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A brain slice experimental model to study the generation and the propagation of focally-induced epileptiform activity

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HIGHLIGHTS

- We propose a model of seizure-like events in temporal cortex slices from young mice.
- In the model local NMDA stimulations induce multiple seizure-like events.
- Electrophysiological, Ca^{2+} imaging and optogenetics are combined in the model.
- Seizures generation and propagation are separately studied.
- The role of specific cell population on these events can be accurately analyzed.

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ABSTRACT

The early cellular events that in a brain network lead to seizure generation and govern seizure propagation are probably based on different cellular mechanisms. Experimental models in which these events can be separately studied would contribute to improve our understanding of epilepsy. We recently described an in vitro model in entorhinal cortex slices from young rats in which focal seizure-like discharges (SLDs) can be induced in spatially defined regions and at predictable times by local NMDA applications performed in the presence of 4-amimopyridine (4-AP) and low extracellular Mg^{2+}. Through the use of single-dual cell patch-clamp and field potential recordings, and Ca^{2+} imaging from large ensembles of neurons, interneurons and astrocytes, we here extend this model to entorhinal and temporal cortex slices of rat and mouse brain, providing evidence that multiple SLDs exhibiting the typical tonic–clonic discharge pattern can be also evoked in these cortical regions by successive NMDA applications. Importantly, the temporal cortex is more accessible to viral vector injections than the entorhinal cortex: this makes it feasible in the former region the selective expression in inhibitory interneurons or principal neurons of genetically encoded Ca^{2+} indicators (GECI) or light-gated opsins. In this model, an optogenetic approach allows to activate specific neuronal types at spatially defined locations, i.e., the focus or the propagating region, and at precise time, i.e., before or during SLD. The NMDA/4-AP model can, therefore, represent a valuable tool to gain insights into the role of specific cell populations in seizure generation, propagation and cessation.

1. Introduction

Epilepsy is a severe neurological disorder characterized by recurrent convulsive or non-convulsive episodes reflecting massive neuronal discharges. Medial temporal lobe epilepsy (MTLE) is one of the most frequent and severe form of epilepsy in adulthood with seizures originating in hippocampal and para-hippocampal regions. As nearly one third of TLE patients are unresponsive to currently available antiepileptic drugs, the need for novel therapeutic tools, possibly with lower side effects, is impelling. The development of new therapeutic strategies relies on our knowledge of the cellular, molecular and network events that lead to seizure maturation, for which experimental animal models are of crucial importance. The most common animal models of epilepsy make use of chemoconvulsants such as pilocarpine and kainic acid (for review see Curia et al., 2008; Levesque and Avoli, 2013). Both toxins are used in rodents to induce status epilepticus which is followed by spontaneous recurring seizures after a latent period recapitulating

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in many aspects human TLE. As the epileptic phenotype develops in vivo, these models are considered valuable to study epileptogenesis (for a recent review on animal models see Kandratavicius et al., 2014).

A different approach uses acute slice preparations from non epileptic brain tissue that can, under certain conditions, develop seizures-like events. For example, slice perfusion with reduced extracellular Ca^{2+}, Mg^{2+}-free solutions (Jefferys and Haas, 1982; Jefferys, 1995; Trevelyan et al., 2006), increased extracellular K^+ (Bear and Lothman, 1993) or the addition of compounds like pentylenetetrazol (PTZ), *N*-methyl-D,L-aspartate (NMDA), tetanus toxin or GABA receptors antagonists, including penicillin, picrotoxin or bicuculline, all cause a dramatic increase in neural network excitability that leads to the generation of epileptiform activity (Pinto et al., 2005; Pitkänen et al., 2006). It is noteworthy that these experimental conditions represent models of acute seizures generation (i.e., ictogenesis) rather than models of epileptogenesis. An additional diffused model of ictogenesis makes use of the K^+ channel blocker 4-aminopyridine (4-AP) that induces epileptiform activity in brain slices in vitro (Voskuyl and Albus, 1985; Perreault and Avoli, 1991, 1992; Avoli et al., 1996; Ziburkus et al., 2006; Uva et al., 2009) and in vivo (Mihaly et al., 1990; Levesque and Avoli, 2013). In this model, after prolonged perfusion of the drug, seizure-like ictal discharges arise spontaneously at unpredictable time and locations (Avoli et al., 1996), but mainly in deep cortical layers (Avoli et al., 2002; Uva et al., 2009).

To study the abnormal activity that at the level of local circuits precedes the generation of focal seizures, as well as the dynamics of the cellular and network events that govern seizure propagation, it is necessary to identify the site of seizure initiation. However, the unpredictable nature of seizures renders the identification of this focal site a daunting challenge, especially in the intact brain. We describe here an experimental model in slice preparations from the entorhinal or temporal cortex of young rats and mice in which seizure-like discharges (SLDs) can be focally induced by challenging small groups of neurons with local NMDA applications. We will provide the experimental procedures in detail and discuss the applicability of the model in specific brain areas. As we show here, NMDA-evoked focal SLDs are indistinguishable from spontaneous events and their site of origin and propagation can be analyzed with unprecedented spatial and temporal accuracy. In the model, single and dual cell patch-clamp recordings, local field potential recordings, Ca^{2+} imaging and optogenetic techniques can be combined to provide insights into the cellular events that govern initiation and spread of SLDs. Explicative experiments are reported.

2. Methods

2.1. Animals

All experiments are carried out in strict accordance with the guidelines established by the European Communities Council Directive and approved by the National Council on Animal Care of the Italian Ministry of Health. All efforts are done to reduce the number of animals used. Brain slices (see Section 2.2) were obtained from Wistar rats, C57BL6J mice, G42 mice (Chattopadhyaya et al., 2004) (kindly donated by Alberto Bacci), and from GCaMP3::Pv-Cre mice obtained by crossing GCaMP3 mice (B6;129S-Gt(ROSA)26Sortm38(CAG-GCaMP3)Hze/J) with B6.Cg-Pvalbtm1.1(cre)Aibs/J mice (id #012358; Jackson Laboratory).

2.2. Brain slice preparations and Ca^{2+} dye loading

Coronal slices are prepared from postnatal day 14–20 animals as previously described (Cammarota et al., 2013). Briefly, rats and mice are deeply anaesthetized with intraperitoneally injected Zoletil (40 mg/kg) and decapitated; the brain is quickly removed and transferred to ice-cold standard ACSF, sACSF (in mM 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 25 glucose, pH 7.4 with 5% CO_2/95% O_2). After brain dissection on the coronal plane, 350 μm-thick slices are cut with a vibratome (VT1000S, Leica Microsystems, GmbH, Wetzlar, Germany). Cutting solution for rat and mice brain are described in Cammarota et al. (2013) and in Dugue et al. (2005), respectively. Slices from mice are transferred for 1 min in a 95% O_2 and 5% CO_2 saturated solution containing (in mM) 225 D-mannitol, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 25 glucose, 0.8 $CaCl_2$, 8 $MgCl_2$, 2 kynurenic acid with 5% CO_2/95% O_2. Slices from both rats and mice are finally transferred in sACSF at 30 °C for 20 min and then maintained at room temperature. Slices are kept in ACSF with Sulforhodamine 101 (SR-101) (0.2 μM, Sigma Aldrich, Milano) at 30 °C for 15 min to selectively stain astrocytes. Loading with the fluorescence Ca^{2+} indicator Oregon Green BAPTA1-AM (10 μM; Life Technologies, Monza) is performed at 30 °C for 50–60 min in the sACSF solution containing pluronic (0.12%, Sigma Aldrich, Milano, Italy) and kynurenic acid (1 mM, Sigma Aldrich, Milano, Italy). After loading, slices are recovered and kept at room temperature.

2.3. Ca^{2+} imaging

Images are acquired with a single-photon upright laser-scanning microscope (TCS-SP5-RS, Leica Microsystems, GmbH, Wetzlar, Germany) with an acquisition time frame of 491 ms (seven line averaging). Oregon Green and GCaMP3 are excited at 488 nm, SR-101 at 543 nm. The microscope is also equipped with a CCD camera for differential interference contrast (DIC) image acquisition.

2.4. Electrophysiology and induction of focal SLDs

Brain slices are continuously perfused in a submerged chamber (Warner Instruments, Hamden, USA) at a rate of 3–4 ml min^{-1} with (in mM): NaCl, 120; KCl, 3.2; KH_2PO4, 1; $NaHCO_3$, 26; $MgCl_2$, 0.5; $CaCl_2$, 2; glucose, 10; at pH 7.4 (with 5% CO_2/95% O_2). Single and dual cell recordings are performed in current-clamp and voltage-clamp configuration using a multiclamp-700B amplifier (Molecular Devices, Foster City, CA, USA). Signals are filtered at 1 kHz and sampled at 10 kHz with a Digidata 1440s interface and pClamp10 software (Molecular Devices, Foster City, CA, USA). Typical pipette resistance was 3–4 MΩ. Access resistance is monitored throughout the recordings and was typically <25 MΩ. Whole-cell intracellular pipette solution is (in mM): K-gluconate, 145; $MgCl_2$, 5; EGTA, 0.5; Na_2ATP, 2; Na_2GTP, 0.2; HEPES, 10; to pH 7.2 with KOH, osmolarity, 280–300 mOsm (calculated liquid junction potential: −14 mV). All patched neurons are from cortical layer V–VI; pyramidal neurons are voltage-clamped at −50 mV (Vh) or current clamped at resting potential as with Pv-FS interneurons. Induction of focal SLDs is performed in the presence of 4-AP (100 μM) and bath temperature maintained at 30–32 °C by an inline solution heater and temperature controller (TC-324B, Warner Instruments, Hamden, USA). A pressure ejection unit (PDES, NPI Electronics, Tamm, Germany) is used to apply a single or double pulse to NMDA (1 mM, Sigma Aldrich, Milano, Italy)-containing pipettes with a 3 s interval, a pressure of 4–10 psi, and a duration of 300–600 ms. In some experiments, the NMDA glass pipette includes an $AgCl_2$ electrode for extracellular local field potential recordings. Field potential signals are filtered at 1 kHz, amplified by an AM-amplifier (AM-systems, Carlsborg, WA, USA) and sampled at 10 kHz.

2.5. Data analysis

In voltage-clamp recordings, the recruitment of principal neurons into propagating ictal events is marked by the transition from

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