Enhanced neuronal regeneration by the combination of cannabidiol (CBD) with CB1 and CB2 antagonists following peripheral nerve axotomy.

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Abstract
We have previously shown that cannabidiol (CBD) is neuroprotective following peripheral axotomy. Nevertheless, the role of endogenous cannabinoid receptors has not been investigated, what may in turn enhance the therapeutic use of substances derived from Cannabis. We show herein that CBD treatment following axotomy downregulates vesicular glutamate transporter 1 (VGLUT-1) and growth associated protein (GAP-43) expression. Such effect is reversed by the application of CB1 and CB2 antagonists, indicating that endogenous cannabinoid receptors are involved in the regenerative response to injury. Interestingly, motor function recovery was improved with the selective combination of CBD and receptor antagonists. The findings of the present work may be used to improve the effects of cannabinoid treatment following nervous system lesion, what may in turn enhance translational medicine application.

Key words: Cannabidiol, Sciatic nerve, Axotomy.

Introduction
Following peripheral nerve lesion, axonal regeneration towards the target muscle is necessary for motor recovery. Regeneration of sensory axons is also important, since numbness and loss of proprioception hampers motor coordination. Pharmacological intervention following nerve repair can improve the regenerative response and several molecules have been tested. In this scenario, cannabidiol (CBD), a component extracted from Cannabis sativa, has shown neuroprotective properties, that are possibly mediated by CB1 and CB2 receptors (endogenous cannabinoid receptors). Therefore, the present study aimed to investigate the neuroprotective and regenerative potential of cannabidiol and its possible action via cannabinoid receptors. For that, C57BL/6J adult male mice were subjected to unilateral sciatic nerve crush at the sciatic notch level. Soon after lesion, mice received daily injections of vehicle solution, CBD alone (15mg/Kg, i.p.) or combined with AM251 (CB1 antagonist) and AM630 (CB2 antagonist) for four weeks. Motor behavior was also monitored up to four weeks post lesion (Catwalk system, Noldus Inc.). Immunohistochemical evaluation was performed in the spinal cord and sciatic nerve four weeks after injury, by using antibodies against growth associated protein (GAP-43), vesicular glutamate transporter 1 (VGLUT-1) and S100 (Schwann cell marker).

Results and Discussion
Nerve regeneration, studied by the walking track test, revealed that CBD treatment did not enhance the pace of motor recovery, as compared to the vehicle. However, the combination of CBD with AM251 resulted in anticipation of full gait recovery. AM631, when combined with CBD led to a significant delay of muscle reinnervation. The immunohistochemical evaluation of the regenerated nerves revealed that CBD alone reduced GAP-43, VGLUT-1 and S100 labeling. Contrarily, the association with CB1 and CB2 antagonists resulted in partial upregulation of such markers (Figure 1). Importantly, the association of AM251 and AM630 with CBD treatment resulted in further labeling enhancement, similar to the vehicle counterpart.

Conclusions
The results of the present work indicate that CBD neuroprotective and regenerative properties are mostly dependent on CB2 receptor and can be enhanced by the selective inactivation of CB1.

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