

Supplementary methods

Implantation of electrodes

To implant electrodes, rats were anesthetized using ketamine (74 mg/kg; Alfasan, Woerden, the Netherlands) and xylazine (11 mg/kg; Bayer AG, Leverkusen, Germany) interperitoneally. Xylocaine spray was applied on the skull and surrounding connective tissue as local pain killer during the procedure. Rats were fixed in a stereotaxic apparatus and their body temperature was maintained using an electrical heating pad. A pair of stimulation electrodes was placed into the left angular bundle (coordinates from bregma: Anterior-Posterior (AP) = 7.2 mm and Medio-Lateral (ML) = 1.7mm) and a pair of hippocampal recording electrodes was placed in the left dentate gyrus of the hippocampus (coordinates from bregma: AP = 3.9 mm and ML = 1.7 mm). Each electrode was made of two insulated stainless-steel wires with a diameter of 70 μm . The tip separation of the recording electrodes was 700 μm and the tip separation of the stimulation electrodes was 500 μm . During surgery, the location of the electrodes was verified by evoking field potentials via the electrodes in the angular bundle and recording via the electrodes in the dentate gyrus. After proper positioning, the electrodes were placed in an eight-pin connector and this connector was attached to the skull using stainless steel screws and dental cement. The rats in the non-kindled groups were sham-operated and received a dummy headstage to control for factors related to the surgical procedure and the presence of a headstage. After surgery, chloramphenicol with lidocaine cream (1 g/g) was applied on the wound, and the rats received a subcutaneous injection of buprenorphine (0.5 mg/kg) and saline (2 ml).

In the kindled groups, two animals with a poor EEG were excluded from the study. Furthermore, one animal was excluded due to a lost headstage and 6 animals died after the surgical procedure.

Rapid kindling stimulation and EEG measurements

One week after the recovery from surgery, rats were connected to a stimulation/EEG recording cage (40 x 40 x 80cm) with a shielded, multistrand cable and electrical swivel (Air precision, Le Plessis Robinson, France). During the EEG recordings, signals from the headstage were carried through a commutator, a custom-designed filter and an amplification unit (BR-20D Breakout Box, NPI electronic GmbH, Tamm, Germany). The signals were then sampled via a computer-controlled digitized card (NI USB-6255, National Instruments Netherlands, Woerden, the Netherlands). The EEG signals were amplified (10x in the headstage), band-pass filtered (0.1 – 1000 Hz), and digitized at 2 kHz (16 bit; 30.5 μ V/bit) by in-house data distribution software in MATLAB (MathWorks, Natick, MA, USA). The stimulations were applied via the same NI USB-6255 apparatus, in the form of biphasic, bipolar voltage stimulation pulses (max. 20V) at microsecond resolution. Via two ERB-24 USB-based 24-channel electromechanical relay interface devices, multiple stimulations were delivered to different rats, in a time-efficient manner. The field potentials were evoked, and the kindling stimulations were delivered by the same distribution software in MATLAB (MathWorks, Natick, MA, USA).

Before the start of the kindling experiment, the stimulation intensity was determined by measuring the threshold for a field potential (minimum voltage) and the intensity necessary to get the maximum field potential amplitude. Each rat was kindled at 70% of the maximum intensity ($\text{min (V)} + (\text{max (V)} - \text{min (V)}) * 0.70$). The EEG was recorded for 24 h/day during the days the animals were connected to the EEG recording/stimulation setup.

Rats were electrically stimulated via the angular bundle (biphasic pulses, 0.2 msec, 50 Hz, total duration 10 sec, max 20V) on four consecutive days, 9 times each day with a 45-minute interval (between 9 AM and 5 PM). After the stimulation, the animals were observed for 3 minutes and behavioral seizures were scored using the Racine scale by trained observers.

Open field test

The open field was conducted during the light (inactive) phase and was deliberately chosen as the first behavioral test as results could have been biased by the Barnes maze and/or novel object recognition task. Animals were placed in the experimental room at least one hour prior to the experiment for habituation. They were then placed in the circular arena with a diameter of 130 cm, opaque walls with a height of 25 cm for 5 minutes. The center of the arena was illuminated with a light intensity of 125 lux and the outer zone with an intensity of 85 lux. The arena was divided into a center and border zone. Rearing frequency was scored manually, and distance moved, velocity, center zone entries and time spent in the center zone were tracked automatically with EthoVision (Noldus, Wageningen, the Netherlands).

Barnes maze

The animals were habituated to the room at least one hour prior to each test phase. All test phases were performed during the light (inactive) phase. Animals a circular arena (1.3 m diameter) that is elevated off the floor without walls, and with 18 evenly spaced circular holes (10 cm) around the edges. One of the holes has an escape box underneath that is always situated in a fixed location in the room. The arena is brightly lit (240 lux) and the animal's innate tendency to avoid the bright and elevated platform motivates them to find and enter the escape box. To facilitate spatial orientation and spatial learning, visual cues were present on the walls around the arena. The first day started with a one-minute habituation phase to the arena in low light conditions. After one minute, the animals were gently guided to the escape box, where they habituated for two minutes. During the five subsequent trials, animals were placed in the center of the arena in an opaque starting cylinder (30 cm diameter) with three minutes to explore and find the escape box, where they then stayed for one minute. The first trial took place on the afternoon of day one, two trials on day two and three trials on day three. The primary latency to find the escape box and the primary errors (number of nose pokes in holes without the escape

box until the escape box was located) were scored manually in EthoVision (Noldus, Wageningen, the Netherlands).

Novel object recognition

The animals were habituated to the room at least one hour prior to the experiment and entered an opaque rectangular arena (39x59 cm) for a 5-minute habituation in the morning. In the afternoon, the animals explored two familiar objects in the arena for 10 minutes. The next day, the animals explored their familiar object and a novel object for 10 minutes. The time and frequency spent exploring both objects were manually scored in EthoVision (Noldus, Wageningen, the Netherlands).

Plasma lipid profiles

A known amount of 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine, (Avanti Polar Lipids, Inc., AL, USA) was added as an internal standard to 75 μ l plasma in a glass tube. The lipids were converted to fatty acid methyl esters (FAME) by adding 2ml methanol and 40 μ l concentrated sulphuric acid (2 v/v %) and heated at 100°C for 60 minutes¹. After cooling down, 2 mL hexane and 0.5 mL 2.5 mol/l sodium hydroxide solution were added to the samples after that vortexed for 2 minutes. After vortexing, the upper layer (hexane with FAME's) was collected and dried using a SpeedVac®. Dried samples were subsequently dissolved in 80 μ l iso-octane and analyzed by gas chromatography (Shimadzu Corporation, Kyoto, Japan) using flame ionization detection with a CP-SIL88 for FAME column (60m \times 0.25 mm id. 0.20 μ m film thickness; Agilent Technologies, Inc., Santa Clara, CA, USA). The carrier gas was hydrogen and the make-up gas was nitrogen. GC oven temperature settings were as follows: initial temperature 60 °C with a hold time of 3 min; temperature increment to 130 °C at 20 °C/min and then a hold time of 7 min; temperature increment to 215 °C at 9 °C/min and then a hold time of 15 min; temperature increment to 230 °C at 30 °C/min and then a hold time of 42 min. (in-house settings). Fatty acids were identified based on retention time using an external

reference standards GLC-569B and GLC-461, (Nu-Chek Prep, Inc., Elysian, MN, USA). Peak area was used as a measure of relative percent. The internal standard was used for absolute quantification of fatty acids (mg/l) in plasma.

Plasma amino acid profiles

After precipitation of proteins and polypeptides with perchloric acid, the sample was centrifuged. Individual amino acid levels were determined by ultra-fast liquid chromatography (0.3ml/min pump, autosampler at 10 °C, column oven at 40 °C and column: Acquity UPLC BEH C18, 1.7 µm, 100x 2.1 mm), using pre-column derivatization with o-phthalaldehyde and fluorimetric detection ($\lambda_{\text{ex}} = 340 \text{ nm}$, $\lambda_{\text{em}} = 455 \text{ nm}$). UFLC eluent A consisted of 1.75 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 3.80 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10 \text{ H}_2\text{O}$ and 0.25 g NaN_3 per liter and 2.5 ml 6 M HCl. For eluent B, 176.9 g methanol was mixed with 178.0 g acetonitrile in water for a total of 500 ml. It was then degassed and filtered over a 0.45 µm filter.

Tissue collection

After decapitation on day 21, the ipsilateral side of the brain was fixated in 4% buffered paraformaldehyde for 1 week and then embedded in paraffin for immunohistochemical stainings. Furthermore, liver tissue samples were collected, frozen on dry ice and stored at -80°C for the liver triglyceride level analysis.

Immunohistochemistry

For immunohistochemistry, the paraffin embedded brains were cut in 5-6 µm sagittal sections. The sections were mounted onto pre-coated object slides (Star Frost, Waldemar Knittel, Braunschweig, Germany) and dried overnight at 37 °C. Sections were deparaffinized using xylene (3 x 3 min) followed by ethanol (100%v/v, 100%v/v, 96%v/v; 3 min each step). In order to block endogenous peroxidase activity, the sections were incubated for 20 minutes with 0.3% hydrogen peroxidase diluted in methanol. After washing the sections with tap-water and

demineralized water, antigen retrieval was performed using a pressure cooker in Tris-EDTA buffer (1.2 g Tris and 0.37 g EDTA/L, pH 9) at 120 °C for 10 minutes. After antigen retrieval, sections were cooled on ice for 10 minutes, washed with tap-water, demi-water and twice with phosphate-buffered saline (PBS, pH 7.4). Incubation with primary antibody took place overnight at 4°C or room temperature, depending on the antibody (1:2000 mouse monoclonal anti-NeuN, Mab377, Merck Millipore, Temecula, CA, USA; 1:500 mouse monoclonal anti-Vimentin, M0725, Dako, Santa Clara, CA, USA; 1:2000 Rabbit polyclonal anti-IBA-1, 019-19741, Wako, Osaka, Japan; 1:1000 Rabbit monoclonal anti-doublecortin, 4604S, Cell Signaling Technology, Danvers, MA, USA; 1:1000 Rabbit polyclonal anti-ZnT3, 197 002, Synaptic Systems, Göttingen, Germany). The following day the sections were washed three times using PBS. Sections were then incubated with a polymer-based peroxidase immunohistochemistry detection kit, containing a post-antibody blocking and a poly-HRP-antibody-anti-mouse/rabbit IgG (Brightvision plus kit, ImmunoLogic, Duiven, the Netherlands), according to manufacturer's instructions. Sections were subsequently washed three times in PBS, and peroxidase activity was detected with 3,3'-Diaminobenzidine (50 mg DAB, Sigma-Aldrich, Zwijndrecht, the Netherlands) and 0.3 % hydrogen peroxide diluted in Tris-HCl (0.05 M, pH 7.6). The reaction was stopped with distilled water. Sections were then dehydrated in ethanol (70%v/v, 96%v/v and 100%v/v; 3 minutes each) and xylene (3 x 3 minutes) and covered using Pertex mounting medium.

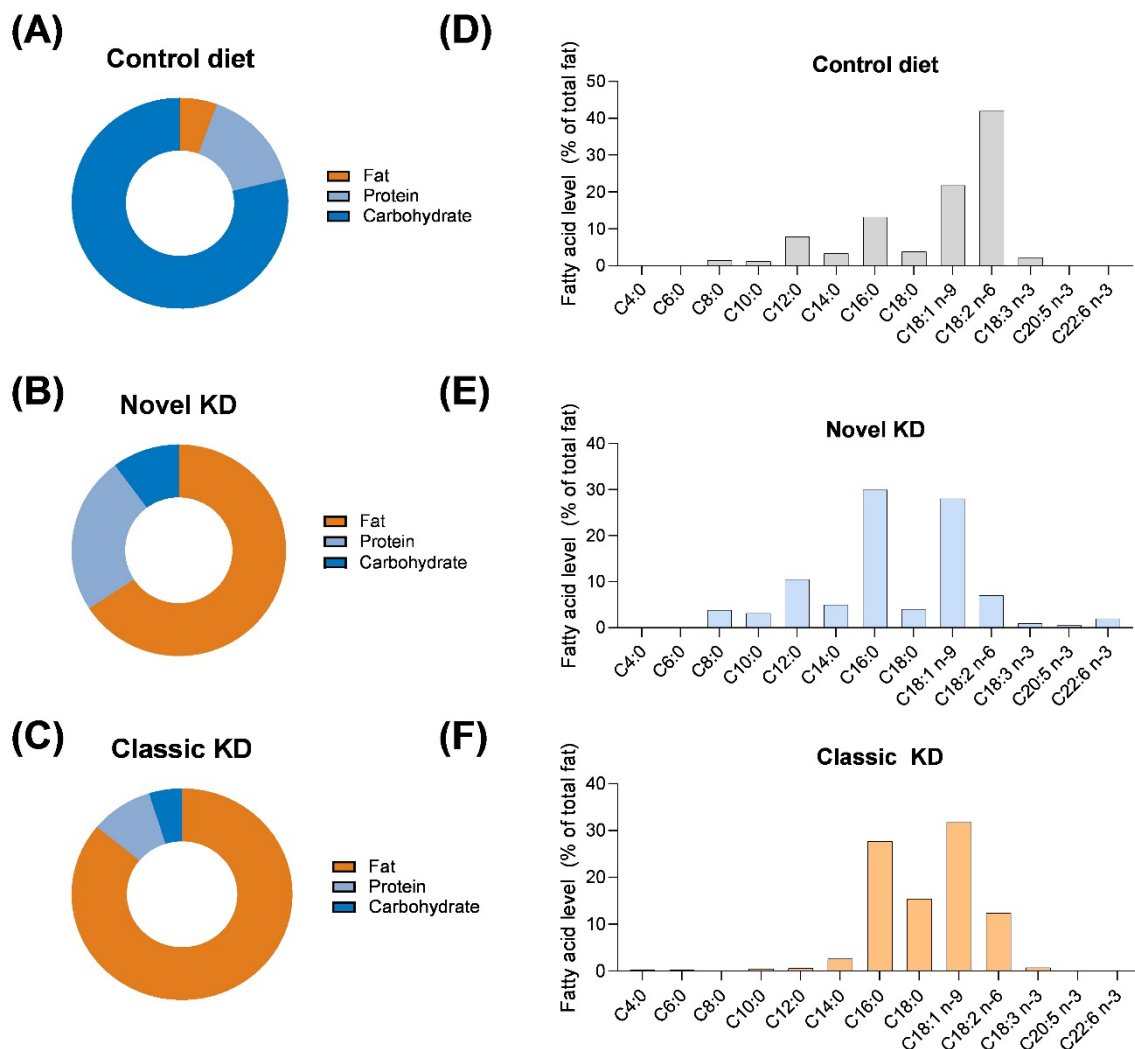
Immunohistochemical quantification

One section per animal per staining was quantitatively evaluated. The number of NeuN-, vimentin-, and IBA1-positive cells were manually counted in the hilus of both the dorsal and ventral hippocampus using a 10 x 10 ocular grid with a 200x magnification. Depending on the size of the hilus, the grid ranged from 64 equivalent squares (8 x 8 rows) to 36 equivalent squares (6 x 6 rows). The density of the cells was then calculated by dividing the number of

NeuN-, vimentin- and IBA1-positive cells by the volume of the grid (cells/0.16mm²). Doublecortin-positive cells in two subgranular zone regions of the dentate gyrus of both the dorsal and ventral hippocampus were manually counted using a 10 x 10 ocular grid with a 200x magnification.

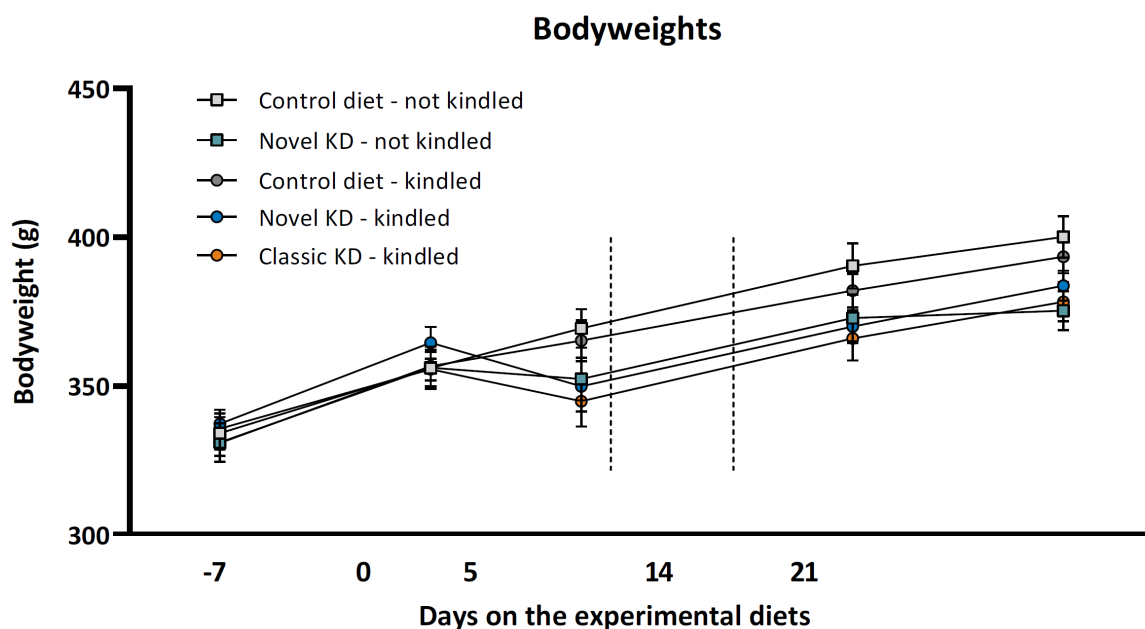
A semi-quantitative analysis was performed on ZnT3 stainings in the molecular layer of the dentate gyrus in the dorsal and ventral regions of the hippocampus. A similar scale as previously used for Timm staining (from 0 to 5²), was used to assess staining of mossy fibers; 0, no granules in the supragranular zone of the dentate gyrus; 1, few granules in an irregular distribution between the tips in the supragranular zone; 2, more granules in an evenly distributed manner between the tips in the supragranular zone; 3, many granules in an evenly distributed manner between tips and crest in the supragranular zone. Patches of confluent dense laminar band between tips can be observed; 4, the granules from a confluent dense laminar between tips in the supragranular zone; 5, the confluent dense laminar band of granules extends into the inner molecular layer.

Supplementary figures

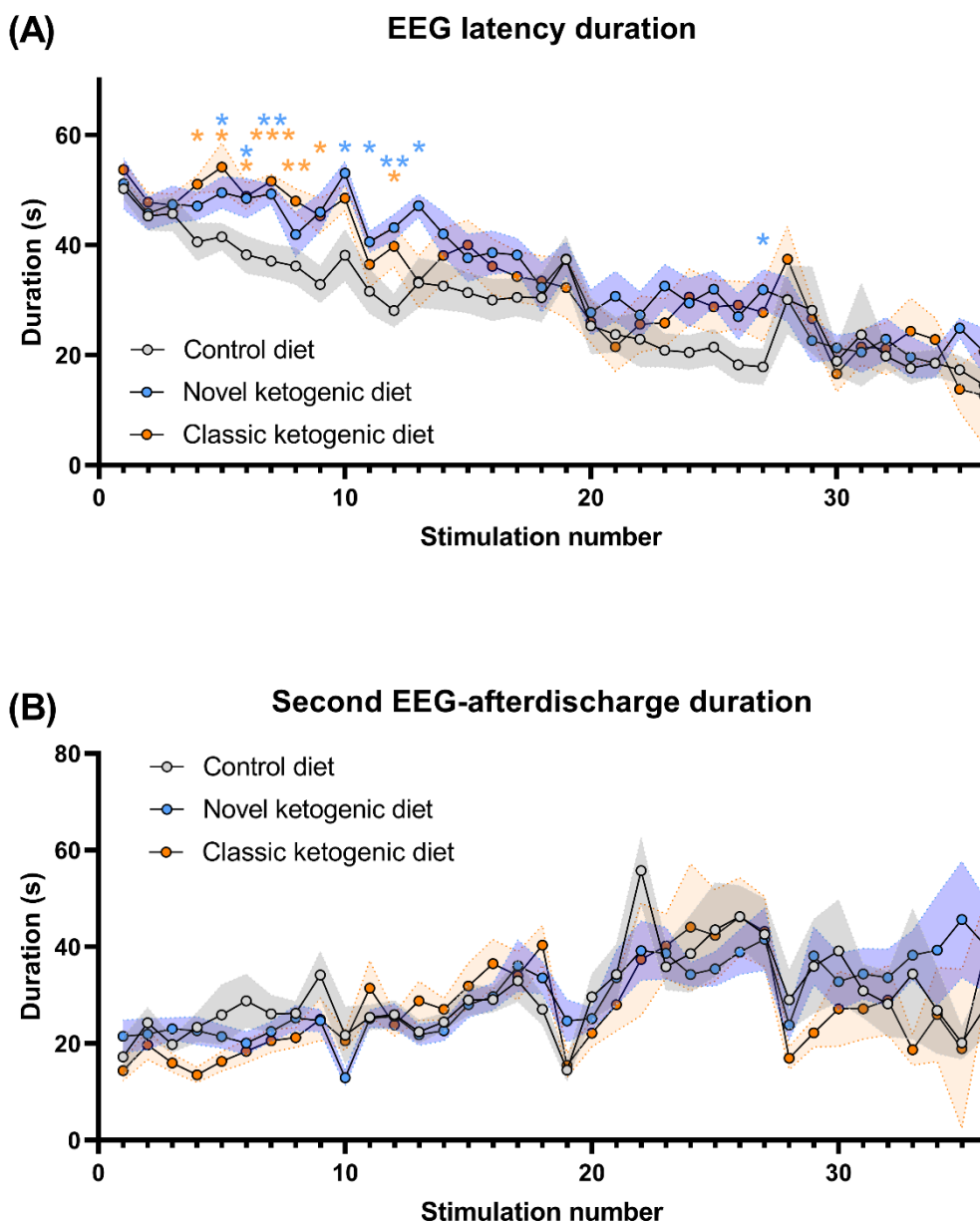


Supplementary figure S1: Diet compositions. The aimed macronutrient content (g/kg diet) is visually represented for (A), the control diet which was aimed to contain 5.5% fat, 15.7% protein and 78.8% carbohydrate (B), the novel KD which was aimed to contain 65.7% fat, 24.1% protein and 10.1% carbohydrate and (C), the classic KD which was aimed to contain 86.0% fat, 9.2% protein and 4.8% carbohydrate. Single fatty acid levels of the three diets were measured to determine the distribution of fatty acids in each diet fat blend. The fatty acids with the highest prevalence are shown, including saturated short-chain (butyrate, C4), medium-chain

(hexanoic acid, C6; octanoic acid, C8; decanoic acid, C10 and dodecanoic acid, C12) long-chain (myristic acid, C14; palmitic acid, C16 and stearic acid, C18) fatty acids and unsaturated ω -9 (oleic acid, C18:1), ω -6 (linoleic acid, C18:2) and ω -3 (α -linolenic acid, C18:3; eicosapentaenoic acid, C20:5; docosahexaenoic acid, C22:6). They were expressed as percentage of the total fat content of each diet for (D), the control diet (E), the novel KD and (F), the classic KD.

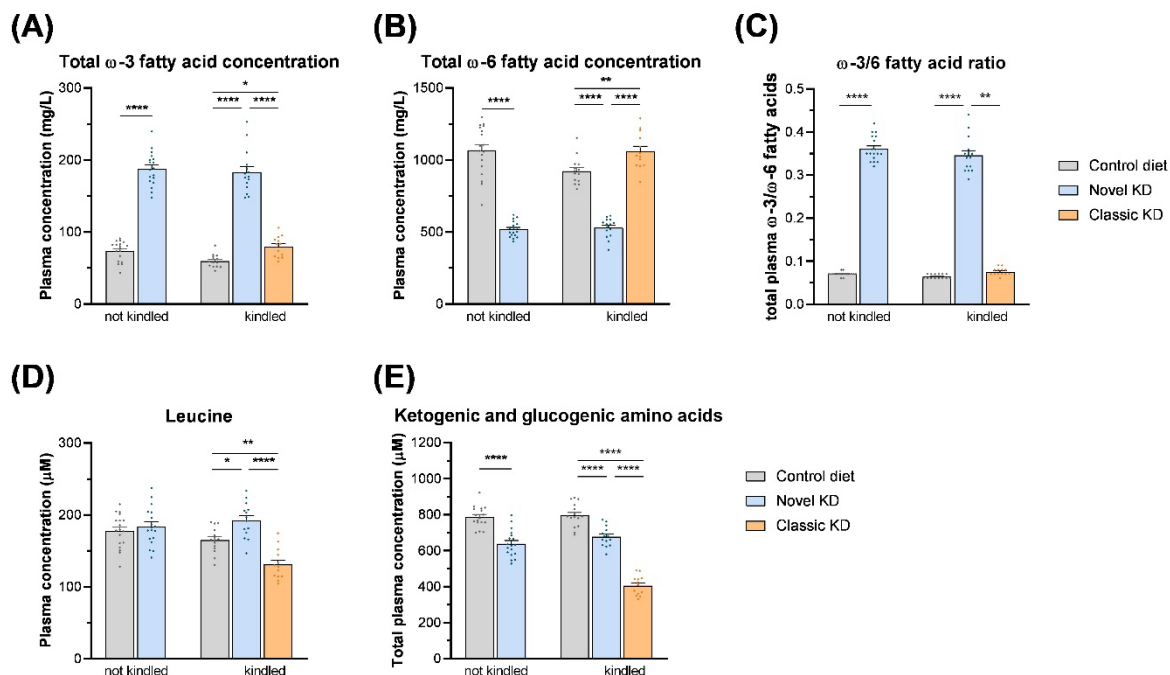


Supplementary figure S2: Bodyweights. The bodyweight was measured at five timepoints spanning the time all animals were switched to the synthetic control diet until the moment the animals were sacrificed. Rapid kindling took place between the dotted lines. There were no differences between groups in a mixed effect ANOVA ($F_{4,83} = 1.112$, $p = 0.357$).



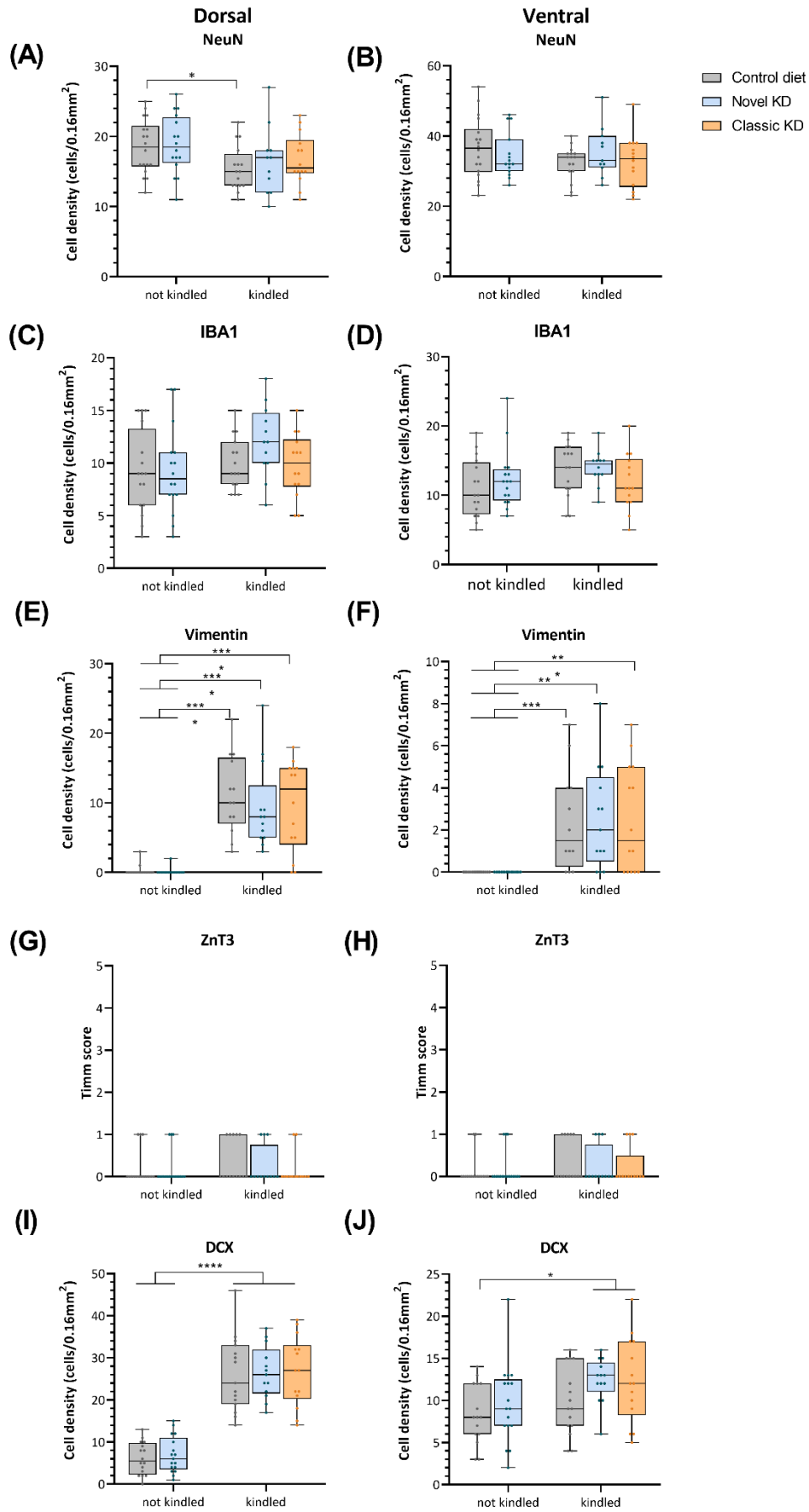
Supplementary figure S3: EEG responses to rapid kindling. (A) A main effect of diet on the duration of the latency between the first and second AD was found ($F_{2,43} = 3.238, p = 0.049$).

For the separate stimuli, latencies are longer for the novel KD in stimuli 5 to 7 and 10-14 and for the classic KD in stimuli 4-9 and 12. (B) No effect of diet on the duration of the second after discharge was found. Data is presented as mean \pm SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (orange, control diet vs. classic KD; blue, control diet vs. novel KD), Mixed effect ANOVA followed by a Holm-Sidak test (A, B).



Supplementary figure S4: Metabolic effects of the three diets. (A) Total omega-3 fatty acid plasma levels were higher for the novel KD compared to the other diets ($F_{4,73} = 160.6$, $*p = 0.0454$, $****p < 0.0001$), while (B), total omega-6 fatty acid levels were lower ($F_{4,73} = 98.29$, $**p = 0.0060$, $****p < 0.0001$), resulting in (C), a higher omega 3-to-6 ratio ($H_2 = 63.41$, $**p = 0.0083$, $****p < 0.0001$). Leucine was supplemented in the novel KD. (D) This was reflected in higher plasma leucine levels compared to both control diet and classic KD ($F_{4,73} = 14.78$, $*p = 0.0115$, $p < 0.0001$) but the difference between control diet and novel KD only presented itself in the kindled groups. Contrarily, classic KD leucine levels were lower compared to the control diet ($F_{4,73} = 14.78$, $**p = 0.0016$). (E) Amino acids that are both ketogenic and glucogenic include Phenylalanine, Isoleucine, Threonine, Tyrosine and Tryptophan. They were grouped because of their similarity in function and group differences, their sum revealed overall lower plasma levels for the novel KD compared to the control diet ($F_{4,74} = 91.28$, $****p < 0.0001$), and lower levels for the classic KD compared to the control diet and novel KD ($F_{4,74}$

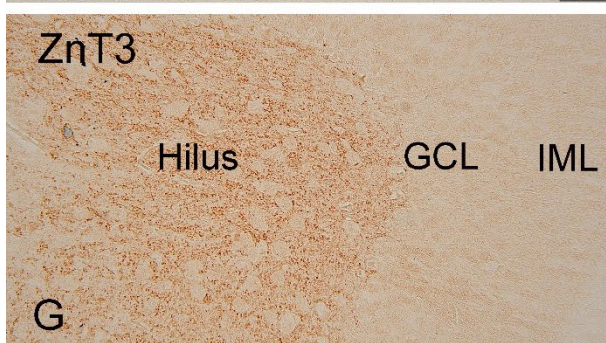
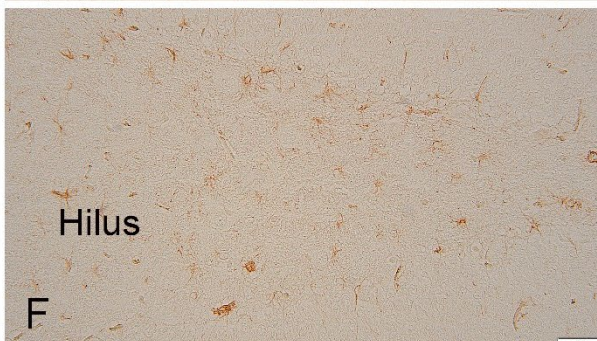
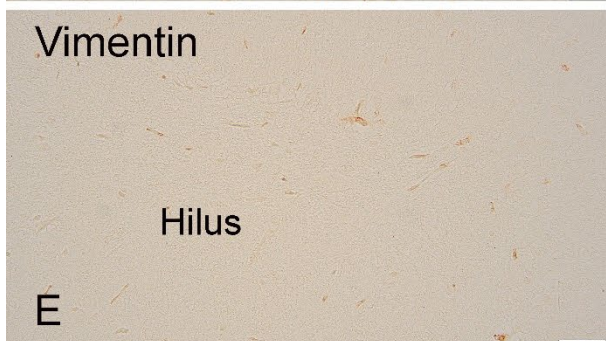
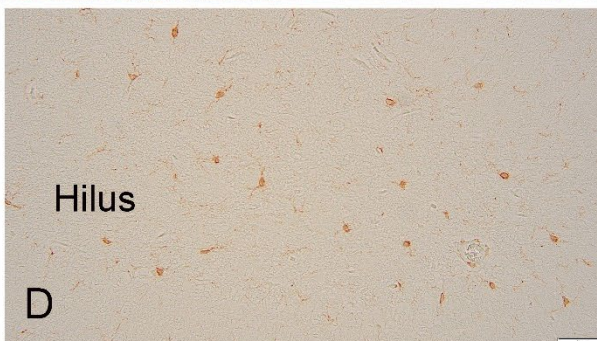
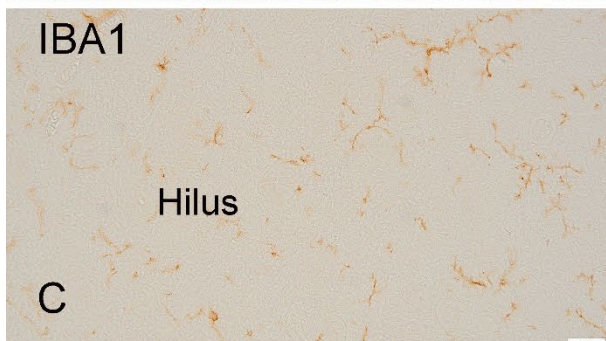
= 91.28, **** $p < 0.0001$). All measurements were taken at day 21 after initiating the experimental diets, and data is presented as mean + SEM, one-way ANOVA and a Holm-Sidak post-hoc analysis (A, B, D and E) and Kruskal Wallis with post-hoc Dunn's test (C).



Supplementary figure S5: Histopathology. (A) Neurons, stained with NeuN, were quantified in the hilus of the dorsal and ventral hippocampus. In the dorsal hilus, the neuronal density in kindled control diet-fed animals was lower compared to non-kindled control diet-fed animals ($p = 0.0172$), this effect was not observed in kindled novel KD-fed animals and classic KD-fed animals. (B) In the ventral hippocampus, neuronal density did not differ between the animals in each group. (C, D) IBA1-positive cells (microglia) in the dorsal and ventral hippocampus were quantified. In both hippocampi, the number of IBA1-positive cells did not differ between the groups. (E, F) Vimentin-positive cells (reactive astrocytes) were quantified in the dorsal and ventral hilus of the hippocampus. The non-kindled animals expressed no vimentin in both the dorsal and ventral hilus. In both hippocampi, the number of vimentin-positive cells of kindled animals was higher in comparison with the non-kindled animals ($p < 0.01$). (G, H) However, both ketogenic diets failed to protect against the seizure-induced astrogliosis. Low Timm scores (0-1) were observed in all groups, indicating that mossy fiber sprouting was not evident and groups did not differ. (I, J) The number of DCX-positive cells was quantified in the subgranular zone of the dorsal and ventral hippocampus. In the dorsal hippocampus, the number of DCX-positive cells in kindled animals is higher compared to non-kindled animals ($p < 0.0001$). However, both diets did not affect seizure-induced neurogenesis. In the ventral hippocampus, kindled animals on both ketogenic diets have more DCX-positive cells compared to non-kindled animals on the control diet ($p < 0.05$). All data was compared using Mann-Whitney U tests, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Non-kindled control

Kindled control



Supplementary figure S6: Immunohistochemistry. Representative images of the dorsal hippocampal dentate gyrus of non-kindled control-fed rats (left) and kindled control-fed (right) for NeuN (A, B), IBA1 (C, D), Vimentin (E, F), ZnT3 (G, H) and DCX (I, J). For quantification see supplementary figure S5. Scale bar = 50 μm . GCL=granule cell layer, IML = inner molecular layer.

References

1. Christie WW. Preparation of Ester Derivatives of Fatty Acids for Chromatographic Analysis. 1993;2:69-111.
2. Cavazos JE, Golijeh, G & Sutula TP. Mossy fiber synaptic reorganization induced by kindling: time course of development, progression, and permanence. *Journal of Neuroscience*. 1991;11(9):2795-2803.