased anti-inflammatory cytokines IL-4 and IL-10 as well as decreased levels of BDNF and TNF α .
- All treatment groups showed significantly higher levels of GAD65/67 compared untreated injured PLP controls.