



Review article

The evolution of the pilocarpine animal model of status epilepticus

Iman Imtiyaz Ahmed Juvala, Ahmad Tarmizi Che Has^{*}

Department of Neurosciences, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

ARTICLE INFO

Keywords:

Pilocarpine
Animal model
Status epilepticus
Seizure
Neuroscience
Behavioral neuroscience
Cellular neuroscience
Cognitive neuroscience
Molecular neuroscience
Systems neuroscience
Health sciences

ABSTRACT

The pilocarpine animal model of status epilepticus is a well-established, clinically translatable model that satisfies all of the criteria essential for an animal model of status epilepticus: a latency period followed by spontaneous recurrent seizures, replication of behavioural, electrographic, metabolic, and neuropathological changes, as well as, pharmacoresistance to anti-epileptic drugs similar to that observed in human status epilepticus. However, this model is also characterized by high mortality rates and studies in recent years have also seen difficulties in seizure induction due to pilocarpine resistant animals. This can be attributed to differences in rodent strains, species, gender, and the presence of the multi-transporter, P-glycoprotein at the blood brain barrier. The current paper highlights the various alterations made to the original pilocarpine model over the years to combat both the high mortality and low induction rates. These range from the initial lithium-pilocarpine model to the more recent Reduced Intensity Status Epilepticus (RISE) model, which finally brought the mortality rates down to 1%. These modifications are essential to improve animal welfare and future experimental outcomes.

1. Introduction

Status epilepticus (SE) is a fatal neurological disorder with a high mortality rate. This condition often occurs as a ramification of stroke (Santamarina et al., 2018; Pauleto et al., 2020), traumatic brain injury (Dhakar et al., 2015; Andrade et al., 2019), infection (Vezzani et al., 2015; Lee and Chi, 2018), metabolic disorder (Sharma and Prasad, 2017; Lin Lin Lee et al., 2018), or alcohol or drug withdrawal (Leach et al., 2012; Chen et al., 2016) and is observed in patients with a history of epilepsy (Trinka et al., 2012). SE was previously defined as either a seizure that lasts ≥ 30 min or two or more consecutive seizures with the absence of complete conscious recovery between them (Milligan, 2010). However, generalised tonic-clonic seizures only last a maximum of 3 min, thus if a seizure surpasses this duration, it becomes self-sustaining (Wray and Knupp, 2011). In a typical clinical setting, 30 min is a long time to wait to assist someone experiencing continuous seizures because a larger scale of neurological damage will occur, resulting in self-sustaining refractory seizures, thus making the use of anti-epileptic drugs (AEDs) futile (Chen and Wasterlain, 2006). As the duration of SE increases, the effectiveness of AEDs gradually decreases, often with a total decline in drug efficacy (Mayer et al., 2002; Wheless and Treiman, 2008) that results in increased comorbidities and poor quality of life. Therefore, the window for diagnosis is further reduced; the current definition of SE according to the International League Against Epilepsy (ILAE) is a

persistent seizure that lasts for ≥ 5 min, with long-term detrimental consequences such as neurodegeneration and formation of aberrant neural networks if the seizure activity exceeds 30 min (Trinka et al., 2015).

2. The animal models of SE

The 30-minute time period is still applicable in experimental animal models, as this is the time required for the models to develop SE (Mazarati et al., 1998a). There is a dire need for a new range of AEDs in patients who exhibit pharmacoresistance, highlighting the necessity of animal models. A good animal model ensures a smooth transition from preclinical testing to human clinical trials, thus establishing a better understanding of the underlying pathophysiology and mechanisms of SE. Animal models also allow good comprehension of the pharmacokinetics, side effects, potency, efficacy, and tolerance of the potential therapeutic agents being tested. Therefore, a successful model must mimic the observed symptoms of SE in humans, such as convulsions (lasting more than 5 min), memory loss, muscle spasms, falling, and confusion. As observed in clinical cases, a "latency period" must occur after the initial injury, which should then be followed by the occurrence of spontaneous recurrent seizures (SRS) (Cavalheiro, 1995; Lévesque et al., 2016). The model must also be able to replicate the neuropathological injuries observed in the brain regions of SE patients; the 30-minute period is

^{*} Corresponding author.

E-mail address: ahmadtarmizi@usm.my (A.T. Che Has).

<https://doi.org/10.1016/j.heliyon.2020.e04557>

Received 5 May 2020; Received in revised form 5 July 2020; Accepted 22 July 2020

2405-8440/© 2020 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

crucial for this development (Fujikawa, 1996; Varelak and Claassen, 2017). Finally, the model must simulate the development of pharmacoresistance to certain anticonvulsants (observed in refractory SE) and allow testing of potential new drugs (Kapur and Macdonald, 1997; Mazarati et al., 1998b; Jones et al., 2002; Chakir et al., 2006). Before creating such an animal model, the investigator must first take the following factors into account: time and effort required to create the model, costs, laboratory skills of the researchers, availability of materials/research environment, strain/susceptibility of animals to seizures, pharmacokinetics, and the mortality rate of animals. The kindling model, for example, is a well-known seizure model created by repeated electrical stimulation via implanted depth electrodes (Song et al., 2018). Although this technique is effective, it is also costly, laborious, and time consuming (Löscher, 1997; Song et al., 2018) making the use of chemoconvulsants a more convenient method. The frequency of SRS is also lower (Brandt et al., 2004) than that of chemoconvulsant models. This technique requires precision and skills on the researcher's part as there is a risk of damaging the implanted electrodes, rendering them unreliable for chronic experiments (Song et al., 2018). Other electrical stimulation models such as the perforant path approach produce less neurodegeneration than chemoconvulsant models such as kainic acid and pilocarpine models (Reddy and Kuruba, 2013), thus making them a less desirable option. Chemoconvulsants such as pilocarpine and kainic acid are much easier to use and do not require sophisticated setups. However, compared with pilocarpine, seizures resulting from intracerebral kainic acid injections are more severe (Kienzler-Norwood et al., 2017); the stages of seizure are also difficult to distinguish because their progression is very rapid (Lévesque et al., 2016), unlike the stages induced by pilocarpine, which can be easily distinguished using the Racine scale (Racine, 1972). Kainic acid-induced animals also show a higher degree of anxiety (Ratté and Lacaille, 2006) and depression-like behaviour (Grötcke et al., 2008). Compared with pilocarpine, it is more difficult to induce SE in mice with systemic administration of kainic acid (McKhann et al., 2003). Studies in mouse models have shown that the C57BL/6, C57BL/10, and F1 C57BL/6×CBA/J strains are resistant to systemic administration of kainic acid, while the FVB/N, ICR, and DBA/2 J strains are vulnerable (McLin and Steward, 2006). Kainic acid models have also proven to be impractical for pharmacoresistance studies because of differences in AED reactions (Reddy and Kuruba, 2013). Another limitation is the excitotoxic effect of kainic acid, which makes separating direct neuronal damage from seizure-induced neuronal damage difficult (Reddy and Kuruba, 2013). As found by Rao and colleagues, while creating a kainic acid model of SE, 77% of their animals developed moderate bilateral neurodegeneration in various hippocampal regions, whereas 23% developed massive neurodegeneration in all regions of the hippocampus (Rao et al., 2006). This was observed even though the kainic acid dosage used, and the frequency of SRS recorded were the same for both groups. Kainic acid is also more expensive to purchase than its competitor, pilocarpine (Lévesque et al., 2016). Unlike kainic acid, pilocarpine can replicate the kindling stages of SE development (Pitkänen et al., 2005), making it a more efficient chemoconvulsant. Studies have also reported differences in SRS frequency between kainic acid and pilocarpine models. Although kainic acid can induce SRS in animal models, these seizures eventually decline within 22–46 days, making this model undesirable (Cavalheiro et al., 1982; Cronin and Dudek, 1988). However, studies have also shown that pilocarpine-induced SRS occur at a constant frequency for an extended length of time (120 days – Cavalheiro et al., 1991; 325 days – Mello et al., 1993). This period has been reported as the “entire observation period”, meaning that if researchers were to extend their observation period, the pilocarpine model would most likely exceed the stated time period, clearly making it a stable model. The pilocarpine model also mimics the “latency period” after the initial insult (Turski et al., 1989; Lévesque et al., 2016). Studies have found similar electrophysiological and morphological abnormalities in the rat hippocampus of the pilocarpine model of SE (Isokawa and Mello, 1991; Mello et al., 1992) and human SE (Scheibel et al., 1974; Babb, 1986; Masukawa et al., 1989;

Isokawa et al., 1991). The pilocarpine model is also capable of replicating several other characteristics such as dentate granule cell dispersion as well as supragranular and intragranular mossy fibre sprouting (Mello et al., 1993), which is commonly observed in clinical cases (Houser et al., 1990; Babb et al., 1991). Thus, although several seizure models have been proposed, ranging from the use of chemoconvulsants to electrical stimulation, the pilocarpine model is one of the oldest and most well-studied animal models of SE.

3. The pilocarpine model: the early days

First described in the early 1980s, the pilocarpine model is characterised by a single large dose of pilocarpine; the dose lies in the range of 300–400 mg/kg for rats (Turski et al., 1983a). The preferred method of administering the drug is usually via intraperitoneal (i.p.) or intrahippocampal injections; both methods elicit identical electrographic and behavioural effects, as well as similar histopathological alterations (Furtado et al., 2002). Intrahippocampal injections, however, have a higher survival rate (71%) than i.p. injections (Furtado et al., 2002). Upon pilocarpine administration, the animals are usually stationary for 10 min, followed by facial twitching (Stage I); head nodding (Stage II); forelimb clonus (Stage III); bilateral forelimb clonus and rearing (Stage IV); and bilateral forelimb clonus with rearing and falling (Stage V) (Racine, 1972). The Racine scale is often used to grade seizure severity; Stage V and above indicate severe brain damage and development of SE (Racine, 1972). After 30 min, these seizures become self-sustaining and occur at 15-minute intervals, persisting for hours until a suitable AED is used to stop the seizures (Curia et al., 2008). SE induced via chemoconvulsants are shown to be more difficult to terminate as compared to electrically induced models (Bankstahl and Löscher, 2008). Thus, several studies utilize a repetitive dose or a combination of AEDs to terminate SE and reduce mortality rates. Pilocarpine is an M1 muscarinic acetylcholine receptor agonist that promotes continuous excitatory activity, resulting in brain tissue damage (Lee et al., 2018). M1 receptor knockout mice show no signs of seizures when administered pilocarpine, and M1 is the only muscarinic receptor subtype that can mediate seizure activity (Hamilton et al., 1997; Bymaster et al., 2003). In addition, when atropine, a competitive antagonist of the muscarinic acetylcholine receptor, is applied to rats prior to pilocarpine treatment, there is an absence of seizure initiation, which is significant for large (150 mg/kg) and small doses (1 mg/kg) of atropine (Honchar et al., 1983; Jope et al., 1986; Birch, 2012). Atropine administration to pilocarpine-treated rats immediately upon forelimb clonus results in termination of seizures, whereas those administered atropine 20 min after forelimb clonus do not indicate any decline in seizure activity, proceeding to SE and eventually death (Jope et al., 1986; Birch, 2012). This finding clearly shows that muscarinic receptors are responsible for the onset of seizure activity in the pilocarpine model of SE, and as observed in clinical cases, anticholinergics are inefficient anticonvulsants for SE. Furthermore, when pilocarpine pre-treated rats are given hemicholinium-3, an acetylcholine synthesis inhibitor, progression of SE is blocked (Jope et al., 1986). This occurs because hemicholinium-3 prevents the re-uptake of choline by high-affinity choline transporters at the presynaptic membrane, causing an 80% reduction in the normal acetylcholine concentration (Freeman et al., 1975). This indirectly reduces the efficiency of the M1 muscarinic acetylcholine receptors on the postsynaptic membrane (Choudhary et al., 2017), highlighting the importance of presynaptic cholinergic activity in the pilocarpine model of SE. Similarly, studies have shown that seizure activity in pilocarpine models can be blocked by a 15-minute pre-treatment with diazepam (a common AED), and initial tonic-clonic seizures can also be terminated via diazepam administration, making this a suitable model for SE (Jope et al., 1986). Seizures induced by pilocarpine begin in the ventral forebrain; the nucleus accumbens is most likely the primary site of injury because of the presence of a high density of muscarinic receptors (Kobayashi et al., 1978; Nonaka and Moroji, 1984). The neocortex suffers the most significant injuries, characterised

by swollen dendrites and cell bodies and pruning of axons (Clifford et al., 1987). Animals often suffer a 10%–20% reduction in body weight post-SE, with eventual recovery to baseline values after 1 week (Turski et al., 1989). Mortality rates in this model are very high, with some studies reporting 30%–40% mortality in male Wistar rats treated with a pilocarpine dose of 300–400 mg/kg (Turski et al., 1983a, 1989; Liu et al., 1994) and other studies reporting 40%–55% mortality with a dose of 320–360 mg/kg (Esclapez et al., 1999; Goffin et al., 2007). A notable observation is the differences in rat strains; Sprague-Dawley rats appear to have lower mortality rates (5%) than Wistar rats (30%) when treated with the same dose of pilocarpine, 380 mg/kg (Leite et al., 1990; Poirier et al., 2000). However, the mortality rate is still a problem, as it is not standardised even within the same rat strain. Thus, using Sprague-Dawley rats does not always ensure a reduction in the number of deaths. For instance, when Sprague-Dawley rats are treated with 400 mg/kg of pilocarpine, SE is generated in 83% of animals, but the mortality rate is 100% (Jope et al., 1986). Another observation in this study is that SE is not induced in all rats. Therefore, when using pilocarpine alone, a higher dose is required to increase the likelihood of replicating the entire disease as well as reducing the latency to SE, which further increases the mortality rate (Clifford et al., 1987; Liu et al., 1994). Studies have also tried to induce SE by splitting the single large dosage of pilocarpine into several smaller doses (Gliem et al., 2001). This however was not fruitful in all animal models. For example, studies have administered single injections of 100 mg/kg of pilocarpine intraperitoneally to induce SE in mice, with the total dosage reaching up to 800 mg/kg (Neumann et al., 2017). Even with such a high overall dose, only 54% of the animals survived the procedure and developed SE. Using such a high dosage of pilocarpine results in an unnecessary wastage of the drug. There is also a limit to the concentration of pilocarpine that can be administered to animals; a higher concentration of pilocarpine results in a higher toxicity level (Pitkänen et al., 2005). To combat this issue, studies have suggested the use of lithium as a pre-treatment before pilocarpine administration (Honchar et al., 1983; Vezzani, 2009).

4. A new hope: the lithium-pilocarpine model of SE

The lithium-pilocarpine model of SE is generally characterised by administration of lithium chloride (LiCl; 127 mg/kg), followed by pilocarpine administration (30 mg/kg) 18 h later (Fan et al., 2020). Methylscopolamine (1 mg/kg in rats) is also given to the animals 30 min before pilocarpine administration to reduce any peripheral effects caused by pilocarpine, which unlike scopolamine, does not cross the blood brain barrier (BBB) (Davis and Berger, 2013). Absence of methylscopolamine results in animals displaying symptoms of peripheral cholinergic activity such as tremor, salivation, piloerection, chromodacryorrhoea, and diarrhoea after pilocarpine administration (Clifford et al., 1987; Vezzani, 2009). However, higher doses of methylscopolamine (≥ 20 mg/kg in rats) can block seizure activity (Turski et al., 1983a, 1983b). Lithium, used previously for psychiatric chemotherapy and bipolar disorder (Duvall and Gallicchio, 2017), potentiates the effect of pilocarpine by indirectly activating T-lymphocytes and mononuclear cells, which in turn results in higher serum IL-1 β levels, thus altering the BBB permeability and increasing pilocarpine uptake (Marchi et al., 2009). Researchers have also found variation in muscarinic receptor binding in the presence of lithium (Hruska et al., 1984; Gibbons et al., 2015). Pre-treatment with LiCl has been reported to increase acetylcholine release, with some studies observing a six-fold increase in the hippocampus (Haas and Ryall, 1977; Jope, 1979; Hillert et al., 2014). This results in more acetylcholine crossing the synaptic cleft and reaching the postsynaptic membrane where it activates M1 muscarinic receptors. LiCl is suggested to play a role in phosphoinositide metabolism, with lithium pre-treatment resulting in a 40-fold increase in myo-inositol-1-phosphate compared with the mere 4-fold increase with pilocarpine alone (Allison et al., 1980; Honchar et al., 1983; Sherman et al., 1985; Clifford et al., 1987). Myo-inositol-1,4,5-trisphosphate (IP $_3$), an inositol phosphate derived from

phosphoinositide hydrolysis, has also been associated with increased seizure activity (Meldrum, 1983; Griffiths et al., 1984). IP $_3$ is a second messenger molecule, which upon binding to IP $_3$ receptors (Ca $^{2+}$ channels), induces Ca $^{2+}$ release from the endoplasmic reticulum (ER) to the cytoplasm, which in turn causes store-operated Ca $^{2+}$ entry in an attempt to refill the depleted ER stores (Kelly et al., 2005). This further increases the concentration of intracellular Ca $^{2+}$, resulting in enhanced vesicular transport and acetylcholine release at the presynaptic membrane (Castellano-Muñoz and Ricci, 2014), which then enhances the activation of muscarinic receptors on the postsynaptic membrane. The increased Ca $^{2+}$ levels are also accompanied by cellular damage and oxidative stress, which increase extracellular lactate, choline, and glycerol; the use of a Ca $^{2+}$ channel blocker reduces SE-induced membrane damage (Imran et al., 2015). Animals pre-treated with LiCl show an increased sensitivity to pilocarpine, with a 20-fold shift in the dose-response curve for seizure generation (Clifford et al., 1987). Thus, the use of LiCl allows for a substantial reduction in the pilocarpine dose required, along with a reduction in the time to SE onset. Both the LiCl-pilocarpine and high-dose pilocarpine models follow the same pattern in seizure initiation and propagation (Clifford et al., 1987; Müller et al., 2009). Both models also generate identical behavioural, electrographic, metabolic, and neuropathological symptoms (Clifford et al., 1987; Müller et al., 2009). The LiCl-pilocarpine model has a higher SE induction rate than pilocarpine alone, with some investigators even reporting 100% SE induction compared with pilocarpine, which only results in 60% SE induction (Goffin et al., 2007; Ahmad, 2013), although the numbers may vary among laboratories. The LiCl-pilocarpine model has a lower mortality rate and has been shown to generate more consistent and prolonged seizures with reproducible results, thus making it a promising model for studying SE.

5. The limitations strike back: imperfections in the lithium-pilocarpine model

Unfortunately, the lower mortality rate is not negligible because it ranges between 92% and 95% (Jope et al., 1986; Morrisett et al., 1987; Huang et al., 2018). Decreasing the pilocarpine dose in the lithium-pilocarpine model reduces the mortality rate, but this is also accompanied with a decline in SE induction (Jope et al., 1986; Huang et al., 2018). Another factor to consider is the reports of alterations in SE induction and mortality rates among different rodent strains (Chen et al., 2005; Winawer et al., 2007a, 2007b). Researchers have observed variations in SE induction and pilocarpine sensitivity between different batches of the same strain of rats (Sprague-Dawley) purchased from different breeders (Bankstahl et al., 2009). These groups indicate strong substrain differences between each other that most likely result in the wide-ranging experimental data reported from different laboratories. These differences between strains and substrains have also been noted in mice (Borges et al., 2003; Müller et al., 2009c; Schauwecker, 2012; Bankstahl et al., 2012). Another study also found remarkable intrastain differences in seizure predisposition between Wistar rats from two different breeding locations as well as rats purchased at different times from the same breeding location (Portelli et al., 2009). Therefore, these genetic differences can have a major impact on the development of SE models, causing difficulty in reproducing results even within the same facility. One possibility for the observed pilocarpine resistance is the presence of the multidrug transporter P-glycoprotein (PGP), which is a transporter that removes pilocarpine from the BBB, serving a protective role. Studies have shown that PGP knockout mice require a lower dose of pilocarpine for SE induction than their wild-type counterparts (Römermann et al., 2015). Studies have revealed that PGP knockout mice injected with a low dose of pilocarpine do not show any increase in brain uptake of the drug, but those injected with a higher dose show significantly increased brain uptake (Sahin et al., 2003; van Vliet et al., 2007; Li et al., 2013). In addition, pre-treatment of wild-type mice with the PGP inhibitor tariquidar causes an increase in pilocarpine brain uptake similar

to that observed in PGP knockout mice, thus emphasizing that pilocarpine is carried by the PGP multidrug transporter (Bankstahl et al., 2013). Female wild-type mice reportedly require a higher pilocarpine dose for SE induction than males (Römermann et al., 2015). The absence of this phenomenon in PGP knockout mice could therefore be explained by a PGP-dependent mechanism (Römermann et al., 2015). Verapamil, a common PGP substrate, showed lower brain uptake in female mice than male mice, suggesting higher PGP expression levels in female mice (Dagenais et al., 2001). Because PGP expression levels are influenced by sexual hormone levels occurring during the oestrus cycle (Schiengold et al., 2006), several studies have reported similar sex-related differences in SE development in both rats and mice (Mejías-Aponte et al., 2002; Morris et al., 2003; Buckmaster and Haney, 2012). Several studies have implicated the excitatory neurotransmitter glutamate in the underlying mechanism that activates PGP (Bauer et al., 2007; Zibell et al., 2009; Hartz et al., 2019; Soldner et al., 2019). As mentioned earlier, animals that reach Stage V on the Racine scale develop SE and no longer show any response to atropine treatment, suggesting activation of other downstream pathways, in addition to cholinergic cells, resulting in SE (Jope et al., 1986; Birch, 2012). Cultured hippocampal neuron studies have revealed that pilocarpine-induced M1 receptor activation results in an imbalance between excitatory and inhibitory signals, which in turn results in the development of SE (Priel and Albuquerque, 2002). Seizures are considered to be initiated by M1 receptors and maintained by N-methyl-D-aspartate (NMDA) receptors (ionotropic glutamate receptors) (Nagao et al., 1996; Smolders et al., 1997). In vivo microdialysis studies have also shown increased hippocampal glutamate levels upon pilocarpine administration (Smolders et al., 1997). Seizures result in NMDA receptor activation and an increase in glutamate release from neurons, which accumulates in the brain interstitial fluid (Holmes, 2002). This contributes to the excitotoxic damage, loss of neurons, and resulting pathophysiology of SE (Barnes and Slevin, 2003; Gardoni and Di Luca, 2006). This increase in glutamatergic signalling increases Cyclooxygenase-2 (COX-2) levels (Strauss and Marini, 2002). The enzyme COX-2 plays an essential role in peroxidation of arachidonic acid and prostaglandin synthesis, which is responsible for inflammation (Pepicelli et al., 2005). Transcriptional upregulation of the COX-2 gene has been observed in both animal (Voutsinos-Porche et al., 2004; Kawaguchi et al., 2005; Takemiya et al., 2006; Lee et al., 2007) and human cases of seizure (Desjardins et al., 2003). Inhibition of COX-2 terminates NMDA receptor-mediated excitotoxic damage of neurons (Manabe et al., 2004; Hewett et al., 2006). Generation of COX products such as prostaglandin E2 can also be blocked by inhibiting the NMDA receptor (Pepicelli et al., 2005), thus suggesting a strong link between NMDA receptor activation and COX-2 expression. Both in vivo and in vitro rodent studies have revealed that glutamate increases PGP expression and transport activity in the brain (Zhu and Liu, 2004), both of which can be blocked using either MK-801, a non-competitive NMDA receptor antagonist, or indomethacin, a non-selective COX inhibitor (Bauer et al., 2007), as COX-2 has also been shown to increase PGP levels (Patel et al., 2002). In addition to pilocarpine, several AEDs are also PGP substrates. Studies have shown that an increase in seizure activity causes an increase in expression of PGP, resulting in an increased rate of AED efflux at the BBB, which might explain why AEDs are ineffective in stopping SE onset (Lösher and Potschka, 2005; Kwan and Brodie, 2006). Studies on pharmacoresistant rats show lower PGP expression levels in the brain than those in AED-responsive rats (Volk and Löscher, 2005). Similar to its effect on pilocarpine uptake, application of tariquidar results in enhanced brain uptake of AEDs as well (Brandt et al., 2006; van Vilet et al., 2006). It could be assumed that PGP has a protective role when activated against pilocarpine in animal models. However, this prolonged activation also occurs as a result of seizures. Thus, an initial protective mechanism becomes the cause of pharmacoresistance.

This effect is the reason why some studies report diazepam as having a positive effect, while others report that it fails to terminate SE (Treiman et al., 1998; Kapur and Macdonald, 1997; Walton and Treiman, 1988;

Jones et al., 2002; Goodkin et al., 2003; Nardou et al., 2011; Aplan et al., 2014; Zhao et al., 2016). In addition to diazepam, several other drugs have also been tested against SE to reduce mortality rates in animal models; diazepam, phenobarbital, phenytoin, valproate, and carbamazepine have been shown to be ineffective in terminating SE in the LiCl-pilocarpine model (Morrisett et al., 1987; Biagini et al., 2001; Kubova et al., 2005). The combination of diazepam with barbiturates such as pentobarbital is reported to be more useful in stopping SE (Lemos and Cavalheiro, 1995; Fujikawa, 1996; Biagini et al., 2001). NS-398, a COX-2 inhibitor, administered along with diazepam has also been shown to reduce SE severity (Trandafir et al., 2015). A study showed that use of MK-801 combined with diazepam was able to terminate all manifestations of SE (Walton and Treiman, 1991). The use of MK-801 alone cannot completely stop seizure activity; however, unlike diazepam, it can block electroencephalogram (EEG) pattern changes that usually occur during SE (Walton and Treiman, 1991). Applying MK-801 alone also allows rats to survive SE; however, the recovery period is much longer than the time required when MK-801 is administered along with diazepam, thus suggesting that MK-801 potentiates the effects of diazepam (Walton and Treiman, 1991). Ketamine, an NMDA receptor antagonist, has also been shown to have a neuroprotective role post-SE (Fujikawa, 1995). An interesting modification of the LiCl-pilocarpine model is the protocol used by Glien et al. (2001), wherein LiCl is injected as per the original protocol; however, the pilocarpine dose to be given the following day is altered. Instead of a single dose of 30 mg/kg, the dose is divided into individual doses of 10 mg/kg that are administered at 30-minute intervals until SE onset. Compared with the original protocol, this revised protocol dramatically reduces the mortality rates by 50%. As animals differ in their tolerance to pilocarpine, this method ensures that animals are not unnecessarily given high doses and improves the induction probability. These results were also observed in several other studies using both pilocarpine and LiCl-pilocarpine models (Brandt et al., 2010; Leung et al., 2015; Neumann et al., 2017; Wu and Wang, 2018).

6. Rise of the pilocarpine model: an improved approach

The most remarkable improvement made to the LiCl-pilocarpine model, however, is the Reduced Intensity Status Epilepticus (RISE) model (Modebadze et al., 2016; Needs et al., 2019). This model is an accumulation of several observations. Built on the same fundamentals as the previous protocol suggested by Glien et al. (2001), this study also divides the single pilocarpine dose into smaller doses that are administered at 30-minute intervals. However, upon animals scoring $\geq$ Stage 3 on the Racine scale, 2.5 mg/kg of xylazine is injected. One hour later, this is followed by administration of a 1 ml/kg drug cocktail consisting of MK-801 (0.1 mg/kg), diazepam (2.5 mg/kg), and 2-methyl-6-(phenylethynyl)pyridine (MPEP) (20 mg/kg). SE terminates within 30 min, with animals fully recovering within 12 h. The frequency of seizures is comparable to those observed with high doses of pilocarpine in previous studies. Animals demonstrate variability and clusters of seizures, which are also observed in human SE, further highlighting the translational significance of this model (French et al., 1993; Arida et al., 1999; Bajorat et al., 2011). Consistent SRS also develop after a latency period. According to Modebadze et al. (2016), there is an absence of overall brain damage compared with other models, accompanied by alterations in the temporal network. The in vitro studies also revealed similar electrophysiological readings as observed in paediatric and adult patients. Features such as fast oscillatory activity and ictal-like events were also observed in in vivo as well as in vitro studies. Xylazine is an adrenergic alpha-receptor agonist that is a common muscle relaxant. Studies show that administration of a low dose of xylazine to animals with SE can minimise the intensity of clonic muscle contractions without changing electrographic seizures (Yang et al., 2006; Thompson et al., 2007). This is important as locomotor hyperactivity and ataxia can cause fatigue in animals, thus increasing their mortality (Modebadze et al., 2016). Although both convulsive and non-convulsive SE share the same EEG

patterns, convulsive seizures are associated with a higher mortality rate (Tatum, 2013). With this reduction in convulsive SE, there is a reduced likelihood of secondary generalization in sub-cortical sites, especially the brainstem (Samoriski and Applegate, 1997). As stated previously, several studies have used NMDA antagonists, alone or combined with diazepam (Walton and Treiman, 1991; Fujikawa, 1995). However, the inspiration to use a combination of MPEP and an mGluR5 antagonist along with MK-801 and diazepam came from Tang et al. (2007). This previous study used the same drug cocktail in a pilocarpine mouse model (300 mg/kg) and was able to terminate the seizures, simultaneously preventing early hippocampal cell death. The RISE model utilises the same drug cocktail with a small modification; the MPEP dose is reduced, and the diazepam dose is increased. Thus, it is able to reduce the mortality rate to 1% but still maintain high morbidity, replicating the epileptogenic characteristics observed in clinical cases of SE and making it a consistent, reproducible, and clinically translatable model for SE. This study on the RISE model was further followed up by another study investigating the underlying biochemical mechanisms involved in the initiation, development and establishment of this model (Needs et al., 2019). Similar to human SE, the study observed alterations in several different receptor expression levels at various time points leading up to SE. These changes also varied depending on the brain regions, for example there were delayed receptor changes in the temporal lobe structures, suggesting that the epileptogenic activity continued long after the initial seizure (Sloviter, 2005; Sloviter and Bumanglag, 2013; Needs et al., 2019). This is beneficial to the model as absence of any ictal activity would make the creation of this model futile. All in all, this proves that the RISE model is reproducible and a good representative model for studying SE.

7. Conclusion

The pilocarpine model of SE has undergone several modifications over the last few decades. From its initial single high dose of 400 mg/kg to its most recent improvement with the RISE model, all of these refinements were essential to ensure reduced mortality rates without compromising the SE induction probability. Even though the use of the original model resulted in higher mortality rates, its use was necessary because of the lack of sufficient clinically translatable models of SE at the time. The high-dose pilocarpine model was the first of its kind that satisfied all of the criteria essential for an animal model of SE: a latency period followed by SRS, replication of behavioural, electrographic, metabolic, and neuropathological changes, and pharmacoresistance to AEDs similar to that observed in human SE. Compared with other models, the original pilocarpine model was also more suitable for investigating the underlying mechanisms of SE and its resulting AED pharmacoresistance, thus for the first time giving researchers hope of designing potential therapeutics. The obvious drawbacks were the high mortality rates and limited SE induction in animals. These problems were soon mitigated with the introduction of the LiCl-pilocarpine model. This model was able to replicate all aspects of the pilocarpine model with the addition of lithium (24 h prior) and a much lower dose of pilocarpine. Although this alteration did improve the model to a certain extent, it did not completely curtail the problems. Several animals did not survive SE onset, and most AEDs were shown to be ineffective in stopping seizure activity. High mortality rates need to be considered because it is animal lives that are being dealt with. In animal studies, scientists must abide by the principles of the 3Rs: replacement, reduction, and refinement. To this end, several researchers have suggested modifications that could be made to the pilocarpine model. Of these, the novel protocol suggested by Glien et al. (2001) was of significant advantage. Dividing the single large dose of pilocarpine into several smaller doses to be administered at 30-minute intervals ensured lower mortality rates than the previous models. This new strategy was also able to generate a larger number of animals with induced SE. However, the most critical advancement was the introduction of the RISE model, which not only reduced the mortality rate (1%) but also increased SE induction and morbidity. This model, using a

combination of previous research, presented a drug cocktail that was able to successfully stop seizures, thus also achieving two of principles of the 3Rs: reduction and refinement. With these improvements, animals are now able to survive longer, thus reigniting the hope for potential new therapeutics and effective drug targets for SE.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

This work was supported by the Ministry of Higher Education, Malaysia (Fundamental Research Grant Scheme (FRGS) 203/PPSP/6171233).

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- Ahmad, M., 2013. Protective effects of curcumin against lithium-pilocarpine induced status epilepticus, cognitive dysfunction and oxidative stress in young rats. *Saudi J. Biol. Sci.* 20 (2), 155–162.
- Allison, J.H., Boshans, R.L., Hallcher, L.M., Packman, P.M., Sherman, W.R., 1980. The effects of lithium on myo-inositol levels of frontal cortex, in cerebellum, and in corpus callosum of the rat. *J. Neurochem.* 34, 456–458.
- Andrade, P., Banuelos-Cabrera, I., Lapinlampi, N., Paananen, T., Ciszek, R., Ndoe-Ekane, X., Pitkanen, A., 2019. Acute non-convulsive status epilepticus after experimental traumatic brain injury in rats. *J. Neurotrauma* 36 (11), 1890–1907.
- Apland, J., Aroniadou-Anderjaska, V., Figueiredo, T., Rossetti, F., Miller, S., Braga, M., 2014. The limitations of diazepam as a treatment for nerve agent-induced seizures and neuropathology in rats: comparison with UBP302. *J. Pharmacol. Exp. Therapeut.* 351 (2), 359–372.
- Arida, R.M., Fulvio, A.S., Clovis de Araujo, P., Cavaleiro, E.A., 1999. The course of untreated seizures in the pilocarpine model of epilepsy. *Epilepsy Res.* 34, 99–107.
- Babb, T.L., 1986. Metabolic, morphologic and electrophysiologic profiles of human temporal lobe foci: an attempt at correlation. *Adv. Exp. Med. Biol.* 203, 115–125.
- Babb, T.L., Kupfer, W.R., Pretorius, J.K., Isokawa, M., Levesque, M.F., 1991. Synaptic recurrent excitation of granule cells by mossy fibers in human epileptic hippocampus. *Neuroscience* 42, 351–363.
- Bajorat, R., Wilde, M., Sellmann, T., Kirschstein, T., Köhling, R., 2011. Seizure frequency in pilocarpine-treated rats is independent of circadian rhythm. *Epilepsia* 52 (9), 118–122.
- Bankstahl, J., Löscher, W., 2008. Resistance to antiepileptic drugs and expression of P-glycoprotein in two rat models of status epilepticus. *Epilepsy Res.* 82 (1), 70–85.
- Bankstahl, M., Brandt, C., Löscher, W., 2009. Differences in pilocarpine-induced epileptogenesis and behavioral alterations of Sprague-Dawley rats from two different breeders. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 379, 48–49.
- Bankstahl, M., Müller, C.J., Wilk, E., Schughart, K., Löscher, W., 2012. Generation and characterization of pilocarpine-sensitive C57BL/6 mice as a model of temporal lobe epilepsy. *Behav. Brain Res.* 230, 182–191.
- Bankstahl, J., Bankstahl, M., Römermann, K., Wanek, T., Stanek, J., Windhorst, A., Fedorowicz, M., Erker, T., Müller, M., Löscher, W., Langer, O., Kuntner, C., 2013. Tariquidar and elacridar are dose-dependently transported by P-glycoprotein and bcpr at the blood-brain barrier: a small-animal positron emission tomography and in vitro study. *Drug Metabol. Dispos.* 41 (4), 754–762.
- Barnes, G.N., Slevin, J.T., 2003. Ionotropic glutamate receptor biology: effect on synaptic connectivity and function in neurological disease. *Curr. Med. Chem.* 10, 2059–2072.
- Bauer, B., Hartz, A., Pekcec, A., Toellner, K., Miller, D., Potschka, H., 2007. Seizure-induced up-regulation of P-glycoprotein at the blood-brain barrier through glutamate and cyclooxygenase-2 signaling. *Mol. Pharmacol.* 73 (5), 1444–1453.
- Biagini, G., Avoli, M., Marcinkiewicz, J., Marcinkiewicz, M., 2001. Brain-derived neurotrophic factor superinduction parallels anti-epileptic-neuroprotective treatment in the pilocarpine epilepsy model. *J. Neurochem.* 76, 1814–1822.
- Birch, N., 2012. *Lithium and the Cell: Pharmacology and Biochemistry*, first ed. Elsevier Science, Kent, pp. 137–138.

- Borges, K., Gearing, M., McDermott, D.L., Smith, A.B., Almonte, A.G., Wainer, B.H., Dingledine, R., 2003. Neuronal and glial pathological changes during epileptogenesis in the mouse pilocarpine model. *Exp. Neurol.* 182, 21–34.
- Brandt, C., Ebert, U., Löscher, W., 2004. Epilepsy induced by extended amygdala-kindling in rats: lack of clear association between development of spontaneous seizures and neuronal damage. *Epilepsy Res.* 62 (2–3), 135–156.
- Brandt, C., Bethmann, K., Gastens, A.M., Löscher, W., 2006. The multidrug transporter hypothesis of drug resistance in epilepsy: proof-of-principle in a rat model of temporal lobe epilepsy. *Neurobiol. Dis.* 24, 202–211.
- Brandt, C., Nozadze, M., Heuchert, N., Rattka, M., Loscher, W., 2010. Disease-modifying effects of phenobarbital and the NKCC1 inhibitor bumetanide in the pilocarpine model of temporal lobe epilepsy. *J. Neurosci.* 30 (25), 8602–8612.
- Buckmaster, P.S., Haney, M.M., 2012. Factors affecting outcomes of pilocarpine treatment in a mouse model of temporal lobe epilepsy. *Epilepsy Res.* 102, 153–159.
- Bymaster, F., Carter, P., Yamada, M., Gomez, J., Wess, J., Hamilton, S., Nathanson, N., McKinzie, D., Felder, C., 2003. Role of specific muscarinic receptor subtypes in cholinergic parasympathomimetic responses, in vivo phosphoinositide hydrolysis, and pilocarpine-induced seizure activity. *Eur. J. Neurosci.* 17 (7), 1403–1410.
- Castellano-Muñoz, M., Ricci, A., 2014. Role of intracellular calcium stores in hair-cell ribbon synapse. *Front. Cell. Neurosci.* 8 (162).
- Cavalheiro, E.A., 1995. The pilocarpine model of epilepsy. *Ital. J. Neurol. Sci.* 16 (1–2), 33–37.
- Cavalheiro, E., Leite, J., Bortolotto, Z., Turski, W., Ikonomidou, C., Turski, L., 1991. Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous I. Recurrent Seizures. *Epilepsia* 32 (6), 778–782.
- Cavalheiro, E., Riche, D., Le Gal La Salle, G., 1982. Long-term effects of intrahippocampal kainic acid injection in rats: a method for inducing spontaneous recurrent seizures. *Electroencephalogr. Clin. Neurophysiol.* 53 (6), 581–589.
- Chakir, A., Fabene, P., Ouazzani, R., Bentivoglio, M., 2006. Drug resistance and hippocampal damage after delayed treatment of pilocarpine-induced epilepsy in the rat. *Brain Res. Bull.* 71 (1–3), 127–138.
- Chen, H., Albertson, T., Olson, K., 2016. Treatment of drug-induced seizures. *Br. J. Clin. Pharmacol.* 81 (3), 412–419.
- Chen, J., Larionov, S., Pitsch, J., Hoerold, N., Ullmann, C., Elger, C.E., Schramm, J., Becker, A.J., 2005. Expression analysis of metabotropic glutamate receptors I and III in mouse strains with different susceptibility to experimental temporal lobe epilepsy. *Neurosci. Lett.* 375, 192–197.
- Chen, J.W., Wasterlain, C.G., 2006. Status epilepticus: pathophysiology and management in adults. *Lancet Neurol.* 5, 246–256.
- Choudhary, P., Armstrong, E., Jorgensen, C., Piotrowski, M., Barthmes, M., Torella, R., Johnston, S., Maruyama, Y., Janiszewski, J., Storer, R., Skerratt, S., Benn, C., 2017. Discovery of compounds that positively modulate the high affinity choline transporter. *Front. Mol. Neurosci.* 10.
- Clifford, D., Olney, J., Maniotis, A., Collins, R., Zorumski, C., 1987. The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. *Neuroscience* 23 (3), 953–968.
- Cronin, J., Dudek, F., 1988. Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. *Brain Res.* 474 (1), 181–184.
- Curia, G., Longo, D., Biagini, G., Jones, R., Avolia, M., 2008. The pilocarpine model of temporal lobe epilepsy. *J. Neurosci. Methods* 172 (2–4), 143–157.
- Dagenais, C., Zong, J., Ducharme, J., Pollack, G.M., 2001. Effect of mdr1a P-glycoprotein gene disruption, gender, and substrate concentration on brain uptake of selected compounds. *Pharm. Res.* 18, 957–963.
- Davis, K., Berger, P., 2013. *Brain Acetylcholine and Neuropsychiatric Disease*, first ed. Springer, New York, NY, p. 227.
- Desjardins, P., Sauvageau, A., Bouthillier, A., Navarro, D., Hazell, A.S., Rose, C., Butterworth, R.F., 2003. Induction of astrocytic cyclooxygenase-2 in epileptic patients with hippocampal sclerosis. *Neurochem. Int.* 42, 299–303.
- Dhakur, M., Sivakumar, S., Bhattacharya, P., Shah, A., Basha, M., 2015. A retrospective cross-sectional study of the prevalence of generalized convulsive status epilepticus in traumatic brain injury: United States 2002–2010. *Seizure* 32, 16–22.
- Duvall, A., Gallicchio, V., 2017. Lithium treatment in clinical medicine: history, current status and future use. *J. Cell Sci. Therap.* 8 (3).
- Esclapez, M., Hirsch, J.C., Ben-Ari, Y., Bernard, C., 1999. Newly formed excitatory pathways provide a substrate for hyperexcitability in experimental temporal lobe epilepsy. *J. Comp. Neurol.* 408, 449–460.
- Fan, J., Shan, W., Yang, H., Zhu, F., Liu, X., Wang, Q., 2020. Neural activities in multiple rat brain regions in lithium-pilocarpine-induced status epilepticus model. *Front. Mol. Neurosci.* 12.
- Freeman, J.J., Choi, R.L., Jenden, D.J., 1975. The effect of hemicholinium on behavior and on brain acetylcholine and choline in the rat. *Psychopharmacol. Commun.* 1, 15–27.
- French, J.A., Williamson, P.D., Thadani, V.M., Darcey, T.M., Mattson, R.H., Spencer, S.S., 1993. Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. *Ann. Neurol.* 34, 774–780.
- Fujikawa, D.G., 1995. Neuroprotective effect of ketamine administered after status epilepticus onset. *Epilepsia* 36 (2), 186–195.
- Fujikawa, D., 1996. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. *Brain Res.* 725 (1), 11–22.
- Furtado, M.de.A., Braga, G., Oliveira, J., Del Vecchio, F., Garcia-Cairasco, N., 2002. Behavioral, morphologic, and electroencephalographic evaluation of seizures induced by intrahippocampal microinjection of pilocarpine. *Epilepsia* 43, 37–39.
- Gardoni, F., Di Luca, M., 2006. New targets for pharmacological intervention in the glutamatergic synapse. *Eur. J. Pharmacol.* 545, 2–10.
- Gibbons, A., Jeon, W., Scarr, E., Dean, B., 2015. Changes in muscarinic M2 receptor levels in the cortex of subjects with bipolar disorder and major depressive disorder and in rats after treatment with mood stabilisers and antidepressants. *Int. J. Neuropsychopharmacol.* 19 (4) pyv118.
- Glien, M., Brandt, C., Potschka, H., Voigt, H., Ebert, U., Löscher, W., 2001. Repeated low-dose treatment of rats with pilocarpine: low mortality but high proportion of rats developing epilepsy. *Epilepsy Res.* 46 (2), 111–119.
- Goffin, K., Nissinen, J., Van Laere, K., Pitkänen, A., 2007. Cyclicity of spontaneous recurrent seizures in pilocarpine model of temporal lobe epilepsy in rat. *Exp. Neurol.* 205, 501–505.
- Griffiths, T., Evans, C., Meldrum, B.S., 1984. Status epilepticus: the reversibility of calcium loading and acute neuronal pathological changes in the rat hippocampus. *Neuroscience* 12, 557–567.
- Grötcke, I., Hoffmann, K., Löscher, W., 2008. Behavioral alterations in a mouse model of temporal lobe epilepsy induced by intrahippocampal injection of kainite. *Exp. Neurol.* 213 (1), 71–83.
- Goodkin, H.P., Liu, X., Holmes, G.L., 2003. Diazepam terminates brief but not prolonged seizures in young, naive rats. *Epilepsia* 44 (8), 1109–1112.
- Haas, H.L., Ryall, R.W., 1977. An excitatory action of iontophoretically administered lithium on mammalian central neurons. *Br. J. Pharmacol.* 60, 185–195.
- Hamilton, S.E., Loose, M.D., Qi, M., Levey, A.I., Hille, B., McKnight, G.S., Idzerda, R.L., Nathanson, N.M., 1997. Disruption of the m1 receptor gene ablates muscarinic receptor-dependent M current regulation and seizure activity in mice. *Proc. Natl. Acad. Sci. U.S.A.* 94, 13311–13316.
- Hartz, A., Rempe, R., Soldner, E., Pekcec, A., Schlittiger, J., Kryscio, R., Bauer, B., 2019. Cytosolic phospholipase A2 is a key regulator of blood–brain barrier function in epilepsy. *FASEB J.* 33 (12), 14281–14295.
- Hewett, S.J., Silakova, J.M., Hewett, J.A., 2006. Oral treatment with rofecoxib reduces hippocampal excitotoxic neurodegeneration. *J. Pharmacol. Exp. Therapeut.* 319, 1219–1224.
- Hillert, M., Imran, I., Zimmermann, M., Lau, H., Weinfurter, S., Klein, J., 2014. Dynamics of hippocampal acetylcholine release during lithium-pilocarpine-induced status epilepticus in rats. *J. Neurochem.* 131 (1), 42–52.
- Holmes, G.L., 2002. Seizure-induced neuronal injury: animal data. *Neurology* 59 (Suppl 5), S3–S6.
- Honchar, M.P., Olney, J.W., Sherman, W.R., 1983. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. *Science* 220, 323–325.
- Houser, C.R., Miyashiro, J.E., Swartz, B.E., Walsh, G.O., Rich, J.R., Delgado-Escueta, A.V., 1990. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. *Neurosci.* 10, 267–282.
- Hruska, R.E., Ludmer, L.M., Pert, A., Bunnev, W.E., 1984. Effects of lithium on 3H-quinuclidinyl benzilate binding to rat brain muscarinic cholinergic receptors. *J. Neurosci. Res.* 11, 171–177.
- Huang, Y., Wu, G., Ren, S., Kang, Y., Wu, J., 2018. A new strategy for developing an animal model of temporal lobe epilepsy. *Neuropsychiatry* 8 (6), 1872–1886.
- Imran, I., Hillert, M., Klein, J., 2015. Early metabolic responses to lithium/pilocarpine-induced status epilepticus in rat brain. *J. Neurochem.* 135 (5), 1007–1018.
- Isokawa, M., Avanzini, G., Finch, D., Babb, T., Levesque, M., 1991. Physiologic properties of human dentate granule cells in slices prepared from epileptic patients. *Epilepsy Res.* 9 (3), 242–250.
- Isokawa, M., Mello, L., 1991. NMDA receptor-mediated excitability in dendritically deformed dentate granule cells in pilocarpine-treated rats. *Neurosci. Lett.* 129 (1), 69–73.
- Jones, D., Esmaeil, N., Maren, S., Macdonald, R., 2002. Characterization of pharmacoresistance to benzodiazepines in the rat Li-pilocarpine model of status epilepticus. *Epilepsy Res.* 50 (3), 301–312.
- Jope, R.S., 1979. Effects of lithium treatment in vitro and in vivo on acetylcholine metabolism in rat brain. *J. Neurochem.* 33, 487–495.
- Jope, R., Morrisett, R., Snead, O., 1986. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. *Exp. Neurol.* 91 (3), 471–480.
- Kapur, J., Macdonald, R.L., 1997. Rapid seizure-induced reduction of benzodiazepine and Zn2+ sensitivity of hippocampal dentate granule cell GABA_A receptors. *J. Neurosci.* 17 (19), 7532–7540.
- Kawaguchi, K., Hickey, R.W., Rose, M.E., Zhu, L., Chen, J., Graham, S.H., 2005. Cyclooxygenase-2 expression is induced in rat brain after kainate-induced seizures and promotes neuronal death in CA3 hippocampus. *Brain Res.* 1050, 130–137.
- Kelly, P., MacKinnon, R., Dietz, R., Maher, B., Wang, J., 2005. Postsynaptic IP3 receptor-mediated Ca2+ release modulates synaptic transmission in hippocampal neurons. *Mol. Brain Res.* 135 (1–2), 232–248.
- Kienzler-Norwood, F., Costard, L., Sadangi, C., Müller, P., Neubert, V., Bauer, S., Rosenow, F., Norwood, B., 2017. A novel animal model of acquired human temporal lobe epilepsy based on the simultaneous administration of kainic acid and lorazepam. *Epilepsia* 58 (2), 222–230.
- Kobayashi, R.M., Palkovits, M., Hruska, R.E., Rothschild, R., Yamamura, H.I., 1978. Regional distribution of muscarinic cholinergic receptors in rat brain. *Brain Res.* 154, 13–23.
- Kubová, H., Rejchrtová, J., Redkozubova, O., Mares, P., 2005. Outcome of status epilepticus in immature rats varies according to the paralytic treatment. *Epilepsia* 46 (Suppl 5), 38–42.
- Kwan, P., Brodie, M.J., 2006. Refractory epilepsy: mechanisms and solutions. *Expert Rev. Neurother.* 6, 397–406.
- Leach, J., Mohanraj, R., Borland, W., 2012. Alcohol and drugs in epilepsy: pathophysiology, presentation, possibilities, and prevention. *Epilepsia* 53, 48–57.
- Lee, B., Dziema, H., Lee, K.H., Choi, Y.S., Obrietan, K., 2007. CRE-mediated transcription and COX-2 expression in the pilocarpine model of status epilepticus. *Neurobiol. Dis.* 25, 80–91.

- Lee, H., Chi, C., 2018. Febrile infection-related epilepsy syndrome (FIRES): therapeutic complications, long-term neurological and neuroimaging follow-up. *Seizure* 56, 53–59.
- Lee, M., Choi, B., Suh, S., 2018. Unexpected effects of acetylcholine precursors on pilocarpine seizure-induced neuronal death. *Curr. Neuropharmacol.* 16 (1), 51–58.
- Leite, J.P., Bortolotto, Z.A., Cavalheiro, E.A., 1990. Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy. *Neurosci. Biobehav. Rev.* 14, 511–517.
- Lemos, T., Cavalheiro, E.A., 1995. Suppression of pilocarpine-induced status epilepticus and the late development of epilepsy in rats. *Exp. Brain Res.* 102, 423–428.
- Leung, A., Ahn, S., Savvidis, G., Kim, Y., Iskandar, D., Luna, M., Kim, K., Cunningham, M., Chung, S., 2015. Optimization of pilocarpine-mediated seizure induction in immunodeficient NodScid mice. *Epilepsy Res.* 109, 114–118.
- Lévesque, M., Avoli, M., Bernard, C., 2016. Animal models of temporal lobe epilepsy following systemic chemoconvulsant administration. *J. Neurosci. Methods* 260, 45–52.
- Li, Y., Wang, Z., Zhang, B., Zhe, X., Wang, M., Shi, S., Bai, J., Lin, T., Guo, C., Zhang, S., Kong, X., Zuo, X., Zhao, H., 2013. Disruption of the blood–brain barrier after generalized tonic-clonic seizures correlates with cerebrospinal fluid MMP-9 levels. *J. Neuroinflammation* 10 (1), 80.
- Lin Lin Lee, V., Kar Meng Choo, B., Chung, Y., Kundap U, P., Kumari, Y., Shaikh, M., 2018. Treatment, therapy and management of metabolic epilepsy: a systematic review. *Int. J. Mol. Sci.* 19 (3), 871.
- Liu, Z., Nagao, T., Desjardins, G.C., Gloor, P., Avoli, M., 1994. Quantitative evaluation of neuronal loss in the dorsal hippocampus in rats with long-term pilocarpine seizures. *Epilepsy Res.* 17, 237–247.
- Löscher, W., 1997. Animal models of intractable epilepsy. *Prog. Neurobiol.* 53 (2), 239–258.
- Löscher, W., Potschka, H., 2005. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat. Rev.* 6, 591–602.
- Marchi, N., Fan, Q., Ghosh, C., Fazio, V., Bertolini, F., Betto, G., Batra, A., Carlton, E., Najm, I., Granata, T., Janigro, D., 2009. Antagonism of peripheral inflammation reduces the severity of status epilepticus. *Neurobiol. Dis.* 33 (2), 171–181.
- Manabe, Y., Anrather, J., Kawano, T., Niwa, K., Zhou, P., Ross, M.E., Iadecola, C., 2004. Prostanoids, not reactive oxygen species, mediate COX-2-dependent neurotoxicity. *Ann. Neurol.* 55, 668–675.
- Masukawa, L., Higashima, M., Kim, J., Spencer, D., 1989. Epileptiform discharges evoked in hippocampal brain slices from epileptic patients. *Brain Res.* 493 (1), 168–174.
- Mayer, S., Claassen, J., Lokin, J., Mendelsohn, F., Dennis, L., Fitzsimmons, B., 2002. Refractory Status Epilepticus: frequency, risk factors, and impact on outcome. *Arch. Neurol.* 59 (2), 205.
- Mazarati, A., Wasterlain, C., Sankar, R., Shin, D., 1998a. Self-sustaining status epilepticus after brief electrical stimulation of the perforant path. *Brain Res.* 801 (1–2), 251–253.
- Mazarati, A., Baldwin, R., Sankar, R., Wasterlain, C., 1998b. Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. *Brain Res.* 814 (1–2), 179–185.
- McKhann II, G.M., Wenzel, H.J., Robbins, C.A., Sosunov, A.A., Schwartzkroin, P.A., 2003. Mouse strain differences in kainic acid sensitivity, seizure behavior, mortality, and hippocampal pathology. *Neuroscience* 122, 551–561.
- McLin, J.P., Stewart, O., 2006. Comparison of seizure phenotype and neurodegeneration induced by systemic kainic acid in inbred, outbred, and hybrid mouse strains. *Eur. J. Neurosci.* 24 (8), 2191–2202.
- Mejías-Aponte, C.A., Jiménez-Rivera, C.A., Segarra, A.C., 2002. Sex differences in models of temporal lobe epilepsy: role of testosterone. *Brain Res.* 944, 210–218.
- Meldrum, B.S., 1983. Metabolic factors during prolonged seizures and their relation to nerve cell death. *Adv. Neural* 34, 261–275.
- Mello, L.E., Cavalheiro, E.A., Tan, A.M., Pretorius, J.K., Babb, T.L., Finch, D.M., 1992. Granule cell dispersion in relation to mossy fiber sprouting, hippocampal cell loss, silent period and seizure frequency in the pilocarpine model of epilepsy. *Epilepsy Res. Suppl.* 9, 51–59.
- Mello, L.E., Cavalheiro, E.A., Tan, A.M., Kupfer, W., Pretorius, J.K., Babb, T., Finch, D.M., 1993. Circuit mechanisms of seizures in the pilocarpine model of chronic epilepsy: cell loss and mossy fiber sprouting. *Epilepsia* 34 (6), 985–995.
- Milligan, T., 2010. Decision Making in Medicine, third ed. Mosby, pp. 430–433.
- Modebadze, T., Morgan, N., Pérès, I., Hadid, R., Amada, N., Hill, C., Williams, C., Stanford, I., Morris, C., Jones, R., Whalley, B., Woodhall, G., 2016. A low mortality, high morbidity reduced intensity status epilepticus (RISE) model of epilepsy and epileptogenesis in the rat. *PLoS One* 11 (2), e0147265.
- Morris, M.E., Lee, H.J., Predko, L.M., 2003. Gender differences in the membrane transport of endogenous and exogenous compounds. *Pharmacol. Rev.* 55, 229–240.
- Morrisett, R.A., Jope, R.S., Snead III, O.C., 1987. Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. *Exp. Neurol.* 97, 193–200.
- Müller, C.J., Bankstahl, M., Grötcke, I., Löscher, W., 2009. Pilocarpine vs. lithium–pilocarpine for induction of status epilepticus in mice: development of spontaneous seizures, behavioral alterations and neuronal damage. *Eur. J. Pharmacol.* 619 (1–3), 15–24.
- Müller, C.J., Grötcke, I., Hoffmann, K., Schughart, K., Löscher, W., 2009c. Differences in sensitivity to the convulsant pilocarpine in substrains and sublines of C57BL/6 mice. *Gene Brain Behav.* 8, 481–492.
- Nagao, T., Alonso, A., Avoli, M., 1996. Epileptiform activity induced by pilocarpine in the rat hippocampal entorhinal slice preparation. *Neuroscience* 72, 399–408.
- Nardou, R., Yamamoto, S., Bhar, A., Burnashev, N., Ben-Ari, Y., Khalilov, I., 2011. Phenobarbital but not diazepam reduces AMPA/kainate receptor mediated currents and exerts opposite actions on initial seizures in the neonatal rat Hippocampus. *Front. Cell. Neurosci.* 5.
- Needs, H., Henley, B., Cavallo, D., Gurung, S., Modebadze, T., Woodhall, G., Henley, J., 2019. Changes in excitatory and inhibitory receptor expression and network activity during induction and establishment of epilepsy in the rat Reduced Intensity Status Epilepticus (RISE) model. *Neuropharmacology* 158, 107728.
- Neumann, A., Abele, J., Kirschstein, T., Engelmann, R., Sellmann, T., Köhling, R., Müller-Hilke, B., 2017. Mycophenolate mofetil prevents the delayed T cell response after pilocarpine-induced status epilepticus in mice. *PLoS One* 12 (11), e0187330.
- Nonaka, R., Moroji, T., 1984. Quantitative autoradiography of muscarinic cholinergic receptors in rat brain. *Brain Res.* 296, 295–303.
- Patel, V.A., Dunn, M.J., Sorokin, A., 2002. Regulation of MDR-1 (P-glycoprotein) by cyclooxygenase-2. *J. Biol. Chem.* 277, 38915–38920.
- Pauleto, G., Bax, F., Gigli, G., Lorenz, S., Verriello, L., Corazza, E., Valente, M., 2020. Status epilepticus mimicking stroke recurrence. *Epilepsy Behav.* 104, 106509.
- Pepicelli, O., Fedele, E., Berardi, M., Raiteri, M., Levi, G., Greco, A., Ajmone-Cat, M.A., Minghetti, L., 2005. Cyclo-oxygenase-1 and -2 differently contribute to prostaglandin E2 synthesis and lipid peroxidation after in vivo activation of N-methyl-D aspartate receptors in rat hippocampus. *J. Neurochem.* 93, 1561–1567.
- Pitkanen, A., Schwartzkroin, P., Moshé, S., 2005. Models of Seizures and Epilepsy, first ed. Elsevier, p. 433.
- Poirier, J.L., Capek, R., De Koninck, Y., 2000. Differential progression of Dark Neuron and Fluoro-Jade labelling in the rat hippocampus following pilocarpine-induced status epilepticus. *Neuroscience* 97, 59–68.
- Portelli, J., Aourz, N., De Bundel, D., Meurs, A., Smolders, I., Michotte, Y., Clinckers, R., 2009. Intrastrain differences in seizure susceptibility, pharmacological response and basal neurochemistry of Wistar rats. *Epilepsy Res.* 87, 234–246.
- Priel, M.R., Albuquerque, E.X., 2002. Short-term effect of pilocarpine on rat hippocampal neurons in culture. *Epilepsia* 43, 40–46.
- Rao, M., Hattiangady, B., Reddy, D., Shetty, A., 2006. Hippocampal neurodegeneration, spontaneous seizures, and mossy fiber sprouting in the F344 rat model of temporal lobe epilepsy. *J. Neurosci. Res.* 83 (6), 1088–1105.
- Racine, 1972. Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogr. Clin. Neurophysiol.* 32 (3), 281–294.
- Ratté, S., Lacaille, J.C., 2006. Selective degeneration and synaptic reorganization of hippocampal interneurons in a chronic model of temporal lobe epilepsy. *Adv. Neurol.* 97, 69–76.
- Reddy, D., Kuruba, R., 2013. Experimental models of status epilepticus and neuronal injury for evaluation of therapeutic interventions. *Int. J. Mol. Sci.* 14 (9), 18284–18318.
- Römermann, K., Bankstahl, J., Löscher, W., Bankstahl, M., 2015. Pilocarpine-induced convulsive activity is limited by multidrug transporters at the rodent blood-brain barrier. *J. Pharmacol. Exp. Therapeut.* 353 (2), 351–359.
- Sahin, D., Ilbay, G., Ates, N., 2003. Changes in the blood-brain barrier permeability and in the brain tissue trace element concentrations after single and repeated pentylenetetrazole-induced seizures in rats. *Pharmacol. Res.* 48, 69–73.
- Samoriski, G.M., Applegate, C.D., 1997. Repeated generalized seizures induce time-dependent changes in the behavioral seizure response independent of continued seizure induction. *J. Neurosci.* 17, 5581–5590.
- Santamarina, E., Abreira, L., Toledo, M., González-Cuevas, M., Quintana, M., Maisterra, O., Sueiras, M., Guzman, L., Salas-Puig, J., Sabin, J., 2018. Prognosis of post-stroke status epilepticus: effects of time difference between the two events. *Seizure* 60, 172–177.
- Schauwecker, P.E., 2012. Strain differences in seizure-induced cell death following pilocarpine-induced status epilepticus. *Neurobiol. Dis.* 45, 297–304.
- Scheibel, M., Crandall, P., Scheibel, A., 1974. The hippocampal-dentate complex in temporal lobe epilepsy. *Epilepsia* 15 (1), 55–80.
- Schiengold, M., Schwantes, L., Ribeiro, M.F., Lothhammer, N., Gonzalez, T.P., Bogo Chies, J.A., Nardi, N.B., 2006. Expression of mdr isoforms in mice during estrous cycle and under hormone stimulation. *Genet. Mol. Biol.* 29, 755–761.
- Sharma, S., Prasad, A., 2017. Inborn errors of metabolism and epilepsy: current understanding, diagnosis, and treatment approaches. *Int. J. Mol. Sci.* 18 (7), 1384.
- Sherman, W.R., Munsell, L.Y., Gish, B.G., Honchar, M.P., 1985. Effects of systemically administered lithium on phosphoinositide metabolism in rat brain, kidney and testis. *J. Neurochem.* 44, 798–807.
- Sloviter, R., 2005. The neurobiology of temporal lobe epilepsy: too much information, not enough knowledge. *Comptes Rendus Biol.* 328 (2), 143–153.
- Sloviter, R., Bumanglag, A., 2013. Defining “epileptogenesis” and identifying “antiepileptogenic targets” in animal models of acquired temporal lobe epilepsy is not as simple as it might seem. *Neuropharmacology* 69, 3–15.
- Smolders, I., Khan, G.M., Manil, J., Ebinger, G., Michotte, Y., 1997. NMDA receptor-mediated pilocarpine induced seizures: characterization in freely moving rats by microdialysis. *Br. J. Pharmacol.* 121, 1171–1179.
- Soldner, E., Hartz, A., Akanuma, S., Pekcec, A., Doods, H., Kryscio, R., Hosoya, K., Bauer, B., 2019. Inhibition of human microsomal PGE2 synthase-1 reduces seizure-induced increases of P-glycoprotein expression and activity at the blood-brain barrier. *Faseb. J.* 33 (12), 13966–13981.
- Song, H., Tufa, U., Chow, J., Sivanenthiran, N., Cheng, C., Lim, S., Wu, C., Feng, J., Eubanks, J., Zhang, L., 2018. Effects of antiepileptic drugs on spontaneous recurrent seizures in a novel model of extended hippocampal kindling in mice. *Front. Pharmacol.* 9.
- Strauss, K.I., Marini, A.M., 2002. Cyclooxygenase-2 inhibition protects cultured cerebellar granule neurons from glutamate-mediated cell death. *J. Neurotrauma* 19, 627–638.
- Takemiya, T., Maehara, M., Matsumura, K., Yasuda, S., Sugiura, H., Yamagata, K., 2006. Prostaglandin E2 produced by late induced COX-2 stimulates hippocampal neuron loss after seizure in the CA3 region. *Neurosci. Res.* 56, 103–110.
- Tang, F.R., Chen, P.M., Tang, Y.C., Tsai, M.C., Lee, W.L., 2007. Two-methyl-6-phenylethynyl-pyridine (MPEP), a metabotropic glutamate receptor 5 antagonist,

- with low doses of MK801 and diazepam: a novel approach for controlling status epilepticus. *Neuropharmacology* 53, 821–831.
- Tatum, W.O., 2013. Artifact-related epilepsy. *Neurology* 80 (1 Suppl 1), S12–25.
- Thompson, S.E., Woodhall, G.L., Jones, R.S.G., 2007. Depression of glutamate and GABA release by presynaptic GABAB receptors in the entorhinal cortex in normal and chronically epileptic rats. *Neurosignals* 15, 202–216.
- Trandafir, C., Pouliot, W., Dudek, F., Ekstrand, J., 2015. Co-administration of subtherapeutic diazepam enhances neuroprotective effect of COX-2 inhibitor, NS-398, after lithium pilocarpine-induced status epilepticus. *Neuroscience* 284, 601–610.
- Treiman, D.M., Meyer, P.D., Walton, N.Y., Collins, J.F., Colling, C., Rowan, A.J., Handforth, A., Faught, E., Calabrese, V.P., Uthman, B.M., Ramsay, R.E., Mamdani, M.B., 1998. A comparison of four treatments for generalized convulsive status epilepticus: veterans Affairs Status Epilepticus Cooperative Study Group. *N. Engl. J. Med.* 339, 792–798.
- Trinka, E., Höfler, J., Zerbs, A., 2012. Causes of status epilepticus. *Epilepsia* 53, 127–138.
- Trinka, E., Cock, H., Hesdorffer, D., Rossetti, A., Scheffer, I., Shinnar, S., Shorvon, S., Lowenstein, D., 2015. A definition and classification of status epilepticus - report of the ILAE task force on classification of status epilepticus. *Epilepsia* 56 (10), 1515–1523.
- Turski, W.A., Cavalheiro, E.A., Schwarz, M., Czuczwar, S.L.J., Kleinrok, Z., Turski, L., 1983a. Limbic seizures produced by pilocarpine I rats: behavioral, electroencephalographic and neuropathological study. *Behav. Brain Res.* 9, 315–335.
- Turski, W.A., Czuczwar, S.J., Kleinrok, Z., Turski, L., 1983b. Cholinomimetics produce seizures and brain damage in rats. *Experientia* 39, 1408–1411.
- Turski, L., Ikonomidou, C., Turski, W.A., Bortolotto, Z.A., Cavalheiro, E.A., 1989. Review: cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. *Synapse* 3, 154–171.
- van Vliet, E.A., van Schaik, R., Edelbroek, P.M., Redeker, S., Aronica, E., Wadman, W.J., Marchi, N., Vezzani, A., Gorter, J.A., 2006. Inhibition of the multidrug transporter P-glycoprotein improves seizure control in phenytoin-treated chronic epileptic rats. *Epilepsia* 47, 672–680.
- van Vliet, E.A., da Costa Araújo, S., Redeker, S., van Schaik, R., Aronica, E., Gorter, J.A., 2007. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. *Brain* 130, 521–534.
- Varela, P.N., Claassen, J., 2017. *Seizures in Critical Care : a Guide to Diagnosis and Therapeutics*. Springer.
- Vezzani, A., 2009. Pilocarpine-induced seizures revisited: what does the model mimic? *Epilepsy Current* 9 (5), 146–148.
- Vezzani, A., Fujinami, R., White, H., Preux, P., Blümcke, I., Sander, J., Löscher, W., 2015. Infections, inflammation and epilepsy. *Acta Neuropathol.* 131 (2), 211–234.
- Volk, H., Löscher, W., 2005. Multidrug resistance in epilepsy: rats with drug-resistant seizures exhibit enhanced brain expression of P-glycoprotein compared with rats with drug-responsive seizures. *Brain* 128 (6), 1358–1368.
- Voutsinos-Porche, B., Koning, E., Kaplan, H., Ferrandon, A., Guenounou, M., Nehlig, A., Motte, J., 2004. Temporal patterns of the cerebral inflammatory response in the rat lithium-pilocarpine model of temporal lobe epilepsy. *Neurobiol. Dis.* 17, 385–402.
- Walton, N.Y., Treiman, D.M., 1988. Response of status epilepticus induced by lithium and pilocarpine to treatment with diazepam. *Exp. Neurol.* 101 (2), 267–275.
- Walton, N.Y., Treiman, D.M., 1991. Motor and electroencephalographic response of refractory experimental status epilepticus in rats to treatment with MK-801, diazepam, or MK-801 plus diazepam. *Brain Res.* 553 (1), 97–104.
- Wheless, J., Treiman, D., 2008. The role of the newer antiepileptic drugs in the treatment of generalized convulsive status epilepticus. *Epilepsia* 49, 74–78.
- Winawer, M.R., Kuperman, R., Niethammer, M., Sherman, S., Rabinowitz, D., Guell, L.P., Ponder, C.A., Palmer, A.A., 2007a. Use of chromosome substitution strains to identify seizure susceptibility loci in mice. *Mamm. Genome* 18, 23–31.
- Winawer, M.R., Makarenko, N., McCloskey, D.P., Hintz, T.M., Nair, N., Palmer, A.A., Scharfman, H.E., 2007b. Acute and chronic responses to the convulsant pilocarpine in DBA/2J and A/J mice. *Neuroscience* 149, 465–475.
- Wray, C., Knupp, K., 2011. *Berman's Pediatric Decision Making*, fifth ed. Mosby, pp. 370–373.
- Wu, Q., Wang, H., 2018. The spatiotemporal expression changes of CB2R in the hippocampus of rats following pilocarpine-induced status epilepticus. *Epilepsy Res.* 148, 8–16.
- Yang, J., Woodhall, G.L., Jones, R.S.G., 2006. Tonic facilitation of glutamate release by presynaptic NR2B-containing NMDA receptors is increased in the entorhinal cortex of chronically epileptic rats. *J. Neurosci.* 26, 406–410.
- Zhao, Z., Wang, H., Wen, B., Yang, Z., Feng, K., Fan, J., 2016. A comparison of midazolam, lorazepam, and diazepam for the treatment of status epilepticus in children. *J. Child Neurol.* 31 (9), 1093–1107.
- Zhu, H.J., Liu, G.Q., 2004. Glutamate up-regulates P-glycoprotein expression in rat brain microvessel endothelial cells by an NMDA receptor-mediated mechanism. *Life Sci.* 75, 1313–1322.
- Zibell, G., Unkrüer, B., Pekcec, A., Hartz, A., Bauer, B., Miller, D., Potschka, H., 2009. Prevention of seizure-induced up-regulation of endothelial P-glycoprotein by COX-2 inhibition. *Neuropharmacology* 56 (5), 849–855.