No persistent effects of intracerebral curcumin administration on seizure progression and neuropathology in the kindling rat model for temporal lobe epilepsy

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\textbf{Purpose:} Curcumin is known for its neuroprotective, anti-inflammatory and anti-oxidant properties and has been investigated as a potential therapeutic drug for Temporal Lobe Epilepsy (TLE). We previously found anti-epileptogenic properties of curcumin in an in vitro brain slice model for epileptogenesis, and inhibitory effects on the MAPK-pathway in vivo after intracerebrally applying curcumin in post-status epilepticus rats. Here, we investigated whether the intracerebral application of curcumin could be anti-epileptogenic in the rapid kindling rat model for TLE.

\textbf{Methods:} Curcumin or vehicle was injected directly into the brain through an intracerebral ventricular cannula at 5 consecutive days during the kindling process. Kindling consisted of repeated electrical stimulations of the angular bundle (12 times a day with a 30 min interval) every other day, until rats were fully kindled or until 36 stimulations were administered. One week after kindling acquisition, additional kindling stimulations were applied in a re-test in the absence of curcumin- or vehicle treatment.

\textbf{Results:} Curcumin-treated rats required more stimulations compared to vehicle-treated rats to reach Racine stage IV seizures, indicating that curcumin delayed seizure development. However, it did not prevent the fully kindled state as shown in the re-test. Increasing the dose of curcumin did not produce a delay in seizure development. Immunohistochemistry showed that kindling produced cell loss, astrogliosis, mossy fiber sprouting and neurogenesis in the dentate gyrus, which were not different between vehicle- and curcumin-treated groups.

\textbf{Conclusion:} Although curcumin’s effects on neuropathology were not detected and the delay of kindling development was transient, the data warrant further exploration of its anti-epileptogenic potential using formulations that further increase its bioavailability.

\section{1. Introduction}

Temporal lobe epilepsy (TLE) is the most common form of epilepsy in adult (Banerjee et al., 2009; Tellez-Zenteno and Hernandez-Ronquillo, 2012). This type of epilepsy has an important risk for high seizure frequency and for the development of pharmaco-resistant epilepsy with about 30–40% of patients with TLE who are medically refractory (Kwan et al., 2010). Therefore, epilepsy researchers are dedicated to find anti-epileptogenic treatments that target underlying processes of seizure development. In TLE, such processes include brain inflammation, mossy fiber sprouting, neuronal death, neurogenesis and blood-brain barrier dysfunction (Pitkanen and Lukasiuk, 2011; Vezzani et al., 2013). One of the compounds that have been under investigation as a potential anti-epileptogenic drug is curcumin. Curcumin, derived from the root of the \textit{Curcuma longa} plant, is a natural compound known for anti-inflammatory and neuroprotective properties (Zhou et al., 2011).
Based on previous findings, we hypothesized that curcumin delays pounds in rodents (De Smedt et al., 2007; Galanopoulou et al., 2021). Curcumin could therefore have anti-epileptogenic potential through multiple actions; it could protect against neuronal loss, which is associated with TLE (Henshall and Murphy, 2008; Swartz et al., 2006), it could be anti-inflammatory (Jobin et al., 1999), possibly related to MAPK inhibition (Kim et al., 2005), and through its suppression of the mTOR pathway it could potentially reduce aberrant growth processes (Beever et al., 2013) associated with epileptogenesis.

In the hippocampal-entorhinal cortex slice culture model, in which seizure-like events develop over the course of three weeks in vitro, we recently found that curcumin is able to suppress seizure development, and that this could be related to anti-inflammatory actions via inhibition of the MAPK pathway (Drion et al., 2019). In vivo however, curcumin has a low bioavailability; it is poorly absorbed in the gut and rapidly degraded in the bloodstream. Therefore, curcumin is unlikely to reach the brain in sufficient concentrations after systemic treatment (Liu et al., 2016). In the present proof of principle study, we circumvented this by injecting curcumin directly into the brain through an intracerebral ventricular (icv) cannula. In a previous study we showed that this treatment inhibited the MAPK pathway in hippocampal tissue (Drion et al., 2018). The aim of the present study was to investigate the anti-epileptogenic potential of curcumin in the rapid kindling rat model for TLE (Lothman and Williamson, 1993) which allows for studying epileptogenesis and screening of potential anti-epileptogenic compounds in rodents (De Smedt et al., 2007; Galanopoulou et al., 2021).

Based on previous findings, we hypothesized that curcumin delays seizure development in the rapid kindling model and reduces neuronal death, brain inflammation, sprouting and neurogenesis in the hippocampus.

2. Methods

2.1. Animals

Adult Male Sprague-Dawley rats (Envigo, Horst, the Netherlands) weighing 300–350 g at the start of the experiment were used. Rats were housed individually in a controlled environment (21 °C ± 1 °C, 60% ± 15% humidity, 12 h light/dark cycle with lights on 8:00 AM to 8:00 PM, with water and food (standard laboratory chew) available ad libitum). All animal procedures were approved by the Animal Ethics Committee of the University of Amsterdam, according to Dutch law, and performed in accordance with the guidelines of the European Community Council Directives 2010/63/EU.

2.2. Electrode and cannula implantation and EEG recording

Rats were implanted with intracranial electrodes for stimulation and EEG recording using the following surgical procedures. Rats were anesthetized with an intraperitoneal (i.p.) injection of ketamine (74 mg/kg); Alfasan, Woerden, the Netherlands) and xylazine (11 mg/kg); Bayer AG, Leverkusen, Germany) and placed in a stereotactic frame. In order to record hippocampal EEG, a pair of insulated stainless-steel electrodes (70 µm) was implanted into the dentate gyrus under electrophysiological control as previously described (Drion et al., 2016). After a minimum of two weeks recovery, rats were placed in individual recording cages (40x40x80 cm) and connected to a recording and stimulation system. For EEG recording, the signals from the headstage were fed through commutators and a custom designed filter and amplification unit (BR 20D Breakout Box, NPI electronic GmbH, Tamm, Germany). Signals were then sampled by a computer-controlled digitized card (NI USB-6255, National Instruments Netherlands, Woerden, the Netherlands) that also controlled the stimulation patterns in a synchronized way. EEG signals were amplified (10x within the headstage), band-pass filtered (0.1–1000 Hz), and then digitized at 2 KHz (16 bit; 30.5 µV/bit) using in-house data acquisition software running under MATLAB (MathWorks, Natick, MA, USA). EEG was recorded continuously during the experiment (24/7), starting 1 day before kindling (baseline). Stimulation was performed by the same NI USB-6255 multifunction I/O device, which was able to deliver biphasic, bipolar voltage stimulation pulses (max 20 V) at microsecond resolution to the selected stimulation channels of the headstage of each individual rat. In-house data acquisition software running under MATLAB (MathWorks, Natick, MA, USA) was used to evoke field potentials, to deliver the kindling stimulus, and to analyze EEG signals.

2.3. Rapid kindling

To determine the proper kindling stimulation intensity, field potential responses (electrically evoked via the angular bundle stimulation electrodes) were determined at minimum (threshold)- and maximum (saturation) intensity for each rat individually. Kindling stimulations were applied at ~75% of the maximum field potential response (determined prior to first kindling stimulation). Average kindling intensities did not differ between treatment groups (vehicle: 8.9 ± 0.4 V, n = 7 and curcumin: 10.1 ± 0.5 V, n = 8).

The rapid kindling method was based on the method described by Lothman and Williamson (Lothman and Williamson, 1993) but the protocol was slightly adapted. The kindling protocol consisted of repeated angular bundle stimulations: 10 s of bipolar, biphasic 1 ms pulses (0.5 ms each), 50 Hz, max amplitude 20 V (~700 μA), applied 12 times a day with an interval of 30 min. On stimulation days, the first kindling stimulation was given 2 h after drug treatment. Stimulations were applied at the first day of stimulation (day 1), day 3 and day 5. Stimulations were applied until rats were fully kindled (see following paragraph) or until 36 stimulations were applied.

2.4. Afterdischarges and behavioral seizure progression

Kindling progression was monitored by assessing seizure development behaviorally and electrophysiologically. EEG recordings were analyzed to determine the duration of afterdischarges (ADs), evoked by the electrical stimulations. EEG recordings were visually screened by trained observers and ADs were manually detected. All stimulations had to result in an AD to be included in the analysis. Following each stimulation, the behavioral response was assessed using an adapted version of the Racine seizure scale (Racine, 1972) with the following classifications: stage I: arrest, wet-dog-shake (WDS) and/or whisker twitching, stage II: facial twitches and/or chewing, head nodding stage III: head nodding with forelimb clonus, stage IV: generalized clonus with rearing, stage V: generalized clonus with rearing and falling.

Rats were considered fully kindled if either i) 5 subsequent stage V seizures, ii) 7 stage V seizures within one day or iii) a total of 10 stage V seizures were observed in response to kindling stimulations. When rats were fully kindled, stimulations were stopped. Otherwise, rats received 36 stimulations (over 3 alternating days). Rats received 6 additional stimulations 1 week after the last kindling day in the absence of curcumin (re-test I). Responses to the re-test stimulations indicate whether the changes induced by kindling are lasting, and therefore the re-test also allows for testing potential anti-epileptogenic effects of curcumin after kindling. 4 days after the re-test rats were sacrificed after 3 additional re-test stimulations (re-test II). Continuous (24/7) EEG recordings were made throughout the experiment (until 11 days after the 36th stimulation) and recordings were screened, to check whether kindling stimulations resulted in spontaneous seizures.

2.5. Drug treatment

During kindling acquisition (on kindling days and rest-days) rats were injected with either vehicle (n = 7) or curcumin (n = 8, of which 1
rat lost its headset after the second kindling day and was therefore excluded from immunohistochemistry analysis). Injections were given 2 h prior to the first kindling stimulation of that day. Curcumin (Sigma-Aldrich, Zwijndrecht, the Netherlands) was stored at −20 °C and freshly prepared (diluted in dimethylsulfoxide (DMSO, Merck, Darmstadt, Germany) on each treatment day. Care was taken to keep the solution in the dark all the time until injection to limit potential degradation of curcumin. Rats were injected into the lateral ventricle via the icv cannula with 2 μl 2 mM curcumin solution. Based on estimates of the cerebro-spinal fluid (CSF) volume in the rat being about 100 μl (Murtha et al., 2014; Partridge, 2011) the end concentration of curcumin was estimated at about 40 μM. In previous in vivo experiments we noticed that this procedure produced a light orange stain around the surrounding brain areas. Although we may assume that concentrations in the nearby brain regions are somewhat lower than the estimated CSF concentrations, previous western blot analysis of hippocampal tissue (using the low dose of curcumin) revealed a significant inhibition of the MAPK pathway by curcumin (Orion et al., 2018). In order to test whether potential effects could be dose-dependent we applied a higher-dose (~80 μM) of icv curcumin in a follow-up experiment (curcumin-treated group: n = 10; vehicle-treated group: n = 10). We sacrificed these subgroups of rats one day after the 24th stimulation in order to evaluate neuropathological characteristics.

### 2.6. Immunohistochemistry

At the end of the experiment, rats were deeply anesthetized with pentobarbital (Euthasol, AST Farma, Oudewater, the Netherlands, 60 mg/kg) and perfused via the ascending aorta with 300 ml 0.37% Na3S and 300 ml 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. After overnight post-fixation the brains were dissected and cryoprotected by overnight incubation (4 °C) in 30% sucrose solution, buffered in 0.1 M phosphate buffer (pH 7.4). Thereafter, brains were frozen in isopentane (between −25 °C and −30 °C) and stored at −80 °C until cutting. Cutting was performed on a sliding microtome and 40 μm thick coronal sections were collected in Tissue Collecting Solution (30% ethylene glycol and 25% glycerol in 0.05 M phosphate buffer) for immunohistochemistry.

For immunohistochemistry, sections containing dorsal hippocampus were selected. Sections were washed in 0.05 M phosphate buffered saline (PBS), pH 7.4 and incubated for 30 min in 0.3% hydrogen peroxide in PBS to inactivate endogenous peroxidase. Sections were then washed (3 times 10 min) in 0.05 M PBS, followed by incubation (60 min) with PBS + 0.5% Triton X-100 + 0.4% bovine serum albumin (BSA). Sections were incubated with the primary antibody of interest in 0.1% Triton X-100 + 0.4% BSA in 0.05 M PBS for 1 h at room temperature and thereafter overnight at 4 °C. Primary antibodies used were mouse-anti neuron specific nuclear protein (NeuN; MAB377, 1:1000; Merck Millipore, Darmstadt, Germany) to detect neurons, mouse-anti-parvalbumin (PV; 1:1000; Sigma-Aldrich, Zwijndrecht, the Netherlands) to detect PV-positive interneurons; rabbit-anti-somatostatin (O1; 1:2500; Peninsula, San Carlos, CA, USA) to detect SOM-positive interneurons; goat-anti-doublecortin (Doublecortin; 1:800; Santa Cruz Biotechnology, Santa Cruz, CA USA) to detect newly born or proliferating neurons (Aynalaja et al., 2017) mouse-anti-vimentin (Vimentin; 1:100; DAKO (Agilent), Santa Clara, CA, USA) to detect reactive astrocytes. After overnight incubation the sections were washed in PBS (3 times 10 min) and then incubated for 1.5 h in secondary antibody; biotinylated sheep anti-mouse, donkey anti-rabbit (1:200; GE Healthcare, Diegem, Belgium) or rabbit anti-goat (1:200; DAKO, Santa Clara, CA, USA) were used. Next, the sections were incubated (60 min) in ABmix (Vectastain ABC kit, Peroxidase Standard pk-4000; Vector Laboratories, Burlingame, CA, USA). After washing (3 times 10 min) in 0.05 M Tris–HCl (pH 7.9), the sections were stained with 0.075% 3,3’-diaminobenzidine tetrahydrochloride (DAB; Sigma-Aldrich, Zwijndrecht, the Netherlands) in 0.05 M Tris–HCl solution with 0.015% hydrogen peroxide. The staining reaction was stopped by washing the sections in 0.05 M PB. After mounting on Superfrost plus slides, the sections were air dried, dehydrated in alcohol and xylene, and coverslipped with Entellan (Merck, Darmstadt, Germany). For analysis, ImageJ (Fiji) software (NIH) was used. NeuN-, PV-, and SOM-positive cells in the dentate gyrus (NeuN and SOM: hilus only, PV: hilus and granule cell layer), were manually counted by 2 independent observers. The number of cells was corrected for the size of the counted area and expressed as cells/mm². For doublecortin and vimentin, a threshold was set to mask the area of marker expression and the area coverage of the masked area in the region of interest (doublecortin: subgranular zone, vimentin: hilus) was calculated. For the doublecortin staining, we also qualitatively analyzed protrusion of newborn granule cell processes (e.g. basal dendrites in the subgranular zone into the hilus).

### 2.7. Timm staining

For Timm staining, sections containing dorsal hippocampus were mounted on Superfrost plus slides and washed for 5 min in distilled water. Sections were stained under dark conditions for 40 min using 5 mM AgNO3 + 121 mM citric acid monohydrate + 91 mM sodium citrate dihydrate + 30% Arabic gum + 154 mM hydroquinone dissolved in distilled water. Slides were then washed twice in distilled water for 5 min, followed by a 10-minute wash step with 5% Na2S2O3. After a final wash step with distilled water for 5 min, slides were dehydrated in an alcohol and xylene series and coverslipped using Entellan (Merck, Darmstadt, Germany). All sections were processed at the same time with the same development time (40 min) to enable comparison between groups. The extent of synaptic reorganization of the mossy fibers was evaluated by two observers according to a standardized 0–5 scale developed by Cavazos et al. (Cavazos et al., 1991). Intermediary values were scored in case the suprapyramidalial blade had a different score than the infrapyramidal blade.

### 2.8. Data analysis

Data were plotted and analyzed using Prism 5 (GraphPad Inc., La Jolla, CA USA) and IBM SPSS Statistics 22. AD duration and touch test scores were compared among treatment groups using repeated measures mixed model (2-way) ANOVA. The number of stimulations required to reach stage III–IV seizures was analyzed using the Mann Whitney U test. Immunohistochemistry results were analyzed with one-way ANOVA or Kruskall Wallis tests, with Bonferroni or Dunn’s post-hoc tests, respectively. Results are expressed as mean ± SEM, unless otherwise indicated and p < 0.05 is considered to indicate a significant difference.

### 3. Results

#### 3.1. Kindling development

The response to kindling stimulations was monitored both electrographically by recording EEG afterdischarge (AD) duration and behaviorally (behavioral seizure score according to the Racine scale) in all kindled rats. We started the experiments with the 40 μM i.c.v. dose. The durations of the AD increased over the first 24 stimulations, which is shown in Fig. 1. There was no effect of curcumin treatment on AD duration development. However, there was a significant slowing down of the kindling development in the curcumin treated group: to quantify and compare behavioral seizure development, the proportion of rats displaying each of the Racine stages was calculated for all stimulations (see Fig. 2). These proportions were compared between curcumin and vehicle-treated kindled groups. For example: at the first stimulation, 2 out of 7 of the vehicle-treated rats responded to the first stimulation with a Racine stage II behavioral seizure, versus none of the curcumin-treated rats (0 out of 8). The number of stimulations required to reach a specific
Fig. 1: Afterdischarge duration. A) Example of an afterdischarge (AD) following stimulation, in 2 EEG traces of the same rat (<stim> indicates the stimulation artefact in the EEG traces). B) Development of AD duration over the first 24 stimulations, for vehicle- (blue circles) and curcumin-treated rats (yellow triangles). Curcumin treatment did not affect AD duration.

Fig. 2: Behavioral seizure development. The proportions of rats responding to kindling stimulations with behavioral seizures are shown for each Racine stage (expressed in different colors, see figure legend) during the 36 kindling stimulations, 6 stimulations of re-test I (1–6) and 3 stimulations of re-test II (1–3). Vehicle (A) and curcumin (B): during the first 12 kindling stimulations curcumin shows a delayed development of behavioral seizure severity.
behavioral seizure score (I-V, fully kindled) are shown in Fig. 3A. For all Racine scores, the average required number of stimulations for reaching stage III or IV was lower for vehicle-treated rats compared to curcumin-treated rats; rats treated with curcumin required more stimulations (median = 15, interquartile range (IQR) = 13.25–16.75, n = 8) compared to vehicle-treated rats (median = 9, IQR = 5–13, n = 7); U = 7, p = 0.02 (Fig. 3B). During the kindling process, 1 rat was excluded after the second kindling day because it lost its headset. Of the remaining 14 rats, 9 (4 out of 7 vehicle- and 5 out of 7 curcumin-treated rats) reached the fully kindled state within 36 stimulations (rats were considered fully kindled if they responded with a stage V seizure to either 5 consecutive stimulations, 7 stimulations within one kindling day, or 10 stimulations in total). Since we had detected this significant delay on kindling development with the low dose of curcumin up to the 24th stimulation, we decided to evaluate the neuropathological characteristics at this earlier phase in the subsequent high dose experiment, in the expectation that this dose would show an even larger effect on slowing down the kindling process, 1 rat was excluded after the second kindling day because it lost its headset. Of the remaining 14 rats, 9 (4 out of 7 vehicle- and 5 out of 7 curcumin-treated rats) reached the fully kindled state within 36 stimulations (rats were considered fully kindled if they responded with a stage V seizure to either 5 consecutive stimulations, 7 stimulations within one kindling day, or 10 stimulations in total). Since we had detected this significant delay on kindling development with the low dose of curcumin up to the 24th stimulation, we decided to evaluate the neuropathological characteristics at this earlier phase in the subsequent high dose experiment, in the expectation that this dose would show an even larger effect on slowing down the kindling development (and reduction of cell death/gliosis). However, treatment with the higher dose (~80 μM) of curcumin did not have a significant effect on behavioral kindling development or seizure duration during the 24 stimulations when compared to the vehicle kindled group (Appendix, A, Supplementary figure 1).

3.2. Re-test

To test whether rapid kindling induced lasting changes and to detect possible anti-epileptogenic effects of curcumin after kindling, six re-test stimulations were given one week after kindling acquisition and curcumin treatment (only the 40 μM group). At the first stimulation, 6 out of 7 vehicle-treated rats and 5 out of 7 curcumin-treated rats responded with a IV-V seizure, indicating that the fully kindled state lasted at least a week after kindling acquisition (Fig. 2). Differences between vehicle- and curcumin-treated rats in the re-tests were not detected (Fig. 2). The screening of 24/7 EEG recordings revealed that kindling did not lead to the occurrence of spontaneous seizures in either group throughout the experiment, which lasted until they were sacrificed (11 days after the last stimulation at the fully kindled stage).

3.3. Neuropathology

Two weeks after kindling and the last curcumin treatment, kindled rats showed neuropathology associated with epileptogenesis: compared to controls, kindled rats showed loss of NeuN-positive cells in the hilus of the dentate gyrus (F (2, 20) = 4.65, p = 0.02, Bonferroni for control versus curcumin: p < 0.05) (Fig. 4A and Table 1 and Table 2). The extent of cell loss (NeuN) was not different between vehicle and curcumin groups. The number of somatostatin- and parvalbumin positive interneurons in the hilus and granule cell layer of the dentate gyrus (Figs. 4B and C, respectively and Tables 1 and 2), did also not differ between vehicle- and curcumin-treated rats.

We observed that the area coverage of doublecortin staining in the subgranular and inner molecular region was higher after kindling (Kruskall Wallis: χ² (2) = 7.22, p = 0.03, Dunn’s for control versus vehicle: p < 0.05) but this was not different between curcumin and vehicle-treated rats (Fig. 5A and Table 1). Vimentin staining was higher in the hilus, which indicates astrogliosis (Kruskall Wallis: χ² (2) = 7.76, p = 0.02, Dunn’s for control versus curcumin: p < 0.05) 2 weeks after kindling (Fig. 5B and Table 1) when compared to controls, but this did not differ between vehicle- and curcumin-treated rats. A higher Timm score was obtained in the inner molecular layer of the dentate gyrus in kindled rats (Kruskall Wallis: χ² (2) = 10, p < 0.01, Dunn’s for control versus vehicle: p < 0.01) (Fig. 5C and Table 1), but this was not different between vehicle- and curcumin-treated rats. The number of NeuN cells in the hilus tended to be lower in the curcumin treated kindled group compared to the vehicle kindled group (Table 2).

4. Discussion

The data show that curcumin initially delayed seizure development in the rapid kindling model for TLE in rats. However, this effect was transient, and curcumin did not have lasting suppressive effects on epileptogenesis. Moreover, this effect appeared to be only present in the lower dosing group. Although intracerebrally applied curcumin in similar doses previously showed to be effective in suppressing the MAPK pathway in the hippocampus, this was not sufficient to reduce kindling-induced neuronal death, astrogliosis and neurogenesis in the dentate gyrus.

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**Fig. 3.** Behavioral seizure development. A) The number of stimulations needed to reach the increasing Racine stages is plotted for vehicle- (blue circles) and curcumin-treated rats (yellow triangles). Shown are the medians (red horizontal lines) with range, and individual data points (vehicle-treated rats: blue circles, curcumin-treated rats: yellow triangles). B) In order to reach stage IV seizures, rats treated with curcumin required more stimulations compared to vehicle-treated rats. * = p < 0.05.(For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Previous experiments in animal models of epilepsy or seizures showed, in general, beneficial effects of curcumin treatment, both with regard to neuroprotection as to anti-epileptic action. For instance, in the pentylenetetrazole (PTZ) kindling rat model, orally administered curcumin had anti-oxidant (Mehla et al., 2010) and anti-inflammatory effects and prevented seizure progression and cognitive decline (Kaur et al., 2015). In mice, oral curcumin treatment also suppressed PTZ–induced kindling development (Agarwal et al., 2011) and reduced seizure severity and depression-like behavior (Choudhary et al., 2013). In amygdala-kindled rats that were intraperitoneally (i.p.) injected with curcumin, more stimulations were needed to reach stage IV / V seizures (Du et al., 2009), which is in line with our results. Similar results were obtained in an iron-induced seizure model, in which curcumin was applied as a diet-supplement (Jyoti et al., 2009). Despite these effects on delaying seizure progression, beneficial neuroprotective and anti-inflammatory effects were not observed in our curcumin treated kindled rats. Moreover, we found that curcumin did not have long-lasting anti-epileptogenic effects since vehicle- and curcumin treated rats responded similarly to the re-test.

Knowing that curcumin is poorly absorbed by the gut and is rapidly metabolized (Anand et al., 2007; Heger et al., 2014), the reported beneficial effects of curcumin treatment in the different animal models of epilepsy or seizures are not easy to understand. Especially after oral administration, curcumin is unlikely to reach the brain in sufficient concentration (Du et al., 2009). In a previous study we found that curcumin (or its related metabolites) was cleared from the blood plasma within 2 h after oral administration, and accordingly, orally applied curcumin, more stimulations were needed to reach stage IV / V seizures (Du et al., 2009), which is in line with our results. Similar results were obtained in an iron-induced seizure model, in which curcumin was applied as a diet-supplement (Jyoti et al., 2009). Despite these effects on delaying seizure progression, beneficial neuroprotective and anti-inflammatory effects were not observed in our curcumin treated kindled rats. Moreover, we found that curcumin did not have long-lasting anti-epileptogenic effects since vehicle-and curcumin treated rats responded similarly to the re-test.

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curcumin did not reduce seizures in a post-status epilepticus (post-SE) rat model for TLE (Drion et al., 2016). When curcumin was applied in an in vitro hippocampal-entorhinal-cortex slice culture model of epileptogenesis (which circumvents the bioavailability problem), we observed that curcumin could suppress seizure development (Drion et al., 2019). This inspired us to test whether curcumin may be anti-epileptogenic in our hippocampal kindling model by injections of curcumin directly into the brain. In our previous study we showed that this intraventricular injection in vivo in post-SE rats is effective in suppressing the MAPK pathway (Drion et al., 2018), so that we expected that this way of curcumin administration would produce an anti-epileptic or anti-epileptogenic action. Indeed, curcumin-treated rats required more stimuli to reach stage III and IV seizures, indicating that curcumin transiently delays behavioral seizure development and delays generalization of seizures. Since the injections were given 2 h before the kindling stimulations, it could be argued that curcumin interfered with the kindling stimulus (electrical stimulation of the angular bundle) or could inhibit seizure activity. However, as shown in Fig. 1, curcumin did not significantly affect hippocampal afterdischarge duration which indicates that curcumin did not have a direct suppressive effect on the induced seizures. In order to obtain a more robust effect, we increased the dose of curcumin in a follow-up experiment. However, contrary to our expectation, the higher dose did not lead to a significant effect on kindling development and tended to lead to more cell death, suggesting that the high dose was suboptimal (Drion et al., 2018) and might therefore have counteracted the significant beneficial effects of the lower dose.

Based on previous reports, we expected curcumin to have anti-epileptogenic effects related to its neuroprotective, anti-inflammatory or anti-oxidant properties (Ezz et al., 2011; Parada et al., 2015; Seyedzadeh et al., 2014; Sumanont et al., 2006). We found that curcumin did not affect the rapid kindling-induced neuronal loss and astrogliosis. Based on its observed property as a mTOR inhibitor, we also investigated whether curcumin could reduce mossy fiber sprouting and neurogenesis as was observed with mTOR inhibition after cortical impact injury induced epileptogenesis in mice (Buckmaster and Lew, 2011). We found that rapid kindling induced mossy fiber sprouting and neurogenesis, but we did not find any differences between the vehicle- and curcumin-treated groups. Whether an earlier start of the treatment (before the start of the kindling procedure) or a longer treatment is necessary to obtain neuroprotective effects will need further investigation.

In conclusion, we show here that intracerebral curcumin treatment transiently delays behavioral seizure development but is not able to reverse or prevent epileptogenesis or to produce neuroprotection in the rapid kindling rat model for TLE. In a recent review, curcumin has been presented as a potential anti-epileptogenic- and anti-seizure drug in animal models and therefore the drug was promoted to be tested clinically soon (Dhir, 2018). Although the effects reported here are limited, they are in the right direction (delaying kindling development) so that we recommend that curcumin should be tested further in epilepsy models with optimized formulations that increase its bioavailability. Recently, curcumin nanoparticles were shown to have promising cognitive, neuroprotective-, and anti-inflammatory effects in PTZ-kindling in mice and micronized curcumin was shown to be anti-convulsant in a PTZ-zebrafish model for seizures (Bertoncello et al.,

Fig. 5: Example images and quantification of immunohistochemistry showing A) Doublecortin (DCX), B) Vimentin (Vim), and C) Timm staining (Timm) in the dentate gyrus of not-stimulated control rats, vehicle-treated kindled rats and curcumin-treated kindled rats, 2 weeks after kindling. * = \( p < 0.05 \), ** = \( p < 0.01 \).
2018). Taken together, this suggests that future studies should be carried out with the newly optimized formulations that can be easily administered over longer periods of time to investigate the anti-epileptogenic potential of curcumin.

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Declaration of Competing Interest

None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eplepsys.2022.106873.

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