Translational potential of the ghrelin receptor agonist macimorelin for seizure suppression in pharmacoresistant epilepsy

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Abstract

Background: Current drugs for epilepsy affect seizures, but no antiepileptogenic or disease-modifying drugs are available that prevent or slow down epileptogenesis, which is characterized by neuronal cell loss, inflammation and aberrant network formation. Ghrelin and ghrelin receptor (ghrelin-R) agonists were previously found to exert anticonvulsant, neuroprotective and anti-inflammatory effects in seizure models and immediately after status epilepticus (SE). Therefore, the aim of this study was to assess whether the ghrelin-R agonist macimorelin is antiepileptogenic in the pharmacoresistant intrahippocampal kainic acid (IHKA) mouse model.

Methods: SE was induced in C57BL/6 mice by unilateral IHKA injection. Starting 24 hours after SE, mice were treated intraperitoneally with macimorelin (5 mg/kg) or saline twice daily for two weeks, followed by a two-week wash-out. Mice were continuously electroencephalogram (EEG)-monitored, and at the end of the experiment neuroprotection and gliosis were assessed.

Results: Macimorelin significantly decreased the number and duration of seizures during the treatment period, but had no antiepileptogenic or disease-modifying effect in this dose regimen. While macimorelin did not significantly affect food intake or body weight over a two-week treatment period, its acute orexigenic effect was preserved in epileptic mice but not in sham mice.

Conclusions: While the full ghrelin-R agonist macimorelin was not significantly antiepileptogenic nor disease-modifying, this is the first study to demonstrate its anticonvulsant effects in the IHKA model of drug-refractory temporal lobe epilepsy. These findings highlight the potential use of macimorelin as a novel treatment option for seizure suppression in pharmacoresistant epilepsy.

1. Introduction

Epilepsy is a neurological disease characterized by the spontaneous manifestation of disproportionate neuronal discharges called seizures[1, 2]. Approximately 30 % of affected patients are resistant to currently available antiseizure drugs (ASDs)[2], and the majority of these individuals are suffering from temporal lobe epilepsy (TLE)[3].

Although TLE is frequently evoked by a single identifiable cause such as a brain insult, classical TLE-related symptoms often develop only a decade after[4]. This transformation of a normal brain into an epileptic one, or so-called epileptogenesis, leads to the occurrence of spontaneous seizures[5], and encompasses multiple irreversible pathological processes, such as hippocampal sclerosis, excessive neuronal loss, granule cell dispersion, gliosis, and aberrant neuronal network formation (reviewed in[6]). Therefore, this silent period following an initial identifiable event might serve as a convenient therapeutic window for preventing epileptogenesis. Current ASDs only provide symptomatic relief by reducing the likelihood of seizures (reviewed in[7]), while there is little evidence available concerning possible antiepileptogenic potentials (reviewed in[8]).

Ghrelin is a peptide that plays a role in food intake, gastric motility, glucose homeostasis, growth hormone (GH) release, cognition, anxiety, motivation and reward (reviewed in[9, 10]). It binds to its G-protein coupled receptor (GPCR), formerly known as the growth hormone secretagogue receptor 1a (GHSR1a)[11], but hereafter referred to as the ghrelin receptor (ghrelin-R). Interestingly, this receptor is highly expressed in the Cornu Ammonis (CA) 3 region and dentate gyrus (DG) of the hippocampus[12], and is associated with a variety of signaling pathways; $G_{q/11}$, $G_{12/13}$ and $G_{i/o}$ signaling, but also β-arrestin recruitment followed by internalization of the ghrelin-R[13, 14].

Ghrelin and ghrelin-R agonists exerted anticonvulsant effects[15-20], increased neuronal survival[21-23] and suppressed inflammation[16, 24] in rodent seizure-, and status epilepticus (SE) models. As these two latter pathological features play a prominent role in the development of epilepsy[25-29], using a compound counteracting these phenomena is a rational option for studying antiepileptogenic effects.

We used the ghrelin-R full agonist macimorelin, which is already approved as a medicinal product in the United States and Europe for the diagnosis of GH deficiency in adults[30, 31], as such promoting swift translation of newly identified preclinical findings to the clinic. Additionally, epilepsy patients who respond well to ASDs showed higher ghrelin plasma levels compared to non-responders, indicating that ghrelin may regulate the response to ASDs[32]. In the search for antiepileptogenic treatments for drug-refractory TLE, only chronic rodent models, such as the intrahippocampal kainic acid (IHKA) mouse model, that show many similarities to epileptogenesis in TLE patients, are clinically relevant and valuable tools[33, 34]. Therefore, the aim of this study was to investigate whether macimorelin exerts antiepileptogenic effects in the IHKA mouse model.

2. Methods

2.1. Animals

A total of 44 ten-week-old male C57BL/6 mice (Janvier Laboratories, France) were used (Table S1). Mice were single housed starting two days prior to the surgical procedures until the end of the experiment. They were kept in a 12/12 h light/dark cycle (7:00 AM-7:00 PM), under temperature (21 ± 2 °C) and humidity (50 ± 20 % relative humidity) controlled conditions, and received regular chow and water *ad libitum*. Animal care and procedures were in accordance with the National Rules on Animal Experimentation and were approved by the Ethical Committee for Animal Experiments of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel, Brussels, Belgium (Ethical approval n°: 17-213-2, license date: May 15th

2017). To the best of our understanding, experiments were planned conform the ARRIVE guidelines[35].

Table S1

2.2. Surgical procedure

Placement of measuring electrodes (E363/3/SPC Invivo 1, Virginia, USA), implantation of the radio-telemetric transmitter (ETA-F10, DSI, Tilburg, The Netherlands) and injection of KA (200 ng in 50 nL; unilaterally in the right CA1 region of hippocampus (anterio-posterior: −2 mm; medio-lateral: −1.5 mm; dorso-ventral: −2.1 mm relative to bregma)) were performed as previously described[19, 36]. Sham-operated control mice received an identical, however non-functional transmitter and electrodes, and saline intrahippocampal injection stereotaxically positioned at the same coordinates. Mice were immediately placed in their home cage in order to start electroencephalogram (EEG) recordings.

2.3. Experimental design

KA mice that experienced SE (verified on EEG) and all sham mice were included in the study. Starting one day after SE, both KA- and sham-operated mice received i.p. administration of macimorelin (5 mg/kg; 10mL/kg body volume; AEZS-130; gift from AEZS, Frankfurt, Germany) dissolved in 0.9 % NaCl (Baxter) or vehicle twice daily (7:00-8:00 AM and 6:00-7:00 PM) for a duration of two weeks. After a two-week wash-out period, mice were sacrificed. Experimenters were blinded for treatment during the experiment, as well as during data analyses. An overview of the experimental design is depicted in Fig. S1.

Figure S1

2.4. Food intake recordings

2.4.1. Chronic assessment

During the two-week treatment period, mice and food pellets were weighed twice daily immediately before the i.p. saline or macimorelin administration.

2.4.2. Acute food intake experiment

On day 13 of the treatment period (7:00-9:00 AM), mice were observed for two hours immediately after macimorelin or saline injection. Mice had access to three pre-weighed pellets in their home cage and the time spent eating was recorded. Afterwards, pellets were weighed again to assess the mass of food consumed during this time period (Fig. S1).

2.5. Immunohistochemistry

At the end of the experiment, mice received an overdose of sodium pentobarbital (Dolethal; Vetoquinol, Aartselaar, Belgium) i.p. and were transcardially perfused with phosphate-buffered saline (PBS; pH 7.4). Brains were removed and post-fixed in 4 % paraformaldehyde overnight at 4 °C, and later kept in PBS without sucrose at 4 °C. Brains were sectioned at 40 µm thickness in the coronal plane using a vibratome (Leica, Wetzlar, Germany). Sections were blocked in PBS containing 10 % donkey serum and 0.1 % Triton-X (T) for one hour at room temperature. The sections were incubated overnight at 4 °C with primary antibodies against neuronal nuclear protein (NeuN; 1:400; host: guinea-pig; 266004, Synaptic systems, Göttingen, Germany), glial fibrillary acidic protein (GFAP; 1:1000; host: chicken; TA309150, Origene, Maryland, USA), and ionized calcium binding adaptor molecule 1 (IbaI; 1:1000; host: rabbit; 019-19741, Fujifilm Wako, Osaka, Japan). After washing with 0.1% Tris-buffered saline (TBS)/T, sections were incubated with Cy2-donkey-α-guinea pig (1:200; 706-225-148), Cy3-goat-α-chicken (1:500; 103-163-155) and Cy5-donkey-α-rabbit (1:400; 711-175-152) (all Jackson ImmunoResearch, Pennsylvania, USA) secondary antibodies for 45 minutes at room temperature. After washing, slices were incubated for 5 minutes with 4',6-Diamidino-2-Phenylindole (DAPI; 1:500; 4083, Cell Signaling Technology, Massachusetts, USA). Sections were mounted on VWR superfrost

slides (VWR, Oud-Heverlee, Belgium) with Dako fluorescence mounting medium (Agilent, California, USA).

2.6. Data analyses

2.6.1. EEG analyses

EEG signals were continuously recorded. Radiofrequency transmitted signals were acquired with receiver plates (RPC-1 receiver, DSI) and sent to the data-exchange matrix (MX2, Matrix 2.0, DSI). EEG signals were sampled at a frequency of 500 Hz using Ponemah software (DSI). EEG recordings were analyzed with Neuroscore software (DSI), after applying a 60 Hz low-pass and a 50 Hz notch filter to the obtained EEG signal. Seizures were detected visually by a blinded experimenter and defined as periods with high frequency (>1 Hz) and strong increase in amplitude (>200% from baseline) with a duration of least 5 seconds and an inter-event interval of at least 1 second[34].

During treatment, six hours of EEG data per day were analyzed, split into two equal time frames of three hours following injection, 8:00-11:00 AM and 8:00-11:00 PM respectively. Data obtained from each three-hour time frame were averaged and presented in the graphs with the denotation AM and PM corresponding to a given day.

During the wash-out period EEG data from 8:00 AM-2:00 PM were used for analysis, also divided in two time frames of three hours each. The three hours counted in such a time frame were averaged and resulted in one value per mouse. These are presented in the graph as the first (8:00-11:00 AM) and second (11:00 AM-2:00 PM) data points corresponding to a given day. Seizure coverage represents the percentage of time mice are experiencing seizures per hour.

2.6.2. Immunohistochemistry processing

Fluorescent images were acquired from the ipsilateral CA1, CA3 and DG regions using a 20 x objective (Axio Observer with LSM 710-6NLO configuration, Zeiss). Quantification was

performed with ImageJ (NIH). Three slices per location per mouse were used for analyses whenever possible, and an average of the obtained values resulted in one value for that mouse. Cells were counted manually and determined as positive for the staining of interest if they were co-localized with DAPI.

2.6.3. Statistical analyses

Data were processed using GraphPad Prism v8.4.1. For longitudinal data (including data with multiple factors), either a three-way ANOVA or a mixed effects analysis was used for analysis. The latter type analysis was used to correct for missing data points (for instance loss of the EEG signal at rare occasions due to technical problems). No multiple comparison's test was applied for these datasets, as our desired outcome was rather to detect an overall effect of macimorelin, and not identifying effects on a specific time point *per se*. Two-way ANOVA was used for non-longitudinal data with either the Tukey's multiple comparisons test (for comparing four different groups with each other) or Sidak's multiple comparisons test (for comparing predefined groups). Data are presented as mean ± SEM.

3. Results

3.1. Macimorelin is anticonvulsant during epileptogenesis

After the induction of SE a latent period occurs in the IHKA model[33], during which we administered saline or macimorelin. After the latent period, chronic epilepsy is established with recurring seizures originating from the temporal lobe[33]. A representative trace is depicted in Fig. 1a. Duration of SE did not differ between both groups (Fig. S2).

There was no significant difference between the number of seizures, seizure coverage, total seizure duration or average seizure duration between macimorelin-treated- and saline-treated mice during the first treatment week (Fig. 1b,d,f,h). Average seizure duration increased

significantly over time in both macimorelin- and saline-treated mice during the first week (Fig. 1h).

However, in the second treatment week, macimorelin-treated mice experienced significantly less seizures per hour compared to saline-treated mice ($P < 0.05$; 7.6 and 17.0 seizures per hour, respectively; Fig. 1c). Macimorelin-treated mice had a significantly lower seizure coverage compared to saline-treated mice ($P < 0.05$; 2.2 % and 4.9 %, respectively; Fig. 1e), and experienced a total seizure duration of 62.4 seconds per hour, while saline-treated mice had a total seizure duration of 156.0 seconds per hour ($P < 0.05$; Fig. 1g). The average seizure duration was significantly shorter in macimorelin-treated mice (6.4 seconds) compared to saline-treated controls (8.2 seconds; $P < 0.05$; Fig. 1i).

Figure 1

3.2. Macimorelin (5 mg/kg twice daily) is not antiepileptogenic in the IHKA model

A two-week long macimorelin treatment did not provide significant seizure relief during the wash-out period. Macimorelin-treated mice experienced 33.7 seizures per hour, while saline-treated mice experienced 34.1 seizures per hour (Fig. 2a,b). Macimorelin-treated mice experienced seizures 10.1 % of the time, and saline-treated mice had a seizure coverage of 10.8 % (Fig. 2c). Total seizure duration was 364.9 seconds for macimorelin- and 387.0 seconds for saline-treated mice (Fig. 2d), and average seizure duration was 10.4 seconds for macimorelin-, and 11.0 seconds for saline-treated mice (Fig. 2e).

Figure 2

3.3. Macimorelin does not affect the KA-induced increase in body weight and differentially affects food intake in KA or SHAM mice

Mice and food pellets were weighed twice daily during the treatment period. KA mice had a significantly increased body weight compared to sham mice, regardless of macimorelin ($P < 0.01$; Fig. 3a). At the end of the treatment period, saline- and macimorelin-treated epileptic mice had an increase in body weight of 15.2 % and 16.7 %, respectively, while saline- and macimorelin-treated sham mice had an increase in body weight of 9.7 %, and 10.4 %, respectively ($P < 0.001$; Fig. 3b) compared to the start of the experiment.

KA mice consumed significantly more food compared to sham mice during the two-week-treatment period, normalized to body weight of mice ($P < 0.05$; Fig. 3c). Accordingly, KA but not macimorelin significantly affected cumulative food intake ($P < 0.01$; Fig. 3d), and at day 13 KA mice had consumed significantly more food (55.9 g and 59.3 g for saline-treated and macimorelin-treated KA mice, respectively) compared to saline-treated- (47.7 g) and macimorelin-treated sham mice (51.0 g; $P < 0.01$; Fig. 3e).

On day 13 of the treatment period, eating behavior of mice was monitored for a duration of two hours after saline- or macimorelin administration in the morning. Saline-treated sham and -epileptic mice spent 236.0 seconds and 231.2 seconds eating, respectively. Macimorelin-treated epileptic mice spent significantly more time eating compared to saline-treated epileptic- or saline-treated sham mice (639.1 seconds), while macimorelin-treated sham mice spent 418.7 seconds eating ($P < 0.0001$; Fig. 3f). During these two hours, saline-treated sham mice, macimorelin-treated sham mice and saline-treated KA mice had consumed 0.17 g, 0.22 g, and 0.18 g of food, while macimorelin-treated epileptic mice had consumed significantly more food (0.43 g; $P < 0.05$; Fig. 3g).

Figure 3

3.4. Macimorelin lacks neuroprotective effects in this dose regimen

Mice were sacrificed two weeks after the wash-out period. KA administration induced a near complete destruction of neurons in the ipsilateral CA1 and CA3, as previously described[33] ($P < 0.001$; Fig. 4a,b; 5a,b). There was a marked, radially oriented, dispersion of neurons in both the supra- and infrapyramidal blade of the DG in KA mice ($P < 0.01$; fig. 6a,b). Macimorelin did not significantly affect the number of neurons in these regions.

The number of astrocytes did not differ significantly in CA1 in epileptic mice (Fig. 4c), also not when CA1 was subdivided into *stratum (s.) oriens* & *s. pyramidale*, and *s. radiatum* (Fig. S3a, S3b). The number of astrocytes was significantly decreased in CA3 and DG of epileptic mice compared to sham mice ($P < 0.001$; Fig. 5c; $P < 0.01$; Fig. 6c). While a decrease in the number of $GFAP^+$ cells in epileptic mice might appear paradoxical at first, KA mice showed altered morphology of astrocytic processes and astrocytic hypertrophy (Fig. 6a; Reviewed in[37]). Therefore, we analyzed staining intensity which reflects immunoreactivity and is relevant for evaluation of astrogliosis. GFAP intensity was significantly increased in KA mice in CA1, CA3 and DG ($P < 0.01$, $P < 0.001$, $P < 0.0001$; Fig. S4a-c). Macimorelin did not significantly affect the number of astrocytes in these areas, nor did it affect staining intensities.

Microglia were significantly increased in epileptic mice compared to sham mice in CA1, CA3 and DG ($P < 0.001$; Fig. 4d, 5d; $P < 0.05$; Fig. 6d). There was an increased number of microglia in *s. oriens* & *s. pyramidale*, and *s. radiatum* of CA1 ($P < 0.01$; Fig. S3c,d). There was no significant effect of macimorelin administration on the amount of microglia in CA1, CA3 nor DG.

Figure 4 – 6

4. Discussion

This is, to the best of our knowledge, the first study that assessed chronic administration of a full ghrelin-R agonist during epileptogenesis in the clinically relevant IHKA mouse model for drug-refractory TLE[34]. Macimorelin significantly decreased the amount of seizures, seizure coverage, total- and average seizure duration in the second treatment week, but not in the first week following KA administration. This can be attributed to the variable and low amount of seizures that IHKA mice display at the onset of epileptogenesis, while in the second week seizure progression intensifies and the frequency of seizures escalates in this model[33].

Previous studies have shown anticonvulsant properties of ghrelin, but only a handful have investigated the effects of the ghrelin-R full agonist macimorelin so far. A single injection of 0.33 mg/kg macimorelin administered 10 minutes prior to pilocarpine in rats did not significantly decrease severity nor delayed the onset of SE[22, 38], while 5 mg/kg macimorelin administered 20 or 30 minutes prior to a 6 Hz stimulus or dopamine 1 receptor agonist SKF81297 decreased seizure duration, amount or seizure severity in mouse kindling models[19, 20]. The discrepancies between the results of these studies could result from different species or epilepsy models used, by variations in the dose and timing of macimorelin administration, or the effects of macimorelin may not be strong enough to interfere with the development of SE. We previously showed that macimorelin required ghrelin-R expression to be anticonvulsive, as macimorelin decreased seizure severity only in fully kindled ghrelin-R wild-type-, but not ghrelin-R knock-out mice[20].

Fundamental pathophysiological mechanisms contributing to epileptogenesis are inflammation, gliosis and neuronal cell loss[6, 33]. The latent phase (including the early establishment of seizures), following an initial precipitating event may be an adequate therapeutic window for establishing prevention of epilepsy. Macimorelin seemed like a rational and promising candidate as its endogenous ligand ghrelin was shown to interfere with inflammation and cell loss in seizure models[16, 21-24]. To identify possible antiepileptogenic and disease-modifying

effects of macimorelin administration, a two week wash-out period was included in this study. However, macimorelin (at the dose regimen of 5 mg/kg twice daily for two weeks) had no significant antiepileptogenic effects in the IHKA mouse model after a two-week washout period.

Additionally, macimorelin did not significantly affect neuronal loss nor the amount of microglia and astrocytes in the affected hippocampus of epileptic mice. Epileptic mice experienced severe neuronal loss in the hippocampus, including pronounced granule cell dispersion in DG. Astrocytic hypertrophy and altered morphology of astrocytic processes was observable throughout the hippocampus, indicating astrogliosis in epileptic mice. The increase in the amount of $IbaI^{+}$-cells in epileptic mice may imply proliferation or increased migration of microglia towards the hippocampus. Previous studies demonstrated that pre-treatment with ghrelin in SE models was neuroprotective, and inhibited microglia and astrocyte activation in CA1 and CA3, assessed three days after SE[21, 23, 24]. Additionally, 0.33 mg/kg macimorelin administered once 10 minutes prior to pilocarpine significantly increased NeuN positivity in the hilus of DG but did not affect the amount of neurons in CA1 nor CA3, assessed four days after SE[22]. The dissimilarities between these studies and our study may result from the timing of ghrelin administration. Whereas animals were pre-treated with ghrelin or macimorelin prior to induction of SE in these previous studies, we did not include pre-treatment with macimorelin in order to increase the clinical translation potential.

Macimorelin was previously shown to induce food intake and increase weight gain[39]. Unexpectedly, we found that not macimorelin, but KA increased overall food intake and weight gain, increases which appeared to be mitigated already early on in epileptogenesis prior to the appearance of chronic seizures. This is in line with previous studies demonstrating increased weight gain in female IHKA mice[40] and in systemic pilocarpine-treated male and female mice[41]. Studying weight gain in epilepsy patients remains challenging because of the well-known

associations between ASDs and their effects on body weight[42, 43]. However, a group of epileptic children that had not yet received ASD treatment, consisted of significantly more overweight patients compared to a control group[44]. Overall, these results indicate that a KA injection early on might be associated to pathophysiological alterations affecting weight but the exact mechanism behind this remains unknown. One possibility is that KA mice may increase their food intake due to an increased need for glucose in the CNS. On the one hand, this higher energy demand may stem from the fact that seizures highly consume energy and that these mice therefore have a higher metabolic rate[45]. Additionally, oxidative glucose metabolism was shown to be impaired in epilepsy, inducing a less efficient energy yield and as such requiring an increased energy need[46].

A diminished sensitivity to macimorelin upon prolonged exposure has been described[39]. C57BL/6 mice that were administered 5 mg/kg macimorelin twice daily showed increased food consumption that normalized after eight days[39]. One of the mechanisms behind this phenomenon may rely on β-arrestin recruitment and subsequent receptor internalization[47]. To assess possible ghrelin-R desensitization resulting from prolonged macimorelin exposure, we performed an acute food intake experiment on day 13.

Macimorelin-treated epileptic mice consumed significantly more food in the two hours after administration compared to saline-treated mice, whereas macimorelin-treated sham mice did not. Moreover, the amount of food that was consumed by macimorelin-treated KA mice in our study corresponds to the amount of food consumed by macimorelin-naïve C57BL/6 mice after a single i.p. injection of 5 mg/kg macimorelin[39]. Our results suggest that macimorelin-treated sham mice become less sensitive to prolonged macimorelin exposure, as previously described[39], whereas this is not the case in epileptic mice, suggesting differential regulation of ghrelin-R availability at the cell surface. Chronic macimorelin administration did not significantly increase 24-hour-food consumption. Fasted mice were shown to display hyperphagia lasting

for several days after fasting, resulting in consumption of the exact amount of calories that were not consumed during fasting[48]. A similar phenomenon may be occurring in macimorelin-treated mice, resulting in an equivalent amount of overall food intake compared to saline-treated mice.

5. Conclusion

Despite the fact that macimorelin was not antiepileptogenic at this treatment regimen, we showed that macimorelin was anticonvulsive in the IHKA mouse model for refractory TLE[34]. Additionally, macimorelin did not significantly induce weight gain nor increased overall food consumption. The results from this study highlight the potential use of macimorelin as a novel treatment option for difficult-to-treat seizures, and open up a new therapeutic avenue for seizure suppression in pharmacoresistant epilepsy.

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Disclosure of conflicts of interest Statement

None of the authors has any conflict of interest to disclose.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Legends

Figure 1: Macimorelin exerts anticonvulsant effects in the second week of epileptogenesis **A)** Representative tracing of hippocampal recording from saline-treated- and macimorelin-treated mouse on day 13 (09:30 AM – 10:00 AM). **B)** Amount of seizures per hour in saline- and macimorelin-treated mice during the first week (Time $P = 0.17$, F (1.421, 12.68) = 2.108; Macimorelin $P = 0.44$, F (1, 9) = 0.6676; Interaction $P = 0.77$, F (13, 116) = 0.6858). **C)** Average amount of seizures mice experienced per hour after treatment administration in the second week (Time $P = 0.07$, F (2.966, 26.24) = 2.612; Macimorelin $P = 0.04$, F (1, 9) = 5.663; Interaction $P = 0.46$, F (13, 115) = 0.9965). **D)** Seizure coverage (% of time mice are experiencing seizures per hour) in the first treatment week (Time $P = 0.21$, F (1.246, 11.12) = 1.784; Macimorelin $P = 0.43$, F (1, 9) = 0.6736; Interaction $P = 0.70$, F (13, 116) = 0.7590). **E)** Effect of macimorelin on **s**eizure coverage in the second treatment week (Time $P = 0.12$, F (3.135, 27.73) = 2.080; Macimorelin $P = 0.05$, F (1, 9) = 5.152; Interaction $P = 0.20$, F (13, 115) = 1.340). **F)** Total seizure duration between saline- and macimorelin-treated mice in the first treatment week (Time $P = 0.21$, F (1.155, 10.30) = 1.844; Macimorelin $P = 0.36$, F (1, 9) = 0.9148; Interaction $P = 0.74$, F (13, 116) = 0.7222). **G)** Effect of macimorelin on total seizure duration in the second treatment week (Time $P = 0.04$, F (3.361, 29.73) = 2.932; Macimorelin $P = 0.04$, F (1, 9) = 5.544; Interaction $P = 0.12$, F (13, 115) = 1.508). **H)** Average seizure duration between saline- and macimorelin-treated mice during the first week (Time $P = 0.001$, F (3.836, 34.23) = 5.737; Macimorelin $P = 0.49$, F (3.836, 34.23) = 5.737; Interaction $P = 0.79$, F (13, 116) = 0.6673). **I)** Effect of macimorelin on average seizure duration in the second treatment week (Time $P = 0.23$, F (4.206, 36.88) = 1.473; Macimorelin $P = 0.04$, F (1, 9) = 5.893; Interaction $P = 0.11$, F (13, 114) = 1.549). Mixed-effects model (REML). The first tick represents the average amount of seizures per hour averaged of three hours counted (8:00 AM -11:00 AM), the second tick corresponds to the average amount of seizures per hour averaged of three hours counted (8:00

PM – 11:00 PM). n_{saline} = 5; $n_{macimorelin}$ = 6. S = seconds; Min = minute; V = volt. Data = mean ± SEM.

Figure 2: Macimorelin is not antiepileptogenic in the IHKA model A) Representative tracing of hippocampal recording from saline-treated- and macimorelin-treated mouse on day 27 (09:30 – 10:00 AM). **B)** Amount of seizures per hour in saline- and macimorelin-treated mice during the wash-out period (Time P = 0.15, F (4.804, 32.21) = 1.785; Macimorelin P = 0.79, F (1, 7) = 0.07976; Interaction P < 0.001, F (27, 181) = 2.918). **C)** There was no significant difference regarding seizure coverage between macimorelin- and saline-treated mice (Time P = 0.25, F (4.157, 27.87) = 1.425; Macimorelin P = 0.68, F (1, 7) = 0.1811; Interaction P < 0.001, F (27, 181) = 2.727). **D)** Total seizure duration did not differ significantly between both groups during wash-out (Time P = 0.26, F (4.180, 28.02) = 1.408; Macimorelin P = 0.68, F (1, 7) = 0.1841; Interaction P < 0.001, F (27, 181) = 2.690). **E)** Average seizure duration did not differ significantly between saline- and macimorelin -treated mice during the wash-out period (Time P = 0.62, F (4.446, 29.81) = 0.6889; Macimorelin P = 0.63, F (1, 7) = 0.2609; Interaction P = 0.29, F (27, 181) = 1.146). Mixed-effects model (REML). The first data point at a given day represents the averaged value assessed in the 8:00 AM – 11:00 AM period, the second data point at a given day corresponds to the averaged value assessed in the 11:00 AM – 2:00 PM period. n_{saline} = 5; $n_{macimorelin}$ = 4. S = second; Min = minute; V = volt. Data = mean ± SEM.

Figure 3: Macimorelin does not significantly affect overall weight gain nor overall food consumption, but acutely induces food intake in KA mice A) Body weight gain of mice during the treatment period. Three-way ANOVA (Time P < 0.001, F (3.327, 113.1) = 206.2; KA P = 0.004, F (1, 34) = 9.306; Macimorelin P = 0.22, F (1, 34) = 1.588; Time x KA P < 0.001, F (13, 442) = 6.098; Time x Macimorelin P = 0.67, F (13, 442) = 0.7932; KA x Macimorelin P = 0.80, F (1, 34) = 0.06764; Time x KA x Macimorelin P = 0.87, F (13, 442) =

0.5753). **B)** Weight gain of mice (expressed in % relative to body weight) on day 14 of the treatment period. Two-way ANOVA with Tukey's multiple comparisons test (KA $P < 0.001$, F (1, 34) = 17.79; Macimorelin $P = 0.40$, F (1, 34) = 0.6290; Interaction $P = 0.80$, F (1, 34) = 0.06336). **C)** Mass of pellets consumed per day in both saline- and macimorelin-treated sham and KA mice, normalized to body weight of mice. Mixed-effects model (REML) (Time $P < 0.001$, F (5.499, 170.5) = 12.57; KA $P= 0.04$, F (1, 34) = 4.481; Macimorelin $P = 0.19$, F (1, 34) = 1.814; Time x KA $P = 0.04$, F (12, 372) = 1.811; Time x Macimorelin $P = 0.66$, F (12, 372) = 0.7941; KA x Macimorelin $P = 0.73$, F (1, 34) = 0.1200; Time x KA x Macimorelin $P = 0.99$, F (12, 372) = 0.3128). **D)** Cumulative food intake during the entire treatment period. Three-way ANOVA (Time $P < 0.001$, F (1.188, 40.40) = 1115; KA $P = 0.03$, F (1, 34) = 5.453; Macimorelin $P = 0.2725$, F (1, 34) = 1.244; Time x KA $P < 0.0001$, F (11, 374) = 6.653; Time x Macimorelin $P = 0.14$, F (11, 374) = 1.479; KA x Macimorelin $P = 0.83$, F (1, 34) = 0.04922; Time x KA x Macimorelin $P > 0.9999$, F (11, 374) = 0.02487). **E)** Food consumed by day 13 of the treatment period. Two-way ANOVA with Tukey's multiple comparisons test (KA $P = 0.008$, F (1, 34) = 7.755; Macimorelin $P = 0.26$, F (1, 34) = 1.297; Interaction $P = 0.99$, F (1, 34) = 4.2e-005). **F)** Time spent eating after saline-or macimorelin administration on day 13 during a two hour observational period. Three-way ANOVA with Tukey's multiple comparisons test (Time $P < 0.0001$, F (2.080, 70.74) = 68.04; KA $P = 0.07$, F (1, 34) = 3.405; Macimorelin $P < 0.0001$ F (1, 34) = 21.06; Time x KA $P = 0.08$, F (4, 136) = 2.128; Time x Macimorelin $P < 0.0001$, F (4, 136) = 11.23; KA x Macimorelin $P = 0.12$, F (1, 34) = 2.534; Time x KA x Macimorelin $P = 0.16$, F (4, 136) = 0.1653). * = $P < 0.05$, KA macimorelin vs. KA saline and SHAM saline. **G)** Mass of food pellets consumed during the two hours immediately following saline- or macimorelin administration. Two-way ANOVA with Tukey's multiple comparisons test (KA $P = 0.06$, F (1, 33) = 3.878; Macimorelin $P = 0.01$, F (1, 33) = 6.815; Interaction $P = 0.09$, F (1, 33) = 3.094). $n_{KA\ -\ saline} = 11$; $n_{KA\ -\ macimorelin} = 11$; $n_{SHAM\ -}$

$_{macimorelin}$ = 7/8; $n_{SHAM - saline}$ = 8. * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$. KA = kainic acid; min = minute; s = seconds. Data = mean ± SEM.

Figure 4: Macimorelin does not significantly affect NeuN$^+$-, GFAP$^+$- or IbaI$^+$- cells in CA1 **A)** Representative images of the staining in CA1. **B)** Epileptic mice have significantly less NeuN$^+$-cells (KA $P < 0.001$, F (1, 9) = 316.8; Macimorelin $P = 0.49$, F (1, 9) = 0.5119; Interaction $P = 0.58$, F (1, 9) = 0.3216). **C)** The amount of GFAP$^+$-cells did not differ significantly between groups (KA $P = 0.82$, F (1, 8) = 0.05728; Macimorelin $P = 0.95$, F (1, 8) = 0.05728; Interaction $P = 0.46$, F (1, 8) = 0.5918). **D)** There were significantly more IbaI$^+$-cells in epileptic mice compared to sham mice (KA $P < 0.001$, F (1, 8) = 69.26; Macimorelin $P = 0.35$, F (1, 8) = 1.005; Interaction $P = 0.11$, F (1, 8) = 3.155). Two-Way ANOVA with Sidak's multiple comparisons test. $n_{KA - saline}$ = 3/4; $n_{KA - macimorelin}$ = 4; $n_{SHAM - macimorelin}$ = 3; $n_{SHAM - saline}$ =2. **** = $P < 0.0001$. GFAP = Glial fibrillary acidic protein; IbaI = Ionized calcium binding adaptor molecule 1; KA = kainic acid; NeuN = Neuronal nuclear protein. Data = mean ± SEM.

Figure 5: Macimorelin does not significantly affect the amount of NeuN$^+$-, GFAP$^+$- or IbaI$^+$- cells in CA3 A) Representative images of the staining in CA3. **B)** Epileptic mice have significantly less NeuN$^+$-cells (KA $P < 0.001$, F (1, 9) = 217.3; Macimorelin $P = 0.94$, F (1, 9) = 0.005578; Interaction $P = 0.34$, F (1, 9) = 1.030). **C)** The absolute amount of GFAP$^+$-cells was lower in epileptic mice compared to sham mice (KA $P < 0.001$, F (1, 9) = 35.89; Macimorelin $P = 0.30$, F (1, 9) = 1.227; Interaction $P = 0.20$, F (1, 9) = 1.885). **D)** There were significantly more IbaI$^+$-cells in epileptic mice compared to sham mice (KA $P < 0.001$, F (1, 8) = 69.26; Macimorelin $P = 0.35$, F (1, 8) = 1.005; Interaction $P = 0.11$, F (1, 8) = 3.155). Two-way ANOVA with Sidak's multiple comparisons test. $n_{KA - saline}$ = 3/4; $n_{KA - Macimorelin}$ = 4; $n_{SHAM - Macimorelin}$ = 2; $n_{SHAM - saline}$ = 3. ** = $P < 0.01$; *** = $P < 0.001$. GFAP = Glial fibrillary acidic protein; IbaI = Ionized calcium binding adaptor molecule 1; KA = kainic acid; NeuN = Neuronal nuclear protein. Data = mean ± SEM.

Figure 6: Macimorelin does not significantly affect the amount of NeuN$^+$-, GFAP$^+$- or IbaI$^+$- cells in DG A) Representative images of the staining in DG. **B)** Epileptic mice have significantly less NeuN$^+$-cells compared to sham mice (KA P = 0.001, F (1, 9) = 21.81; Macimorelin P = 0.90, F (1, 9) = 0.01783; Interaction P = 0.09, F (1, 9) = 3.597). **C)** Epileptic mice have a decreased amount of GFAP$^+$-cells compared to sham mice (KA P = 0.004, F (1, 9) = 15.35; Macimorelin P = 0.32, F (1, 9) = 1.128; Interaction P = 0.22, F (1, 9) = 1.719). **D)** Epileptic mice have an increased amount of IbaI+-cells in epileptic mice compared to sham mice (KA P = 0.01, F (1, 9) = 9.694; Macimorelin P = 0.90, F (1, 9) = 0.01596; Interaction P = 0.93, F (1, 9) = 0.007246). Two-way ANOVA with Sidak's multiple comparisons test. $n_{KA - saline}$ = 4; $n_{KA - Macimorelin}$ = 4; $n_{SHAM - Macimorelin}$ = 2; $n_{SHAM - saline}$ = 3. * = P < 0.05; ** = P < 0.01. GFAP = Glial fibrillary acidic protein; IbaI = Ionized calcium binding adaptor molecule 1; KA = kainic acid; NeuN = Neuronal nuclear protein. Data = mean ± SEM.

Table S1: overview of animals used in the study. Five mice were discarded from the study early on because of technical reasons (for instance lack of status epilepticus (SE)). Later, five mice lost their head stage and were subsequently discarded. Of the 19 KA mice used for *ex vivo* analyses, 13 mice were used for EEG analyses. EEG = electroencephalographic; KA = kainic acid.

Figure S1: Overview of experimental design CA = Cornu Ammonis; D = Day; DG = Dentate gyrus; EEG = Electroencephalography; GFAP = Glial fibrillary acidic protein; IbaI = Ionized calcium binding adaptor molecule 1; IHKA = Intrahippocampal kainic acid; NeuN = Neuronal nuclear protein; SE = Status epilepticus. Created with BioRender.com

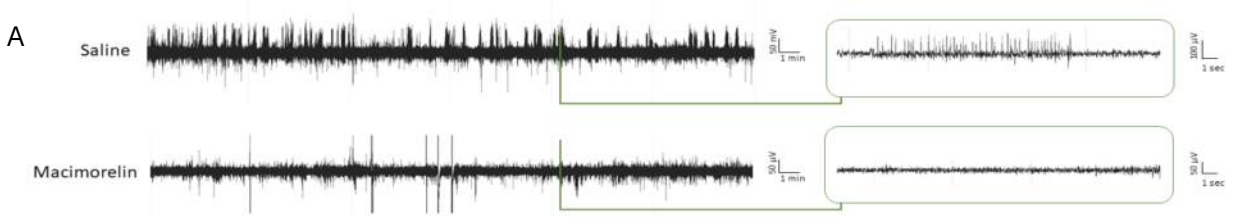
Figure S2: SE duration did not differ between macimorelin- and saline-treated mice. SE duration was defined as the amount of time (minutes) between the beginning of recordings (immediately after the surgery), until the last seizure in the SE (with no seizures occurring for

at least 30 minutes after this last seizure). Two-tailed unpaired t-test (P = 0.16, t = 1.57, df = 8).

Figure S3: Macimorelin does not significantly affect GFAP$^+$- or IbaI$^+$- cells in subregions of CA1 A) There is no significant difference in the amount of astrocytes in *s. oriens* and *s. pyramidale* between the groups (KA P = 0.06, F (1, 8) = 4.806; Macimorelin P = 0.72, F (1, 8) = 0.1352; Interaction P = 0.57, F (1, 8) = 0.3525). **B)** There is no significant difference in the number of astrocytes in *s. radiatum* between the groups (KA P = 0.02, F (1, 8) = 2.486; Macimorelin P = 0.68, F (1, 8) = 0.1805; Interaction P = 0.13, F (1, 8) = 2.885). **C)** There are significantly more IbaI$^+$-cells *s. oriens* and *s. pyramidale* of KA mice compared to sham mice (KA P = 0.002, F (1, 8) = 19.54; Macimorelin P = 0.27, F (1, 8) = 1.377; Interaction P = 0.10, F (1, 8) = 3.493). **D)** KA mice have significantly more IbaI$^+$-cells in *s. radiatum* compared to sham mice (KA P = 0.002, F (1, 8) = 22.45; Macimorelin P = 0.90, F (1, 8) = 0.01689; Interaction P = 0.42, F (1, 8) = 0.7290). Two-way ANOVA with Sidak's multiple comparisons test. $n_{KA - saline} = 3$; $n_{KA - Macimorelin} = 4$; $n_{SHAM - Macimorelin} = 3$; $n_{SHAM - saline} = 2$. ** = $P < 0.01$. KA = kainic acid. Data = mean ± SEM.

Figure S4: There is a significant increase in GFAP immunoreactivity in CA1, CA3 and DG, indicating astrogliosis in KA mice A) Epileptic mice have significantly increased GFAP staining intensities in CA1 compared to sham mice (KA P = 0.0016, F (1, 8) = 21.66; Macimorelin P = 0.89, F (1, 8) = 0.02092; Interaction P = 0.09, F (1, 8) = 0.03490). **B)** Epileptic mice have significantly increased GFAP staining intensities in CA3 compared to sham mice (KA P = 0.0002, F (1, 9) = 36.12; Macimorelin P = 0.33, F (1, 9) = 1.080; Interaction P = 0.50, F (1, 9) = 0.4824). **C)** Epileptic mice have significantly increased GFAP staining intensities in DG compared to sham mice (KA $P < 0.0001$, F (1, 10) = 77.28; Macimorelin P = 0.76, F (1, 10) = 0.099; Interaction P = 0.728, F (1, 10) = 3.597). Two-way ANOVA with Sidak's multiple

comparisons test. $n_{KA - saline} = 3/4$; $n_{KA - Macimorelin} = 4$; $n_{SHAM - Macimorelin} = 2/3$; $n_{SHAM - saline} = 2/3$. ** = $P < 0.01$. KA = kainic acid. Data = mean ± SEM.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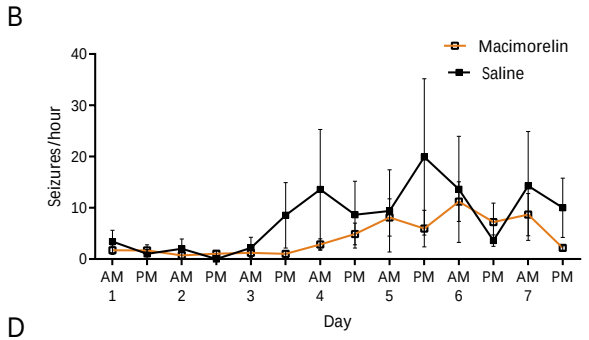
Saline
Macimorelin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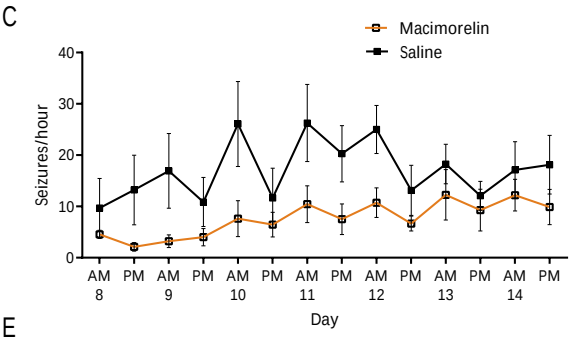
Macimorelin
Saline
Seizures/hour
Day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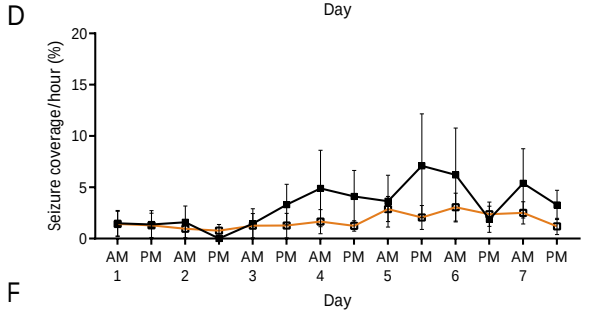
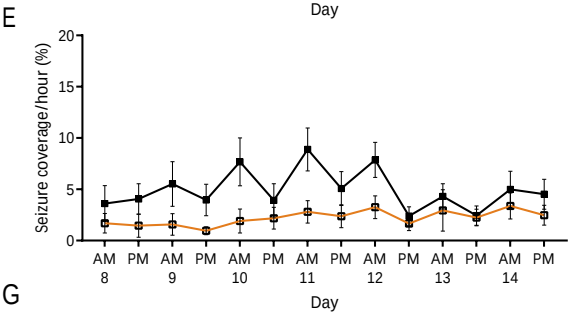

D
Seizure coverage/hour (%)
Day


E
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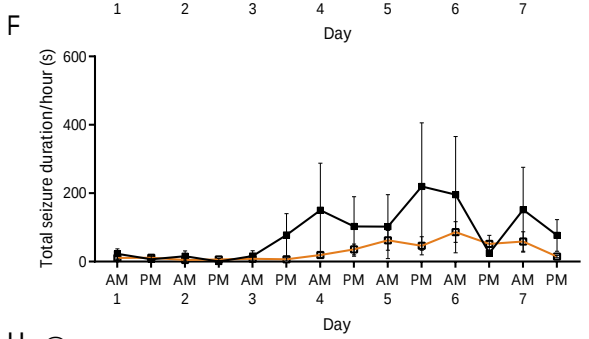
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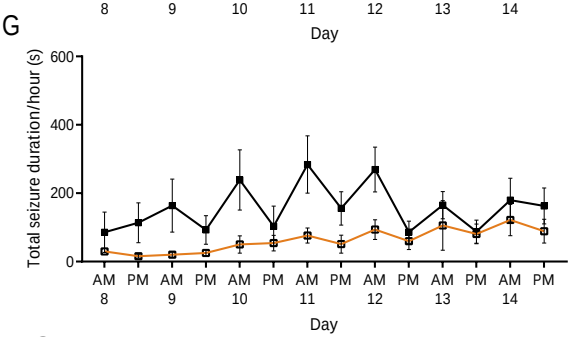
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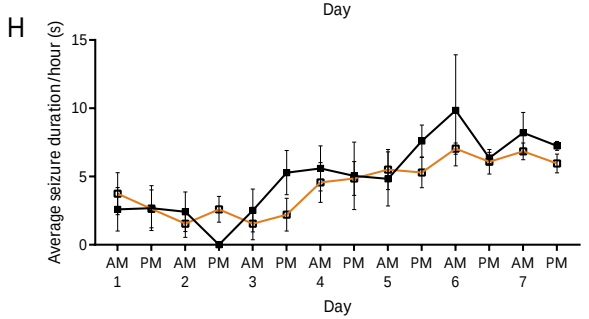
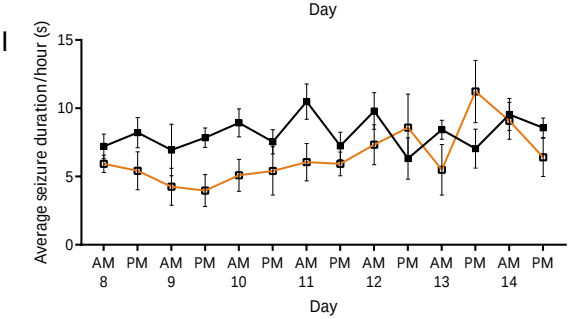
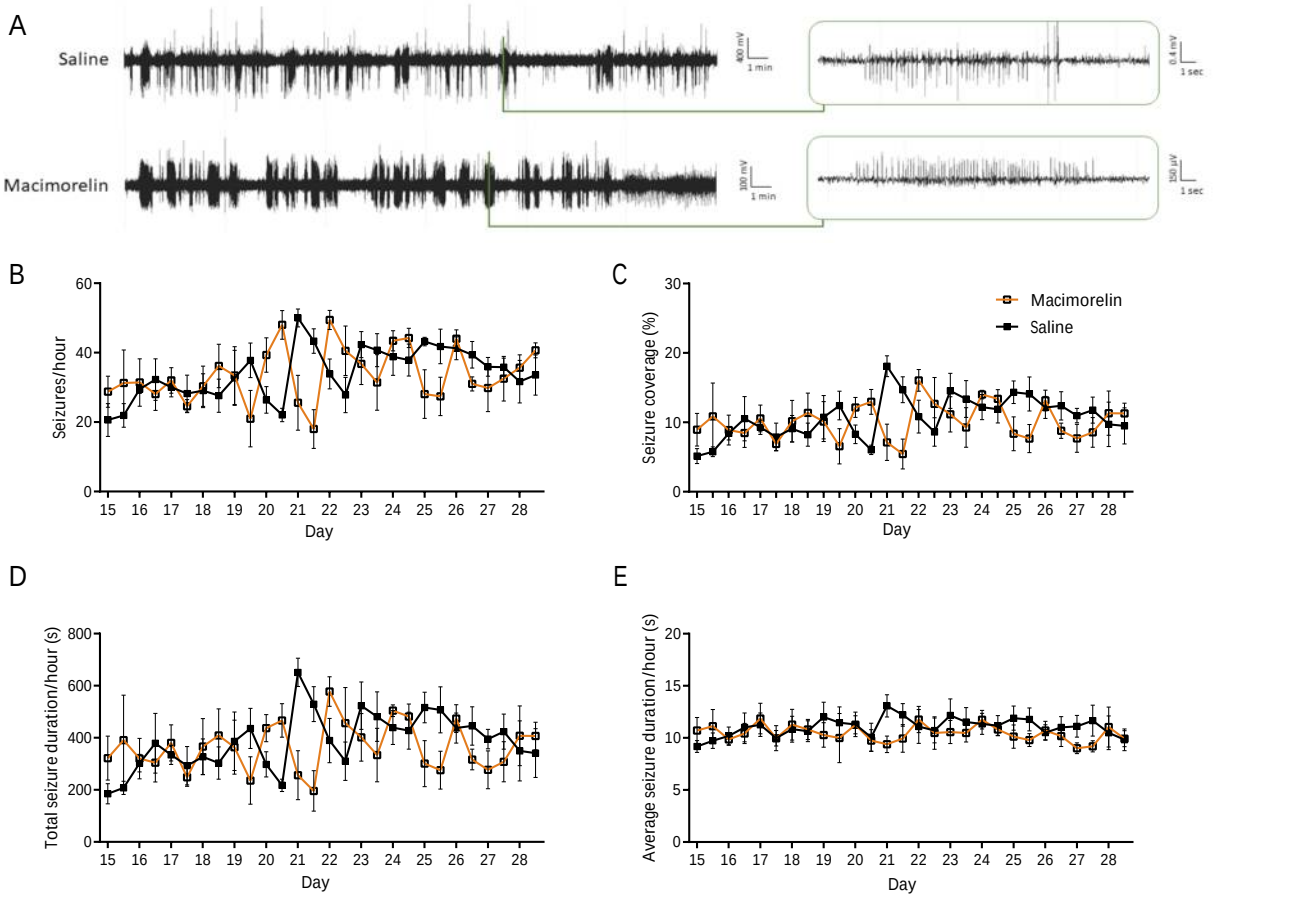
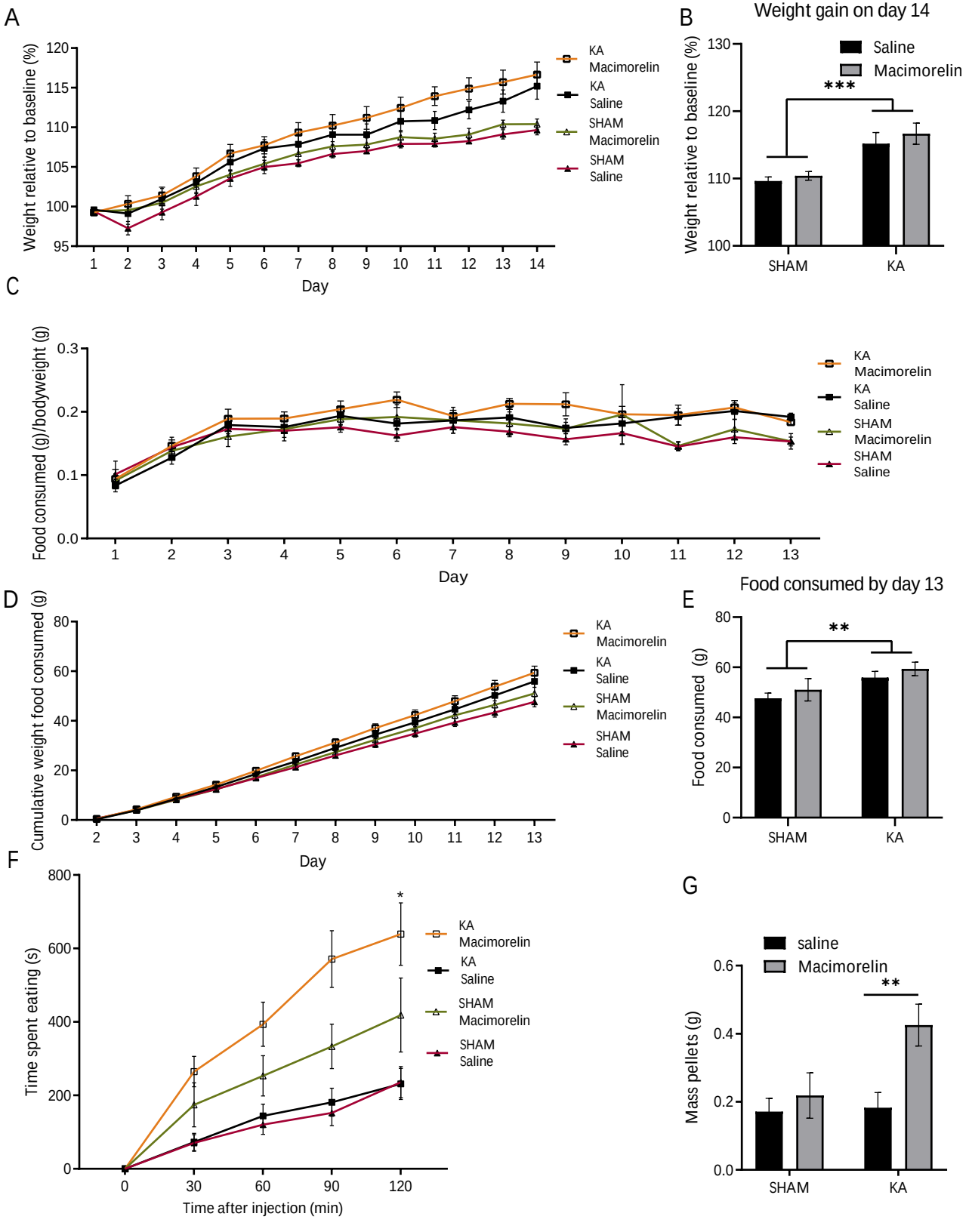
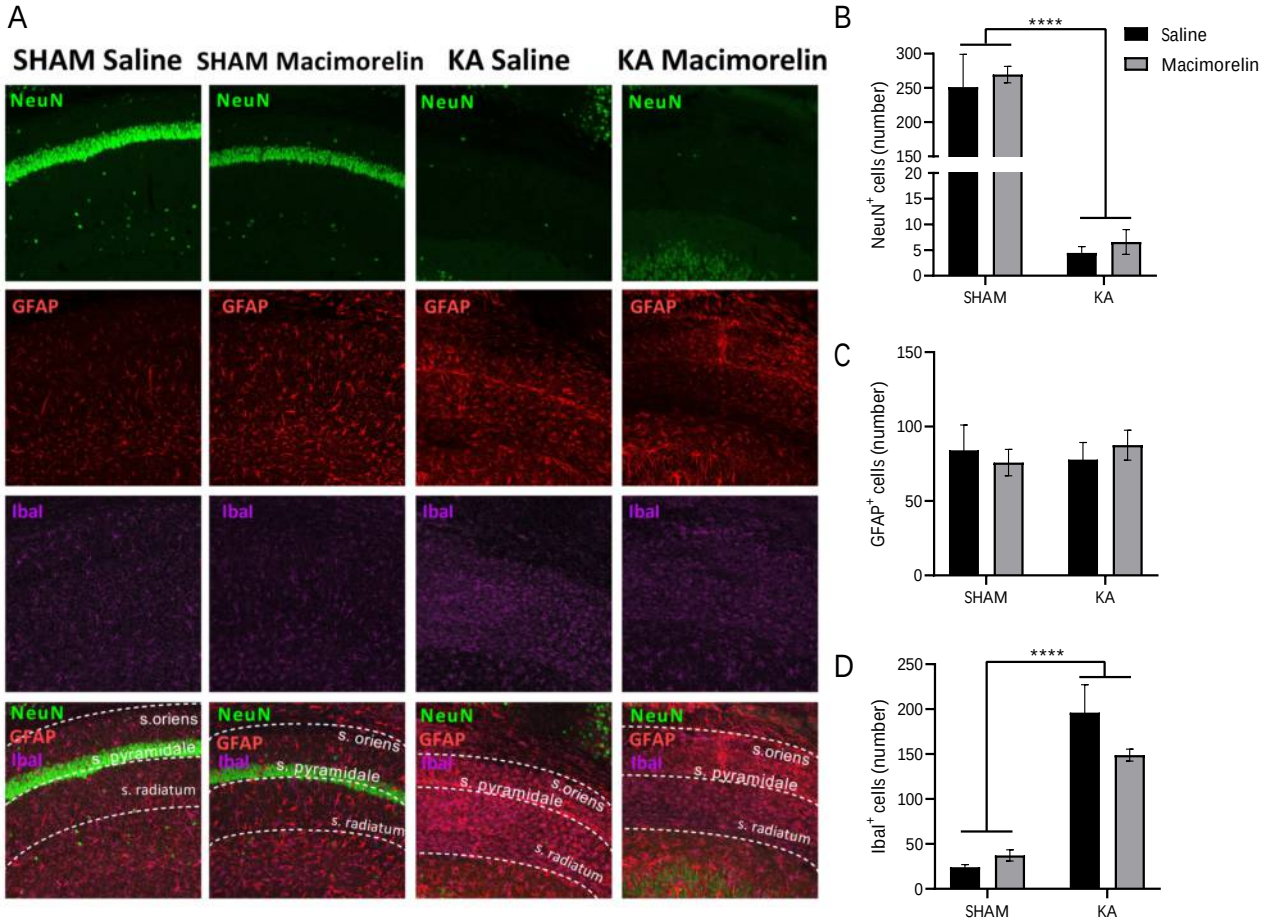
Total seizure duration/hour (s)
Day

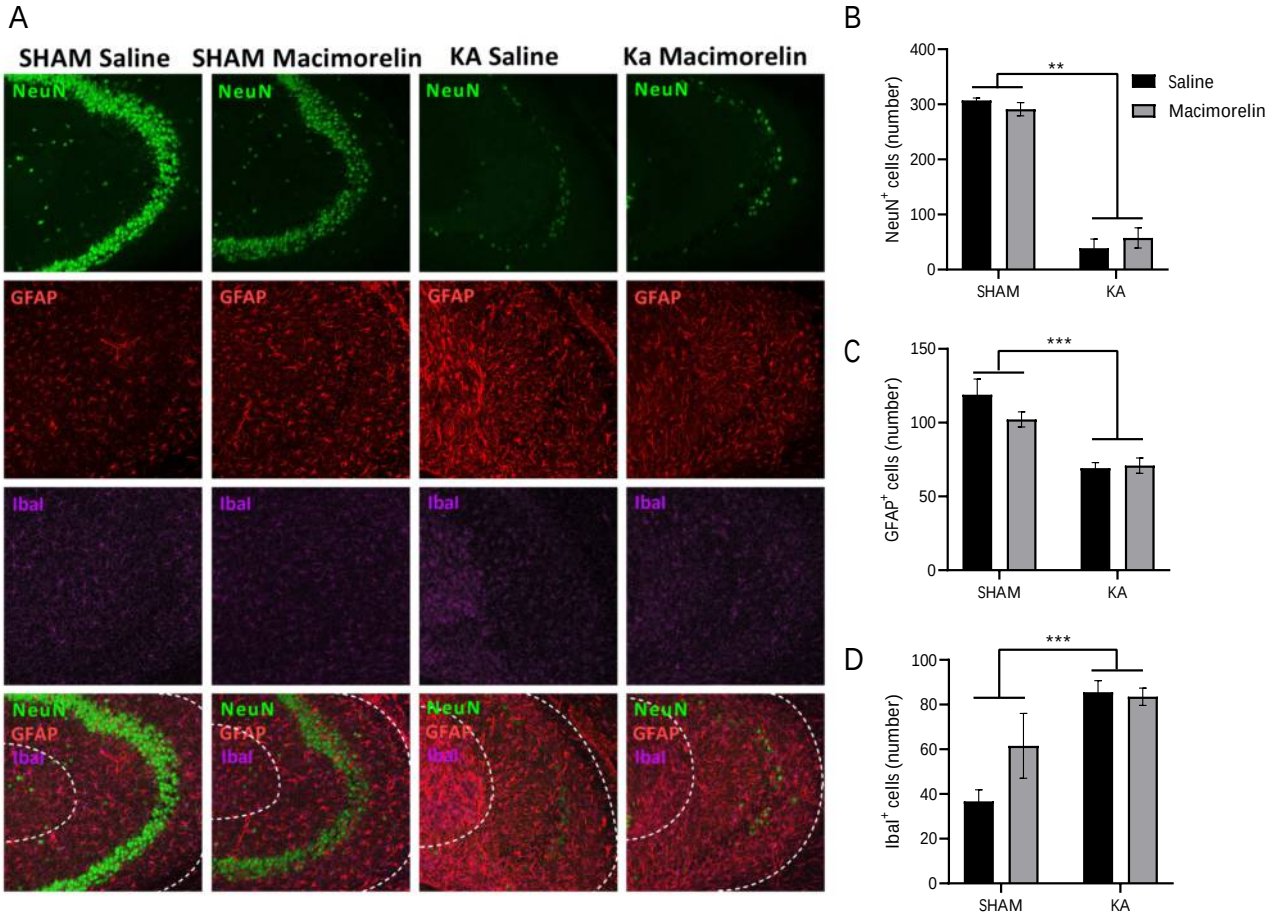

H
Average seizure duration/hour (s)
Day

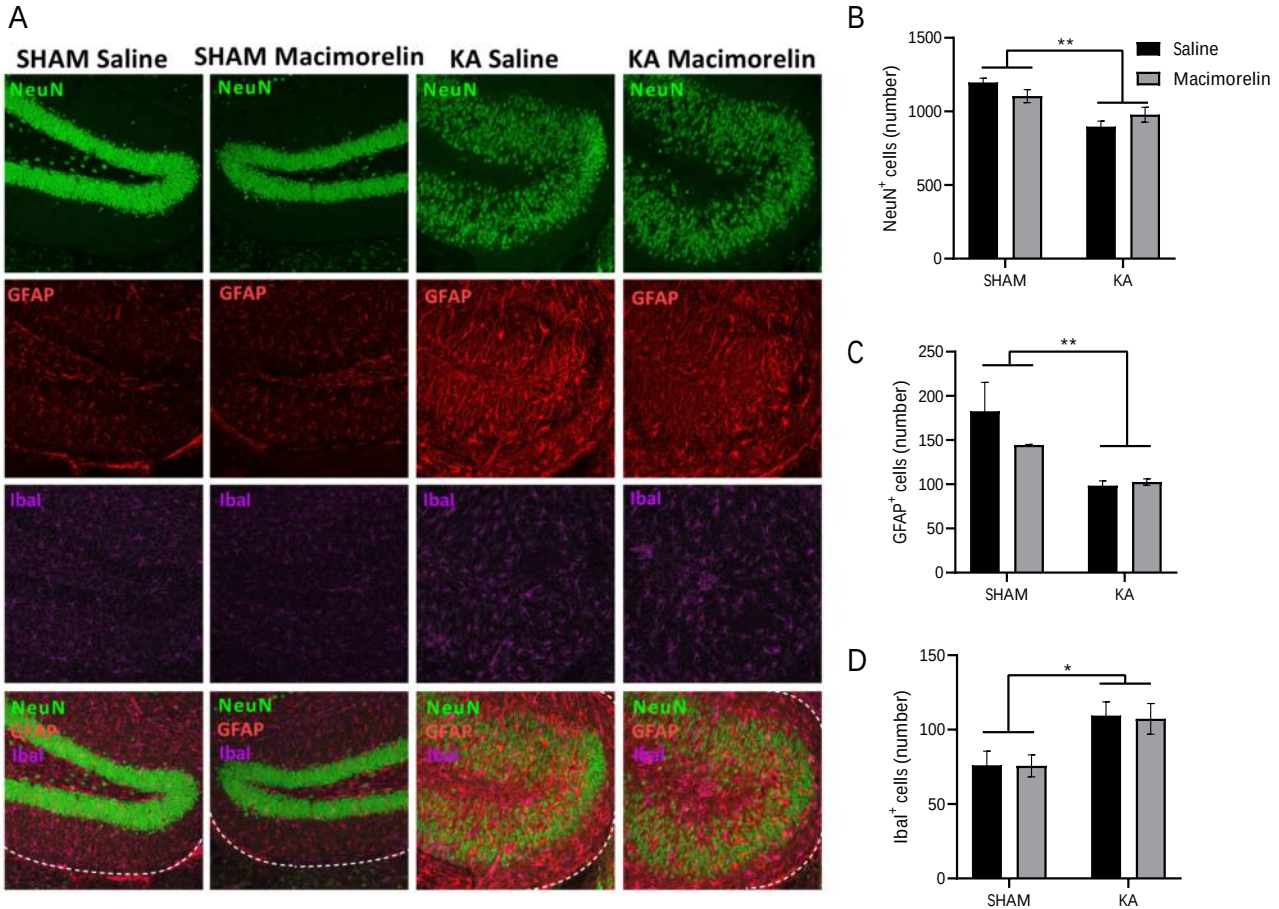

I
Average seizure duration/hour (s)
Day


A
Saline
Macimorelin
400 mV
1 min
0.4 mV
1 sec
100 mV
1 min
150 µV
1 sec
B
Seizures/hour
Day
C
Seizure coverage (%)
Macimorelin
Saline
Day
D
Total seizure duration/hour (s)
Day
E
Average seizure duration/hour (s)
Day

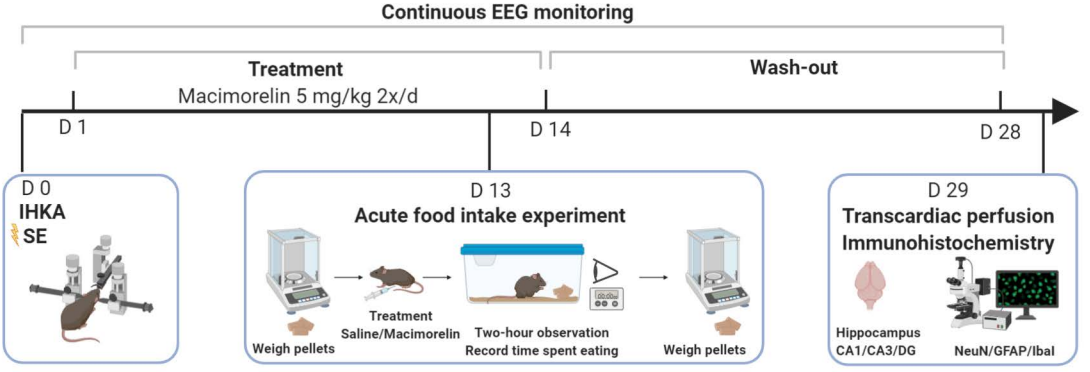

A
Weight relative to baseline (%)
Day
KA Macimorelin
KA Saline
SHAM Macimorelin
SHAM Saline
B
Weight gain on day 14
Weight relative to baseline (%)
Saline
Macimorelin
***
SHAM
KA
C
Food consumed (g)/bodyweight (g)
Day
KA Macimorelin
KA Saline
SHAM Macimorelin
SHAM Saline
D
Cumulative weight food consumed (g)
Day
KA Macimorelin
KA Saline
SHAM Macimorelin
SHAM Saline
E
Food consumed by day 13
Food consumed (g)
**
SHAM
KA
F
Time spent eating (s)
Time after injection (min)
*
KA Macimorelin
KA Saline
SHAM Macimorelin
SHAM Saline
G
Mass pellets (g)
saline
Macimorelin
**
SHAM
KA

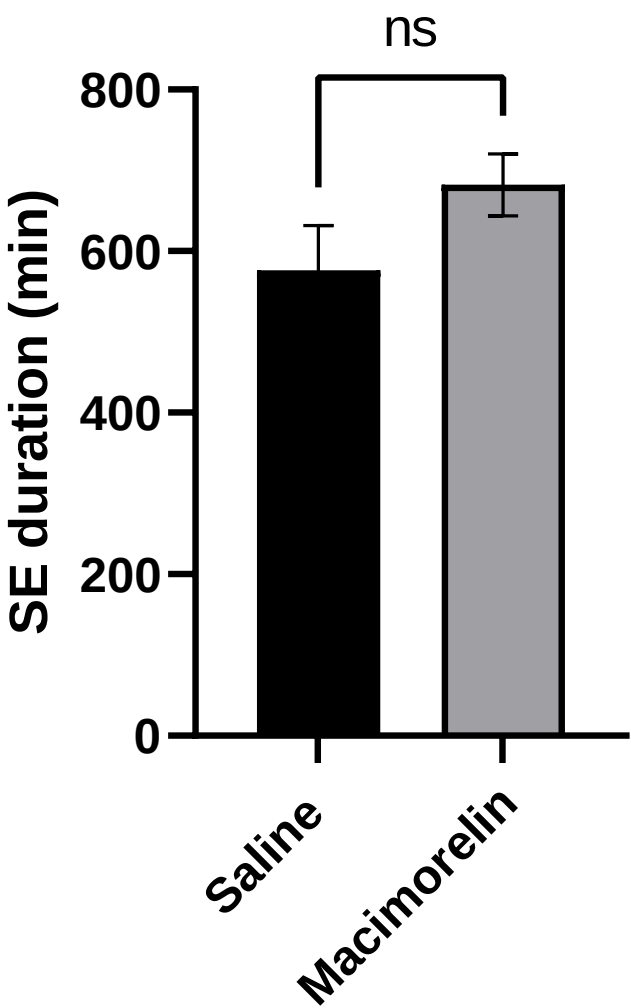

A
SHAM Saline
SHAM Macimorelin
KA Saline
KA Macimorelin
NeuN
GFAP
Iba1
s.oriens
s. pyramidale
s. radiatum
B
****
Saline
Macimorelin
NeuN+ cells (number)
300
250
200
150
20
15
10
5
0
SHAM
KA
C
GFAP+ cells (number)
150
100
50
0
SHAM
KA
D
****
Iba1+ cells (number)
250
200
150
100
50
0
SHAM
KA

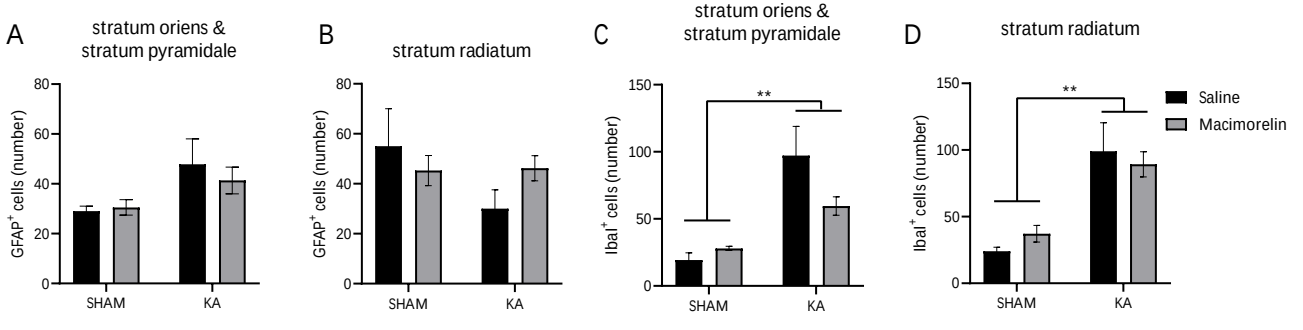

A
SHAM Saline
SHAM Macimorelin
KA Saline
Ka Macimorelin
NeuN
GFAP
Iba1
B
NeuN+ cells (number)
Saline
Macimorelin
SHAM
KA
**
C
GFAP+ cells (number)
***
D
Iba1+ cells (number)
***

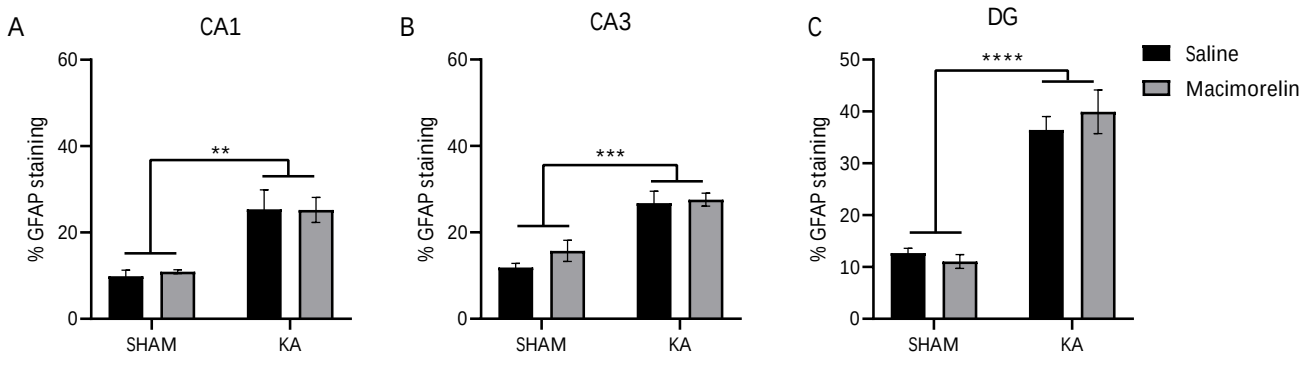

A
SHAM Saline
SHAM Macimorelin
KA Saline
KA Macimorelin
NeuN
GFAP
Iba1
B
Saline
Macimorelin
NeuN+ cells (number)
1500
1000
500
0
**
SHAM
KA
C
GFAP+ cells (number)
250
200
150
100
50
0
**
SHAM
KA
D
Iba1+ cells (number)
150
100
50
0
*
SHAM
KA

44 mice					
28 KA				16 SHAM	
19 used for ex vivo analyses	13 used for EEG analyses	4 loss head stage	5 discar-ded	15 used for ex vivo analyses	1 loss head stage


Continuous EEG monitoring
Treatment
Macimorelin 5 mg/kg 2x/d
Wash-out
D 1
D 14
D 28
D 0
IHKA
SE
D 13
Acute food intake experiment
Weigh pellets
Treatment
Saline/Macimorelin
Two-hour observation
Record time spent eating
Weigh pellets
D 29
Transcardiac perfusion
Immunohistochemistry
Hippocampus
CA1/CA3/DG
NeuN/GFAP/Iba1


ns
800
600
400
200
0
SE duration (min)
Saline
Macimorelin


A
stratum oriens &
stratum pyramidale
GFAP+ cells (number)
80
60
40
20
0
SHAM
KA
B
stratum radiatum
GFAP+ cells (number)
80
60
40
20
0
SHAM
KA
C
stratum oriens &
stratum pyramidale
Iba1+ cells (number)
150
100
50
0
**
SHAM
KA
D
stratum radiatum
Iba1+ cells (number)
150
100
50
0
**
SHAM
KA
Saline
Macimorelin


A
CA1
% GFAP staining
60
40
20
0
**
SHAM
KA
B
CA3
% GFAP staining
60
40
20
0
***
SHAM
KA
C
DG
% GFAP staining
50
40
30
20
10
0
****
SHAM
KA
Saline
Macimorelin