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Review article

The anticonvulsant effects of cannabidiol in experimental models of epileptic seizures: From behavior and mechanisms to clinical insights

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ABSTRACT

Epilepsy is a neurological disorder characterized by the presence of seizures and neuropsychiatric comorbidities. Despite the number of antiepileptic drugs, one-third of patients did not have their seizures under control, leading to pharmacoresistance epilepsy. *Cannabis sativa* has been used since ancient times in Medicine for the treatment of many diseases, including convulsive seizures. In this context, Cannabidiol (CBD), a non-psychoactive phytocannabinoid present in *Cannabis*, has been a promising compound for treating epilepsies due to its anticonvulsant properties in animal models and humans, especially in pharmacoresistant patients. In this review, we summarize evidence of the CBD anticonvulsant activities present in a great diversity of animal models. Special attention was given to behavioral CBD effects and its translation to human epilepsies. CBD anticonvulsant effects are associated with a great variety of mechanisms of action such as endocannabinoid and calcium signaling. CBD has shown effectiveness in the clinical scenario for epilepsies, but its effects on epilepsy-related comorbidities are scarce even in basic research. More detailed and complex behavioral evaluation about CBD effects on seizures and epilepsy-related comorbidities are required.

1. Introduction

Epilepsies are neurological disorders characterized by *"an enduring predisposition to generate epileptic seizures, and by the neurobiological, cognitive, psychological, and social consequences of this condition"* (Fisher et al., 2014). In that sense, epilepsy is not more defined exclusively as a brain disorder, but also by the presence of neuropsychiatric comorbidities (Kanner, 2017). In its turn, epileptic seizures are defined as a transient occurrence of symptoms and signs associated with abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005).

According to the International League Against Epilepsy (ILAE), approximately 65 millions of people worldwide have epilepsies (Ngugi et al., 2010; Thurman et al., 2011), while the prevalence of active epilepsy was estimated in 6,4 per 1000 people (Fiest et al., 2017). Most of these patients do not receive the appropriate neurological and psychiatric treatment, mainly at low and middle income countries (Meyer et al., 2010; Thompson et al., 2012), which contributes not only to the social stigma but also to civil and economic difficulties (Boer et al., 2008). Additionally, despite the diversity of anticonvulsant drugs available for the epilepsies treatment, pharmacological approaches are not capable of controlling seizures in one-third of the cases. Therefore, those patients are considered pharmacoresistant (Kwan et al., 2010; Kwan and Brodie, 2010). In this sense, due to biological, psychological and social aspects associated to the epilepsies (Johnson et al., 2004; Kanner, 2017; Tellez-Zenteno et al., 2007; Thompson et al., 2012; Thurman et al., 2011), patients and their relatives, continue to seek for a better treatment capable to improve their quality of life.

Animal models have been necessary for the study of epilepsies and anticonvulsant drugs since the discovery of phenytoin as an anticonvulsant drug in cats (Putnam and Merritt, 1937). In the preclinical research context, animal models with an appropriate behavioral analysis are essential tools to study normal and pathological brain functions (Krakauer et al., 2017), and in the particular case of epilepsy research, it is not different (Löscher, 2017). Additionally, heavy and rationally executed behavioral analysis are extremely important in the contemporary neuroscience research, especially because it is difficult to characterize the neuronal basis of a specific behavior if we do not

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understand in details the behavioral phenomenon (Krakauer et al. (2017). However, in the epilepsies research scenario, most of the preclinical models are thought to be models of epileptic seizures and not necessarily models of the epilepsies (Löscher, 2011). In this manner, understanding not only the advantages but also the limitations of each animal model may provide more reliable information about human epilepsies, which in turn should increase the knowledge of underlying mechanisms, improving science's quality and, consequently, the clinical translation (Kandratavicius et al., 2014).

Since quite a long time, Marijuana (*Cannabis*) has been used in different ancient cultures not only as a recreational drug but also as a medicine (Zuardi, 2006). Nowadays, given the failure of current anticonvulsant drugs in the treatment of the epilepsies (Kwan et al., 2010) and the need for alternative therapies, cannabidiol (CBD), a *Cannabis* compound devoid of psychoactive effects, has been received special attention due to its anticonvulsant properties in both, experimental models (Carlini et al., 1973; Kaplan et al., 2017; Mao et al., 2015) and humans (Cunha et al., 1980; Devinsky et al., 2017; Porter and Jacobson, 2013; Press et al., 2015).

The purpose of this study was to review the effects of CBD, a non-psychoactive *Cannabis* compound, in animal models of epileptic seizures, and to develop a critical discussion about the scientific evidence associated to CBD anticonvulsant effects in the treatment of seizures and epilepsies. Nowadays, neuroscience has a large set of new technologies available to study the healthy brain and neurological diseases that affect the central nervous system. Although the brain is a complex system made of connected networks, neurotransmitters and neuromodulators, specific neural cells (neurons and glia), and micro and nanoscale components such as membranes, cell organelles, ions, and their diffusion and transport channels, the main final outcome of the activity of the brain are the behavioral sequences. Therefore, there are no reasons to look only for molecular alterations and biomarkers, if the behavioral outcome is not precisely evaluated.

2. A brief history of *Cannabis* and CBD in medicine

Although the economic boom of *Cannabis* production was firstly associated to the cultivation and international market of the hemp fiber (cañamo) for more than 10.000 years, the first writing description of *Cannabis* for treatment of human diseases was present in the first century of this Era at the oldest Chinese pharmacopeia. Moreover, since ancient times, Oriental Medicine used *Cannabis* for treating rheumatic pain, malaria, intestinal constipation, and also convulsive seizures (Zuardi, 2006). More recently, in the middle of the 19th century, William B. O'Shaughnessy described the use of *Cannabis* for the treatment of many illnesses, such as seizures and muscular spasms (O'Shaughnessy, 1843). Then, the first clinical conference about medical *Cannabis* took place in America and was organized by the Ohio State Medical Society, in 1860 (Zuardi, 2006).

However, during the last two centuries, improvements on Western medicine, boosted by the development of new drugs for pharmacological therapy, associated to the variation between the efficacy of different *Cannabis* samples have both contributed to its reduced use in medicine (Zuardi, 2006). In addition, because of the presence of delta Δ9-tetrahydrocannabinol (THC-9, THC) and its psychoactive properties, *Cannabis* was criminalized, and many legal restrictions lead to its removal from the American Pharmacopeia in 1941 (Friedman and Sirven, 2017; Pain, 2015; Zuardi, 2006). Nevertheless, many cultural changes happened during the second half of the 20th century, when *Cannabis* reached its greatest social importance with its consumption for recreational purpose (Zuardi, 2006), as an example, between 1967 and 1980, the percentage of young adults using *Cannabis* in the USA increased from 5% to 68% (Kandel, 1984).

The sudden increase in *Cannabis* use, during the 60′s to the 80′s, contributed to the scientific interest in this plant and its compounds. At this time, the THC, the main psychoactive compound present in *Cannabis,* was isolated and its chemical structure was decoded by Mechoulam's research group (Gaoni and Mechoulam, 1964). Curiously, CBD had already been isolated by two different research groups in 1940 (Adams et al., 1940; Jacob and Todd, 1940), although its chemical structure was decoded only after two decades (Mechoulam and Shvo, 1963). Additionally, the discovery of the endocannabinoid system, classically composed by cannabinoids receptors type 1 (CB1) and type 2 (CB2) (Matsuda et al., 1990; Munro et al., 1993) and its endogenous bindings, arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG) (Devane et al., 1992; Mechoulam et al., 1995; Sugiura et al., 1995), reinforced the current and growing medical interest in *Cannabis* compounds.

Nowadays, it has been demonstrated that *Cannabis* contains more than 400 compounds, among which we can emphasize the phytocannabinoids, such as CBD and THC (Mechoulam and Hanuš, 2000; Turner et al., 1980). Generally, different *Cannabis* extracts present different proportions between CBD and THC, which may modify pharmacological and psychological effects (Potter et al., 2008). In that context, CBD has been shown to be a promising compound due to its hypnotic (Carlini and Cunha, 1981; Monti, 1977; Pickens, 1981), anti-inflammatory (Costa et al., 2004; Esposito et al., 2011), antioxidants (Hampson et al., 1998, 2000), antipsychotic (Zuardi et al., 1991; 2006) and neuroprotective (Hampson et al., 1998, 2000; Iuvone et al., 2004) properties. Additionally, CBD presents low toxicity, high tolerability and the lack of psychoactive effects (Bergamaschi et al., 2011; Carlini and Cunha, 1981; Cunha et al., 1980), supporting its safe pharmacological use.

During the early 1970′s, a pioneer group led by Professor Elisaldo Carlini in Brazil developed studies investigating cannabinoids effects on epilepsies. At this time, CBD effects on seizures were studied not only in animal models (Carlini et al., 1973; Consroe et al., 1982; Izquierdo and Tannhauser, 1973; Leite et al., 1982), but also in humans (Carlini and Cunha, 1981; Cunha et al., 1980). However, in recent years, a substantial increase in this research field was observed (Fig. 1), probably it was due to many case reports regarding health improvement in patients with epilepsies receiving CBD-enriched *Cannabis* extracts or only a

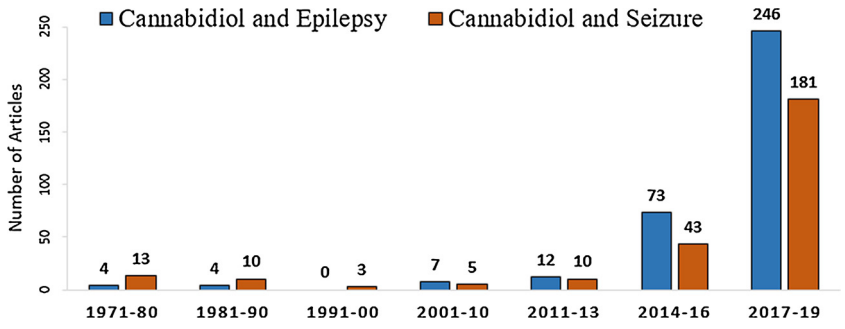

Fig. 1. Number of articles retrieved in the PubMed by using the terms: "Cannabidiol and Epilepsy" and "Cannabidiol and Seizure". Articles grouped by year of publication (from January 01, 1971 to December 31, 2019).

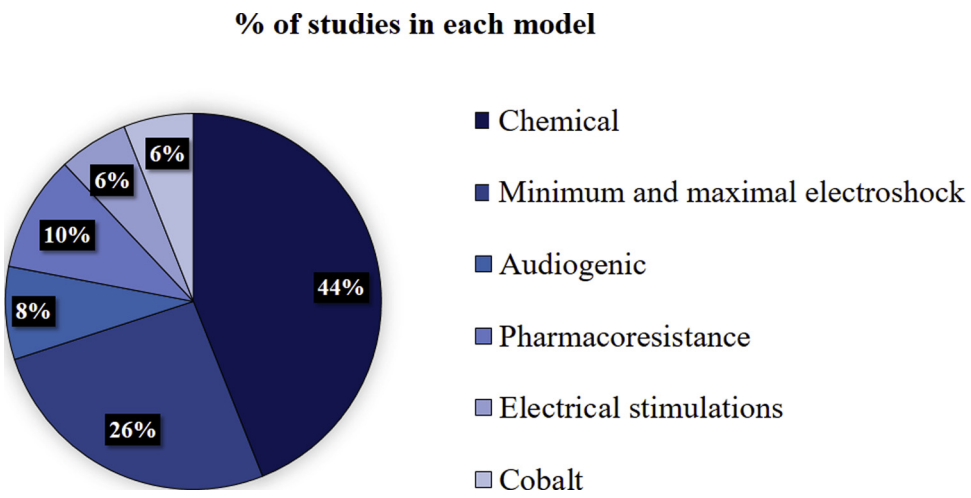

Fig. 2. Percentage of studies evaluating CBD anticonvulsant effects in animal models of epileptic seizures. Total of studies: 50. A single article can presents more than one animal model.

purified CBD extract (Devinsky et al., 2016, 2017; Hegde et al., 2012; Mortati et al., 2007; Porter and Jacobson, 2013; Press et al., 2015).

3. Cannabidiol in animal models of epileptic seizures

In the pre-clinical research, the CBD anticonvulsant effects were demonstrated in a great diversity of *in vivo* experimental models, such as electrical, chemical, genetic, among others (Fig. 2). However, since epilepsies are some of the most diverse human disorders, it is important to note that each animal model has its own limitations. In this manner, the basis for potential translational correlations between animal models and human epilepsies were discussed below, certainly considering the particularities of each model. In that scenario, it is important to note that some of the selected studies present only behavioral data, but issues related to electrophysiological, neural alterations, and possible CBD mechanisms of action associated with its anticonvulsant activity are also present in many studies. See Table 1 for details and the main results from each one of the reviewed studies

3.1. Maximal electroshock

The Maximal Electroshock (MES) is a model classically used to study seizure and antiepileptic drug effects (Toman et al., 1946). In the MES test, seizures are elicited by a transauricular electrical stimulation and it is considered that those events are associated with the activation of brainstem structures. The MES model is thought to mimic generalized tonic-clonic seizures in humans (Löscher, 2011). Usually, the MES has some fixed parameters, for example, the electrical current (50 mA for mice and 150 mA for rats), the frequency (50 − 60 Hz) and the stimulus duration (0,2 s) (Castel-Branco et al., 2009). It is important to notice that, a variation of the MES is the Minimal Electroshock, in which the current is applied by transcorneal electrodes to induce clonic seizure behaviors (Browning and Nelson, 1985).

In the early 70's Izquierdo and Tannhauser (1973) published a study using the MES model to investigate the potential use of CBD in seizures. Rats should present hyperextension of hindlimbs before receiving intraperitoneal (i.p.) injection of CBD and, to be considered protective, CBD should block this tonic behavior. In this study, CBD (12 mg/kg) was able to protect almost 100% of animals (Izquierdo and Tannhauser, 1973). Another study showed similar protective results with higher dosages of CBD (> 100 mg/kg) in animals that received repeated electroshock stimulation for six times, hourly after CBD administration (Karler et al., 1973).

Chesher and Jackson (1974) had orally administered CBD in high doses (≥50 mg/kg) in mice before animals been hourly exposed to MES four times. Single CBD administration did not change the seizure pattern of MES, curiously when CBD was administered together with THC and cannabinol (50 mg/kg each one) a reduction in the tonic extension behavior was observed. Additionally, using the same tonic extension parameter, when CBD was administered immediately before phenytoin, it was capable of reducing phenytoin's median effective dose (ED50) necessary to protect animals from MES seizures (Chesher and Jackson, 1974). Chesher et al. (1975) investigated the effects of CBD orally administered before phenobarbitone on seizures, and evaluated CBD co-administration with THC (25 and 50 mg/kg, 1:1) before phenobarbitone. When CBD was administered before phenobarbitone, a reduction on the phenobarbitone ED50 was observed (Chesher et al., 1975). Additionally, when both CBD and THC were co-administered, seizure protection was evidenced by hindlimb extension time reduction and a decrease of phenobarbitone ED50 (Chesher et al., 1975). In another study, when CBD (ED50: 12 mg/kg) was orally administered in rats exposed to the MES, similar effects to those of phenytoin were observed, with prevention of hindlimb extension (Consroe and Wolkin, 1977).

CBD anticonvulsant activity was also compared in three different species of animals exposed to the MES model: mice, rats, and frogs. CBD was capable of preventing hindlimb extension when acutely administered (Karler and Turkanis, 1981). Furthermore, results indicated that CBD anticonvulsant effects are more potent in frogs (0,1 mg/kg), than in rats (50 mg/kg), and subsequently in mice (120 mg/kg) (Karler and Turkanis, 1981). The MES model was also used in association with convulsant drugs (*i.e.*, bicuculline and pentylenetetrazole) or transcorneal stimulation to evaluate CBD effects. CBD demonstrated a dose-dependent protection against tonic seizure behaviors, but not clonic, when chemical or transcorneal stimulations were applied before the MES test (Consroe et al., 1982).

Recently, Shirazi-zand et al. (2013) showed that the central administration of CBD (200 μg) into the lateral ventricle blocked hindlimb hyperextension in 100% of the mice tested in the MES. Recently, CBD was capable of protecting mice (83,5 mg/kg) and rats (88,9 mg/kg) submitted to MES from tonic-clonic seizures in a dose-dependent manner (Klein et al., 2017). Moreover, with a similar dose (80 mg/kg; i.p.), Patra et al. (2019) observed that CBD pretreatment blocked the tonic behaviors in mice submitted to the MES.

Data about CBD and MES model strongly suggest that this phytocannabinoid may present anticonvulsant activity against generalized tonic-clonic seizures. Additionally, in the MES test CBD seems to be more effective to protect animals from tonic than from clonic seizure behaviors in both, rats and mice.

Table 1
Anticonvulsant effects of CBD in epilepsy animal models studies.

CBD administration	Experimental Seizure Model	Subjects	Cannabidiol Seizure Effects	References
10, 50, 200 mg/kg, i.p.; 1 h, post	Leptazol	Male mice	↓ number of seizure	Carlini et al. (1973)
20,25 mg/kg i.p.; 30 min, pre	Audiogenic	Male Wistar rats	TC+; wild running+	
1.5, 3, 6, 12 mg/kg-[1] i.p.; 1 h, pre	MES	Female Albino Adults Rats	T + E/F+	Izquierdo and Tannhauser (1973)
ED50: 105 mg/kg; 200 mg/kg i.p., pre	MES	Male Charles River Mice	E/F+	Karler et al. (1973)
150 mg/kg orally 30 min pre	PTZ	Male mice QS strain and C57	NE	Chesher and Jackson (1974)
50, 100, and 200 mg/kg i.p.	MES	Male mice QS strain and C57	E/F: NE	
50 mg/kg orally by gavage 2 h pre	MES	Male mice QS strain	E/F+	Chesher et al. (1975)
ED50:12 mg/kg orally	MES	Adult male rats	E/F+	Consroe and Wolkin (1977)
ED50: 17 mg/kg	Audiogenic	Adult male rats	E/F+	
CBD 5, 50,100 mg/kg; i.p.	Electrical stimulations	Male Sprague-Dawley Rats	After-discharge activity: NE	Turkanis et al. (1977)
1–50, 200 mg/kg; i.p.	Cobalt; Subconvulsant PTZ	Male Sprague-Dawley Rats	C +, able to block seizure spread activity; Frequency of focal epileptic potentials: NE	Chiu et al. (1979)
0,3–15 mg/kg; 0,3 − 3 mg/kg; i.p.	Electrical stimulations	Male Sprague-Dawley rats	C+; ↑ AD; ↓ threshold duration and amplitude	Turkanis et al. (1979)
82,4 mg/kg; i.p. 2 h pre; 14,9 mg/kg; i.v. 15 min pre	Audiogenic	Adult male audiogenic Rats	↓ number of animals TC+	Consroe et al. (1981)
mice: 120 mg/kg, i.p.; rat: 50 mg/kg, i.p.; Frog: 0,1 mg/kg, into ventral lynph sac	MES; 6-Hz; PTZ; Electrical stimulations; Cobalt	Sprague-Dawley Rats Male Charles River Mice; Male and Female *Rana pipiens*	Anticonvulsant +; AD: ↑ threshold, ↓ duration and amplitude	Karler and Turkanis (1981)
0,1–80 mg/kg, i.p.	Electrical stimulations	Sprague-Dawley Rats	AD: ↓ duration and amplitude, ↑ threshold	Turkanis and Karler (1981)
60 mg/kg; i.p. 2 times/day.	Cobalt	Male Sprague-Dawley Rats	Polyspike periods and seizures: NE	Colasanti et al. (1982)
50–400 mg/kg; i.p.	MES associated to: PTZ, 3-mercaptoproprionic, isonicotinic acid hydrazine picrotoxin, bicuculline, strychnine sulfate	Albino Male Mice	T+ (except for strychnine sulfate), Running, C and lethally: NE Mechanisms: Could potentiates GABA effects	Consroe et al. (1982)
1, 10 and 100 mg/kg; i.p. 1 h pre	PTZ	Male *Wistar* Kyoto Rats	1 mg/kg: ↓ latency to TC; 100 mg/kg: ↓ % of rats with TC	Jones et al. (2010)
1, 10, 100 mg/kg; i.p., 1 h pre	PILO	Male *Wistar* Kyoto Rats	C+; ↓ elevation/fall; TC+	Jones et al. (2012)
1, 10,100 mg/kg; i.p. 1 h pre	Penicillin	Male *Wistar* Kyoto Rats	↓ % rats with TC 10 and 100 mg/kg ↓ lethally	
CBD + CBDV (diverse combinations);	PTZ; PILO;	Male *Wistar* Kyoto Rats	TC+; TC+; wild running+;↓ seizure severity;	Hill et al. (2013)
CBD ED50: 80 mg/kg	Audiogenic	DBA/2 J Mice	Mechanisms: Independent of CB1	
0,2, 2, 20, 200* ng/mice; i.cv. 26 ng/mice	PTZ	Male NMRI Mice	↑ threshold to TC+; C: NE; Mechanisms: dependent of BK channels activity; Postsible ↓ intracellular Ca^{2+}	Shirazi-Zand et al. (2013)
0,2, 2, 20, 200 ng/mice; i.c.v.	MES	Male NMRI Mice	Hind limb extension+ Mechanisms: dependent of BK channels activity	
15, 30, 60, 90 mg/kg; 30 min pre	Cocaine	Male Swiss Mice	↓ seizure duration ↑ seizure latency Mechanism: ↓ glutamate release; independent of CB1 and CB2	Gobira et al. (2015)
30, 60, 90 mg/kg; i.p.; 30 min pre	Cocaine	Male Swiss Mice	↓ seizure duration, ↑ seizure latency FAAH inhibitor: anticonvulsant	Vilela et al. (2015)
10, 20, 50 mg/kg; i.p.; 1 h pre, during 28 days	PTZ	Male Sprague-Dawley rats	↓ seizures severity Hippocampus: ↓ neurons loss; ↓ astrocytic hyperplasia Mechanism: ↓ NMDA-NR1 subunit. iNOS: NE	Mao et al. (2015)
100, 200 ng/animal; i.c.v.1 or 5 days post chronic phase	PILO	Male and female Wistar rats	↓ head nodding, C+ CBD 5 days: ↑ latency chronic phase; ↑ autophagy and antioxidant related proteins (ATg7, ATg5/12, ATg12, LC3II/LC3I)	Hosseinzadeh et al. (2016)

(*continued on next page*)

Table 1 (*continued*)

CBD administration	Experimental Seizure Model	Subjects	Cannabidiol Seizure Effects	References
ED50: 88,9 − 164 mg/kg. mice 83,5 mg/kg; rats 88,9 mg/kg	MES; 6-Hz 44 mA; PTZ; Corneal Kindling; Lamotrigine Amygdala Kindling	Male Albino CF-1 Mice Male albino Sprague-Dawley Rats	MES: TC+ 6-Hz: C+ PTZ: C+ Corneal Kindling + Lamotrigine Amygdala Kindling: NE	Klein et al. (2017)
30,60, 90 mg/kg; i.p., 30 min pre	PTZ; PTZ kindling	Male Swiss mice-20–30 g	↓ seizures severity and EEG seizure activity; Delayed PTZ kindling Mechanism: dependent of CB1, CB2, TRPV1; Prevented ↑ IL-6 in the PFC	Vilela et al. (2017)
100 mg/kg; i.p., 60 min pre	PTZ	Adolescent Wistar-Kyoto rats	↓ % of seizures; TC+ Mechanism: independent of 5-HT_{1A} and 5-HT_{2A}	Pelz et al. (2017)
Sigle dose 10, 20, 50, 100, 200 mg/kg; i.p.; 1 h pre Repeated dose 100 200 mg/kg; 2x/ day for 8 days	Acute Thermal Seizure/Dravet-Syndrome model	Mutant Mice for Scl1a	Sigle dose: ↓ duration and seizure severity; Repeated dose: ↓ frequency of spontaneous seizures; ↑ GABA activity in interneurons; Mechanism: independent of CB1; GPR55 antagonist mimics CBD effects; Epilepsy-related comorbidities +	Kaplan et al. (2017)
10 mg/kg i.p. 1 h pre SE or pre and post SE;	PILO	Adult male Wistar rats	↑ SE latency, after-discharge latency, neuron density; ↓ SE severity; relative powers in delta and theta bands, and neurodegeneration; Epilepsy-related comorbidities +	Do Val-Da Silva et al. (2017)
3 nmol/mice; i.c.v.	NMDA-induced seizures (i.c.v. 0,3 − 1 nmol/mice)	Male Albino Mice	T+; wild running+ ; C+; ↓ lethality Mechanism: dependent of sigma 1 receptors and 5-HT_{1A} receptors	Rodríguez-Muñoz et al. (2018)
ED50: 80 mg/kg; i.p.; 50 − 200 mg/kg orally;	MES; PTZ (85 mg/kg; s.c.; 0,01 ml/g body weight); 6-Hz 44 mA; Corneal kindling; PILO;	Mice Rat	MES: T+ PTZ: C+ 6-Hz 44 mA: C+ PILO: C+ Epilepsy-related comorbidities +	Patra et al. (2019)

Abbreviations: maximal electroshock (MES); pentylenetetrazole (PTZ); electroencephalogram (EEG); pilocarpine (PILO); intraperitoneal (i.p.); subcutaneous (s.c.); intracerebroventricular (i.c.v.); ntravenous (i.v.); intrahippocampal (i.h.); cannabidivarin (CBDV); cannabidiol (CBD); CBD pretreatment (pre); CBD postt treatment (post); minutes (min.); hour (h.); median effective dosage (ED50); postitive effect (+); no effective (NE); increases (↑); decrease (↓); tonic-clonic seizure (TC); tonic seizure (T); clonic seizure (C); extension/flexion (E/F); after-discharge (ADSE); prefrontal cortex (PFC).

3.2. Pentylenetetrazole

Pentylenetetrazole (PTZ) is a $GABA_A$ antagonist (Macdonald and Barker, 1977), classically used to induce epileptic seizures in rodents (Swinvard et al., 1955) and, together with the MES, is one of the main animal models used to verify anticonvulsant effects of new drugs and compounds (Lüttjohann et al., 2009). Usually, in this model rats receive lower doses (70–90 mg/kg) than mice (80–100 mg/kg), and seizure behavioral manifestation depends on both, PTZ dose and route of administration, besides the time course of PTZ effects (Löscher et al., 1991). In general, drugs that are effective against generalized absence seizures in humans, also present anticonvulsant activity in the PTZ model. This parallel can be made because, in low doses (20 − 30 mg/kg), PTZ can be a model of absence seizures, presenting well clinical correlates, such as bilateral synchronous activity with 7 − 9 Hz frequencies (Cortez et al., 2016; White et al., 1995).

Chesher and Jackson (1974) used intravenous (i.v.) PTZ administration (6 mg/ml; 19 mg/minute) to induce minimal clonic seizures in mice. CBD was orally administered 30 min before PTZ, but it was not capable of protecting mice and no changes in the PTZ dose necessary to induce seizures were observed (Chesher and Jackson, 1974). Other authors showed that CBD was capable of abolishing hindlimb extension but it had no effects on forelimb and jaw clonus (Karler and Turkanis, 1981). Consroe et al. (1982) showed that CBD was capable of blocking the tonic behavior in a dose-dependent manner after subcutaneous (s.c.) PTZ (135,9 mg/kg) administration. Moreover, high CBD doses also reduced the lethality and percentage of animals that exhibited running behaviors (Consroe et al., 1982).

Jones et al. (2010) demonstrated CBD protective effects in rats using a higher dose of CBD (100 mg/kg) in the PTZ (80 mg/kg. i.p.) test, reducing the mean of seizure severity in a scale for generalized seizures from 5 (fully developed tonic-clonic seizure with loss of righting reflex) to 3 (forelimb clonus with tonic components). This result is associated with a reduction in the percentage of animals that reached the maximum tonic-clonic seizure severity from 53% to 7%, when the CBD is compared to the control group. Furthermore, the percentage of rats with tonic-clonic seizures and mortality were both reduced. However, in the same test, CBD in dose low dose (1 mg/kg) decrease the latency to tonic-clonic seizure (Jones et al., 2010).

CBD together with its analog cannabidivarin (CBDV) was also evaluated in the PTZ test (85 mg/kg; i.p.). This co-administration (CBDV 200 mg/kg : CBD 47 mg/kg) reduced the median of maximum seizure severity from 5 (fully developed tonic-clonic seizure) to 2 (atypical clonic seizure) and the mortality dropped from 40% to almost zero (Hill et al., 2013). Here it is worth to note that CBDV has already been demonstrated to be anticonvulsant in the PTZ test, even when administered alone, reducing the mean seizure severity, mortality, and increasing the percentage of rats free from seizures (Hill et al., 2012). Additionally, the same authors also showed CBDV anticonvulsant properties on *in vitro* assays (for details see Hill et al., 2012).

Recently, CBD anticonvulsant effects were also investigated using i.v. PTZ (1% at 0,25 ml/ min) administration (Shirazi-zand et al., 2013). Animals that had received intracerebroventricular (i.c.v.) administration of CBD (200 ug/mice) presented a decrease in PTZ threshold necessary to induce the tonic seizure, but no alteration in clonic behaviors was observed (Shirazi-zand et al., 2013). Klein et al. (2017) demonstrated that CBD (159 mg/kg) protected mice from acute clonic seizures induced by s.c. PTZ (85 mg/kg) (Klein et al., 2017). Differently from Shirazi-zand et al. (2013), in this study, CBD attenuated only clonic, but not tonic behaviors, and effective doses were two times less potent in the PTZ than in the MES model (Klein et al., 2017).

Vilela et al. (2017) evaluated the pretreatment with CBD in different PTZ protocols: 60 mg/kg (i.p. and s.c.); 10 mg/ml (i.v.); and PTZ-induced kindling (15 injections of PTZ 35 mg/kg, i.p. on alternate days). In all experiments, authors observed that CBD (60 mg/kg; i.p.) attenuated seizures, moreover, latency and seizure duration were both reduced in the i.p. and s.c. PTZ protocols. This protective effect was also observed in a video-EEG analysis (Vilela et al., 2017). Moreover, in the i.v. PTZ protocol, CBD pretreatment increased the threshold to induce seizures (Vilela et al., 2017). Pelz et al. (2017) also demonstrated CBD anticonvulsant effects in rats submitted to PTZ (85 mg/kg; i.p.). CBD pretreatment significantly reduced the percentage of animals that developed any type of seizures such as tonic-clonic, besides reducing the mortality induced by PTZ (Pelz et al., 2017). Finally, clonic behaviors were attenuated in mice when CBD was given before s.c. PTZ (85 mg/kg) administration (Patra et al., 2019).

Except for only one study that did not observe any effect after CBD administration (Chesher and Jackson, 1974), all PTZ-induced seizures studies had shown CBD anticonvulsant activities with behavioral improvements, such as increased latency to seizure onset and reduction of clonic behaviors. Curiously, one study observed that a low dose of CBD was capable of decreasing the latency to seizures (Jones et al., 2010). We have not found articles that evaluated effect of CBD on absence-like seizures induced by PTZ. Potential studies using this model should provide information from distinct methods, such as behavioral, EEG, and pharmacological approaches.

3.3. Electrical stimulation

An important characteristic in this method is that seizure responses are dependent on both, the area to be stimulated and the properties of electrical discharges, mimicking specific seizure behaviors (Kandratavicius et al., 2014). In this model, seizures should be induced without generating brain damage (however cellular damage is not always evaluated), and also with reduced mortality, although this method is not neuron-type specific (Kandratavicius et al., 2014).

CBD was effective against limbic seizures in rats submitted to a kindling procedure with repeated electrical stimulations of the left subiculum every other day, during a period between 1 and 4 months (Turkanis et al., 1979). Electrophysiological improvements were observed after CBD (0,3 − 3 mg/kg) administration, such as reduction of afterdischarge amplitude into subiculum, hippocampus, and cortex. Additionally, CBD increased the afterdischarge threshold and decreased its duration. Although CBD presents low toxicity, effects associated with motor phenotype were observed (CBD: 173 mg/kg) in the bar-walk test, but no excitatory effects were observed, not even when doses up to 200 mg/kg were used. Authors also mentioned that limbic seizures, with jaw and forelimb clonus, were usually present and CBD treatment reduced or abolished these behavioral manifestations, but there is no additional information associated with behavior analysis (Turkanis et al., 1979). Using the same protocol, CBD (0,3 − 3 mg/kg) blocked limbic seizure behaviors and induced electrophysiological improvements, increasing the threshold and reducing duration and amplitude of electrically induced limbic seizures (Karler and Turkanis, 1981). Similar anticonvulsant effects were observed in conscious rats with chronically implanted electrodes into the parietal cortex, with a reduction of the amplitude of cortical evoked response after i.p. CBD (0,1 − 3 mg/kg) treatment (Turkanis and Karler, 1981). However, there is only electrophysiological evaluation in this study, which makes difficult any association between EEG data and seizure behavior.

While two studies indicate positive effects of CBD on afterdischarges parameters associated with behavioral protection (Karler and Turkanis, 1981; Turkanis et al., 1979), one observed electrophysiological improvements with no behavioral evaluation (Turkanis and Karler, 1981). Differently, in a protocol of afterdischarge potentials of the visually evoked response in conscious rats, CBD did not induce changes in afterdischarge properties (Turkanis et al., 1977). Although these studies provide insights about CBD treatment and EEG improvements, behavioral analysis was almost absent. Thus, there is a clear necessity to create additional and better links between behavioral and electrophysiological effects of CBD in *in vivo* animal models of epileptic

seizures, this type of approach may certainly bring more detailed and reliable data for clinical translations.

3.4. Pilocarpine

Pilocarpine (PILO) is an alkaloid agonist of acetylcholine M1 receptors. The muscarinic receptors M1 are involved in the initiation of seizures, whereas the N-methyl-D-aspartic acid (NMDA) receptors are involved in seizures maintenance (Smolders et al., 1997; Turski et al., 1983). After the original descriptions by Turski et al. (1983) using i.p. administration of PILO, the main original observation and which raised the interest in its universal use, was the presence of spontaneous recurrent seizures after the *Status Epilepticus* (SE) and the so-called latent period (Cavalheiro et al., 1991). PILO administration could also be made focally, for example, by intrahippocampal microinjection. In fact, this protocol is associated with low mortality as compared to systemic PILO route (Furtado et al., 2002). Regarding its translation to human, seizures induced by PILO are thought to mimic the human temporal lobe epilepsy (TLE) (Curia et al., 2008). However, this concept needs to be reviewed because the amount of lesioned areas and associated networks (even in SE induced by intrahippocampal PILO) (Castro et al., 2011; Bertram et al., 2009), indicate that extra-temporal and subcortical structures are involved in the expression of behavioral, EEG, cellular and molecular alterations, well beyond those classically associated to TLE.

Pharmacological studies of CBD in the PILO model have been performed more recently in the literature. Jones et al. (2012) observed that different doses of CBD, one hour before PILO (380 mg/kg; i.p.), was capable of attenuating seizure behavior in many parameters. CBD reduced the percentage of animals which developed Racine class 5 (bilateral clonus with elevation and fall), the percentage of tonic-clonic seizures and the mean occurrences of seizures (Jones et al., 2012).

Hill et al. (2013), also used the PILO model (380 mg/kg; i.p.), but in this study CBD was i.p. co-administered with CBDV (CBD 27 mg/kg and CBDV 116 mg/kg). This treatment was capable of reducing the maximum seizure severity from 4 (bilateral forelimb clonus with rearing and falling) to 2 (unilateral forelimb clonus). In a similar study, only animals that had displayed forelimb clonus or more severe limbic seizures were selected for further analysis. Then, rats were divided into two groups with i.c.v. CBD (100 ng/rat) injections during one or five days. CBD attenuates PILO effects, delaying the onset of the chronic phase and reducing the number of head nodding and both mono and bilateral forelimb clonus. However, using a higher CBD dose (200 ng/rat), the mortality was increased (Hosseinzadeh et al., 2016).

Do Val-da Silva et al. (2017) investigated the behavioral and electrophysiological effects of CBD in the intrahippocampal PILO-induced SE. CBD was administered before, or before and after PILO-induced SE. CBD injection before PILO reduced the number of rats that developed SE, doubled the latency to SE onset and attenuated SE severity. Using the Racine (1972) scale, it was observed that the mean severity index reduced from class 4 (forelimb clonus followed by elevation) to class 3 (forelimb clonus). Moreover, 36% of rats submitted only to SE presented Racine class 5 (forelimb clonus followed by elevation and fall), whereas none of the pretreated rats reached this index. CBD also increased the contralateral latency to epileptiform discharges and decreased relative powers in delta (0.5–4 Hz) and theta (4–10 Hz) oscillations, being these oscillations features of ictal electrographic activity (Do Val-da Silva et al., 2017). Moreover, i.v. CBD (10 mg/kg) administration before systemic PILO (380 mg/kg), reduced the maximum seizure severity from Racine class 5 to class 3 in rats. Moreover, after the SE induced by PILO, CBD was orally administered (200 mg/kg) during 8 weeks and, in the chronic phase of the PILO model, epileptic seizures were worse in the vehicle than in the CBD group with a decrease in seizure severity in 70% of animals treated with CBD (Patra et al., 2019).

As mentioned above, although for decades the PILO has been considered a model of human TLE (Curia et al., 2008; Raol and Brooks-Kayal, 2012), in a recent comparison between neurodegeneration patterns, both systemic and intrahippocampal PILO-induced SE induced quite significant encephalic lesions in dozens of areas (Castro et al., 2011). This information is important for the use of CBD, for example, as a potential anticonvulsant substance, particularly when comparing its effects in models such as amygdala electrical kindling, MES, PTZ or chronic audiogenic seizures.

3.5. Audiogenic seizures in susceptible strains

There are rodent strains with genetically susceptible animals capable to developing seizures in response to intense sound stimulation (Jobe et al., 1973; Reigel et al., 1986; Doretto et al., 2003; Poletaeva et al., 2017). In general, when an audiogenic, or susceptible, animal is exposed to acoustic sound stimulation (~120 dB), seizures begin with a wild running, followed by tonic seizure, and then by generalized clonic seizures (Garcia-Cairasco et al., 1996; Galvis-Alonso et al., 2004) however, different audiogenic strains may express different types and patterns of seizures (Ross and Coleman, 2000; Garcia-cairasco et al., 2017). Differently from what could be supposed, audiogenic seizures may not be a model of reflex seizures in humans (Wolf, 2017), but rather generalized tonic-clonic seizures in the acute protocol (Terra and Garcia-Cairasco, 1992; Garcia-Cairasco et al., 1993) and of TLE in the audiogenic seizures kindling protocol (Naritoku et al., 1992; Garcia-Cairasco et al., 1996; Moraes et al., 2000; Romcy-Pereira and Garcia-Cairasco, 2003).

The first study that investigated CBD effects on audiogenic models was the one from Carlini's research group. Authors offered to normal Wistar rats a solution of sodium barbitone diluted in water with its concentration increasing (up to 4 mg/ml) along 38 days. After two days of sodium barbitone withdrawal, animals received CBD before the acoustic stimulation. In this investigation, CBD reduced the number of animals that developed audiogenic seizures from six to only one (Carlini et al., 1973). Still in the 1970's, another study showed that CBD treatment was capable of preventing audiogenic seizures similarly to the latter study, although behavioral analysis has been restricted only to the presence or the absence of seizures c. Similar results were obtained by Consroe et al. (1981) in another study in which the number of susceptible rats with generalized tonic-clonic seizure was reduced after prior i.p. (82 mg/kg) or i.v. (14,9 mg/kg) CBD administration.

After approximately 30 years, a new study investigating the antiepileptic properties of CBD in audiogenic tests was made in susceptible DBA/2 J mice using CBD and its analog CBDV. Administration of both compounds was capable of reducing wild running, clonic and tonic seizure, whereas isolated CBD administration was capable of reducing only the incidence of clonic seizures (Hill et al., 2013).

Although few studies investigated CBD antiepileptic potentials in audiogenic models, these studies found similar results with CBD attenuating or blocking acute audiogenic seizure behaviors, suggesting a potential anticonvulsant effect against generalized tonic-clonic seizure.

4. Cannabidiol in historically unusual animal models of seizures

4.1. Cobalt-Epilepsy rat model

The Cobalt-Epilepsy Rat is a model in which initially a small piece of cobalt wire is implanted into the motor cortex, mono or bilaterally, and this procedure leads to the development of spontaneous focal aware seizure that were observed behaviorally and by EEG (Colasanti et al., 1974; Chang et al., 2004). Additionally, it has been already demonstrated that focal to bilateral tonic-clonic seizures and focal impaired awareness seizures may also appear between the first and third week after cobalt implantation, reflecting the recruitment of cortical brain areas. Thus, the cobalt-induced seizures is thought to be a model of neocortical chronic seizures with both, focal and focal to bilateral

seizures (Chang et al., 2004). At this point, it is important to note that the seizures classification were recently revised by the ILAE and the spontaneous focal aware seizures corresponds to the previously called simple partial seizures, the focal impaired awareness seizures represents the traditional complex partial seizures, and the focal to bilateral tonic-clonic seizures replace the secondary generalized seizures (Fisher et al., 2017). One of the mechanisms associated with cobalt-induced epileptiform activity in brain tissue preparations is through the facilitation of gap junction activity (He et al., 2009).

CBD effects on cobalt-epilepsy rat model are scarce, with CBD being able to block limb and jaw clonus, but with no changes in excitatory effects on focal epileptic potentials. Authors supposed that these effects should be due to the central blockade of seizure spreading (Chiu et al., 1979). The same research group described that CBD blocked spontaneous recurrent seizures in cobalt-implanted rats (Karler and Turkanis, 1981). Cobalt implantation was also applied bilaterally into the frontal cortex, however, different from those studies mentioned above, CBD did not affect neither the seizure phenotype nor the frequency of focal polyspikes (Colasanti et al., 1982).

Besides the capability of the cobalt model to induce spontaneous recurrent seizures, verified by behavior and EEG, little was made in the epileptology field employing cobalt-protocols to study CBD anticonvulsant properties. This limited use of this model, may be due, at least in part, to the difficulty to implant an identical cobalt piece in each animal (Chang, 2004). Consequently, protocols using powder cobalt application in brain regions (Cesa-Bianchi et al., 1967; Fujii et al., 2012) would increase the accuracy of this model for further CBD anticonvulsant evaluation.

4.2. Cocaine-induced seizures

Cocaine intoxication is known to induce clonic seizures in rodents (Gasior et al., 1999) and it seems to mimic also generalized tonic-clonic seizures in humans after cocaine intoxication (Koppel et al., 1996). The mechanism by which cocaine induces epileptic seizures is unknown. However, the glutamatergic system is thought to play a key role, probably by modulation of multiple mechanisms such as nitric oxide, dopamine, and voltage-dependent receptors (Lason, 2001; Planeta et al., 2013).

Recently, cocaine was administered (75 mg/kg; i.p.) to mice to study CBD anticonvulsant effects. CBD pretreatment, in different doses, reduced seizure duration, and CBD (30 mg/kg; i.p.) also increased latency to the seizure onset (Gobira et al., 2015). Vilela et al. (2015) found the same CBD protective effects and, besides this, CBD prevented hepatic injury induced by cocaine, suggesting CBD protective effects on drug intoxication.

4.3. Penicillin-induced seizures

Penicillin is a $GABA_A$ antagonist (Macdonald and Barker, 1977) capable of inducing seizures with bilateral and synchronous spontaneous generalized spike and wave discharge. Penicillin-induced seizures were described as a model of human generalized seizures without motor manifestation (absence), however, in experimental animals, not only absence seizures, but also clonic and tonic-clonic seizures can be observed depending on the dose of penicillin (Avoli, 1995; Cortez et al., 2016).

Only one study investigated the anticonvulsant effects of CBD in penicillin-induced seizures. Jones et al. (2012) used penicillin into the right lateral ventricle (1,5 μl) to induce focal seizures. They demonstrated that CBD reduced the lethality and the percentage of animals reaching tonic-clonic seizures without postural control, but had no effect on seizure severity and the number of tonic-clonic seizure occurrences. However, little motor impairment was observed after high CBD dose (100 mg/kg) on the rotarod test (Jones et al., 2012).

4.4. NMDA-induced seizures

Only one study evaluated the CBD effects on seizures induced by NMDA (i.c.v.) administration. CBD pretreatment (i.c.v.), 30 min before NMDA, reduced the percentage of mice with tonic seizures from 95% to 20% and dropped lethality from 20% to zero. Additionally, NMDA induced wild running and clonic behaviors in all mice and CBD was capable of protecting more than 50% of animals from these behaviors (Rodríguez-Muñoz et al., 2018).

5. Mechanisms of Action of the Cannabidiol anticonvulsant activity

The best possible explanation of CBD anticonvulsant effects implies to verify the mechanisms of action involved in this phenomenon. Nevertheless, there are few studies using animal models of epileptic seizures to test CBD pharmacological mechanisms (for main results see Table 1).

Chesher and Jackson (1974) showed that CBD potentiated phenytoin anticonvulsant effects in mice when both were co-administered before the MES test. Additionally, Chiu et al. (1979), using cobalt-induced seizures focus in the left motor cortex, showed that CBD could attenuate clonic seizures, but was ineffective against focal epileptic potentials, presenting similar results to phenytoin, suggesting better effects on seizure spreading than in focal activity. These data suggest that CBD presents similar mechanisms of action to phenytoin. However, phenytoin presents affinity for a great variety of targets (Hesselink, 2017), as well as CBD (Devinsky et al., 2014), which makes difficult to make statements about CBD mechanisms of action on the epilepsies based only on these findings.

In the case of the GABAergic system, CBD was capable of preventing seizures induced in mice by convulsant drugs that inhibit GABAergic neurotransmission (Consroe et al., 1982). In a model of focal seizures induced by i.c.v. injection of penicillin (Jones et al., 2012) and in PTZ-induced seizures, both $GABA_A$ antagonists, CBD showed a clear anticonvulsant activity (see Sections 3.2 and 4.3). In this context, in a model of Dravet-Syndrome, GABAergic neurotransmission is reduced in the dentate gyrus, and CBD treatment reverts this deficit, while a $GABA_A$ antagonist prevented CBD effects (Kaplan et al., 2017). These authors also observed that CBD was capable of increasing the frequency of inhibitory postsynaptic currents and reduced spontaneous excitatory postsynaptic currents in the dentate gyrus (Kaplan et al., 2017). These effects were blocked by $GABA_A$ and also by GPR55 receptor antagonist, but not by a CB1 antagonist (Kaplan et al., 2017). Taken together, these data suggest that, at least part of the CBD anticonvulsant effects may be dependent on GABAergic modulation.

The GPR55 receptor is a G_{13}-protein-coupled receptor that is activated by endocannabinoids and antagonized by CBD (Ryberg et al., 2007). In that scenario, this receptor has been recently suggested to be a member of the endocannabinoid system (Pertwee, 2007). Concerning GPR55 receptor signaling, its activation may lead to intracellular calcium increase, through the mobilization of both intracellular and extracellular calcium (Lauckner et al., 2008). GPR55 antagonist pretreatment mimics CBD anticonvulsant effects, and no additional effect was observed when CBD was administered after the GPR55 antagonist, suggesting that CBD anticonvulsant effects may be, at least in part, due to the antagonism of GPR55 receptors (Kaplan et al., 2017). Then, GPR55 receptors are suggested to be potential pharmacological targets capable of attenuating seizures.

Still regarding calcium mobilization, pretreatment with paxilline, a long conductance calcium-activated potassium (BK) channel antagonist, blocked CBD anticonvulsant effects in mice submitted to the PTZ test (Shirazi-zand et al., 2013). Once BK channels are calcium and voltage-activated receptors, as well as potassium selective, increasing BK channels activity, may lead to increase in potassium conductance, which in turns reduces membrane potential (Vergara et al., 1998),

while its blockade can reduce the spike broadening during a burst (Shao et al., 1999). Since BK channels activity is dependent of intracellular calcium levels and CBD is capable of interfering with calcium homeostasis mobilizing intracellular calcium stores in neuronal tissue (Drysdale et al., 2006), it is possible that the CBD mechanism of action associated to BK channels is dependent of intracellular calcium regulation. Supporting this idea, recently, Rodríguez-Muñoz et al. (2018) demonstrated that the manipulation of calcium signaling of the endoplasmic reticulum is capable of attenuating CBD anticonvulsant effects.

Additionally, CBD interaction with NCX, a mitochondrial sodium/calcium exchanger, supports a mechanism in which CBD regulates intracellular calcium levels. Through this mechanism, CBD prevents epileptic-like activity in cultured hippocampal neurons, increasing the calcium influx from the cytoplasm (Ryan et al., 2009). In this context, another mechanism that could be involved in CBD and calcium modulation is the mitochondrial CB1 receptor (mtCB1) (Busquets-Garcia et al., 2018). Taken together, these findings suggest that calcium modulation could be involved, at least in part, on CBD anticonvulsant effects. However, this mechanism of action associated with calcium modulation needs to be further investigated in order to directly or indirectly, measure neuronal calcium levels in response to *in vivo* CBD administration in animal models of epileptic seizures.

Moreover, in respect to endocannabinoids signaling, intracellular calcium mobilization may lead to anandamide production (Van Der Stelt et al., 2005) and have already been demonstrated that CBD inhibits anandamide hydrolysis, which in turns increases the endocannabinoid availability (Bisogno et al., 2001). Through these mechanisms, endocannabinoids can interact with the presynaptic CB1 receptors and then excitatory neurotransmission could be modulated (Di et al., 2003; Lutz, 2004). In this regard, Vilela et al. (2015) observed CBD behavioral protective effects against seizure induced by cocaine, while the pretreatment with an inhibitor of fatty acid amide hydrolase (URB597) induced the same anticonvulsant effects of CBD and no additional anticonvulsant effect were observed when both compounds were co-administered (Vilela et al., 2015). This suggests that an increase in endocannabinoid signaling, either by an inhibition of its hydrolysis or by an increase in calcium signaling, both mechanisms modulated by CBD (Ryan et al., 2009; Van Der Stelt et al., 2005), could be associated with its anticonvulsant effects.

Still supporting the endocannabinoid and calcium signaling in CBD anticonvulsant effects, using the PTZ model, behavioral and EEG protection induced by CBD (see Section 3.2) were prevented by pretreatment with selective antagonists of CB1 (AM251), or CB2 (AM630), or transient receptor potential vanilloid 1 (TRPV1) (SB366791) (Vilela et al., 2017). Additionally, it has been already demonstrated that only with a blockade of CB1 receptors, audiogenic seizures become more severe (Vinogradova et al., 2011). However, future studies should be done to verify if CBD treatment is involved in CB1 receptors signaling transduction by an indirect pathway, such as endocannabinoid signaling potentiation.

On the other hand, but still concerning CB1 receptors, using mouse brain membranes for binding assays, it was demonstrated that CBD anticonvulsant activity was independent of CB1 receptors (Hill et al., 2013). Gobira et al. (2015) investigated CBD protective effects in a cocaine-induced seizure model (see Section 4.2) and observed that CBD prevented hippocampal glutamate increase induced by cocaine. Pretreatment with CB1 and CB2 antagonists were not effective to block CBD anticonvulsant effects, but curiously, rapamycin pretreatment blocked the protection induced by CBD (Gobira et al., 2015).

Finally, the PTZ model was used to verify possible interactions between CBD and the serotonergic system. Firstly, rats received WAY-100635, a $5HT_{1A}$ antagonist, or MDL-100907, a specific $5HT_{2A}$ antagonist, 20 min before CBD administration and 80 min before PTZ (85 mg/kg; i.p.). CBD treatment significantly attenuated seizure severity and reduced lethality. However, administration of both serotonergic antagonists did not modify CBD effects on seizures (Pelz et al., 2017). These results showed that in the PTZ model the anticonvulsant effects of CBD do not depend on 5-HT_{1A} or 5-HT_{2A} receptors, although the interaction between CBD and 5-HT_{1A} have already been demonstrated (Russo et al., 2005; Resstel et al., 2009; Espejo-Porras et al., 2013). On the other hand, recently it was demonstrated that WAY-100635 administration previously to CBD, was capable of attenuating the CBD anticonvulsant effects in a model of seizures induced by i.c.v. NMDA administration (Rodríguez-Muñoz et al., 2018), supporting a role for the serotonergic system in the CBD anticonvulsant effects.

Since the epilepsies present a diverse etiology, seems to be a *naïve* thought to believe that a single drug target would be able to improve epilepsies and their related comorbidities. Hence, in situations of a multifactorial disease, the best approach should not be a drug which blocks or activates a single target, but rather a promiscuous compound capable to rebalancing many physiological, pharmacological, and molecular systems (Mencher and Wang, 2005). Indeed, studies suggested that the anticonvulsant effects of CBD may result from multiple mechanisms of action highlighting the GABAergic modulation, endocannabinoid signaling *via* CB1 receptors, and calcium mobilization related to GPR55 receptors, BK channels, TRPV1 receptors, and mitochondrial receptors. However, it is possible to assume that CBD promising effects on the epilepsies may be due to the combination of different mechanisms of action. Therefore, we summarize the reviewed CBD mechanisms of action that support its anticonvulsant activity in a schematic representation (Fig. 3).

Besides those mechanisms directly evaluated in *in vivo* animal models, there are *in vitro* data supporting those anticonvulsant mechanisms associated with intracellular calcium signaling through TRPV1 receptors (Iannotti et al., 2014). Additionally, CBD restores changes in the hippocampal CA1 long-term potentiation in mice submitted to PILO-induced SE through a mechanism dependent of 5-HT_{1A} and intracellular calcium stores, but independent of CB1 signaling (Maggio et al., 2018). However, contradictory results from Blair et al. (2006) indicate anticonvulsant effects dependent on CB1 receptors in hippocampal neuronal culture models of SE. These results reinforce the necessity of more studies for better explain the CBD anticonvulsant activity on both *in vivo* and *in vitro* animal models.

6. Cannabidiol neuroprotective effects in the epilepsies

Effective pharmacological neuroprotection after seizure needs different therapeutic strategies for preventing or treating chronic epilepsy. Thus, prevent seizure-induced neuronal damage and network reorganization are potent tools to fighting against deteriorating epileptogenic process (Acharya et al., 2008; Hoffman et al., 2003). However, it is possible that neuroprotective effects of a specific drug have no substantial anti-seizure potential against neurodegenerative mechanisms (Velisek et al., 2013). In that context, identification of potential drugs that could induce neuroprotection, like CBD, certainly has an impact on epilepsies treatment, for this purpose preclinical studies are required. Neuroprotective CBD effects are observed in numerous neurodegenerative disorders in preclinical research, such as in Huntington, Parkinson, and Alzheimer diseases (Campos et al., 2016; Fernández-Ruiz et al., 2013; Iuvone et al., 2004). Nevertheless, few data were found associated with neuroprotection in preclinical studies of the epilepsies.

Mao et al. (2015) investigated the effects of various concentrations of CBD in daily pretreatment in a chronic protocol of PTZ along 28 days (see section 7). Morphological changes were evaluated by means of the measurement of the expression levels of glial fibrillary acidic protein (GFAP), inducible nitric oxide synthase (iNOS), and mRNA expression levels of NMDA receptor subunits NR1 and NR2B in hippocampal neurons. CBD decreased astrocyte hyperplasia, NR1 expression, and neuronal damage in the hippocampus, however, despite these improvements CBD has not affected iNOS expression. These data reveal

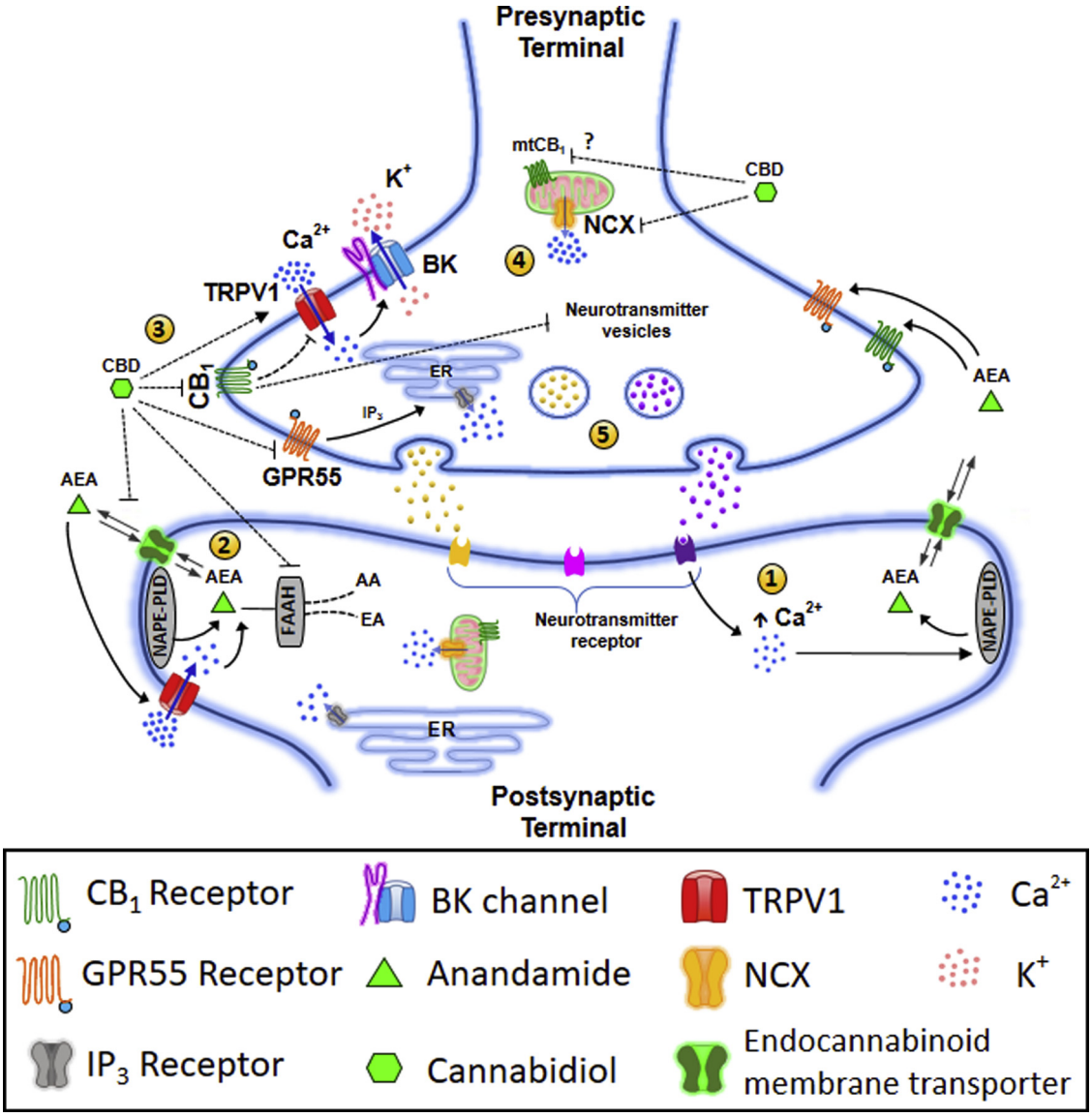

Fig. 3. Schematic representation of cannabidiol mechanism of action based upon data from animal models of epileptic seizures. (1) Signal transduction promoted by the activation of neurotransmitter receptors leads to increased intracellular calcium at the postsynaptic terminal by different mechanisms. This increase in calcium levels promotes endocannabinoid anandamide (AEA) biosynthesis, catalyzed by N-acyl-phosphatidylethanolamines-specific phospholipase-D (NAPE-PLD). Endocannabinoids are transported bidirectionally *via* the endocannabinoid membrane transporter (EMT). **(2)** In the extracellular space, the AEA can interact with different targets, such as the G-protein-coupled CB_1 receptor, GPR55 receptor, and the TRPV1 channels, or be internalized and degraded. AEA is hydrolyzed by fatty acid amide hydrolase (FAAH) into arachidonic acid (AA) and ethanolamine (EA). The increase of AEA leads to the activation of TRPV1 channels, promoting calcium influx, this increase in calcium levels leads to increased production of AEA by NAPE-PLD, high levels of AEA activate its degradation by FAAH, a negative feedback mechanism. **(3)** Cannabidiol (CBD) has multiple mechanisms of action, involving CB_1 and GPR55 receptors, TRPV1 and BK channels and mitochondrial Na^+/Ca^{2+} exchanger (NCX). CBD would function as a partial antagonist of CB_1, whose activity involves the release of $G_{i/o}$ protein which results in the reduction of TRPV1 activity. CBD also interacts directly with TRPV1 promoting calcium influx. The increase in intracellular calcium levels leads to activation of the BK channels, increasing the potassium efflux, causing hyperpolarization of the presynaptic terminals. Activation of GPR55 by AEA leads to increased intracellular calcium *via* inositol 1,4,5-triphosphate (IP_3) signaling, whereas CBD acts as an antagonist. **(4)** On the other hand, CBD can act on the NCX, reducing or elevating cytosolic calcium. Through mechanisms not yet elucidated, CBD can act on the mitochondrial CB_1 receptor ($mtCB_1$), modulating calcium signaling and bioenergetic processes. **(5)** All these effects inhibit the release of the presynaptic vesicles, disrupting the release of neurotransmitters (*i.e.*, glutamate and GABA).

that CBD exhibits neuroprotective effects in a protocol of chronic seizures.

Besides behavioral and electrophysiological effects of CBD in the intrahippocampal PILO model (see Section 3.4), Do Val-da Silva et al. (2017) also investigated neuropathological effects of CBD. This study also demonstrated that CBD induced a decrease in neurodegeneration in the hilus and CA3, moreover higher neuron density in granule cell layer, hilus, CA1, and CA3 were observed in animals treated with CBD in comparison to the control group. These results revealed neuroprotective effects in the acute phase of the intrahippocampal PILO model (Do Val-da Silva et al., 2017). In the same model, Hosseinzadeh et al. (2016) studied not only seizure behavior during the chronic phase after a single CBD treatment (see Section 3.4), but also investigated autophagy pathways and antioxidant effects. During the chronic phase, CBD induced autophagy, increased catalase enzyme activity, and reduced glutathione content. These results suggest that CBD post-treatment could be neuroprotective in the chronic phase of the PILO model.

Corroborating these previous data, a neuronal reconstruction study showed that kainic acid treatment in rats was capable of reducing the density of parvalbumin-expressing neurons in the dentate gyrus, CA1,

and CA3. Dendritic arborization and soma size were also reduced in kainic-acid-rats. CBD (100 mg/kg; i.p.) treatment after kainic acid SE onset restores morphological alterations promoted by kainic acid in hippocampal parvalbumin-interneurons, observed two weeks after SE (Khan et al., 2018). These results suggest that CBD may present neuroprotective effects in different animal models of epileptic seizures. However, studies should be done to better understanding CBD effects and its mechanisms underlying neuroprotection.

7. Cannabidiol on chronic protocols of seizures: an open window of possibilities for epilepsy-related comorbidities

In the context of the epilepsies related neuropsychiatric comorbidities, anxiety and depression seem to be the most frequent psychopathologies in people with epilepsies. Additionally, psychopathologies are associated with more severe seizures, pharmacological tolerance, and increased suicide rates and in people with epilepsies (Johnson et al., 2004; Kanner et al., 2012; Kanner, 2016; Sarkisova et al., 2017; Tellez-Zenteno et al., 2007; Verrotti et al., 2014). Rodent strains genetically susceptible to seizures also present comorbidities associated with epilepsies, such as depressive- (Jones et al., 2008; Sarkisova and van Luijtelaar, 2011; Sarkisova et al., 2017), and anxiety-like behaviors (Garcia-Cairasco et al., 1998; Jones et al., 2008; Sarkisova and van Luijtelaar, 2011; Sarkisova et al., 2017). Additionally, hormonal alterations, like high corticosterone levels, are directly related to increased epileptiform activities recorded using EEG in chronic epileptic mice (Castro et al., 2012).

Acute seizure protocols are not capable of discriminating and evaluating modifications in neuronal tissue or comorbidities associated with epileptic seizures, as well as possible adverse or toxic effects related to chronic pharmacological therapy. Consequently, chronic protocols are essential to the epilepsies research scenario (Simonato et al., 2014). Different from some years ago, nowadays there are studies that investigated the potential therapeutic use of CBD on chronic models of epileptic seizures.

In chronic protocols of PTZ and PILO, CBD presented behavioral protection (see Section 3.2 and 3.4 for more details). Moreover, in the corneal kindling model, seizure severity increases along the protocol (Matagne and Klitgaard, 1998) and when acute CBD (119 mg/kg; i.p.) was administrated in fully corneal kindled mice, the number of limbic seizures was reduced in a dose-dependent manner, indicating protective effect against both, focal aware clonic and focal impaired awareness seizures (Klein et al., 2017). Similar results in the corneal kindling model were observed by Patra et al. (2019).

Mao et al. (2015) evaluated chronic CBD effects in rats submitted to a PTZ kindling model (35 mg/kg; i.p.) during 28 days. Before each daily PTZ injection, animals received CBD (50 mg/kg; i.p.), which significantly attenuated the daily seizure, suggesting a long-term effect against generalized seizures induced by chronic PTZ (Mao et al., 2015). Furthermore, in the PTZ-induced kindling (PTZ 35 mg/kg; i.p.; 15 injections on alternate days), CBD (60 mg/kg; i.p.) delayed the kindling progression, even though it did not prevent mice from becoming fully kindled (expressing tonic-clonic seizure or generalized tonic-clonic seizure) until the end of the protocol (Vilela et al., 2017).

Only one study evaluated if CBD presents some tolerance along with a chronic protocol of seizures. In a chronic protocol of 22 days, CBD induced tolerance on MES threshold. However, concerning clonic behaviors, called minimal seizures in the MES test, CBD induced reverse tolerance, and no signs of excitability were observed along 22 days (Karler and Turkanis, 1981). However, this study did not provide additional information about seizure profile in those animals chronically treated with CBD.

Even though new studies have employed chronic protocols for CBD anticonvulsant activity, in general, a description of CBD effects on behavioral seizures and their comorbidities are scarce. In this context, Dravet-Syndrome mice with spontaneous recurrent seizures exhibit social alterations mimicking those presented by patients with Dravet Syndrome, among them social deficits and hyperactivity. It was observed that CBD at low doses (10 − 20 mg/kg) reverted the social deficits, while high doses (100 mg/kg) were effective against hyperactivity and also reduced seizure duration (Kaplan et al., 2017). Locomotor exploratory activity and post-ictal changes were evaluated in the open field test in rats submitted to intrahippocampal PILO-induced SE. CBD pretreatment increased vertical exploratory activity one day after SE, suggesting that CBD could modulate exploratory input after SE (Do Valda Silva et al., 2017). Motor deficits associated with PILO- induced SE observed 6 weeks after PILO administration were prevented in those rats chronically treated with oral CBD (200 mg/kg). Additionally, in the same protocol, working memory was impaired after PILO-induced SE, but these alterations were not observed in the CBD chronically treated group (Patra et al., 2019).

Although studies with chronic protocols observed CBD anticonvulsant activity, little has been done to better understand the CBD effects on epilepsy-related comorbidities, mainly on the neuropsychiatric sphere. Despite the contributions of acute models of epileptic seizures, there is still a necessity to evaluate CBD effects in chronic protocols of epileptic seizures. Then, for future progress, it will surely be necessary to understand brain modifications along with chronic seizures (Simonato et al., 2014), as well as the chronic CBD treatment and its effects on the central nervous system. Chronic protocols like those based on kindling and long-lasting SE modifications, as well as genetic models of epilepsies, could be effective approaches to a reliable and precise evaluation of CBD effects on epilepsy-related comorbidities.

8. The clinical scenario of Cannabidiol in the treatment of epilepsy: how can preclinical research improve the clinic?

Since the epilepsies have a quite diverse etiology, it is challenging to incorporate all variables in only one animal model. Thus, the selection of the model to be used in a specific study depends on the questions to be answered (Raol and Brooks-Kayal, 2012). Once each model provides restricted information about human epilepsy, translational aspects (based on the ILAE Commission for Classification and Terminology) should be considered (see Table 2).

Table 2

During the early 1980s, CBD has already been used in the clinic for the treatment of patients with epilepsy. It was observed that chronic CBD (200 − 300 mg/kg; daily, during 4–5 months) was capable of attenuating generalized seizures in pharmacoresistant patients, and some of them became seizure free (Carlini and Cunha, 1981; Cunha et al., 1980).

More recently, Mortati et al. (2007) described a case of a patient who developed seizures every night along 27 years. The patient started to smoke *Cannabis* before bed and then the seizures did not occur anymore, but when he did not smoke, the seizures reappeared. Another case report described a patient with cortical dysplasia treated with carbamazepine, and even so, seizures were present five to six times per night. After smoking *Cannabis* nightly, seizure frequency decreased to one to two per night (Hegde et al., 2012). Even though these case reports are about patients who were smoking *Cannabis* (therefore not exposed only to CBD), these findings certainly aroused the interest in medical cannabinoids, such as CBD, for the treatment of the epilepsies.

In a parent survey report, *Cannabis* extracts were orally administered in pharmacoresistant children. A reduction in the frequency of convulsive behaviors was observed, although improvements in the interictal EEG activity were not correlated with behavioral seizure modification (Press et al., 2015). This lack of EEG correlation with behavioral improvements reinforces the necessity to better analyze complex and sequential seizure behaviors. In a similar study, CBD-enriched extracts were effective in the treatment of pharmacoresistant children. It was reported that half of the children presented a reduction of > 80% in the frequency of seizures (Porter and Jacobson, 2013). Finally, pure

Table 2
Experimental animal model and its behavioral correlates with human epilepsies.

Experimental Model	Behavioral Animal Correlates	Human Seizure Correlates*
MES	Tonic-clonic seizures	Generalized tonic-clonic seizures
PTZ	Clonic, tonic, tonic-clonic, and absence seizures	Generalized myoclonic and absence seizures
Electrical stimulations, Kainic Acid, PILO	Clonic and tonic-clonic seizures	Focal impaired awareness seizures – TLE
Audiogenic seizures	Tonic, tonic-clonic (acute; brainstem dependent), and clonic seizures (chronic; forebrain dependent)	Generalized tonic-clonic seizures/ Focal Impaired Awareness seizures – TLE
Penicillin	Absence seizure, mild clonic behaviors and tonic seizures	Generalized absence seizures
Cocaine	Clonic seizures	Generalized tonic-clonic seizures
Cobalt	Clonic seizures	Focal aware, focal impaired awareness, and focal to bilateral toni-clonic seizure – neocortical seizures
MES 6 Hz 44 mA, mouse cornel kindling, lamotrigine-resistant amygdala kindling, Dravet-Syndrome model	Tonic, tonic-clonic, and clonic seizures	Therapy-resistant epilepsy

Abbreviations: maximal electroshock (MES); pentylenetetrazole (PTZ); pilocarpine (PILO); temporal lobe epilepsy (TLE).
* Human Seizure Correlates: seizures classification according to ILAE Commission for Classification and Terminology (Fisher et al., 2017).

CBD was orally administered in children and young adults resistant to pharmacological treatments. In this clinical study, the frequency of seizures was reduced, and some patients who received CBD became seizure-free after CBD treatment (Devinsky et al., 2016, 2017).

Strong evidence of CBD anticonvulsant efficacy for pharmacoresistant children came from studies in patients with Dravet Syndrome and Lennox-Gastaut Syndrome (O'Connell et al., 2017; Perucca, 2017). These studies have a high quality of therapy design, such as powered placebo-controlled and randomized trials. CBD showed a decrease ($\geq$ 50%) in the frequency of tonic-clonic, tonic, clonic, and atonic behavior of seizures in patients with Dravet Syndrome, and drop seizures in patients with Lennox-Gastaut Syndrome (O'Connell et al., 2017). Similar results were obtained in a long-term study in patients with Lennox-Gastaut and Dravet Syndromes that received CBD for approximately 2 weeks (Laux et al., 2019). Additionally, recently it was demonstrated that pharmacoresistent patients chronically treated with CBD (25 mg/kg/day) presented seizure and mood improvements associated with CBD anxiolytic effects. Moreover, cognitive improvements associated with modulation of attention control and reduction of the fMRI activity in the right superior frontal gyrus and the right middle frontal gyrus were also observed in those patients (Allendorfer et al., 2019).

Previously, clinical information about CBD on epilepsies was limited to case reports, small series, and surveys reporting on the use of CBD in high doses (100 – 300 mg/day) with 50% of adults patients free of seizures (Cunha et al., 1980; Mechoulam and Carlini, 1978). However, in most recent years, there was an increase in the use of CBD in clinical trials with accumulative evidence of seizure improvement in humans with lower doses than before (2 – 50 mg/kg/day), especially for pharmacoresistant patients (Devinsky et al., 2016, 2017; Laux et al., 2019; O'Connell et al., 2017; Porter and Jacobson, 2013). Furthermore, reinforcing the clinical translation, the recent CBD effectiveness doses (up to 50 mg/kg) for patients with epilepsies are similar to those used in most of the pre-clinical studies.

It is important to note that recent studies have evaluated the adverse effects associated with CBD treatment for epilepsies. Fatigue, somnolence, diarrhea, and appetite alterations were some of the main side effects observed in patients with epilepsies (Devinsky et al., 2016, 2017; Press et al., 2015; Thiele et al., 2018; Wheless et al., 2019). These adverse CBD effects reinforce the necessity to further evaluation of motor, social, psychological, and physiological effects associated with pharmacological CBD treatment for epilepsies.

In that context, quantitative behavioral studies such as neuroethological analysis with flowcharts and graph analysis provide integrative and reliable information about seizure generation, neuronal structures, and the sequential behavioral expression, not only in animal models (Etholm and Heggelund, 2009; Garcia-Cairasco et al., 1996, 2004), but also in humans with TLE (Bertti et al., 2010; Dal-Cól et al., 2006) or frontal lobe epilepsies (Bertti et al., 2014). This type of methodological approach, including graph analysis to the clinical semiology, could be an interesting approach to evaluate specific and sequential behavioral alterations associated with CBD treatment in humans and rodents. Those tools are strongly used in studies of MRI and quantitative EEG studies in humans (Chauvel and Mcgonigal, 2014; Mcgonigal et al., 2018), adding the view of multifactorial and complexity evaluation to the clinical scenario and reinforcing the importance to look not only to seizure score parameters or EEG data but also to complex seizure behaviors displayed by patients. A natural consequence is that a combination of semiology, fMRI and EEG tools needs to be integrated to evaluate CBD effects in patients with epilepsies.

Finally, it is extremely important to identify possible pharmacological interactions between CBD and conventional anticonvulsant drugs. Pre-clinical studies demonstrated that CBD potentiated the anticonvulsant effects of phenobarbitone (Chesher et al., 1975) and phenytoin (Chesher and Jackson, 1974. Conversely, CBD decrease clonazepam, trimethadione and ethosuximide effects in the MES test (Consroe and Wolkin, 1977). However, due to methodological limitations in previous studies, it is unclear how CBD modifies the anticonvulsant activity of those drugs. Recent data from clinical studies in pharmacoresistant patients demonstrated a CBD-induced increase on blood concentration of different anticonvulsant drugs, such as rufinamide, topiramate, zonisamide, eslicarbazepine, and *N*-desmethylclobazam, the clobazam active metabolite, although blood concentration of other anticonvulsant drugs (valproate, levetiracetam, phenobarbitone, clonazepam, phenytoin, among others) were not modified by CBD (Geffrey et al., 2015; Gaston et al., 2017). Therefore, pharmacological and pharmacokinetics interaction between CBD and conventional anticonvulsant drugs should be better investigated. Consequently, animal models may provide evidences and insights to clinical treatment.

8.1. Cannabidiol in Therapy-Resistant Epilepsy Models: building a bridge between basic and clinical research

Since one-third of people with epilepsies are considered pharmacoresistant, due to the difficulty to achieve suitable control of seizures (Kwan et al., 2010; Kwan and Brodie, 2010), a better understanding of the mechanisms underlying pharmacoresistance, as well as the development and screening of new anticonvulsant drugs, may improve the clinical treatment. In that context, *Scn1a* gene mutation mimics seizures, morphological, and social alterations presented by patients with Dravet Syndrome in mice. Thus, using a model of Dravet Syndrome, authors observed that acute CBD (100 and 200 mg/kg; i.p.), before thermal-onset induction, had attenuated both, duration and severity of seizures. Furthermore, repetitive CBD administration (twice a day for eight days) was capable of reducing spontaneous recurrent seizures frequency by 70% (Kaplan et al., 2017). Thus, CBD was capable of

protecting *Scn1a* mutant mice from febrile seizures and future spontaneous seizures in a Dravet Syndrome model (Kaplan et al., 2017). Improvements on comorbidities, such as social deficits and hyperactivity (see Section 7) were also observed after CBD treatment.

Still, regarding pharmacoresistance, the MES-6 Hz (44 mA) stimulation model is thought to be a therapy-resistant epilepsy model (Barton et al., 2001). The behavioral manifestation of 6 Hz-induced seizures are distinct from the conventional MES test, rather than the tonic extension, minimal clonic and stereotyped behaviors are present (Barton et al., 2001). Using the 6-Hz stimulation, authors observed that CBD (120 mg/kg) presented anticonvulsant activity against jaw and front limb clonus (Karler and Turkanis, 1981). In recent studies using the 6-Hz 44 mA seizure model, it was observed that CBD (ED50:164; mg/kg) was able to protect mice from forelimb and facial clonus seizures in a dose-dependent manner (Klein et al., 2017). Patra et al. (2018) observed similar results with CBD (144 mg/kg; i.p.) in the 6-Hz test. Finally, in a different manner, using the lamotrigine-resistant amygdala kindling model, a protocol in which animals become pharmacoresistant, it was observed that CBD did not generate protection neither in behavioral nor in electrographic seizures (Klein et al., 2017).

9. Conclusions and future perspectives

Essentially, CBD has shown effectiveness against tonic, clonic, generalized tonic-clonic seizures, and on therapy-resistant epilepsy models, presenting behavioral, EEG, and neuroprotective effects in both, acute and chronic protocols. CBD anticonvulsant effects in experimental models are in agreement with clinical data that showed prominent results in drug-resistant patients. Consequently, studies with CBD for epilepsies treatment in experimental models of epilepsy support the use of this phytocannabinoid for future studies in epileptology and neuropsychiatric comorbidities. Concerning the mechanism of action, this review showed that CBD anticonvulsant effects are related to a great variety of mechanisms among them, endocannabinoid modulation, potentialization of GABAergic/inhibitory neurotransmission, and calcium mobilization from BK channels, TRPV1, mitochondrial and GPR55 receptors. Thus, it is possible to assume that the CBD anticonvulsant activity may not be due to exclusively one, but rather to multifactorial mechanisms underlying the behavioral, electrophysiological and neuroprotective effects presented by CBD in many epileptic seizure models and even in pharmacoresistant patients.

Some studies presented behavioral analysis restricted only to the presence or absence of seizures, with no additional information, reinforcing the divergence of methodological improvements in electrophysiological and molecular mechanisms in comparison with behavioral analysis. Therefore, neuroethological approaches are essential tools that could provide crucial and reliable information about CBD effects on complex and sequential seizure behaviors improving translational aspects between basic and clinical science. Moreover, pharmacokinetic, pharmacodynamics and molecular mechanisms of CBD in each preclinical model remain all unclear, with restricted information. Furthermore, considering the neuroprotective effects of this drug, designed studies in epileptogenesis are scarce. Finally, the effects of chronic CBD treatment on seizures and epilepsy-related comorbidities, mostly those alterations on the neuropsychiatric sphere, need to be also urgently clarified.

Author contributions

WLL conceived the original design and wrote the manuscript. RAVS and RMPSJ assisted in the writing, background research, critical revisions, and discussions to incorporate important intellectual content. RMPSJ and WLL created the figures. WLL an RAVS created the Tables. JPL and NGC provided critical advice and revisions of the manuscript. All authors reviewed and approved the final version.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neubiorev.2020.01.014.

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