ANNALS of Clinical and Translational Neurology
Open Access
AMERICAN NEUROLOGICAL ASSOCIATION

BRIEF COMMUNICATION

Modulation of GABAergic dysfunction due to *SCN1A* mutation linked to Hippocampal Sclerosis

Gabriele Ruffolo[1,a], Katiuscia Martinello[2,a], Angelo Labate[3,4], Pierangelo Cifelli[5], Sergio Fucile[1,2], Giancarlo Di Gennaro[2], Andrea Quattrone[3], Vincenzo Esposito[2], Cristina Limatola[1,2], Felice Giangaspero[2], Eleonora Aronica[6,7], Eleonora Palma[1,b] & Antonio Gambardella[3,4,b]

[1]Department of Physiology and Pharmacology, Istituto Pasteur-Fondazione Cenci Bolognetti, University of Rome Sapienza, Rome, Italy
[2]IRCCS Neuromed, Pozzilli, Isernia, Italy
[3]Institute of Neurology, University Magna Græcia, Catanzaro, Italy
[4]Institute of Molecular Bioimaging and Physiology of the National Research Council, Catanzaro, Italy
[5]Department of Biotechnological and Applied Clinical Sciences (DISCAB), Amsterdam UMC, University of L'Aquila, L'Aquila, Italy
[6]Department of (Neuro)Pathology, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
[7]Stichting Epilepsie Instellingen Nederland, Heemstede, the Netherlands

Correspondence
Antonio Gambardella, Institute of Neurology, University *Magna Græcia* of Catanzaro, Viale Europa, 88100 Catanzaro, Italy. Tel: +39-961-3647270; Fax: +39-0961-3647177; E-mail: a.gambardella@unicz.it;
Eleonora Palma, Department of Physiology and Pharmacology, Istituto Pasteur-Fondazione Cenci Bolognetti, University of Rome Sapienza, Rome, Italy. Tel: +39-0649910059; Fax: +39-0649910851; E-mail: eleonora.palma@uniroma1.it

Received: 11 April 2020; Revised: 11 July 2020; Accepted: 16 July 2020

***Annals of Clinical and Translational Neurology* 2020; 7(9): 1726–1731**

doi: 10.1002/acn3.51150

[a]These authors equally contributed to this work.

[b]Shared corresponding authorship

Abstract

We compared GABAergic function and neuronal excitability in the hippocampal tissue of seven sporadic MTLE patients with a patient carrying a *SCN1A* loss-of-function mutation. All had excellent outcome from anterior temporal lobectomy, and neuropathological study always showed characteristic hippocampal sclerosis (Hs). Compared to MTLE patients, there was a more severe impairment of GABAergic transmission, due to the lower GABAergic activity related to the $Na_V1.1$ loss-of-function, in addition to the typical GABA-current rundown, a hallmark of sporadic MTLE. Our results give evidence that a pharmacological rescuing of the GABAergic dysfunction may represent a promising strategy for the treatment of these patients.

Introduction

Mesial temporal lobe epilepsy (MTLE) with hippocampal sclerosis (Hs) represents the most common type of focal drug-resistant epilepsy.[1] Although MTLE is now routinely treated with neurosurgery, over a third of MTLE patients do not achieve seizure freedom,[2] and surgery can have important adverse consequences. Better treatment options, or even prevention, of MTLE and Hs are therefore needed, but rational therapy remains elusive because their causes remain unclear. A large body of evidence indicates the biologic processes that are relevant in the pathogenesis of Hs include glial activation, immune response, synaptic transmission, signal transduction, ions transport, and synaptic plasticity.[3,4] In the hippocampus, GABAergic inhibitory dysfunction mostly contributes to hyperexcitability in MTLE with Hs.[5–7]

Of interest, recent studies illustrate *SCN1A* involvement in the epileptogenic neuronal network underlying MTLE associated with Hs.[8] Accordingly, we already illustrated that MTLE with febrile seizures may be part of the epileptic phenotype encountered in a large family carrying a *SCN1A* mutation.[9,10] This M145T mutation of *SCN1A* cosegregated in all affected individuals of a large family affected by simple febrile seizures (FS), three of whom later developed MTLE.[10] Most

important, the M145T mutation is located in a highly conserved amino acid in the first transmembrane segment of domain I of the *SCN1A*, and functional studies in mammalian cells demonstrated that the M145T mutation causes a 60% reduction of current density and a 10 mV positive shift of the activation curve.[9] More recently, one family member with refractory MTLE and Hs has benefited from surgery, and now we wish to report the extensive neurophysiological study from the resected hippocampal tissue of this patient, in comparison with seven sporadic patients with refractory MTLE and Hs. We believe that this study may provide important new directions to the physiopathological comprehension of MTLE with Hs and FS, and ultimately may pave the road to develop new therapeutic approaches in MTLE.

Patients and Methods

Patients

The clinical data of seven sporadic MTLE patients with Hs, whose hippocampal tissues were used for electrophysiological recordings, are described in detail in Supplemental Material and summarized in Table S1. The patient (mutated, $mNa_V1.1$) carrying a loss-of-function missense mutation (M145T) of *SCN1A* is a 31-year-old man with MTLE, whose electroclinical data were reported in detail elsewhere.[9,10] He had simple FS every 3–6 months from the age of 8 months until 70 months. At the age of 13 years, he began to have afebrile seizures characterized by behavioral arrest, some lip smacking and gestural automatisms, or nocturnal secondary generalized motor seizures. Over the years, he also began to experience epigastric auras with some experiential phenomena prior to the loss of awareness. Since seizures became refractory to antiepileptic drugs (AEDs) that included oxcarbazepine, phenobarbital, topiramate, and valproate, and he underwent an extensive presurgical evaluation. Interictal awake and sleep EEG recording showed bilateral mesiotemporal epileptiform spikes, which strongly predominated (>80%) on the right side. On intensive video-polygraphic monitoring, several typical seizures with onset in the right midinferomesial temporal region were recorded. Brain MRI revealed right *Hs* and, at the age of 27, he underwent surgery, in the form of right anterio-temporal lobectomy. Histopathological findings disclosed type 2 Hs with the characteristic pattern of predominant neuronal loss in area CA1.[3,4] Since surgery, in the last 50 months, he has only had non-disabling auras (Engel's outcome classification Class 1B), and he has been taking a dual therapy with carbamazepine plus valproate.

Electrophysiology

See Supplemental Material for details. The study was approved by the local Ethics Committees.

Results

Patch-clamp experiments in human slices

To highlight the functional implications of mNa_V 1.1 expression, patch-clamp experiments were performed on pyramidal cells and interneurons of the resected hippocampus of the $mNa_V1.1$ patient, comparing the results with those obtained from sporadic MTLE patients (patients, # 4-8, Table S1). Current steps (100–150 pA, 500 ms duration) applied to the cells were able to elicit one or more action potentials (APs), with pyramidal neurons from all patients exhibiting APs with similar characteristics, in particular with no change in AP threshold (MTLE: -53 ± 3 mV; n = 10; # 4-8, Table S1, mNa_V 1.1 patient: -53 ± 3 mV; n = 5; $P = 0.64$; Fig. 1A,B). By contrast, three interneurons from the $mNa_V1.1$ patient showed a significantly depolarized AP threshold (-40 ± 3 mV) in comparison to interneurons from MTLE patients (-58 ± 12 mV, n = 5; # 4-8, Table S1; $P = 0.04$; Fig. 1A,B, see Table S2 for electrophysiological parameters). These data suggest that $mNa_V1.1$ expressing interneurons may have a reduced intrinsic excitability as expected from M145T mutation of *SCN1A*.[9]

GABA currents rundown

To investigate how GABA currents respond in $mNa_V1.1$ patient's hippocampus, we performed microtransplantation experiments injecting oocytes with hippocampal tissues from sporadic MTLE patients (without known genetic mutations and with Hs, Fig. 2A), one control individual (Fig. 2B) and mNa_V 1.1 patient (Fig. 2C-E). The results confirmed that a strong, pathological GABA-current rundown is present in drug-resistant MTLE hippocampi ($49.0 \pm 9.1\%$; n = 33; patients #1,2,4,5 Table S1; Fig. 2A), whereas the same phenomenon was absent in the control (75.8 ± 7.0 %; n = 10; # 3, Table S1; Fig. 2B) as previously shown.[5–7]

Interestingly, the sclerotic hippocampus of mNa_V 1.1 patient also showed a GABAergic rundown (43.2 ± 3.1 %; n = 19; Fig. 2C) similar to sporadic MTLE patients. This current rundown was mitigated by pretreatment of 2 hours with endogenous factors, as BDNF, or exogenous agents, as the cannabis derivative cannabidivarine (CBDV, Fig. 2D; Table S3) showing again a strong similarity with data previously published for MTLE patients.[5,11]

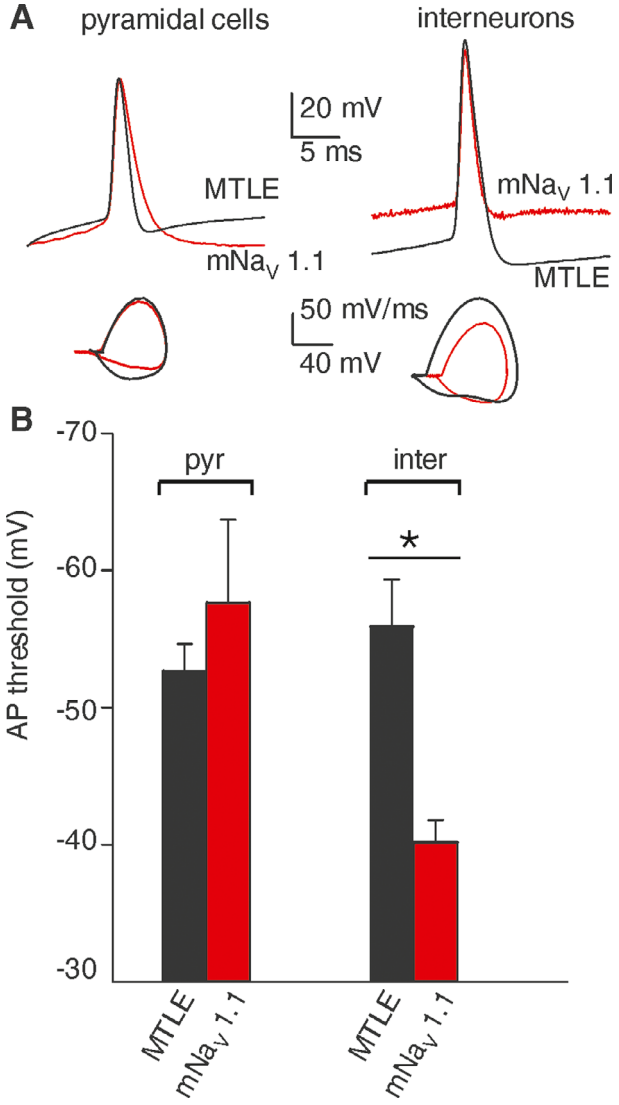

Figure 1. mNaV 1.1 patient exhibits depolarized action potential (AP) threshold in hippocampal interneurons but not in pyramidal cells. (A) Left top, superimposed AP traces recorded from one pyramidal neuron of the mNa$_V$ 1.1 patient (red trace) and from one MTLE patient (black trace, #5); left bottom, superimposed phase-plane plot obtained from APs showed on top. Right top, superimposed AP traces recorded from one interneuron of mNa$_V$ 1.1 patient (red trace) and from the same MTLE patient (black trace); right bottom, superimposed phase-plane plot obtained from APs showed on top. (B) Bar graph representing the mean action potential threshold values recorded from pyramidal neurons (pyr; n = 10) and interneurons (inter; n = 5) in resected hippocampi of patients with MTLE (black bars, #4-8, Table S1) and from pyramidal cells (pyr; n = 5) and interneurons (inter; n = 3) of mNa$_V$ 1.1 patient (red bars). *P = 0.044; One-way ANOVA Test, and post hoc Holm–Sidak test.

Interestingly, we found that acute co-application of cannabidiol (CBD, 5 μM, GABA 50 μM), which has been reported to be clinically effective in Dravet syndrome,[12] potentiated both GABA-current amplitude from mNa$_V$ 1.1 patient (I_{GABA}; +29.8 ± 4.1 %; n = 13; Fig. 2E, Table S3) and from sporadic MTLE patients (I_{GABA}; +27.5 ± 8.8 %; n = 22; #1,2, Table S3).[13]

Discussion

The novelty of our study is that we performed electrophysiological experiments from an exclusive hippocampal tissue obtained from a patient with MTLE and Hs, who also carried the M145T *SCN1A* loss-of-function mutation. Compared to hippocampal tissue from MTLE patients, there was a more severe impairment of GABAergic transmission, due to the lower GABAergic activity related to the Na$_V$1.1 loss-of-function, in addition to the characteristic GABA-current rundown, a hallmark of MTLE.[5–7,11]

We recorded valuable APs on both pyramidal neurons and interneurons from mNa$_V$ 1.1 and five (of seven) sporadic MTLE patients. In all of them, the histopathological study showed the features of Hs related to MTLE, with the characteristic pattern of neuronal loss in CA1 (see Table S1).[3,4] Nonetheless, only in hippocampal mNa$_V$ 1.1 slices, AP threshold was more depolarized in interneurons than in pyramidal neurons, reflecting a reduced excitability of GABAergic interneurons related to *SCN1A* mutation.[14,15] In spite of the limited number of recordings on mNav 1.1 interneurons, this type of AP responses paralleled the functional data obtained from cells transfected with M145T mutated cDNA.[9]

Because of the limited quantity of fresh tissue from the patient and technical difficulty of recordings from acute hippocampal slices, part of the tissue was snap-frozen and used for recordings in microtransplanted oocytes.[6,16] Notably, we found a GABA current rundown that was very similar to the one recorded from hippocampi of sporadic MTLE patients.[5–7,11] The GABA current rundown is considered an electrophysiological dysfunction of refractory human MTLE,[5–7] which may explain, at least in part, the hyperexcitability underlying the ictogenesis in these patients. Interestingly, we previously showed in epileptic rats that GABA current rundown arises from the hippocampal region (during status epilepticus) then spreading to temporal cortex in the chronic phase of seizures.[7,17]

We already emphasized that the pharmacological modulation of the GABAergic impairment (i.e., GABA current rundown) underlying MTLE may represent an interesting therapeutic approach in pharmacoresistant MTLE.[17] In this way, it is not surprising that BDNF could ameliorate the GABA current rundown both in the mNa$_V$ 1.1 and sporadic MTLE patients, as previously reported for both refractory human MTLE[5] and preclinical animal models of MTLE.[7] Notably, CBDV continues to show its potential in recovering the mNav 1.1 GABAergic rundown as

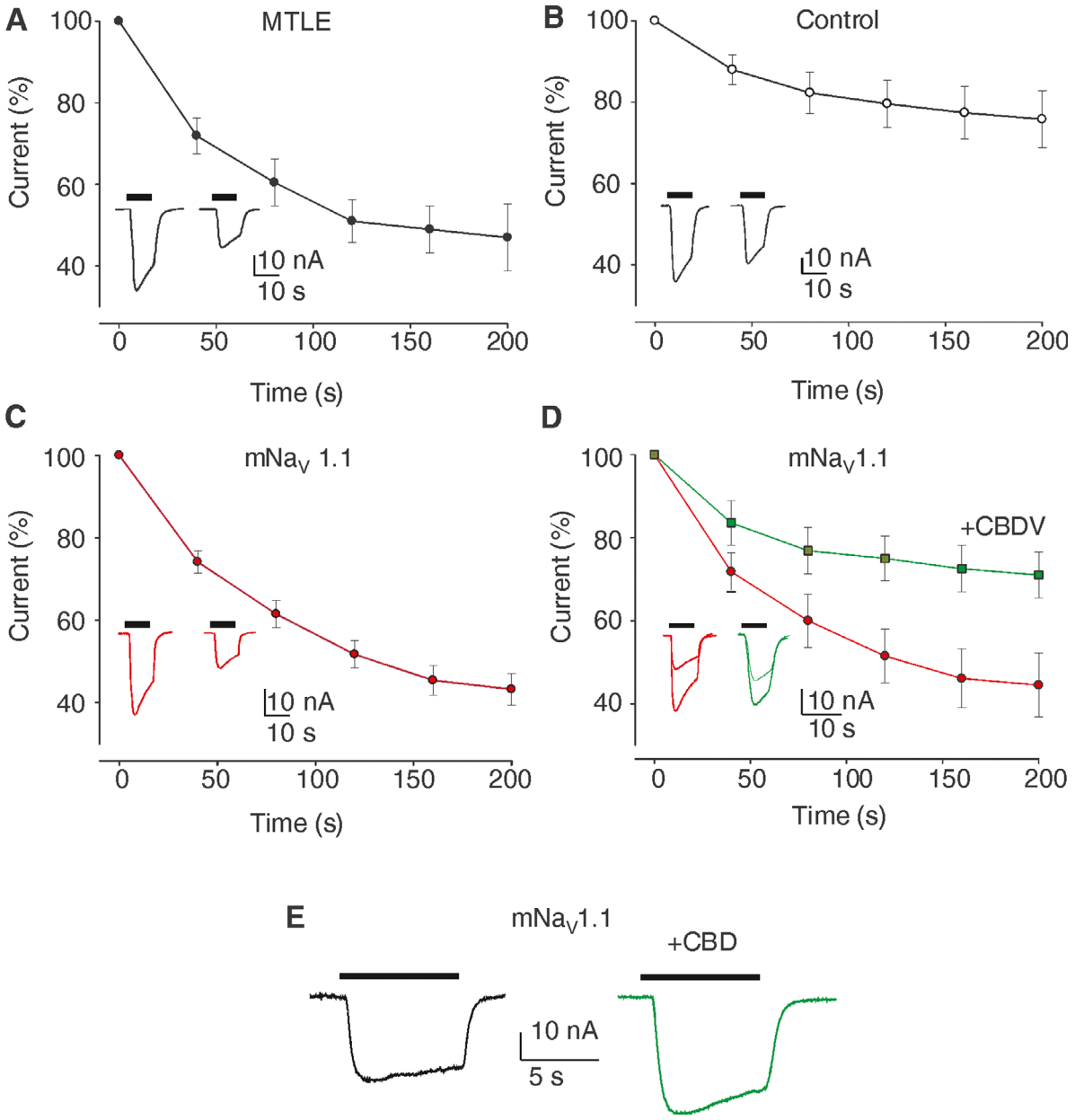

Figure 2. mNaV 1.1 patient exhibits a GABA current rundown similar to MTLE hippocampal tissues. (A) Oocytes injected with hippocampal membranes of MTLE patients (n = 33; I_{max} = 40.5 ± 20 nA; #1,2, 4,5 Table S1). Black dots (●) represent the amplitude of consecutive GABA currents as % of the first response (GABA 500 μM). Data points represent means ± SEM. In this and following panels all the currents are normalized to the first current (I_{max}) and black horizontal bars represent the timing of GABA application. In all experiments, the holding potential was −60 mV. *Inset:* Representative current traces elicited by the first and sixth GABA application (500 μM, horizontal bar); (B) Oocytes injected with hippocampal membranes from a non-epileptic control (n = 10; I_{max} = 49.5 ± 17.3 nA). White dots (○) represent the amplitude of consecutive GABA currents as % of the first response (GABA 500 μM). *Inset*: Representative current traces as in (A). (C) Oocytes injected with hippocampal membranes from mNa$_V$ 1.1 patient (n = 19; I_{max} = 30.8 ± 5.7 nA). Red dots (●) represent the amplitude of consecutive GABA currents as % of the first response (GABA 500 μM). *(Inset)* Representative current traces as in (A). Note the current rundown similar to MTLE patients. (D) Oocytes injected with hippocampal membranes from the mNa$_V$ 1.1 patient before and after CBDV treatment. Red dots (●) represent the amplitude of consecutive GABA currents as % of the first response (GABA 500 μM) while the green squares ■) represent the amplitude of consecutive GABA currents on the same cells after 2 hours of incubation with CBDV 50 nM. Data points represent means ± SEM [●) I_{max} = 30.7 ± 8.0 nA; (■) I_{max} = 27.8 ± 7 nA)]. *Inset*: Representative GABA current traces as in (A), before (red traces) and after 50 nM CBDV application (green traces) as indicated. P = 0.002, n = 19 by Shapiro–Wilk test and Student's t-test. (E) Representative GABA current traces evoked by 50 μM GABA in one oocyte of 13 injected with membranes of mNa$_V$ 1.1 before (black trace) and after CBD co-application (5 μM, green trace).

previously shown for MTLE.[11] Furthermore, CBD was capable to increase GABA current amplitude in mNa_V 1.1 and MTLE patients, as reported in Dravet syndrome and tuberous sclerosis complex.[12,13,18] Altogether our results emphasize the role of cannabis derivatives as treatment for refractory epilepsies.

In our patient, the overall clinical, pharmacological, and neuropathological findings were consistent with those observed in sporadic MTLE with Hs, including the excellent outcome from anterior temporal lobectomy. In this way, our findings reinforce the belief that known genetic defect, which is present diffusely in the brain, do not necessarily preclude a good prognosis following epilepsy surgery, if surgery is a reasonable option based on the concordance of other data during presurgical evaluation. It cannot be excluded, however, that the milder loss-of-function impairment of SCN1A related to the M145T mutation could also influence the better postsurgical outcome.

Important, as previously reported in Dravet syndrome and tuberous sclerosis complex,[13,18] CBD was capable to increase GABA current amplitude in mNa_V 1.1 and MTLE patients, that was recently approved as treatment for Dravet and Lennox–Gastaut syndromes.[12] Thus, in accordance with the "interneuron hypothesis,"[14] our findings support the assumption that seizures in mNa_V 1.1 patient could arise from a minor GABA release from interneurons together with a reduction of GABAergic postsynaptic efficacy after repetitive stimulation (i.e., rundown). Major therapeutic implication of these findings is that by rescuing the GABAergic inhibitory activity it is possible to improve the clinical outcome of this kind of patients. In this way, this study ultimately may pave the road to develop new antiepileptic approaches in epileptic patients with Hs.

Acknowledgments

The work was supported by grants from AICE-FIRE; UCB Pharma, Brussels, Belgium (IIS-2014-101617, E.P.); "Bando di Ateneo (Sapienza University)," grant no RM1181642D8BD87B. G.R. was supported by a fellowship from "Istituto Pasteur Italia – Fondazione Cenci Bolognetti." We are grateful to all the families and patients who were recruited in the studies.

Conflicts of Interest

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

References

1. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005–2009. Epilepsia 2010;51:676–685.
2. De Tisi J, Bell GS, Peacock JL, et al. The long-term outcome of adult epilepsy surgery, patterns of seizure remission, and relapse: a cohort study. Lancet 2011;378:1388–1395.
3. Blumcke I, Thom M, Aronica E, et al. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: a task force report from the ILAE Commission on Diagnostic Methods. Epilepsia 2013;54:1315–1329.
4. Thom M. Hippocampal sclerosis in epilepsy: a neuropathology review. Neuropathol Appl Neurobiol 2014;40:520–543.
5. Ragozzino D, Palma E, Di Angelantonio S, et al. Rundown of GABA type A receptors is a dysfunction associated with human drug-resistant mesial temporal lobe epilepsy. Proc Natl Acad Sci USA 2005;102:15219–15223.
6. Palma E, Esposito V, Mileo AM, et al. Expression of human epileptic temporal lobe neurotransmitter receptors in Xenopus oocytes: an innovative approach to study epilepsy. Proc Natl Acad Sci USA 2002;99:15078–15083.
7. Cifelli P, Palma E, Roseti C, et al. Changes in the sensitivity of $GABA_A$ current rundown to drug treatments in a model of temporal lobe epilepsy. Front Cell Neurosci 2013;7:108. DOI: https://doi.org/10.3389/fncel.2013.00108. eCollection 2013.
8. Kasperaviciute D, Catarino CB, Matarin M, et al. Epilepsy, hippocampal sclerosis and febrile seizures linked by common genetic variation around SCN1A. Brain 2013;136:3140–3150.
9. Mantegazza M, Gambardella A, Rusconi R, et al. Identification of an Nav1.1 sodium channel (SCN1A) loss-of-function mutation associated with familial simple febrile seizures. Proc Natl Acad Sci USA 2005;102:18177–18182.
10. Colosimo E, Gambardella A, Mantegazza M, et al. Electroclinical features of a family with simple febrile seizures and temporal lobe epilepsy associated with SCN1A loss-of-function mutation. Epilepsia 2007;48:1691–1696.
11. Morano A, Cifelli P, Nencini P, et al. Cannabis in epilepsy: from clinical practice to basic research focusing on the possible role of cannabidivarin. Epilepsia Open 2016;1:145–151.
12. Arzimanoglou A, Brandl U, Cross JH, Other members of The Cannabinoids International Experts Panel. Epilepsy and cannabidiol: a guide to treatment. Epileptic Disord 2020;22(1):1–14. https://doi.org/10.1684/epd.2020.1141.
13. Ruffolo G, Cifelli P, Roseti C, et al. A novel GABAergic dysfunction in human Dravet syndrome. Epilepsia 2018;59:2106–2117.

14. Chopra R, Isom LL. Untangling the Dravet syndrome seizure network: the changing face of a rare genetic epilepsy. Epilepsy Curr. 2014;14(2):86–89.
15. Cheah CS, Yu FH, Westenbroek RE, et al. Specific deletion of NaV1.1 sodium channels in inhibitory interneurons causes seizures and premature death in a mouse model of Dravet syndrome. Proc Natl Acad Sci USA 2012;109 (36):14646–14651.
16. Eusebi F, Palma E, Amici M, et al. Microtransplantation of ligand-gated receptor-channels from fresh or frozen nervous tissue into Xenopus oocytes: a potent tool for expanding functional information. Prog Neurogibol 2009;88:32–40.
17. Gambardella A, Labate A, Cifelli P, et al. Pharmacological modulation in temporal lobe epilepsy: current status and future perspectives. Pharmacol Res 2016;113:421–425.
18. Palma E, Ruffolo G, Cifelli P, et al. Modulation of GABAA Receptors in the Treatment of Epilepsy. Curr Pharm Des 2017:23(37), 5563–5568.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

MTLE Patients. Detailed description of patients.
Table S1. Clinical characteristics and neurophysiological findings of MTLE patients.
Electrophysiology. Patch-clamp in human slices; Membrane Preparation, Injection Procedure, and voltage-clamp Recordings in Oocytes.
Table S2. Electrophysiological parameters in patch-clamped human neurons.
Table S3. Effects of pharmacological agents on $mNa_V1.1$ and MTLE patients.
Figure S1. Firing of hippocampal interneurons.
Statistics. Detailed description of the statistical analysis.

Modulation of GABAergic dysfunction due to *SCN1A* mutation linked to Hippocampal Sclerosis

Supplemental material

MTLE Patients

The clinical cases and controls included in this study were selected from the Departments of Neuropathology of the University Medical Center (UMC, University of Amsterdam) and the Neuromed Neurosurgery Center for Epilepsy (Pozzilli, Italy). The clinical characteristics derived from the patients' medical records are summarized in Table S1. All patients underwent presurgical evaluation with non-invasive tests. Patients who underwent implantation of strip and/or grid electrodes for chronic subdural invasive monitoring before resection were excluded from the study. In MTLE patients, the surgery consisted of an extensive temporal lobectomy (ETL) including microsurgical resection of the amygdala and parahippocampal gyrus and en-bloc excision of the hippocampal formation. The interventions differed in the extent of the neocortical resection. Nondominant ETL included excision of approximately 4 –7 cm or more cm of the superior, middle and inferior temporal gyrus, depending on intraoperative EEG measurements, whereas dominant ETL included excision of approximately 3-7 cm of the superior, middle and inferior temporal gyrus, depending on language mapping and intraoperative EEG measurements. The predominant seizure types were medically intractable focal impaired awareness seizures (FIAS), and all patients had seizures which were resistant to maximal doses of different anti-epileptic drugs (Table S1). Epilepsy duration was calculated as the interval in years from age at seizure onset to age at surgery; no patients included in our series had seizures in the 24 h before surgery. Patients #1-2, 4-8 (Table S1) had hippocampal sclerosis (Hs) with predominant neuronal loss in CA1 (Hs, ILAE type 2). All cases were reviewed independently by two neuropathologists, and the diagnosis was confirmed according to the international consensus classification (Blumcke et al., 2013). As control tissue, we used a specimen from an autopsy of an individual without any neurological disease (#3, Table S1; death by myocardial infarction). The autopsy was performed within 10 to 24 h of death obtaining

tissue with preserved immunoreactivity (Roseti et al., 2013). All tissue was snap-frozen over liquid nitrogen and stored at -80 °C until use. Frozen tissue was shipped by courier to University of Rome. Tissue was obtained and used in accordance with the Declaration of Helsinki and the UMC Research Code provided by the Medical Ethics Committee. The Ethics Committee of the University of Rome "Sapienza" and Neuromed I.R.C.C.S approved the technical procedures. Informed consent was obtained from all individuals involved in this study.

Table S1. Clinical characteristics and neurophysiological findings of MTLE patients

P#	Age (yrs)/sex	Epilepsy onset (yrs)	Surgical zone	Seizure type	no. seizures/month	Pathology	Medications	Prognosis after surgery (follow-up)
#1	36/F	6	R-T	FIAS	10	HS	CBZ	Seizure free (five years)
#2	27/M	10	R-T	FIAS	12	HS	CBZ, LCS	Seizure free (six years)
#3	31/M control	--	--	--	--	--	--	myocardial infarction
#4	56/F*	12	R-T	FIAS	2	HS	CNP	Seizure free (five years)
#5	51/M*	16	R-T	FIAS	2	HS	CBZ, PHB, VGB	Rare disabling seizures (six years)
#6	29/M*	4	L-T	FIAS/GS	32	HS	LMT, TPM	Seizure free (four years)
#7	48/M*	30	L-T	FAS	32	HS	LMT,VPA	Seizure free (five years)
#8	39/M*	20	L-T	FIAS	14	HS	CBZ	Seizure free (six years)

P#, patients; T, temporal; HS, hippocampal sclerosis, FAS, focal aware seizure; FIAS, focal impaired awareness seizure; GS, generalized seizures; CBZ, carbamazepine; CNP, clonazepam; LCS, lacosamide; LMT, lamotrigine; PHB, phenobarbital; TPM, topiramate; VGB, vigabatrin; VPA, valproic acid; *, tissues used for patch-clamp recordings.

Electrophysiology

Patch-clamp in human slices

Immediately after surgery, transversal hippocampal slices (350 μm) were cut in glycerol-based artificial cerebro-spinal fluid (ACSF) with a vibratome (Leica VT 1000S; Leica Microsystems);

placed in a slice incubation chamber at room temperature with oxygenated ACSF and transferred to a recording chamber within 1–8 h after slice preparation. ACSF had the following composition (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1.25 NaH_2PO_4, 1 $MgCl_2$, 26 $NaHCO_3$, 10 glucose, 0.1 Na-pyruvate (pH 7.35; 5% CO_2).

Whole-cell patch clamp recordings were performed on cells in stratum pyramidale and in stratum oriens, at 24– 25 °C. Pyramidal cells were distinguished from interneurons by localization and morphological properties. Furthermore, the action potential analysis confirmed cell identification, with significantly different after-hyperpolarization values: -4.1 ± 0.6 mV in pyramidal cells and -12 ± 1 mV in interneurons (n=12 and 8, $p<0.001$). Action potentials (AP) were recorded from neurons applying depolarizing current steps (100-150 pA, 500 ms) using glass electrodes (3-4 MΩ) filled with (in mM): 140 KCl 10 Hepes, 5 BAPTA, 2 Mg-ATP (pH 7.3, with KOH). Data analyzed with ANOVA. For more details, see Ragozzino et al., 2005.

Membrane Preparation, Injection Procedure, and voltage-clamp Recordings in Oocytes.

Membrane preparation and injection was performed as already described (Eusebi et al., 2009).

Briefly, human tissues were homogenized using a Teflon glass homogenizer with 2 ml of glycine buffer of the following composition (in mM): 200 glycine, 150 NaCl, 50 EGTA, 50 EDTA, 300 sucrose; plus 20 μl protease inhibitors (Sigma); pH 9 adjusted with NaOH. The homogenate was centrifuged for 15 min at 9.500 x g. The supernatant was collected and centrifuged for 2 h at 10^5 x g at 4 °C. The pellet was washed, re-suspended in assay buffer (glycine 5 mM) and used directly, or aliquoted and stored at –80 °C for later use.

The rundown of the GABA-evoked currents was elicited by 6 applications of GABA (500 μM) interspaced by 40 s intervals and was defined as the percent decrease of the sixth GABA current peak amplitude (I_{GABA}) after five previous applications. Cannabidivarine (CBDV) or brain derived neurotrophic factor (BDNF) were applied as previously described (Ragozzino et al., 2005; Morano et al., 2016) . GABA-current potentiation by cannabidiol (CBD) was tested in previously validated

conditions (Ruffolo et al., 2018) and I_{GABA} expressed as a percentage of the currents evoked before drug application. For comparison, in some experiments, we used one control individual that is described in Table S1. In the text, the number of patients used in each experiment is reported and referred to Table S1, using the symbol # and numbers (n) referring either to oocytes or neuronal cells used in each experiment. Animal protocols were approved by the Italian Ministry of Health (authorization no. 78/2015-PR). From 12 to 48 h after injection, membrane currents were recorded from voltage-clamped *Xenopus laevis* oocytes using two microelectrodes filled with 3M KCl as previously described (Miledi et al., 2006). The oocytes were placed in a recording chamber (0.1ml volume) and perfused continuously with oocyte Ringer solution (OR in mM: 82.5 NaCl; 2.5 KCl; 2.5 $CaCl_2$; 1 $MgCl_2$; 5 Hepes, adjusted to pH 7.4 with NaOH) at room temperature (20-22°C). To apply GABA or OR we used a gravity driven multi-valve perfusion system (9-10 ml/min) controlled by computer (Biologique RSC-200; Claix, France) to ensure the exact duration of each application. Using this system, 0.5 to 1s are sufficient to reach the complete replacement of applied solution. GABA current rundown was defined as the percentage decrease of the current peak amplitude after six 10s-applications of GABA at 40s intervals (Eusebi et al., 2009; Roseti et al., 2013). All the salts were purchased by Sigma Aldrich (USA), GABA was purchased by Tocris Bioscience (Bristol, UK) while cannabis derivatives were purchased by THC Pharma (Frankfurt, Germany). BDNF (Sigma) was dissolved in H_2O, stored as frozen stock solutions (50 μg/ml) until use. CBD and CBDV were dissolved in ethanol and then diluted to the desired concentration in OR. The final dilution of ethanol was always lower than 1:5000.

Table S2. Electrophysiological parameters in patch-clamped human neurons

	MTLE pyr	$Na_V1.1$ pyr	MTLE int	$Na_V1.1$ int
resting membrane potential (mV)	-68 ±1	-68 ± 2	-74 ± 2	-63 ± 8
AP half width (ms)	2.3±0.4	2.2±0.4	1.6±0.2	1.9±0.3
afterhyperpolarization (mV)	-4.1 ± 0.6	-4.0 ± 1	-11 ± 2	-13 ± 2

Data represent means ± SEM; AP, action potential; pyr, pyramidal cells; int, interneurons.

Table S3. Effects of pharmacological agents on mNa$_V$1.1 and MTLE patients

patients	I_{GABA} rundown (%)				I_{GABA} increase (%)		
	control	+CBDV 50 nM	*n*	+BDNF 0.5µg/ml	*n*	+CBD 5 µM	*n*
mNav 1.1	43.2±3.1	71.0±5.5 p<0.01	19	72.5±3.2 p<0.01	8	+29.8±4.1 p<0.01	13
MTLE	49.0±9.1	70.1±5.7 p<0.01	33	74.7±4.5 p<0.01	9	+27.5±8.8 p<0.01	22

Data represent means ± SEM; *n*, number of cells; CBDV, cannabidivarine; CBD, cannabidiol. GABA concentration was 500 µM in rundown experiments, and 50 µM in CBD experiments.

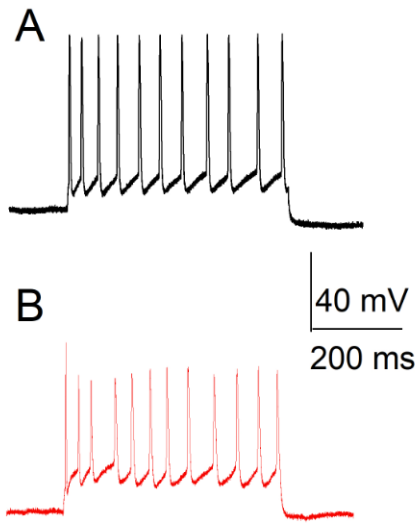

Figure S1. ***Firing of hippocampal interneurons***

A, typical trace showing action potential (AP) properties in a current-clamped MTLE hippocampal interneuron. Current intensity, 100 pA. **B**, typical trace showing AP properties in a current-clamped mNa$_V$1.1 hippocampal interneuron.

Statistics

Before data analysis, normal distribution was assessed with Shapiro-Wilk test. According to the result parametric (Student's t-test; ANOVA and post hoc Holm–Sidak test), or non-parametric (Wilcoxon signed rank test, Mann-Whitney rank sum test) tests have been used. The statistical analysis of the data was performed with Sigmaplot 12 software, and differences between two data sets were considered significant when $p < 0.05$.

Supplementary References

1) Blumcke I, Cross JH, and Spreafico R. The international consensus classification for hippocampal sclerosis: an important step towards accurate prognosis. Lancet Neurol 2013; 12, 844–846.
2) Roseti C, Fucile S, Lauro C, et al. Fractalkine/CX3CL1 modulates GABAA currents in human temporal lobe epilepsy. Epilepsia 2013; *54*, 1834–1844.
3) Miledi R, Palma E, and Eusebi F. Microtransplantation of neurotransmitter receptors from cells to Xenopus oocyte membranes: new procedure for ion channel studies. Methods Mol. Biol 2006; 322, 347–355.
4) Eusebi F, Palma E, Amici M, et al. Microtransplantation of ligand-gated receptor-channels from fresh or frozen nervous tissue into Xenopus oocytes: a potent tool for expanding functional information. Prog. Neurobiol. 2009; 88, 32–40.
5) Ragozzino D, Palma E, Di Angelantonio S, et al. Rundown of GABA type A receptors is a dysfunction associated with human drug-resistant mesial temporal lobe epilepsy. Proc Natl Acad Sci U S A. 2005;102, 15219–15223.
6) Eusebi F, Palma E, Amici M, et al. Microtransplantation of ligand-gated receptor-channels from fresh or frozen nervous tissue into Xenopus oocytes: a potent tool for expanding functional information. Prog. Neurobiol. 2009; 88, 32–40.
7) Morano A, Cifelli P, Nencini P, et al. Cannabis in epilepsy: From clinical practice to basic research focusing on the possible role of cannabidivarin. Epilepsia Open 2016; 1, 145–151.
8) Ruffolo G, Cifelli P, Roseti C, et al. A novel GABAergic dysfunction in human Dravet syndrome. Epilepsia 2018; 59, 2106–2117.