Cannabinoid (CBD) is reported to have therapeutic potential for treating neurodegenerative disorders that affect the nervous system, including epilepsy and Alzheimer’s disease. Despite this, the mechanisms by which it affects certain aspects of brain physiology in health and disease are poorly understood. To address this gap in knowledge, we have begun a detailed examination of the dose-dependent effects of CBD on three different measures of synaptic transmission in the amygdala and hippocampal CA1 regions of mouse brain slices. First, we evaluated the effect on the evoked compound action potential. Next, we evaluated the effect on the magnitude of the post-synaptic response to neurotransmission. Finally, we measured the effects on the paired-pulse ratio, PPR, a parameter that evaluates the dynamics of Ca<sup>2+</sup>-dependent presynaptic neurotransmitter release. Results showed that CBD exerts very reproducible, although subtle effects on each of these parameters in both the amygdala and hippocampus, with the effect on action potential latency being the most pronounced. This would suggest potential efficacy of CBD as a therapeutic for diseases involving over-excitability, such as epilepsy.

**Electrophysiological methods**

Field potential recording in the mouse brain slice

Thirty-five to 45 day old male C57BL/6 mice (Charles River Laboratories) were housed in groups of 4 under a 12 hour-dark light cycle and given ad libitum food and water. Electrophysiological experiments were conducted in accordance with the Colorado State University-Pueblo Institutional Animal Care and Use Committee (IACUC) guidelines. Mice were sacrificed by decapitation, and a Larvco Vibratome Series 1000 was used to make 350-μm horizontal brain slices. The brain slices were incubated in artificial cerebrospinal fluid (ACSF) before being transferred to the recording chamber. ACSF contained (in mM) 124 NaCl, 2.5 KCl, 2 MgSO<sub>4</sub>, 1 CaCl<sub>2</sub>, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 10 D-glucose, and 25 Na<sub>2</sub>SO<sub>4</sub>. The pH was adjusted with 1 M HCl or 1 M NaOH to between 7.35 and 7.40 and was periodically monitored throughout the course of experimentation. During recording, slices were perfused with oxygenated ACSF at a rate of 3-4 mL per minute at room temperature.

To measure the field excitatory post-synaptic potential (fEPSP), a 200-μm diameter bipolar concentric stimulating electrode (FHC CBAEC75) and a sharp borosilicate glass recording electrode filled with 2M NaCl were placed in the stratum radiatum of the CA1 region of the hippocampus or in the internal capsule (stimulator) and cell layer of the dorsal lateral amygdala with a 250-500 μm interelectrode distance. The slice was stimulated with a square pulse of 0.1 ms duration every 30 seconds.

The magnitude of the post-synaptic field potential was measured as either the falling slope or the peak, normalized to the internal control condition (unstimulated) for each slice. The latency of the fiber volley was measured from the onset of the stimulus artifact. The paired-pulse ratio (PPR) was measured at a 50ms inter pulse interval and calculated by dividing the slope of the second fEPSP by the first and converting the ratio to a percentage.

**Conclusions**

Cannabidiol (CBD) perfused over living brain slices from mice mildly inhibited synaptic transmission in both the dorsal lateral amygdala and hippocampal CA1 synaptic fields. In both brain regions, the pre-synaptic action potential was reversibly slowed, suggestive of an anesthetic-like effect. Also, both regions showed a decrease in the magnitude of the post-synaptic field potential, however, this effect was only reversible in the hippocampus, and continued to grow in the amygdala after washout at which point the effect became statistically significant in this brain region. Pre-synaptic calcium-dependent processes were also mildly affected by CBD in both brain regions. Combined, potential efficacy of CBD as a therapeutic for diseases involving over-excitability, such as epilepsy. More work is needed to evaluate the detailed molecular mechanisms behind these phenomena.

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