

RESEARCH ARTICLE



TRPM3 regulates fear memory encoding and seizure susceptibility in the lateral amygdala

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Abstract

Background and purpose: TRPM3 is best known for its role as a peripheral sensor of pain and heat. Recent studies reporting gain-of-function TRPM3 mutations in patients with neurodevelopmental and epilepsy-associated disorders suggest a previously unrecognized role of this channel in the brain. However, the function of TRPM3 in neurons of the brain remains largely unexplored.

Experimental approach: Using a battery of *in vivo* and *in vitro* electrophysiological and histological experiments, we studied the effect of TRPM3 channel on amygdala-related functions.

Key results: Here, we identify TRPM3 expression in excitatory neurons of the lateral nucleus of the amygdala (LA), a brain region critical for fear learning also implicated in neuropsychiatric and epileptic disorders. We demonstrate that TRPM3 is functionally active in LA neurons and regulates their intrinsic electrophysiological properties. Consistent with this role, genetic ablation of TRPM3 in mice impairs fear memory formation. Moreover, in a kainic acid-induced epilepsy model, loss of TRPM3 reduces seizure severity, whereas pharmacological activation of the channel exacerbates seizure phenotypes. Finally, we demonstrate the presence of TRPM3 transcripts in neurons of the human LA.

Conclusions and implications: Together, these findings uncover a central role for TRPM3 in amygdala function and identify this ion channel as a modulator of fear memory and seizure susceptibility.

KEYWORDS

amygdala, epilepsy, fear memory, TRPM3

Abbreviations: KA, kainic acid; LA, lateral nucleus of the amygdala; TRPM3, Transient Receptor Potential Melastatin 3.

Attila Gyéresi, Laura Mundrucz, Erzsébet Kövesdi and Miklós Kecskés contributed equally to this work.

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1 | INTRODUCTION

Transient receptor potential melastatin 3 (TRPM3) is identified as a Ca^{2+} -permeable channel expressed in various cell types, including vascular smooth muscle cells or pancreatic beta cells; however, it is mainly studied in the somatosensory neurons of dorsal root and trigeminal ganglia (Grimm et al., 2003; Vriens et al., 2011). A recent study in mice clarified that TRPM3, together with two other members of the **TRP family** of ion channels, **TRPV1** and **TRPA1**, act as a molecular sensor for noxious heat detection in the skin. Therefore it helps the animal to avoid burn injury (Vandewauw et al., 2018). Moreover, TRPM3 contributes to the development of inflammatory hyperalgesia, as well (Mulier et al., 2020; Zhao et al., 2022). Consequently, recent drug developmental studies focus on TRPM3 as a potential painkiller (Biohaven, n.d.).

Besides the well-established role of TRPM3 in peripheral nociception, exciting studies in recent years suggested the potential function of the channel in brain (patho)physiology. One of the first reports relating TRPM3 to a neurological disorder described an exon deletion of the channel in a family with autism (Pagnamenta et al., 2011). Moreover, a recent case study reported two *de novo* mutations in the TRPM3 gene as the cause of developmental and epileptic encephalopathies (DEE) in eight patients (Dyment et al., 2019). Besides other neurological symptoms, seven out of eight patients experienced epileptic seizures during their life and four out of eight patients showed autism-like symptoms. Later, two independent research teams performed detailed electrophysiological characterizations of the two reported TRPM3 disease mutations *in vitro* (Van Hoeymissen et al., 2020; Zhao et al., 2020). Both reports clearly showed that the mutations are associated with gain-of-function (GoF) phenotype in TRPM3, which resulted in elevated channel activity upon the application of the endogenous agonist **pregnenolone sulphate (PregS)** (Wagner et al., 2008) and an increased sensitivity to heat stimuli. Since the original report, several additional studies have been published in recent years linking either the same or novel TRPM3 mutations to similar pathologies (Roelens et al., 2024). These publications clearly indicate a fundamental role of TRPM3 in (patho)physiological functions of neurons in the brain, however the cell type-specific expression of TRPM3 in the central nervous system is still unclear.

Here, we show that *Trpm3* is expressed in the lateral nucleus of the amygdala (LA) and plays a crucial role in the excitability of these neurons. Amygdala is a group of brain nuclei involved in the processing of many emotional processes, including fear, anxiety and aggression. Within the amygdala, LA has been defined as the main input structure and the place of associative fear learning (Sears et al., 2014). Its plasticity is thought to comprise a classic Hebbian mechanism in

What is already known

- Mutations in the thermosensitive TRPM3 channel cause developmental and epileptic encephalopathy in humans.

What does this study add

- TRPM3 is expressed in the lateral amygdala and contributes to fear memory encoding in mice.
- TRPM3 regulates seizure susceptibility in kainic acid-induced models of epilepsy in mice.

What is the clinical significance

- TRPM3 represents a promising drug target in seizure management.

the form of long-term potentiation (LTP). Additionally, the amygdala has been implicated in several neurological disorders, including autism, post-traumatic stress syndrome (PTSD) and temporal lobe epilepsy (TLE) (Aroniadou-Anderjaska et al., 2008).

In this study, we investigated the novel hypothesis that TRPM3 in the LA neurons acts as an important player in cellular excitability both in physiological and pathological conditions. With a battery of *in vitro* and *in vivo* experiments, we showed that loss of TRPM3 resulted in impaired fear memory and LTP formation in mice and decreased seizure susceptibility in the kainic acid epilepsy model. Remarkably, we detected a high level of TRPM3 transcripts in neurons of the mouse and human LA, as well. Altogether, our results shed light on a previously unrecognized role of TRPM3 in the (patho)physiological function of the LA.

2 | METHODS

2.1 | Experimental animals

We used male C57BL/6N (wild type [WT]) and *Trpm3* knock-out (hereafter *Trpm3*^{-/-}) mice for our experiments. WT mice were obtained from the in-house breeding colony of the Animal Facility of the Preclinical Research Center, University of Pécs. This colony was originally

established from mice purchased from Charles River Laboratories. *Trpm3*^{-/-} mice were obtained from collaboration partners (KU Leuven, Belgium). Female mice were not used in the study because of the known sex differences in status epilepticus (SE) induction and mortality (Scharfman & MacLusky, 2014). The *Trpm3*^{-/-} mouse line generated earlier (Vriens et al., 2011) was bred in homozygous form and after every fifth generation they were backcrossed into the C57BL/6N background used as WT. The following primer pairs were used for genotyping: - WT and knock out (KO) reverse: 5'-cct caa gac tcc caa tcc aag-3'; KO forward: 5'-gca gcg cat cgc ctt cta tc-3'; WT forward: 5'-ctg tgg ctg cca agc acc gtg ac-3'. Mice were kept in standard mouse cages (size: 332 × 160 × 133 mm, 3–5 mouse/cage) with a 12-h light/12-h dark cycle and allowed water and standard rodent food (SAFE 132-Innovo) ad libitum. The animal housing facility is maintained at a temperature of 22 ± 2°C and a moderate relative humidity (55 ± 10%), regulated by a central automated system. Sample size estimation was performed using GPower 3.1 software. Assuming a normal distribution, an alpha level of 0.05 and 95% statistical power to generate groups of equal size.

All animal experiments were performed according to the European Community Council Directive and approved by the local ethics committee (Ethics Committee on Animal Research of Pécs, Hungary, approval No.: BA02/2000-17/2025). In addition, all animal studies are reported in compliance with the ARRIVE guidelines (Percie du Sert et al., 2020) and with the recommendations made by the British Journal of Pharmacology (Lilley et al., 2020).

2.2 | Behaviour tests

Behavioural experiments were performed using the same cohort of animals (WT: *n* = 10, *Trpm3*^{-/-}: *n* = 11, 10–12 weeks old). All mice underwent the complete behavioural test battery. Tests were conducted on separate days with 24-h intervals between each test, except the fear conditioning test, where we examined the behaviour of mice on two consecutive days. The order of behavioural tests was arranged to minimize the impact of stress and avoid interference with subsequent learning-based paradigms. Animals were brought into the experimental room 1 h before each test for acclimatization. The tests were carried out on different days. Ethovision software (Noldus Information Technology) was used to evaluate behaviour.

2.2.1 | Open field test (OFT)

Mice were placed individually in a predetermined corner of a 45 × 45 cm open-top chamber for 10 min, and their locomotor activity and anxiety-related behaviour were recorded. Activity was characterized by total distance travelled (cm). Anxiety level was characterized by the time spent in the centre area (22.5 × 22.5 cm) divided by the total testing time (10 min). This parameter is considered to inversely proportional to the anxiety level.

2.2.2 | Elevated plus maze test (EPM)

Mice were allowed to freely explore the two open arms (50 cm × 10 cm) and two closed arms (50 × 10 × 40 cm) of the cross shaped maze, which was located 1 m above the floor. At the beginning of the test, mice were placed in the middle of the intersection of the four arms. The number of entries and the time spent in the open arms were measured during the 10-min test period (Ethovision software, Noldus Information Technology).

2.2.3 | Light-dark box test (LDB)

During light-dark box, the free movement of the animals is monitored in a custom made two-compartment (40 × 40 × 40 cm) wooden box containing an open, light and a closed, dark chamber connected via a small opening. Mice were placed in the centre of the light chamber and were allowed to freely explore both chambers through the small opening for 10 min of the test period. During the measurement, time spent on the light side and the number of entries to the light chamber were recorded (Ethovision software, Noldus Information Technology).

2.2.4 | Fear conditioning test

Day 1: Training

The mice were placed in a fear conditioning apparatus (Ugo Basile) with the light and the fans turned on. The test started with a 5-min baseline period of video observation without sound or electric shock. Then, 30-s-long, 80-dB sound signals combined with a 2-s-long, 0.45-mA electric shock were applied three times. The electric signals ended at the same time as the sound signals and 120-s intervals were set between repetitions. During testing, the freezing level (duration of freezing time/total testing time) was measured (ANY-maze program, Stoelting).

Day 2, morning: Context test

The setup was the same as used during the training. The mice were placed individually in the fear conditioning apparatus for 8 min. Neither electric shock nor sound effect was applied. During testing, the freezing level (duration of freezing time/total testing time) was measured (ANY-maze program, Stoelting).

Day 2, afternoon: Cued fear test

The cued fear test took place 5 h after the context test. Compared to the previous tests, the context was changed by altering the pattern of one of the side walls of the apparatus. After the 5-min baseline period, the mice hear a sound signal three times, as described in the training session, with 120-s intervals, during which the duration and number of freezing were measured. Electric shock was not presented during this part of the test (ANY-maze program, Stoelting). During testing, the freezing level (duration of freezing time/total testing time) was measured.

2.3 | Electric shock threshold determination

For threshold determination, we tested different current intensities from 0.05 to 0.45 mA (ramp: 0.05 mA) with the following setup of the apparatus. The mice (WT or *Trpm3*^{-/-}) were placed in the fear conditioning apparatus with the light and the fans turned on. The test started with a 1-min baseline period (no sound or electric shock). Then, it was followed with a 2-s-long electric shock. After a 30-s waiting period, the animals received another electric shock, which was 0.05 mA greater than the previous one. This procedure continued until 0.45 mA was reached. At the end of the test, the animals were returned to their home cage. Based on the recorded video the lowest current intensity, where the mice reacted with behaviour signs (jumping) were determined for each mouse.

2.4 | Implantation of electrodes for EEG measurement

Before surgery, we prepared the EEG electrodes containing two recordings (tungsten, 0.05 mm, insulated, GoodFellow), a reference and a ground (silver) wire. The wires were soldered to a custom-made six-pin connector. For the electrode implantation, mice were placed under deep anaesthesia by inhalation of oxygen-containing isoflurane (5% induction, 2% maintenance). The body temperature of mice were controlled with a heated plate. The head of the animal was placed in a stereotaxic frame, the hair on the top of the head was shaved and part of the skin was removed. The stereotaxic locations of the craniotomies for the electrodes were the following: coordinates of the right amygdala AP: -1.5 mm, ML: 3.0 mm and DV: -3.3 mm; coordinates of the left hippocampus: AP: -2.0 mm, ML: 1.00 mm and DV: -1.5.

First, using a dental drill, three craniotomies were made (each smaller than 1 mm in diameter) in accordance with the above-mentioned locations so that the EEG electrodes could be implanted. The fourth electrode (ground electrode) was placed under the skin.

To induce status epilepticus, 100 nl of kainic acid (KA, dose: 20 mM dissolved in sterile water) was injected into the right amygdala at the coordinates described above using a Hamilton syringe (25 G). After waiting for 2 min, the Hamilton syringe was slowly withdrawn from the craniotomy. Then, the previously prepared electrodes were implanted through the craniotomies into the amygdala and hippocampus and then secured to the skull surface using dental cement. Following the experiments, the condition of mice were monitored daily until the day of perfusion. During monitoring, changes in the following parameters are observed and scored: 1. external appearance, 2. food/fluid intake, 3. clinical signs (body temperature, circulation, respiration), baseline behaviour.

In a subset of experiments, either 300 nl KA (1 mM) or a mixture of 300 nl KA (1 mM) and [CIM0216](#) (250 μ M) were injected.

2.5 | Video-EEG monitoring

2.5.1 | Examination of acute status epilepticus

After injecting KA and implanting the electrodes, we waited until the animals wake up after the surgery, and then the mice subjected to video-EEG to monitor the seizures. For the video-EEG monitoring, mice were placed into a 25 $\times$ 25 cm transparent cage. For recording the EEG waves from the amygdala and hippocampus, a preamplifier was inserted into the six-pin connector via a multichannel commutator (Moflon Technology LTD, MC190), allowing the mice to freely explore and move throughout the entire cage. EEG signal was acquired at 1 kHz and band-pass filtered at 1.6–2000 Hz (Supertech BioAmp, AD Instruments PowerLab). The EEG monitoring was performed for 90 min. We recorded EEG waves with LabChart software (AD Instruments) and captured simultaneous behavioural activities/seizures with a webcam (Alcor AWC1080). At the end of the measurement, any further seizures were inhibited by injecting i.p. of 5 mg·kg⁻¹ [diazepam](#). In addition, 5 mg·kg⁻¹ s.c. [carprofen](#) (for 2 days) is used for pain relief and mice returned to their home cages. All mice in our study experienced status epilepticus after KA injection.

2.5.2 | Examination of chronic status epilepticus

Two to three weeks after the injection of KA, mice were again subjected to video-EEG monitoring, during which spontaneous seizures were recorded for 24 h.

2.6 | Slice preparation

In vitro patch clamp recordings were performed in acute coronal brain slices from C57BL/6N and *Trpm3*^{-/-} mice. In deep isoflurane anaesthesia, mice were decapitated, brain was quickly removed and coronal slices (300 μ m thick) were cut using a vibratome (Leica VT1200) in ice-cold external solution containing 93 mM NMDG, 2.5 mM KCl, 25 mM glucose, 20 mM Hepes, 1.2 mM NaH₂PO₄, 10 mM MgSO₄, 0.5 mM CaCl₂, 30 mM NaHCO₃, 5 mM L-ascorbate, 3 mM Napyruvate and 2 mM thiourea, bubbled with 95% O₂ and 5% CO₂. After an incubation period of 10 min at 34°C in the external solution, the slices were maintained at 20°C–22°C in ACSF containing 2.5 mM KCl, 10 mM glucose, 126 mM NaCl, 1.25 mM NaH₂PO₄, 2 mM MgCl₂, 2 mM CaCl₂ and 26 mM NaHCO₃ bubbled with 95% O₂ and 5% CO₂ until use. In some cases, the sections were immersed into fixative (4% paraformaldehyde in 0.1 M phosphate buffer) after recordings, for overnight fixation.

2.7 | *In vitro* electrophysiological recordings

Patch pipettes were pulled from borosilicate glass capillaries with filament (1.5 mm outer diameter and 1.1 mm inner diameter; Sutter

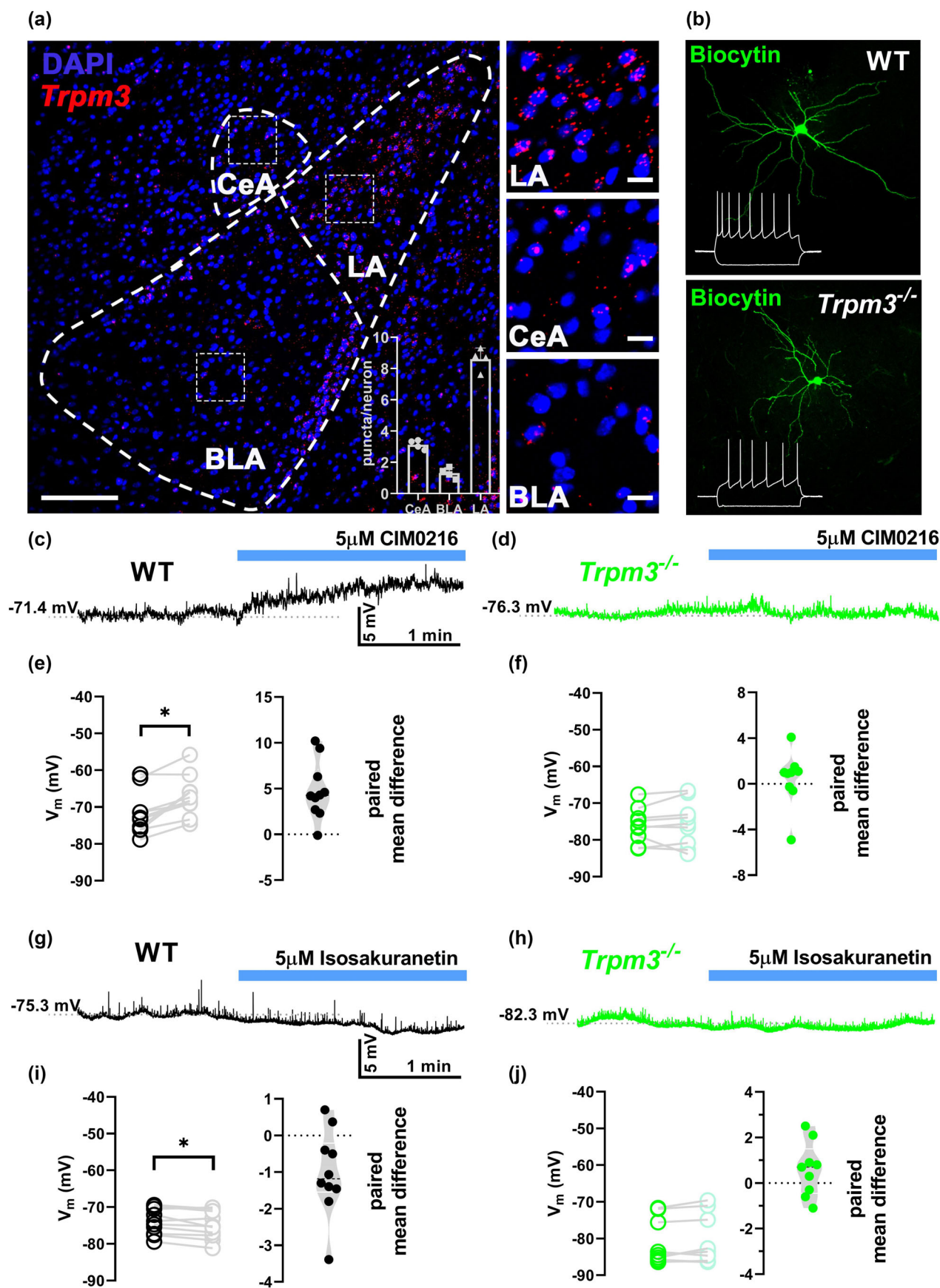


FIGURE 1 Legend on next page.

FIGURE 1 Exploratory data: *Trpm3* is present and functionally active in lateral amygdala excitatory neurons. (a) Representative confocal images of *Trpm3* (red) mRNA expression in the mouse amygdala. Inset shows averaged *Trpm3* puncta/cell. BLA, basolateral amygdala; CeA, central nucleus of the amygdala; LA, lateral nucleus of the amygdala. (b) Representative biocytin-filled neurons in the LA from WT and *Trpm3*^{-/-} mice. Insets show representative hyper- and depolarizing current steps. (c,d) Representative whole-cell membrane potential traces before and during 5 μ M CIM0216 application. (e,f) Summary plots of the effect of 5 μ M CIM0216 on membrane potential ($n = 10$ for WT and 9 for *Trpm3*^{-/-}). On the right side of the plots, the paired mean differences between conditions are shown. (g,h) Representative whole-cell membrane potential traces before and during 5 μ M Isosakuranetin application. (i,j) Summary plots of the effect of 5 μ M isosakuranetin on membrane potential ($n = 10$ for WT and 10 for *Trpm3*^{-/-}). On the right side of the plots, the paired mean differences between conditions are shown. Scale bar: 100 μ m and 10 μ m, *Each data point represents individual neurons.

Instruments, BF150-110-7.5HP) with a resistance of 2–3 M Ω . The recording pipette solution contained 10 mM KCl, 130 mM K-gluconate, 1.8 mM NaCl, 0.2 mM EGTA, 10 mM HEPES, 2 mM Na-ATP and 0.2% Biocytin, pH 7.3 adjusted with KOH; 290–300 mOsm. Whole-cell recordings were made with Axopatch 700B amplifier (with Clampex 10.7 and Axoclamp1.1, Molecular Devices) using an upright microscope (Nikon Eclipse FN1, with 40 $\times$, 0.8 NA water immersion objective lens) equipped with differential interference contrast (DIC) optics. DIC images were captured with an Andor Zyla 5.5 sCMOS camera. All recordings were performed at 33°C in ACSF bubbled with 95% O₂ and 5% CO₂. Cells with <20 M Ω access resistance (continuously monitored) were accepted for analysis. Signals were low-pass filtered at 5 kHz and digitized at 20 kHz (Digidata 1550B, Molecular Devices). *In vitro* data analysis was performed with the help of Clampfit 10.7 (Molecular Devices) and Prism 8 (Graph Pad). When it is indicated, 5 μ M CIM0216 (5,521) or 5 μ M isosakuranetin was applied into the bath solution.

2.8 | *In vitro* LTP induction

Excitatory postsynaptic potentials were recorded from identified LA neurons in current clamp mode. Basal synaptic responses were evoked by a brief pulse (1 ms) of constant current delivered by a stimulus isolation unit (Supertech Instruments, BioStim) every 30 s with bipolar electrodes. Stimulation electrodes were positioned at axonal bundles from the cortex. Synaptic strength was quantified as the peak amplitude of EPSPs. Baseline responses were collected with a stimulation intensity that yielded 30%–40% of the maximal response. Slices displaying unstable baseline recording were discarded. LTP was induced by four trains of high-frequency stimulation (HFS $\times$ 4, 100 Hz for 1 s per train; 10-s intervals).

2.9 | RNAscope in situ hybridization in mouse and human samples

Sections containing the LA from naïve C57BL/6 mice were used for RNAscope ISH. Human amygdala tissue blocks were coronally oriented and the sections (illustrated in Figure 6) were subjected to RNAscope ISH. The RNAscope staining was optimized for paraformaldehyde fixed tissue, as described earlier (Nemes et al., 2021). RNAscope experiments were performed based on the supplier instruction (ACD, Hayward, CA, USA) to visualize *Trpm3* (ACD; Cat. No.: 459911), *Vglut1* (ACD; Cat. No.:

501101-C2), *Vglut2* (ACD; Cat. No.: 319171-C3), *Gad1* (ACD; Cat. No.: 400951-C3), *TRPM3* (ACD; Cat. No.: 530591-C3), *VGLUT1* (ACD; Cat. No.: 415611) and *VGLUT2* (ACD; Cat. No.: 319171-C2). Mouse (ACD; Cat. No.: 320881) and human (ACD; Cat. No.: 320861) 3-plex positive control probes specific to *Polr2a* mRNA (fluorescein), *Ppib* mRNA (Cy3) and *Ubc* mRNA (cyanine 5, Cy5) and 3-plex negative (ACD; Cat. No.: 320871) control probes to bacterial *dabP* mRNA were tested on mouse and human amygdala. The 3-plex positive control probes produced clear signals in the human and mouse amygdala, while the negative control probes did not give any detectable RNAscope signal in the samples.

2.10 | Human amygdala sample

Subject studied in this project had no diagnosed neurological or psychiatric disorders or brain trauma, died from any not-brain-related cause and displayed no signs of brain neuropathologies.

After the brain removal, perfusion via cannula inserted into the internal carotid and vertebral arteries was performed within 2 h post-mortem. Perfusion was performed with 1.5 L of 0.33% heparin containing physiological saline for 30 min, then with 4–5 L of a Zamboni fixative solution containing 4% paraformaldehyde and 0.2% picric acid in phosphate buffer (pH 7.4) over a duration of 1.5–2 h. After perfusion, 0.5–1-cm-thick blocks were cut from the brain. Cortical tissue blocks of multiple Brodmann areas were collected for other studies. The mediobasal temporal lobe dorsal to the anterior lateral entorhinal cortex in the anterior parahippocampal gyrus and the rostral part of Brodmann area 35 in the rostral part of temporal fusiform (occipito-temporal) gyrus was isolated and post-fixed in Zamboni solution overnight (Maglóczy et al., 1997). This tissue block contained the amygdala including its lateral division as shown in the Allen Brain Atlas (Allen Institute for Brain Science) plate 112282763, structure 10367 (<https://atlas.brain-map.org/atlas?atlas=138322605#atlas=138322605&plate=112282763&structure=10367>) and in Figure 6.

The brain samples were sectioned for 30 μ m thickness using a Leica VT1000S vibratome (Leica Biosystems, Wetzlar, Germany); then, the sections were collected and stored in sterile PBS, containing sodium azide (0.01%) at 4°C. For long-term storage at –20°C, they were transferred into anti-freeze solution. The studies on perfused human brain samples received ethical approval from the Regional and Institutional Committee of Science and Research Ethics of the Scientific Council of Health (Hungary) (ETT TUKEB 15032/2019/EKU) and was conducted in adherence to the principles of the Declaration of Helsinki.

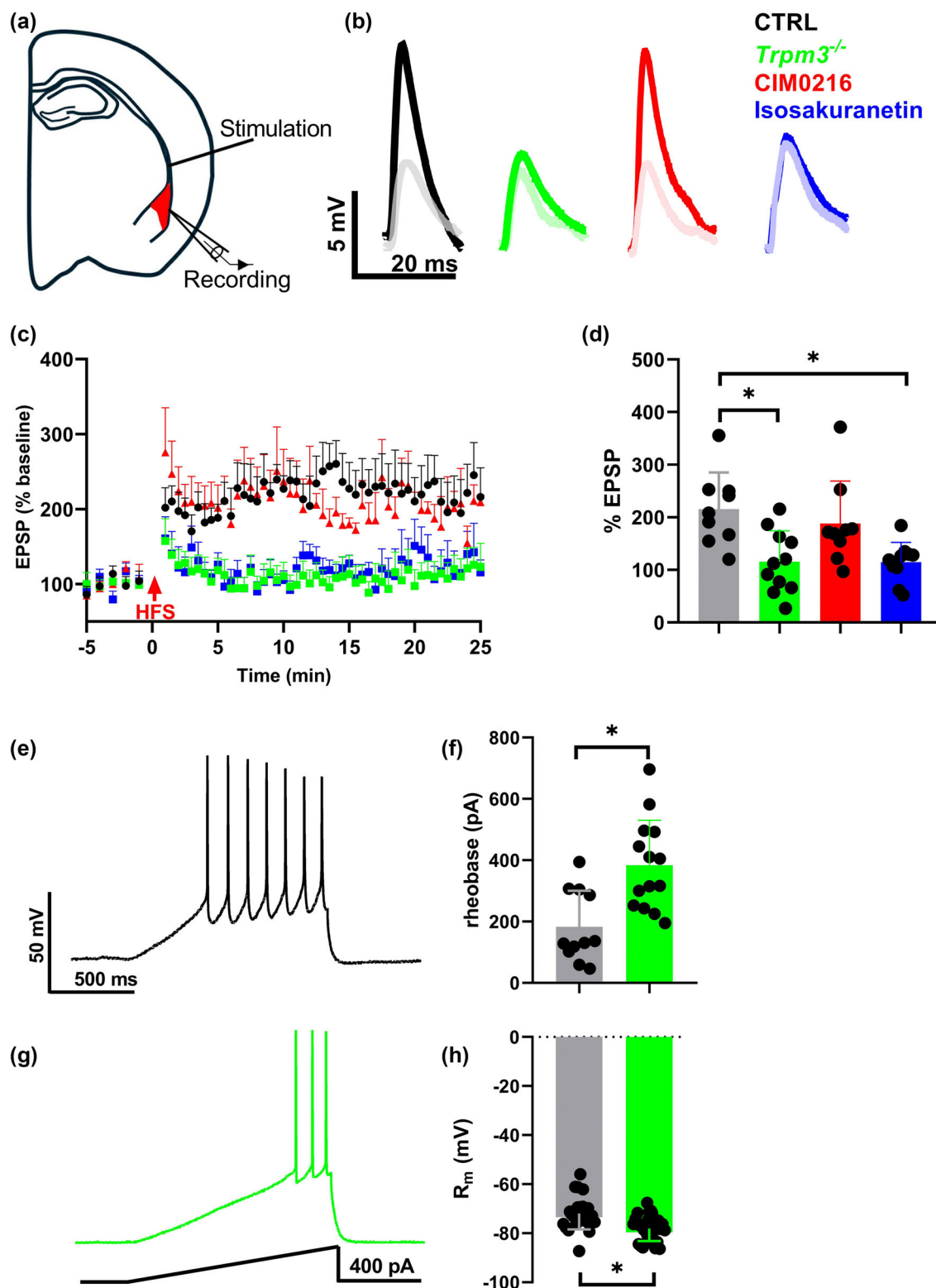


FIGURE 2 LTP is altered in the lateral nucleus of the amygdala (LA) neurons of *Trpm3*^{-/-} mice. (a) In the experimental configuration, synaptic responses were measured for cortex → LA neuron synapses. (b) Representative EPSP traces before (light colour) and after (dark colour) high-frequency stimulation (HFS) control (CTRL; WT, black), *Trpm3*^{-/-} (green), CIM0216 (WT) (red) and isosakuranetin (WT) (blue). (c) Averaged synaptic responses (normalized to baseline) plotted against time. (d) Exploratory data showing the LTP magnitude after HFS in CTRL (black) and *Trpm3*^{-/-} (green) neurons and during CIM0216 (red) or isosakuranetin (blue) application (*n* = 9 for CTRL, 11 for *Trpm3*^{-/-}, 8 for CIM0216 and 10 for isosakuranetin). (e,g) Representative voltage responses to a depolarizing ramp current recorded in LA neurons from WT (black) and *Trpm3*^{-/-} (green) mice. (f,h) Statistics showing rheobase (*n* = 11 for WT and 14 for *Trpm3*^{-/-}) and resting membrane potential values (*n* = 26 for WT and 30 for *Trpm3*^{-/-}) of WT and *Trpm3*^{-/-} LA neurons. **P* < 0.05, one-way ANOVA and unpaired *t* test. Each data point represents an individual neuron.

2.11 | Data and statistical analysis

The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology (Curtis et al., 2025). Statistical analyses were performed with Prism 8 (Graph Pad). Data are represented as mean $\pm$ SD. All statistical analyses were only performed on data sets where the group size is at least $n = 5$. Statistical analysis was performed using independent values (i.e. not treating technical replicates as independent values). No outlier data points were excluded. For experiments where this threshold was not met, results must be described as 'exploratory' or 'preliminary' and no inferential statistics can be applied. The normality of samples was tested with Shapiro–Wilk test. Normally distributed samples were compared with two sample or paired sample Student's *t* test; non-normally distributed data were compared with Mann–Whitney test. In case of ANOVA, post-hoc tests were conducted only if *F* was significant and there was no variance inhomogeneity. A *P*-value of less than 0.05 was considered statistically significant. For spike analysis, we first determined the standard deviation (SD) of the signal intensity for each recording from a spike free 'baseline' period at the beginning of the recordings using LabChart software (AD Instruments). Spikes were considered as fast events (>10 Hz) with amplitude higher than 7 SDs of the given recording. Seizure was considered as train of spikes with a frequency of at least 0.5 Hz and a duration of at least 5 s. Seizures were considered status epilepticus if appeared in both recording electrodes and persisted ≥ 5 min (Mundrucz et al., 2023).

The analysis of the behaviour tests and the EEG experiments were carried out blind to the phenotype or to the drug application.

For quantification of *Trpm3* puncta, a standardized rectangular region of interest (ROI) was randomly placed within the central nucleus of the amygdala (CeA), basolateral amygdala (BLA) or LA. *Trpm3* mRNA puncta (red) located in close proximity to all DAPI-stained nuclei (blue) within the selected area were counted. The average number of *Trpm3* puncta per cell was then calculated for each brain region in each animal.

2.12 | Materials

The following drugs were obtained from Sigma-Aldrich (Germany), kainic acid (KA; 420318), glucose, NaCl, NaH_2PO_4 , MgCl_2 , CaCl_2 , NaHCO_3 , K-gluconate, EGTA, HEPES, Na-ATP, Biocytin, KOH, NMDG, MgSO_4 , L-ascorbate, Na-pyruvate, paraformaldehyde, heparin, picric acid, sodium azide, DAPI, Isoflurane was obtained from CP Pharma (Germany), CIM0216 (5521) were from Tocris Bioscience (United Kingdom) and isosakuranetin (HY-N213) from MedChemExpress (USA).

Details of other materials and suppliers are provided in the specific sections.

2.13 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY <http://www.guidetopharmacology.org>

and are permanently archived in the Concise Guide to PHARMACOLOGY 2025/2026 (Alexander et al., 2025).

3 | RESULTS

3.1 | Exploratory Data: *Trpm3* is expressed in the mouse lateral nucleus of the amygdala (LA)

To examine the expression of *Trpm3* in the mouse brain, we used the ultrasensitive RNAscope in situ hybridization technology since commercially available antibodies are poorly selective for TRPM3. Interestingly, among few other brain regions, we have found abundant expression of *Trpm3* in the amygdala. Investigating the different anatomical subdivisions of the amygdala clearly the LA displayed the highest expression while expression in the basolateral (BLA) and central nucleus of the amygdala (CeA) was relatively low (average number of *Trpm3* puncta per cell: CeA = 3.13 ± 0.29 , BLA = 1.31 ± 0.33 , LA = 8.6 ± 0.71 , from 4 mice) (Figure 1a). To clarify which cell type in the LA is expressing *Trpm3*, we counter stained with molecular markers and found that *Trpm3* is mainly co-expressed with the excitatory neuronal markers vesicular glutamate transporter 1 (VGLUT1) and 2 (VGLUT2) while it is mostly absent in the glutamate decarboxylase 1 (GAD1) positive inhibitory interneurons (Figure S1a–c). Consequently, our histological studies clearly showed that *Trpm3* is highly expressed in the excitatory neurons of the LA.

3.2 | Exploratory Data: TRPM3 contributes to the electrophysiological properties of LA excitatory neurons

To test the functional presence of TRPM3 in the LA neurons, we performed whole-cell patch clamp recordings in acute coronal brain slices containing the amygdala. Randomly selected biocytin-filled neurons were visualized for post hoc anatomical identification (Figure 1b). Inhibitory and excitatory neurons were identified based on their action potential parameters (AP half width) and only the excitatory neurons were used in the statistical analysis. In order to test the activity of TRPM3 in LA neurons, we applied the potent agonist CIM0216 (Held, Kichko, et al., 2015) ($5 \mu\text{M}$) on WT and *Trpm3*^{−/−} neurons. In WT neurons, activation of TRPM3 resulted in membrane depolarization (control = -71.86 ± 5.88 mV, CIM0216 = -67.07 ± 5.47 mV), while in case of the *Trpm3*^{−/−} neurons, CIM0216 application had no effect on the resting membrane potential (control = -76.01 ± 4.79 mV, CIM0216 = -75.59 ± 6.18 mV, $n = 10$ from 4 mice for WT and 9 from 3 mice for *Trpm3*^{−/−}; Figure 1c–f). Interestingly, in similar experimental settings, application of the TRPM3 antagonist isosakuranetin resulted in membrane hyperpolarization in WT neurons (control = -74.01 ± 3.5 mV, Iso. = -75.03 ± 3.78 mV); in contrast, no change in the resting membrane potential has been observed in case of the *Trpm3*^{−/−} neurons (control = -81.2 ± 6.25 mV, Iso. = -80.61 ± 6.81 mV, $n = 10$ from 4 mice for WT and 10 from 3 mice for *Trpm3*^{−/−}; Figure 1g–j).

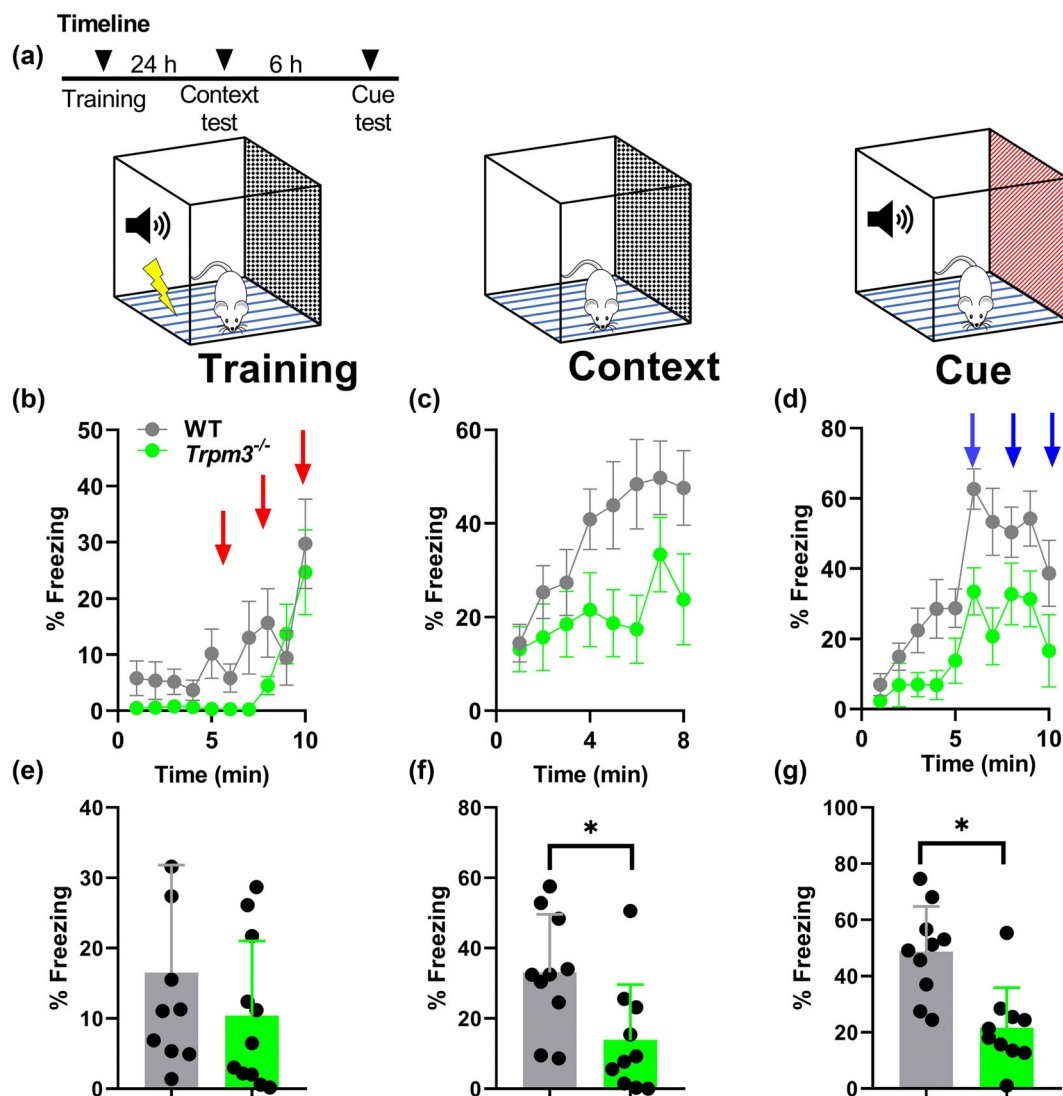


FIGURE 3 *Trpm3*^{-/-} mice display reduced fear memory formation. (a) Cartoons showing experimental design and timeline. During 'training', 90x, and after a 5-min habituation period, three 30-s-long sound signals terminated by a 2-s-long foot shock were delivered. Each sound signal + foot shock session was separated by a 120-s interval. During 'context' test, 24 h after training, mice were placed into the same environment and monitored for 8 min; no sound or shock was presented. During 'cue' test, 30 h after training, mice were placed into a different environment, and after a 5-min habituation, three 30-s-long sound signals not combined with foot shocks were delivered with 120-s intervals. (b-d) Freezing levels during 'training', 'context' and 'cue' tests in WT and *Trpm3*^{-/-} mice. (e-g) Statistics showing the total freezing levels during the 'training', the 'context' and the 'cue' test (n = 10 for WT and 10 for *Trpm3*^{-/-}). *P < 0.05, unpaired t test. Data are presented as mean ± SD (b-d). Each data point represents an individual animal (e,g). Red arrows: sound + electric signal, blue arrows: only sound signal.

Altogether, these data indicate that TRPM3 is functionally active in LA excitatory neurons since its activation leads to membrane depolarization, while its inhibition to membrane hyperpolarization. Of note, our experiments with isosakuranetin suggest that a fraction of TRPM3 in LA neurons are most likely constitutively active therefore it may contribute to the resting membrane potential of LA neurons.

3.3 | TRPM3 deletion impaired LTP at LA synapses

LA plays an important role in fear memory formation which thought to comprise a classic Hebbian mechanism in the form of LTP in the

LA (Trent et al., 2025). Therefore, in the next set of experiments, we tested whether deletion of TRPM3 has any effect on the plasticity of cortex → LA synapses. To assess synaptic plasticity, we applied high-frequency stimulation (HFS) to cortical inputs and recorded excitatory postsynaptic potentials (EPSPs) in LA excitatory neurons (Figure 2a,b). In WT slices, HFS induced a robust increase in EPSP amplitude as it was described earlier (Rogan et al., 1997); however, LTP was strikingly reduced in case of *Trpm3*^{-/-} slices (WT = 215.6 ± 69.51%, *Trpm3*^{-/-} = 115.5 ± 58.72%, n = 9 from 4 mice for WT and 11 from 4 mice for *Trpm3*^{-/-}; exploratory data). Furthermore, application of isosakuranetin in WT slices to block TRPM3 resulted in reduced LTP (isosakuranetin = 114.7 ± 37.61%, n = 10 from 4 mice; exploratory data). Interestingly, activation of TRPM3 using

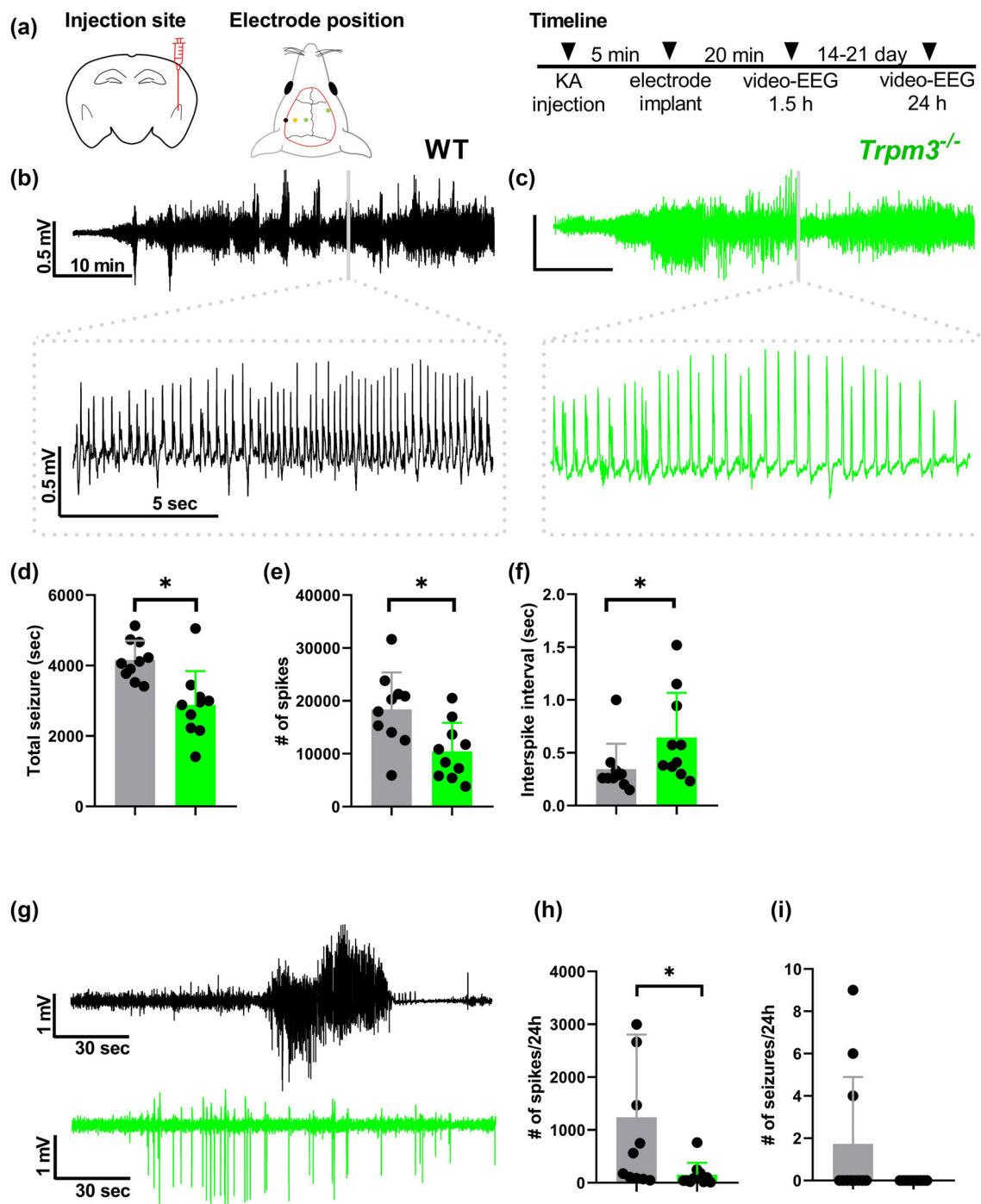


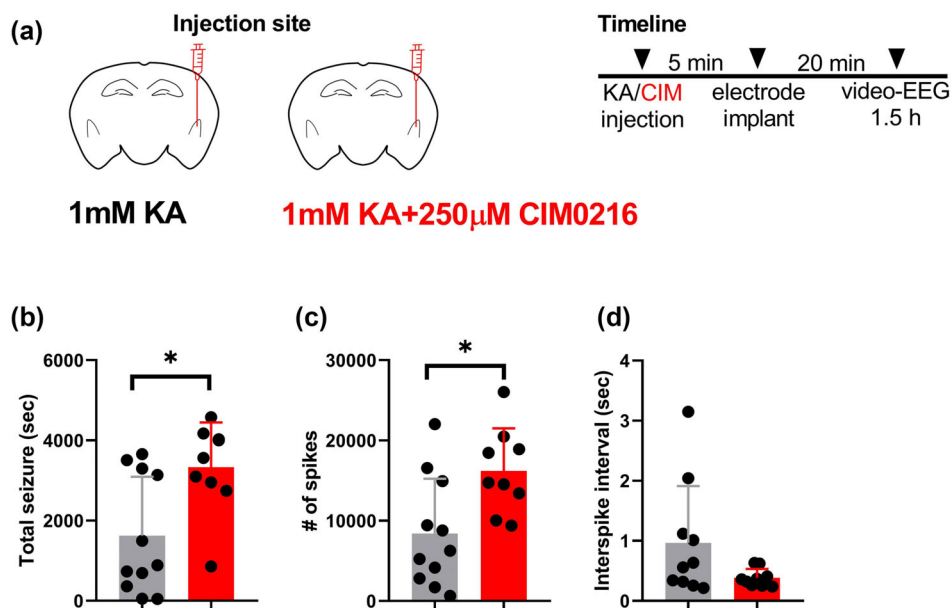
FIGURE 4 *Trpm3*^{-/-} mice display decreased seizure susceptibility compared to WT mice. (a) Left: Cartoons showing the injection site and the electrode position (green: right lateral nucleus of the amygdala (LA) and left hippocampus, yellow: reference electrode, black: ground electrode). Right: Timeline of the experiment. (b,c) Representative EEG recordings during status epilepticus (SE; upper panel) and a magnified view (lower panel) in control (black) and *Trpm3*^{-/-} (green) mice after KA injection into the lateral amygdala. Statistics showing the (d) total time spent with seizure (e) number of spikes and the (f) interspike interval during the 90-min recording period after KA injection ($n = 10$ for WT and 10 for *Trpm3*^{-/-}). (g) Representative EEG recordings of spontaneous spiking activity in control (black) and *Trpm3*^{-/-} (green) mice 3 weeks after KA injection into the lateral amygdala. Statistics showing the (h) number of spikes and the (i) number of seizures during a 24-h recording period $n = 10$ for WT and 10 for *Trpm3*^{-/-}. * $P < 0.05$, Mann-Whitney and unpaired t test. Data are presented as mean $\pm$ SD. Each data point represents an individual animal.

CIM0216 did not affected LTP (CIM0216 = $188.1 \pm 81.01\%$, $n = 8$ from 4 mice; exploratory data) (Figure 2c,d). These results suggest that TRPM3 is required for normal synaptic plasticity in the excitatory neurons of the LA.

To further explore the effect of TRPM3 on the excitability of LA neurons we applied a 1-s current ramp on WT and *Trpm3*^{-/-} neurons. The minimal current needed to elicit an action potential (rheobase) was significantly higher in *Trpm3*^{-/-} neurons compared to WT ($WT = 182.7$

FIGURE 5 TRPM3 activation increases seizure susceptibility.

(a) Left: Cartoons showing the injection sites and drug concentrations right: timeline of the experiment. Statistics showing the (b) total time spent with seizure (c) number of spikes and the (d) interspike interval during the 90-min recording period after KA (black) or KA + CIM0216 (red) injection ($n = 11$ for KA and 9 for KA + CIM0216). * $P < 0.05$, unpaired t test. Data are presented as mean $\pm$ SD. Each data point represents individual animals.



± 117.5 pA, $Trpm3^{-/-}$ = 383.7 ± 146.8 pA, $n = 11$ from 5 mice for WT and 14 from 5 mice for $Trpm3^{-/-}$ (Figure 2e,f). Application of CIM0216 or isosakuranetin increased or decreased the resting membrane potential of these neurons, respectively (see Figure 1). Accordingly, TRPM3 activation resulted in a decrease (control [CTRL] = 316.6 ± 124.4 pA, CIM0216 = 270.3 ± 127.4 pA, $n = 6$ from 3 mice, exploratory data), while inhibition resulted in a significant increase of rheobase (control = 363.7 ± 130.1 pA, isosakuranetin = 409.9 ± 123.8 pA; $n = 10$ from 5 mice) in WT neurons (no separate graph shown).

Furthermore, resting membrane potential was significantly more hyperpolarized in $Trpm3^{-/-}$ neurons (WT = -71.94 ± 6.5 mV, $Trpm3^{-/-}$ = -78.14 ± 5.07 mV, $n = 26$ from 12 mice for WT and 30 from 8 mice for $Trpm3^{-/-}$), indicating that TRPM3 activity can modulate resting membrane potential and therefore excitability in LA neurons (Figure 2h). Of note, other electrophysiological properties including input resistance (WT = 120 ± 52.46 M Ω , $Trpm3^{-/-}$ = 95.54 ± 49.28 M Ω , $n = 26$ from 12 mice for WT and 28 from 8 mice for $Trpm3^{-/-}$), membrane time constant (WT = 26.74 ± 11.96 ms, $Trpm3^{-/-}$ = 23.59 ± 11.42 ms, $n = 26$ from 12 mice for WT and 28 from 8 mice for $Trpm3^{-/-}$) and cell capacitance (WT = 84.32 ± 29.38 pF, $Trpm3^{-/-}$ = 96.95 ± 32.62 pF, $n = 26$ from 12 mice for WT and 28 from 8 mice for $Trpm3^{-/-}$) were not significantly different between the groups (Figure S3a-c).

3.4 | $Trpm3^{-/-}$ mice showed unchanged anxiety-related behaviour

Next, we sought to investigate the possible role of TRPM3 in LA-related behavioural tests. In general, the amygdala is involved in the processing of emotions including fear, anxiety and aggression, while LA has been

also defined as the place of associative fear learning (Sears et al., 2014). Therefore, WT and $Trpm3^{-/-}$ mice were investigated in a variety of anxiety-related behaviour tests to see whether TRPM3 has any physiological relevance in the neural coding of anxiety.

First, we examined the effect of TRPM3 on anxiety-related behaviour using elevated plus maze (EPM) test. Each mouse was given the choice to spend time in the unprotected open arms or in the protected closed arms of the maze. In this test, we detected no differences between WT and $Trpm3^{-/-}$ mice in the time spent in the open arm (WT = 9.962 ± 3.76 s, $Trpm3^{-/-}$ = 9.972 ± 6.47 s, $P = 0.9967$, unpaired t test). However, $Trpm3^{-/-}$ mice walked significantly longer distance in elevated plus maze, compared to WT (WT = 2471 ± 521.4 cm, $Trpm3^{-/-}$ = 2964 ± 545.5 cm, $n = 10$ for WT and 11 for $Trpm3^{-/-}$) (Figure S2a-c).

Next, we exposed the animals to a novel environment in the light-dark box test with protected (dark side) and unprotected (light side) areas. Similarly to elevated plus maze, both groups spent the same time in the unprotected area (WT = 202.3 ± 44.49 s, $Trpm3^{-/-}$ = 212.4 ± 30.23 s, $P = 0.5468$, unpaired t test), but the $Trpm3^{-/-}$ mice entered this zone significantly more frequently than their WT counterparts (WT = 26.5 ± 5.19 , $Trpm3^{-/-}$ = 32.36 ± 6.05 , $n = 10$ for WT and 11 for $Trpm3^{-/-}$) (Figure S2d-f).

Last, we tested both strains in open field test (OFT) test, where one can test anxiety levels based on the time spent in the central zone of the apparatus. Again, both genotypes spent the same amount of time in the central zone (WT = 18.56 ± 6.34 s, $Trpm3^{-/-}$ = 13.38 ± 6.95 s) and $Trpm3^{-/-}$ mice travelled significantly longer distance in the maze, compared to WT animals (WT = 3853 ± 442 cm, $Trpm3^{-/-}$ = $5352 \pm 1,129$ cm, $n = 10$ for WT and 11 for $Trpm3^{-/-}$) (Figure S2g-i).

Based on these anxiety-related tests, $Trpm3^{-/-}$ mice did not show altered anxiety level in under basal conditions. However, they display significantly increased locomotor activity.

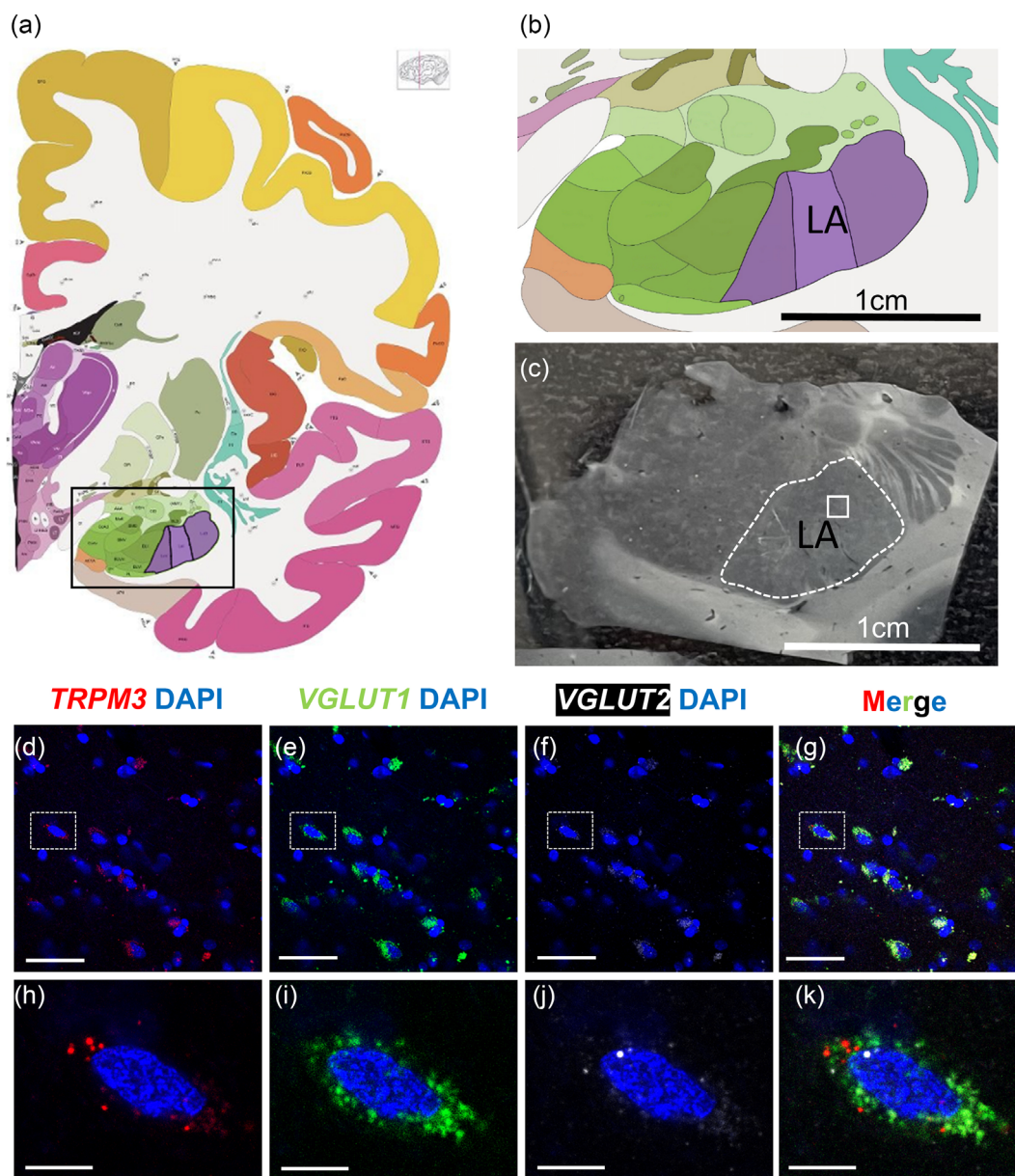


FIGURE 6 *TRPM3* mRNA is expressed in glutamatergic cells of the human lateral amygdala. (a) Panel was adapted from Allen Human Brain Reference Atlas (Allen Institute for Brain Science) Plate 112282763, structure 10367, and shows a coronal section of the human brain at the plane of the amygdaloid nucleus (boxed area). Panel (b) illustrates the boxed area in (a), with boundaries of subdivisions within the amygdaloid nucleus. The purple highlighted area corresponds to the lateral nucleus of amygdala (LA). (c) Photograph of a native free-floating coronal section from a brain tissue block in the plane illustrated by the reference atlas; LA is indicated by dashed borderline. This representative section was subjected to RNAscope in situ hybridization for *TRPM3*, *VGLUT1* and *VGLUT2*, and the boxed area indicates the histological images (d–k). Red colour represents *TRPM3* mRNA, green illustrates *VGLUT1* mRNA, and white indicates *VGLUT2* mRNA signal. All channels are superimposed in images (g,k) with nuclear counterstaining by 4',6-diamidino-2-phenylindole (DAPI, blue) to illustrate the co-expression of these RNAs in the human lateral amygdala. One representative glutamatergic cell indicated by the dashed bordered box is depicted in panels h–k. Scale bars: d–g: 100 μ m, h–k: 50 μ m, l–o: 10 μ m.

3.5 | *Trpm3*^{−/−} mice display reduced fear memory formation in fear conditioning test

Pavlovian fear conditioning test has become an important model for investigating the role of LA neurons in fear-related learning and memory processes (Maren, 2001). We tested both the context-dependent and

independent fear learning. In the fear conditioning test, we exposed mice to a tone as conditioned stimulus (CS) and paired with moderate foot shock as an aversive unconditioned stimulus (US) which allowed us to test fear memory formation (Figure 3a). *Trpm3*^{−/−} and WT mice exhibited comparable freezing levels during training sessions (WT = 16.53 $\pm$ 15.27 s, *Trpm3*^{−/−} = 10.41 $\pm$ 10.59 s), indicating that the foot shock

represented a comparable aversive stimulus for both genotypes (Figure 3b,e). However, when the same context was presented 24 h after the training session, *Trpm3*^{-/-} mice displayed significantly lower levels of freezing than control mice (WT = 33.09 ± 16.52 s, *Trpm3*^{-/-} = 13.91 ± 15.74 s) (Figure 3c,f). Additionally, during cued testing (when context is changed but the same tone is presented as on training) 30 h after training session, *Trpm3*^{-/-} mice displayed significantly lower levels of freezing than control mice (WT = 48.75 ± 16.06 s, *Trpm3*^{-/-} = 21.59 ± 14.25 s, n = 10 for WT and 10 for *Trpm3*^{-/-}) (Figure 3d,g).

Because of the well-established role of TRPM3 in peripheral pain sensation (Vriens et al., 2011), we tested their threshold for the electric foot shock used in the tests. Importantly, WT and *Trpm3*^{-/-} mice displayed similar pain threshold regarding the electric foot shock (WT = 0.16 ± 0.04; *Trpm3*^{-/-} = 0.17 ± 0.04; see methods), highlighting that the observed alteration in fear memory formation in *Trpm3*^{-/-} mice does not related to different pain threshold.

3.6 | *Trpm3*^{-/-} mice display decreased seizure susceptibility compared to WT mice

Since the amygdala is frequently the focus of epileptic seizures (Aroniadou-Anderjaska et al., 2008) and the recently described human gain-of-function mutations also leads to seizures (Dyment et al., 2019), in a next set of experiments, we intended to explore the involvement of TRPM3 in experimental epilepsy. First, KA was injected into the right LA of WT and *Trpm3*^{-/-} mice to induce status epilepticus and then electrodes were inserted into the left dorsal hippocampus and into the right LA (Figure 4a). Immediately after the surgery, mice were connected to a video-EEG recording system to monitor seizures (Figure 4b,c). These experiments revealed that *Trpm3*^{-/-} mice displayed significantly lower spike numbers (WT = 18,370 ± 7007, *Trpm3*^{-/-} = 10,446 ± 5393) and therefore longer interspike interval (WT = 0.346 ± 0.24 s, *Trpm3*^{-/-} = 0.6453 ± 0.423) during the recording session (Figure 4e,f). Furthermore, total time spent with seizure during the recording period was also significantly shorter in case of the *Trpm3*^{-/-} mice (WT = 4156 ± 552 s, *Trpm3*^{-/-} = 2,885 ± 959.3 s) n = 10 for WT and 10 for *Trpm3*^{-/-} (Figure 4d).

Next, we defined whether deletion of TRPM3 also effects the occurrence and severity of chronic seizures. Previously KA injected WT and *Trpm3*^{-/-} mice were used for 24-h EEG recordings 2–3 weeks after status epilepticus. In these experiments, three out of 10 WT while zero out of 10 *Trpm3*^{-/-} mice experienced spontaneous seizures (WT = 1.27 ± 3.16, *Trpm3*^{-/-} = 0.0 ± 0.0). Notably, spike numbers during the 24-h recording session (ictal and inter-ictal spikes) were significantly lower in case of the *Trpm3*^{-/-} mice compared to WT (WT = 1240 ± 1564, *Trpm3*^{-/-} = 150.8 ± 228.5; n = 10 for WT and 10 for *Trpm3*^{-/-}; Figure 4h,i).

To further investigate the role of TRPM3 in seizure susceptibility, we intended to activate TRPM3 in the LA neurons to see whether this leads to detectable seizures. Interestingly, injection of the selective TRPM3 agonist CIM0216 (250 μM) directly into the LA of WT mice did not result in electrographic seizures (data not shown). Therefore,

in a next set of experiments, we injected WT mice with either a low concentration of KA (1 mM) or with a mixture of KA (1 mM) and CIM0216 (250 μM) (Figure 5a). As we expected, the low concentration of KA resulted in detectable but less severe seizures compared to our original protocol (see Figure 4). However, injection of CIM0216 together with KA resulted in significantly increased seizure parameters, including the number of spikes (KA = 1625 ± 1469, KA + CIM = 3332 ± 1113) and the total seizure time (KA = 8417 ± 6820, KA + CIM = 16,222 ± 5301) (Figure 5b–d). The interspike interval was decreased upon injection of CIM0216 together with KA; however, it was not significantly different (KA = 0.96 ± 0.94 s, KA + CIM = 0.38 ± 0.14 s, n = 11 for KA and 9 for KA + CIM0216).

3.7 | TRPM3 is expressed in the human LA

The recently described human *TRPM3* mutations clearly suggest the functional presence of the channel in the human brain; however, our present knowledge is highly limited about the cell type specific expression pattern of TRPM3 in human neurons (Roelens et al., 2024). Therefore, in the next set of experiments, we performed RNAscope *in situ* hybridization on brain samples containing the amygdala from perfused post-mortem human brain. These experiments clearly showed high expression of *TRPM3* in the *VGLUT1* and *VGLUT2* positive excitatory neurons of the lateral amygdala (Figure 6 and Figure S4).

4 | DISCUSSION

Here, we report that *Trpm3* is expressed in the excitatory neurons of the lateral nucleus of the amygdala (LA), a structure that plays an important role in the neural coding of fear and is affected by brain pathologies including autism and temporal lobe epilepsy (Aroniadou-Anderjaska et al., 2008; Trent et al., 2025). At the cellular level, we demonstrated that pharmacological activation or inhibition of the channel leads to membrane depolarization or hyperpolarization, respectively, indicating the functional presence of TRPM3 on LA neurons. Interestingly, TRPM3 deletion resulted in impaired LTP formation on LA synapses *in vitro*, leading to reduced fear memory formation *in vivo*. Furthermore, we showed that *Trpm3*^{-/-} mice are characterized by less severe seizure parameters in the kainic acid epilepsy model, while activation of the channel *in vivo* can worsen seizure outcomes. Finally, we provided the first direct evidence for the expression of TRPM3 on human excitatory LA neurons.

Recently, several *de novo* mutations in the *TRPM3* gene were identified in patients with developmental and epileptic encephalopathy resulting in a gain-of-function of the channel, suggesting a pivotal role of TRPM3 in neurons of the brain (Dyment et al., 2019; Gauthier et al., 2021; Kang et al., 2021; Lines et al., 2022; Van Hoeymissen et al., 2020; Zhao et al., 2020). To clarify by what means the characterized gain-of-function mutation of TRPM3 can lead to neurological symptoms, we must answer two main questions: where and how?

Where TRPM3 is expressed in the brain and how TRPM3 activity modulates those neurons and the surrounding circuit.

The interesting but so far unresolved question regarding TRPM3 in the brain is the cell type specific expression pattern and the (patho) physiological function of the channel in neurons. The expression of TRPM3 in the CNS has been proposed since the first description of the channel (Grimm et al., 2003), but functional characterization of TRPM3 in CNS neurons is still lacking.

Interestingly, a publicly available single-cell RNAseq database suggests high expression of TRPM3 in the human amygdala especially in clusters (*LA_RORB*, *LA_ZBTB20*) linked to the lateral division (Totty et al., 2025). Our RNAscope experiments further supported the expression of TRPM3 in the VGLUT1–2 positive excitatory LA neurons both in mice and human. To our best knowledge, this is the first direct evidence showing the presence of TRPM3 in the amygdala which raises the question - “What is the function of TRPM3 in this brain region?” One can speculate that TRPM3 as a non-selective cation channel may act as an important regulator of cellular excitability. Indeed, our results showed that pharmacological activation of TRPM3 resulted in a significant depolarization of the resting membrane potential, pointing to a functional cation channel. In contrast, pharmacological blockade of TRPM3 resulted in membrane hyperpolarization, indicating that the channel is at least partially active in resting conditions. A possible explanation for the basal activity of TRPM3 in LA neurons is activation by heat (33°C in our *in vitro* experiments). Given the temperature sensitivity of TRPM3 and evidence that the channel can be partially activated at temperatures in this range (Held, Voets, & Vriens, 2015), it is possible that temperature-dependent TRPM3 activity contributes to the regulation of neuronal excitability under physiological conditions.

Our results also demonstrated that besides the partial heat activation, TRPM3 can be further activated by chemical ligands suggesting the role of an endogenous agonist, like pregnenolone sulphate (Wagner et al., 2008). Unfortunately, the source and exact mechanism of action of such endogenous agonist are still unknown.

Amygdala is a cluster of brain nuclei involved in the processing of emotional memory including fear, anxiety and aggression. Within the amygdala, the LA has been defined as the main input structure and the place of associative fear learning (Sears et al., 2014). Interestingly, the lack of TRPM3 not effected the basal anxiety level in our behavioural tests; however, *Trpm3*^{−/−} mice displayed altered fear memory formation both in context-dependent and independent tests. It must be noted however that in the context-dependent tests, we observed a somewhat different temporal freezing profile from that reported in previous studies, with a gradually increasing freezing levels in the first half of the test. This might arise from the relatively long baseline exploration period during the training session. Moreover, contextual fear memory depends also on the hippocampus, which is required for the encoding and retrieval of contextual representations (Phillips & LeDoux, 1992). Thus, although our findings identify a role for TRPM3 in the LA, we cannot exclude the possibility that TRPM3-dependent mechanisms in the hippocampus may also contribute to the impaired contextual fear memory observed in *Trpm3*^{−/−} mice.

Overall, these findings suggest that TRPM3 may be required for the synaptic plasticity underlying fear memory formation in the LA. This plasticity is thought to involve a classic Hebbian mechanism in the form of LTP (Sears et al., 2014). Accordingly, we found reduced theta burst stimulation evoked LTP in slices from *Trpm3*^{−/−} mice and after pharmacological blockade of the channel. Altogether, these data indicate that TRPM3 as a Ca²⁺-permeable cation channel might be an important player regulating presynaptic plasticity in the LA. Interestingly, the characterized human gain-of-function mutations among other symptoms were reported to manifest in autism spectrum disorders and aggressive behaviour (Jolitz et al., 2025), all which can be linked to a malfunctioning amygdala (Wang & Li, 2023), further supporting the key role of TRPM3 in this brain region.

Besides the general developmental delay, the second most common phenotype among the *TRPM3* mutant patients was seizures. Importantly, amygdala is also often the focus of seizures in temporal lobe epilepsy (Aroniadou-Anderjaska et al., 2008; Tilelli et al., 2021). Our results clearly showed that in the intra-amygdaloid KA epilepsy model, *Trpm3*^{−/−} mice display reduced seizure parameters both in the acute and chronic phases of the model. In contrast, direct injection of a TRPM3 agonist (CIM0216) into the LA resulted in more severe seizure parameters, suggesting that high TRPM3 activity might lead to over-excitation and seizures as it happens in the patients with gain-of-function *TRPM3* mutation. These results suggest that TRPM3 activity can directly regulate cellular excitability during seizures. Indeed, in our *in vitro* experiments we observed more hyperpolarized resting membrane potential and increased rheobase in case of *Trpm3*^{−/−} LA neurons. These electrophysiological parameters can lead to reduced excitability explaining the less severe seizure parameters upon TRPM3 ablation. Although the present study demonstrates that TRPM3 influences seizure susceptibility in the intra-amygdala kainic acid model, it remains to be determined whether this effect is specific to seizures initiated in the amygdala. To directly test this possibility, an LA-specific Cre-mouse line would be convenient to specifically manipulate TRPM3 and therefore more precisely characterize its role in the LA. However, to our best knowledge, no such mouse line exists to date. Therefore, we used global *Trpm3*^{−/−} mice in our *in vivo* experiments and stereotactically targeted LA where it was applicable. It should be noted that the homozygous *Trpm3*^{−/−} mice used in this study were intended to define the physiological role of TRPM3 in the lateral amygdala rather than to model the human TRPM3-associated neurological disorders. Another important aspect in our study is that all experiments were conducted in male mice. This might be important given that sex differences in epilepsy have been documented in both clinical studies and experimental models. These differences are thought to arise, at least in part, from the modulatory effects of sex hormones, including oestrogen, progesterone and androgens (Scharfman & MacLusky, 2014). Therefore, it would be important in future studies to investigate whether genetic deletion or pharmacological activation of TRPM3 exerts comparable effects on seizure parameters in female mice as well.

Taken together, we report here that TRPM3 is expressed both in mouse and human lateral nucleus of the amygdala (LA) excitatory

neurons and as a cation channel controls their resting membrane potential and excitability. Furthermore, TRPM3 is a regulator of synaptic plasticity in LA neurons. Thus the deletion of the channel leads to reduced LTP and fear memory formation. Finally, TRPM3 deletion can reduce, while activation of the channel can worsen seizure parameters. All these results point to a so far unrecognized role of TRPM3 in the cellular excitability of LA neurons both in physiological and pathophysiological conditions. Thus, better understanding the role of TRPM3 in these neurons could lead to novel strategies for treating amygdala-related disorders.

AUTHOR CONTRIBUTIONS

Attila Gyéresi: Writing—original draft; data curation. **Laura Mundrucz:** Writing—original draft; data curation. **Máté Deák:** Data curation. **Angéla Kecskés:** Data curation. **Éva Borbély:** Data curation. **Viktória Kormos:** Data curation; writing—original draft. **Balázs Gaszner:** Writing—original draft; data curation. **Orsolya Mihály:** Data curation. **Balázs István Tóth:** Conceptualization; writing—original draft. **Joris Vriens:** Conceptualization; writing—original draft. **Thomas Voets:** Conceptualization; writing—original draft. **Erzsébet Kövesdi:** Conceptualization; writing—original draft; visualization; data curation. **Miklós Kecskés:** Conceptualization; funding acquisition; writing—original draft; visualization; data curation; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interest.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article and its [Supporting Information](#) files. Raw data are available from Miklós Kecskés upon reasonable request.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the BJP guidelines for [Design and Analysis](#) and [Animal Experimentation](#) and as recommended by funding agencies, publishers and other organizations engaged with supporting research.

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