Spatiotemporal Dynamics of Optogenetically-Induced and Spontaneous Seizure Transitions in Primary Generalized Epilepsy

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Running Head: Neural Dynamics of Absence Seizure Transitions

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ABSTRACT

Transitions into primary generalized epileptic seizures occur abruptly and synchronously across the brain. Their potential triggers remain unknown. We used optogenetics to causally test the hypothesis that rhythmic population bursting of excitatory neurons in a local neocortical region can rapidly trigger absence seizures. Most previous studies have been purely correlational, and it remains unclear whether epileptiform events induced by rhythmic stimulation (e.g. sensory/electrical) mimic actual spontaneous seizures, especially regarding their spatiotemporal dynamics. Here, we used a novel combination of intracortical optogenetic stimulation and microelectrode array recordings in freely moving WAG/Rij rats, a model of absence epilepsy with a cortical focus in the somatosensory cortex (SI). We report three main findings: (1) Brief rhythmic bursting, evoked by optical stimulation of neocortical excitatory neurons at frequencies around 10 Hz, induced seizures consisting of self-sustained spike-wave discharges (SWDs) for about 10% of stimulation trials. The probability of inducing seizures was frequency-dependent, reaching a maximum at 10 Hz. (2) Local field potential power before stimulation and response amplitudes during stimulation both predicted seizure induction, demonstrating a modulatory effect of brain states and neural excitation levels. (3) Evoked responses during stimulation propagated as cortical waves, likely reaching the cortical focus, which in turn generated self-sustained SWDs after stimulation was terminated. Importantly, SWDs during induced and spontaneous seizures propagated with the same spatiotemporal dynamics. Our findings demonstrate that local rhythmic bursting of excitatory neurons in neocortex at particular frequencies, under susceptible ongoing brain states, is sufficient to trigger primary generalized seizures with stereotypical spatiotemporal dynamics.
INTRODUCTION

The transition into seizures in primary generalized epilepsy occurs abruptly and almost synchronously across the brain, with a particular involvement of the thalamocortical circuitry (Destexhe et al., 1999; Panayiotopoulos, 2005). Several studies, however, have revealed focal cortical features in human patients with primary generalized epilepsy (Lombroso, 1997; Holmes et al., 2004; Ferrie, 2005; Westmijse, 2009) and in rat models of absence epilepsy, specifically the inbred Wistar Albino Glaxo Rats from Rijswijk (WAG/Rij) and Genetic Absence Epilepsy Rats from Strasbourg (GAERS) (Meeren et al., 2002; Klein et al., 2004; Gurbanova et al., 2006; Polack et al., 2007; Polack et al., 2009; Lüttjohann et al., 2011; Zheng et al., 2012). Yet it remains unknown what events within these potential cortical foci may actually trigger generalized seizures.

Previously, an increased corticothalamic feedback has been suggested to initiate absence seizures based on biophysical computational models (Destexhe, 1998) and in vitro electrical stimulation studies (Blumenfeld and McCormick, 2000). In vivo intracellular recordings in GAERS rats have also shown that 9 to 11-Hz rhythmic membrane oscillations and firing of layer 5/6 neurons in the facial primary somatosensory cortex (SI) tend to precede seizure onset (Polack et al., 2007). However, a causal demonstration that rhythmic firing or bursting actually triggers absence seizures is still lacking. It is possible that these rhythmic oscillations preceding seizure onset have no causal role, and simply reflect an underlying process driving the transition into the ictal state (e.g. Jirsa et al., 2014).

Sensory- and electrically-evoked seizures support a causal relationship between rhythmic stimulation and seizure initiation. For example, photoparoxysmal responses to intermittent light stimulation can cause various types of generalized seizures in photosensitive epilepsy (Topalkara et al., 1998; Guerrini and Genton, 2004). Electrical stimulation can also trigger self-sustained epileptiform events commonly referred to as afterdischarges and classified as seizures when associated with behavioral effects. For
example, early work from Steriade and colleagues has shown that self-sustained SWDs, the hallmark of absence seizures, can be elicited by single-shock or 10-Hz periodic electrical stimulation of the thalamus in behaving monkeys with spontaneous absence seizures (Steriade, 1974), and by either thalamic or cortical periodic stimulation in cats under light barbiturate anesthesia (Steriade and Yossif, 1974; Steriade et al., 1976). More recent studies have investigated cortically-induced afterdischarges in different rat strains. Cortical stimulation in immature rats can induce spike-wave afterdischarges, but without any specific difference between WAG/Rij rats and other strains with minimal incidence of seizures, suggesting different mechanisms for spontaneous seizures and electrically-induced afterdischarges in immature animals (Mares and Tolmacheva, 2007). In adult rats, afterdischarges induced by double-pulse electrical stimulation of deep cortical layers are specific to WAG/Rij rats and more pronounced in the SI cortical region (Lütjohann et al., 2011). Similarly, in GAERS rats, periodic (7-Hz) electrical stimulation in SI but also in the secondary somatosensory (SII) and insular (IC) cortices can induce behavioral spike-wave seizures (Zheng et al., 2012).

Here, we tested the hypothesis that rhythmic population bursting of excitatory cells in a local neocortical region can trigger absence seizures in epileptic rats. A limitation of the above studies is that electrical stimulation activates cells in a non-physiological manner, making it difficult to draw conclusions about the neuronal mechanisms underlying transition into spontaneous seizures. For example electrical stimulation affects all cell types, including not only excitatory and inhibitory neurons but also glial cells such as astrocytes (Vedam-Mai et al., 2012), which are thought to play an important role in seizure generation (Clasadonte and Haydon, 2012). It can also stimulate fibers of passage and result in antidromic neuronal activation (David et al., 2010), likely resulting in the activation of sparse and distributed neuronal populations (Histed et al., 2009). Optogenetic techniques, or the control of genetically targeted neuronal populations by light (Yizhar et al., 2011a), enable to circumvent these limitations.
A second limitation of previous studies is the lack of comparison between the spatiotemporal dynamics of induced epileptiform activity and spontaneous seizures. Our hypothesis implies that the induced seizures should originate from the same cortical location as spontaneous seizures, not from the stimulated area, and that they should share the same spatiotemporal features. This comparison is crucial to demonstrate that rhythmic bursting in local cortical regions may indeed trigger spontaneous generalized seizures. To monitor the spatiotemporal dynamics of SWDs during spontaneous and induced seizures, we used a novel combination of optogenetic stimulation and microelectrode array (MEA) recordings (Wang et al., 2012) in freely moving WAG/Rij rats, a well-established model of absence epilepsy (Coenen and van Luijtelaar, 2003).

We first asked (1) whether frequency-specific rhythmic population bursting of excitatory neurons in neocortex can rapidly lead to seizures consisting of self-sustained SWDs. Next, we investigated (2) whether ongoing brain states and neural excitation levels modulate the probability of seizure induction. Finally, we asked (3) whether induced and spontaneous seizures involved the same spatiotemporal network dynamics, as predicted by our hypothesis.

MATERIALS AND METHODS

Subjects

All procedures were approved by the Brown University Institutional Animal Care and Use Committee. Subjects used in this study were five male WAG/Rij rats (referred to as H2, H10, H11, H12 and H13; respectively 7, 12, 14, 16 and 17 months old). We also carried out additional experiments in five male Wistar rats, in two groups of age (Group 1: 6-7 months, referred to as C1, C2, C3. Group 2: 3-4 months, referred to as C5, C6). All animals were acquired commercially (WAG/Rij: Charles River, Germany; Wistar: Charles River, USA).
We injected recombinant adeno-associated viral vectors (serotype 2 pseudotyped with serotype 5) carrying genes for the red-shifted opsin C1V1(T/T) and the yellow fluorescent protein EYFP under the control of the CaMKIIα promoter (rAAV5/CaMKIIα-C1V1(E122T/E162T)-TS-EYFP), with a titer of $2 \times 10^{12}$ genome copies / ml. Viral constructs were kindly shared by Dr. Karl Deisseroth at Stanford, and were packaged and distributed through the University of North Carolina Vector Core.

During our preliminary experiments, one of the animals was injected with a lentivirus expressing ChR2 under the control of the synapsin promoter (VSV-G-Synapsin-hChR2(H134R)-EYFP-WPRE). This animal (H2) was included only in the part of the study related to spatiotemporal dynamics, to illustrate the variability of propagation patterns across subjects. However, the overall results and claims did not seem to depend on the type of opsin.

We used commercially available 32-channel ‘Utah arrays’ (1-mm long, 400-μm pitch, Blackrock Microsystems) where one electrode had been laser-ablated, and 64-channel laminar probes (200-μm pitch, Neuronexus), customly integrated with an optical fiber as described previously for planar MEAs (Wang et al., 2012). Briefly, a plastic cannula (Plastics One) was glued to the back of the planar MEAs using 5-Minute Epoxy (Devcon), while the array was temporarily inserted into a 2 % agarose gel. A 50 μm-core / 125 μm-cladding multimode optical fiber (0.22 NA, Thorlabs), previously tapered with a fiber puller (P-2000, Sutter Instruments) and connectorized to a 1.25 mm multimode ceramic ferrule (Thorlabs), was then inserted through the cannula. We took care that the fiber extended at the desired length (~ 1mm like neighboring electrodes) by visual inspection with a microscope, and then fixed it in place using dental cement (C&B Metabond, Parkell). Laminar arrays were built similarly, without the need for the agarose gel and using a flattened cannula. A few additional experiments in Wistar rats also employed platinum-iridium microwires.
Most subjects were implanted with a planar opto-MEA (H2, H10, H11, C1, C6). H13 was implanted with a laminar silicon probe and H12 with the combined planar / laminar arrays. C2, C3 and C5 were implanted with microwires.

**Surgical Procedures**

Animals underwent stereotactic surgery under isoflurane anesthesia. The temporal muscle was partially resected, a craniotomy was performed above the left SI (stereotaxic coordinates: -3-1 mm AP, 2-6 mm ML) and the dura was carefully removed. A viral vector was injected (1 and 2 mm deep, 1 μl each), using a 25-μl syringe cemented to a 32-gauge hypodermic needle (model 702 SN, Hamilton) connected to a microinjection pump (UltraMicroPumps III, World Precision Instruments) and positioned with a stereotaxic alignment system (David Kopf Instruments). A recording device was then chronically implanted at approximately the same location based on vascular landmarks, either right after the injections or four weeks later. Craniotomies were sealed with silicon elastomer (Kwik-Cast, World Precision Instruments) and covered with dental cement (C&B Metabond, Parkell).

The implantation procedure was preferentially performed during a second surgery, four weeks after the injections, in order to maximize the period of time with both high-quality recordings and opsin expression (subjects H10, H11, H12, C1, C5, C6). To perform controls, a few subjects were injected and implanted during the same procedure (H2, H13, C2, C3), in order to compare the effect of optical stimulation before (sham stimulation) and after opsin expression in the same animals. All the experiments used for statistical analysis were performed at least four weeks after viral injections, to obtain significant expression levels (for an evolution of the expression levels over time using a similar construct in rats, see Diester et al., 2011).
Electrophysiological Recordings and Optical stimulation

Experiments were performed using a custom data acquisition and control system built with amplifiers from Plexon (32-ch VLSI headstage 20x gain, model HST/32V-G20; 32-channel amplifiers, 0.7 Hz - 8 kHz, 50x gain, model PBX3/32wb-G50), multifunction DAQ from National Instruments (NI PCI- or PCIe -6259, M Series DAQ, 32 AI, 48 DIO, 4 AO) and custom software (Labview, National Instruments). Green or blue DPSS lasers (532 nm: LaserGlow Technologies; 561 or 473 nm: OptoEngine LLC) were modulated directly or via an acousto-optic modulator (AOM, model 48058-5-.55-5W, Gooch & Housego), which significantly improved pulse shapes. We report optical powers estimated in the brain given coupling efficiencies measured before implantation, typically 30-80 mW with short (10-ms) pulses.

Stimulation Protocol

In each animal, we first confirmed the presence of optically-induced effects by manually triggering the laser. For statistical analysis, we then used a randomized automatic protocol, in which the stimulation was automatically triggered at a random time every 30 to 60 s. The rationale was to wait long enough after each stimulation trial to avoid potential adaptation mechanisms, and to vary the inter-trial interval to account for possible slow rhythms affecting the probability of seizure induction. The choice of a 30-s minimum was based on the preliminary observation that a seizure could be induced even 4 s after the end of another spontaneous or induced seizure, and that seizures lasted on average less than 10 s. We also note that the stimulations occurring during ongoing seizures had very little effect and in particular did not interrupt them. During each session, one parameter (frequency, pulse width, train duration or optical power) was varied, with its different values randomly interleaved across trials. Sessions contained ~ 100 trials per parameter value, lasting ~ 8 h.
Histology

Following a period of 3 to 6 weeks after implantation, subjects were deeply anesthetized with pentobarbital (Beuthanasia-D) and perfused transcardially with phosphate buffer saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Brains were further fixed in 4% phosphate-buffered paraformaldehyde at 4°C overnight and then cryoprotected in 30% phosphate-buffered sucrose for 48 hours. 50 μm thick sections were prepared using a freezing microtome and mounted on glass slides using VectaShield Mounting Medium (Vector Laboratories).

Data Analysis

Inclusion criteria

For each animal, we pooled together all recording sessions where the same stimulation parameters (protocol, optical power, laser) were used. For H13, we pooled together days at 30 mW with one day at 80 mW, because there was no difference in optically-induced amplitudes and probabilities of seizure induction. The datasets used for H11, H12 and H13 were the same throughout the study. For H10, we used two different datasets. The dataset used in Figure 3 did not have 10-ms pulses. To be consistent across animals, we used another dataset containing 10-ms pulses for the rest of the study. In H11, the pia under the medial part of the array was partially damaged during surgery, but recordings on the lateral side of the array were unaffected. For this animal, we decided to use only the electrodes with good electrophysiological signals in the analyses presented here, yielding results consistent with the other animals, but excluded it from the spatiotemporal analysis.

Seizure detection algorithm

To detect seizures, we developed a custom algorithm, which detected local field potential (LFP) ‘spikes’ on each channel, grouped them together into potential epileptiform events and, for each of these events, defined its earliest onset across channels. Specifically, a spike was detected when the LFP first derivative
crossed a negative and positive threshold within a certain time window (typically 50 ms). Lower and upper thresholds on the amplitude were also used to avoid high-derivative low-amplitude spikes (lower threshold) and scratching artefacts (upper threshold). All thresholds were proportional to the signal median and on a coefficient adjusted to each animal (typ. 3-6). Next, if two spikes occurred within a certain time window (typ. 250 ms), they were grouped together. Different groups of spikes were further combined if they occurred close enough (typ. within 500 ms) and were considered a valid event only if they lasted more than 1 s. Valid events obtained individually on each channel were then compared across channels, so that the multichannel event onset (resp. end) was the earliest onset (resp. latest end) observed across electrodes. Multichannel events longer than 2.2 s were retained as seizures. Visual inspection was finally used to validate these seizures, but the onset times were fully determined by the automatic algorithm just described.

**Definition of induced and spontaneous seizures**

Seizures were classified as spontaneous or induced, depending on their coincidence with optical stimulation. A coincidence was detected if the seizure onset occurred during stimulation or shortly before, using a time delay adapted to each animal (H13: 500 ms, others: 150 ms). This time delay was justified because the grouping of individual threshold crossings in the seizure detection algorithm could result in detected onset times that shortly preceded the actual onset as assessed by visual inspection. These time delays were chosen by visual inspection of one part of the data (first hour of recordings for each animal) and are typically related to the thresholds used for seizure detection. We discarded spontaneous seizures containing light stimulation, induced seizures that did not outlast stimulation by > 1 s, and stimulation trials that followed a seizure by < 10 s (to account for a potential post-ictal state). We also discarded stimulation trials containing movement artefacts during the time windows of interest.
Perievent LFP and eMUA preprocessing

LFPs were filtered between 1 and 300 Hz (forward-backward 3rd-order Butterworth filter), an artifact removal algorithm was applied, and signals were downsampled at 1 kHz. We extracted the multi-unit activity (MUA) envelope (eMUA) as described previously (Stark and Abeles, 2007): we filtered raw signals between 300 Hz and 8 kHz, took their absolute value, applied an artifact removal algorithm, low-pass filtered below 300 Hz and downsampled at 1kHz. To remove artefacts, we interpolated a time segment around stimulation, consistently for all electrodes (1 ms before and after pulse for planar MEAs, 3 ms before and 8 ms after for laminar probes, shape-preserving piecewise cubic interpolation for LFPs and linear interpolation for eMUA).

Spectral analysis

The Chronux toolbox (http://chronux.org/; Mitra and Bokil, 2007) was used to obtain the multitaper spectrum (‘mtspectrunc’) and multitaper time-frequency spectrum (‘mtspecgramc’) of LFPs between 1 and 300 Hz. For all analyses, we used a time window of 1 s, a time half bandwidth product TW = 2, and number of tapers K = 3. These parameters were chosen to obtain a reasonable degree of smoothness while maintaining enough band separation.

Spectrograms comparing the different types of stimulation trials and seizures (Figure 5A) were represented by normalizing each time-frequency spectrum by the average frequency spectrum in the 2-s time window preceding trials with no seizure (the common reference allowed visual comparison between the three cases). Power spectral densities (PSD) (Figures 5B and 7A) represent the frequency spectra without normalization (in mV^2 / Hz) in a particular time window, and are displayed between 1 and 30 Hz to illustrate the main differences that we identified. Spectral features used for subsequent classification / prediction performance analysis (Figure 7C-D) were computed by averaging the power of the frequency spectrum in the following frequency bands, based on previous literature (e.g. Menzer et al., 2010): delta (1-3.5 Hz), theta (3.5-8 Hz), alpha (8-14 Hz), beta (14-30 Hz), low-gamma (30-60 Hz), mid-gamma (60-
90 Hz), high-gamma (90-130 Hz) and high frequencies (130-300 Hz). Finally, analyses based on the
phase at 10 Hz (Figure 7C) relied on the Hilbert transform to extract the instantaneous phase of the signal
20 ms before the beginning of the optical stimulation.

Optical flow analysis

Directions of propagation of LFP waves were extracted based on the Horn-Shunk method (Horn and
Schunck, 1981), a computer vision algorithm classically used to extract the optical flow, or apparent
motion, between two consecutive frames. Optical flow methods (Horn-Shunk or similar) have been used
previously to study the spatiotemporal dynamics of neural signals (Lefèvre and Baillet, 2009; Slater et al.,
2012; Mohajerani et al., 2013). Briefly, we normalized LFPs, interpolated spatially, applied a mask at a
negative threshold, ran the Horn-Shunk algorithm, and took the average optical flow, yielding a
directionality vector for each time. The propagation direction of a given discharge was defined as the
angle of the directionality vector when its norm reached a maximum within a certain time window ([−20,
5] ms) around the population peak. More precisely, LFPs were first z-scored based on the signals between
5 and 0 s before stimulation during trials with no seizure. Next, interpolation was used to create smooth
maps. Missing or noisy channels were interpolated by iteratively averaging (10 times) the values of their
nearest neighbors. Then, we applied a 2D linear interpolation on a refined grid formed by repeatedly
dividing the intervals 3 times in each dimension. Finally, we restricted our analysis to the negative spike
components by applying a mask on the interpolated data, taking into account only pixels that were more
negative than 2 standard deviations. We ran the Horn-Shunk algorithm with 100 iterations and a
smoothness parameter $\alpha = 10$.

Neural signals prediction performance

We tested the hypothesis that different features of neural signals during ongoing brain dynamics or in
response to stimulation could predict whether a given trial would lead to a self-sustained seizure after the
end of the stimulation. This question was addressed by building classifiers that could distinguish between
trials which succeeded (‘ind. sz’) or failed (‘no sz’) to induce a seizure, based on either spectral or
amplitude LFP features (for more details about features, see ‘Spectral analysis’ section) in a period either
prior or during optical stimulation. We used support vector machine (SVM; see e.g. Hastie et al., 2009)
classifiers with a Gaussian radial basis function (RBF) kernel, i.e. \( K(x, x') = \exp(-\frac{\|x-x'\|^2}{2\sigma^2}) \).

Hyperparameters (kernel parameter \( \sigma \) and soft margin parameter \( C \)) were selected using a coarse
logarithmic grid search (\( \log_{10}(C) \in [-3, 3] \), \( \log_{10}(\sigma) \in [0, 3] \)) and 10-fold cross-validation. For a given
set of hyperparameters, we combined scores from all the cross-validation folds and computed a receiver
operating characteristic (ROC) curve. As a performance metric, we used the area under the ROC curve
(AUC) for the best set of hyperparameters (\( \sigma \) and \( C \)) and reported a normalized version of it as the
prediction performance (\( 2 \times AUC - 1 \)), with 0 indicating chance level and 1 perfect classification. To
estimate the robustness of the obtained metric, this entire procedure was repeated 10 times over randomly
chosen cross-validation folds. 95 % chance levels and p-values associated with the prediction
performance (\( 2 \times AUC - 1 \)) were computed by permutation tests. Specifically, the labels (‘ind. sz’ or
‘no sz’) were randomly shuffled, a classifier was trained on the shuffled data, its performance evaluated,
and this procedure was repeated 100 times for 95% chance levels and 1,000 times for p-values.

**Monte-Carlo statistical tests**

Monte-Carlo tests were used in Figures 2C and 3 to test if the number of coincidences between light
stimulation and seizures could be explained by chance alone. Specifically, surrogate stimulation times
were generated based on the parameters of the randomized stimulation protocol. For each surrogate
dataset, the number of coincidences in the surrogate data (\( n_{ind_{MC}} \)) was then compared to the number of
coincidences in the observed data (\( n_{ind_{obs}} \)), yielding a p-value: \( p = 1 + \frac{N}{N_{MC}+1} \), where \( N \) is the number
of datasets with \( n_{ind_{MC}} \geq n_{ind_{obs}} \), and \( N_{MC} \) is the number of Monte-Carlo surrogate datasets.
**Other statistical methods**

Unless stated otherwise, all pooled data are represented by their means and 95% confidence intervals obtained by bootstrap. For bootstrapping time or frequency series, we sampled with replacement the entire vector (series) across trials, yielding 1,000 vector bootstrap estimates, calculated the mean vector of each bootstrap estimate, and then computed the 95% confidence interval of the distribution of these mean vectors. Welch’s t-tests were employed to compare the means between two groups. Permutation tests were used to compare two groups in cases where t-tests could not be used. To correct for multiple hypothesis testing, we performed a false discovery rate (FDR) correction for either independent (Benjamini and Hochberg, 1995) or dependent tests (Benjamini and Yekutieli, 2001).

**Simulations of Light and Heat Propagation**

We estimated light-induced heating as described previously in the context of optogenetics (Ozden et al., 2013). We first used the Monte-Carlo method of photon transport in a scattering medium (Wang et al., 1995) to obtain the distribution of absorbed volume power density, and then numerically solved the heat equation. Specifically, we launched $10^7$ photon packets from a 50-μm diameter aperture (NA = 0.22) on a three-dimensional Cartesian grid of step size $dx = dy = dz = 10$ μm. We used a brain refractive index of 1.36, absorption and scattering coefficients of 42.3 m$^{-1}$ and 10,600 m$^{-1}$ respectively and an anisotropy factor of 0.887, values which were previously obtained from optical measurements of human grey matter at 532 nm (Yaroslavsky et al., 2002). These simulations yielded the absorbed volume power density $P_V$.

We then used a finite difference method (forward in time and central second-order in space with zero-Laplacian boundary conditions) to solve the heat equation $\rho c \frac{\partial T}{\partial t} = k \Delta T + P_V$, where $T$ is the temperature in the brain, $P_V$ the absorbed volume power density obtained from the above simulations, $\rho = 1.07 \times 10^6$ g.m$^{-3}$ the brain mass density, $c = 3.6$ J.g$^{-1}$.K$^{-1}$ the specific heat capacity and
$k = 0.56 \text{ W.m}^{-1}\text{.K}^{-1}$ the thermal conductivity. These parameter values have been used previously for heat simulations in the brain (see e.g. Elwassif et al., 2006).

RESULTS

Frequency-specific optically-evoked rhythmic population bursting of excitatory cells in neocortex triggers self-sustained seizures in absence epileptic rats

To evoke cortical bursting, we first expressed the opsin C1V1(T/T), which enables cell depolarization under green light illumination, in cortical excitatory cells using the CaMKIIα promoter. To observe whether pulsed optical stimulation of these excitatory cells was able to trigger primary generalized absence seizures, we implanted devices for combined optical stimulation and multisite electrical recordings, consisting of an optical fiber integrated with 32-channel planar MEAs, 64-channel laminar probes or both (Figure 1A-C). These devices were implanted at the site of viral injections in SI, located in the vicinity of the presumed cortical focus (Figure 1D-E). The injection site showed strong expression of the C1V1(T/T)-EYFP construct as assessed by histology at the end of experiments (Figure 1F). Despite slight variations between animals, we consistently observed the largest spread in deep layers (V-VI), over several millimeters both in the coronal and horizontal planes. This pattern could be due to the depth of our injections (1-2 mm) or to a particular tropism of the virus (AAV5), as observed previously in non-human primates (Diester et al., 2011).

To induce seizures, we delivered optical pulse trains (green light, 532 nm) at a constant frequency of 10 Hz for 1 s, with a typical pulse width of 10 ms (Figure 2). This pattern was designed to mimic the ~ 10-Hz SWDs seen at seizure onset in rats. For sufficiently high optical powers (typically 30-80 mW in the brain) and under appropriate brain states, each optical pulse evoked a burst of neuronal spiking activity, characterized by one or more negative deflections in LFPs, followed by a period of neuronal silence.
(Figure 2A), reminiscent of SWDs during spontaneous seizures (Pollen, 1964; Steriade, 1974) but with more variable morphologies (Figure 2B). Importantly, the effect of optical stimulation was dependent on the cortical location and on the behavior of the animal, likely indicating a dependence on ongoing brain states as detailed later. Strongly negative LFP deflections were associated with neuronal bursting as seen in the spiking activity, while more moderate or positive LFP deflections were associated with irregular spiking (Figure 2A). We also note that these deflections were physiological and not related to photo-induced artefacts, which affected only a subset of electrodes and could be removed by a custom algorithm (see Materials and Methods). As detailed later, they were also mediated by the opsin and not by non-specific effects such as heating.

Following the 1-s train of stimuli, two types of behavior occurred: either a quick return of neuronal activity to its background level, or self-sustained trains of SWDs outlasting the stimulation period by up to more than 10 s (Figure 2A, ‘no seizure’ and ‘induced seizure’ respectively). SWDs during these induced epileptiform events appeared similar in morphology and amplitude to SWDs occurring during spontaneous seizures (Figure 2B). We refer henceforth to the responses directly following each light pulse as optically-evoked responses / bursts, different from the self-sustained SWDs that followed the 1-s stimulation period during induced epileptiform events.

Absence seizures in rats have very mild behavioral manifestations: a simple freezing behavior, sometimes associated with rhythmic whisker movements. They are also known to occur mostly during drowsiness, when the rat is already still, making their behavioral identification challenging. Although we did not systematically quantify behavior, all induced self-sustained epileptiform events observed during wakefulness were also associated with behavioral arrest and sometimes whisker movements (Supplemental Movie 1). Therefore, we define these episodes of self-sustained SWDs as induced seizures in the rest of this study.
To quantify the probability of inducing seizures, we employed an automated stimulation protocol where the stimulation was triggered at a random time every 30 to 60 s with randomly interleaved parameters, and we developed an amplitude-based algorithm for seizure detection, able to define seizure onset in an unbiased manner (see Materials and Methods). These events were then automatically labeled as spontaneous or induced seizures, depending on whether they coincided with stimulation. This annotation process led to the definition of three types of events: stimulation trials with no seizure, induced seizures, and spontaneous seizures.

We characterized the probability of inducing seizures for stimulation frequencies between 6 and 18 Hz, over several hours per session (typically 8 h), across at least three sessions per animal. The resulting datasets contained hundreds of stimulation trials for each animal and each stimulation parameter. The probability of inducing seizures was defined as the ratio between the number of stimulation trials that induced a seizure and the total number of stimulation trials. Although we conservatively discarded trials when a few LFP spikes occurred before stimulation onset, some of the seizures classified as ‘induced’ could be due to random coincidences between stimulation trials and spontaneous seizures (hundreds of spontaneous seizures per day). To quantify the chance level of such coincidences, we performed Monte-Carlo statistical tests (see Materials and Methods). In all WAG/Rij rats tested (N = 4), we found that the probability of inducing seizures was always statistically significant (p < 0.05 with FDR correction for multiple tests) for stimulation frequencies of 8, 10 and 12 Hz (Figure 2C and Figure 3). Additionally, the probability of inducing seizures was strongly dependent on the stimulation frequency and achieved a peak at 10 Hz, reaching a maximal probability of 6-20% depending on the animal (Figure 3). Although resonant frequency responses have been observed with wild-type channelrhodopsin 2 (Tchumatchenko et al., 2013), C1V1(T/T) does not have such intrinsic properties (Yizhar et al., 2011b). The observed frequency tuning therefore originated from the brain. It did not develop as a result of repeated stimulation, as there was no apparent correlation with the number of days of stimulation.
Finally, optically-evoked LFP responses and MUA bursts were mediated by the opsin and not by thermal
effects or visual stimulation, as seen by the absence of evoked responses prior to opsin expression and by
their progressive increase over four weeks following viral injections (Figures 4A-B). Even at 30-80 mW,
temperature changes induced by short pulses at 10 Hz were safe and did not exceed 1-2 °C at the end of
the train (Figure 4C), as assessed by simulations of light and heat propagation in brain tissue (Ozden et
al., 2013). The fiber was located at a depth of ~ 1 mm, but light scattering in the brain probably resulted
in illumination across a few millimeters based on irradiance levels from simulations of light propagation,
and on electrophysiological results described later (Figure 9A).

In the rest of this study, we will consider exclusively stimulation at 10 Hz because of its maximal
probability of inducing seizures.

To investigate in more details the similarities between induced and spontaneous seizures in epileptic
WAG/Rij rats, we compared their duration and spectral characteristics using multitaper methods (Figure
5). Optically-evoked responses were characterized by several harmonics of the 10-Hz stimulation
frequency. During trials with induced seizures, a high-power band slightly below 10 Hz followed, looking
similar to spontaneous seizures (Figure 5A), which are known to start at 10 Hz and evolve to 8 Hz once
established (Coenen and van Luijtelaar, 2003). We computed the LFP multitaper power spectral density
(PSD) in a 1-s time window following the end of the stimulation period. The PSDs of induced and
spontaneous seizures were strikingly similar and had their main peak in the 5-12 Hz frequency range,
with a maximum at 8 Hz (Figure 5B). Confirming visual inspection, the PSDs of induced and
spontaneous seizures were almost perfectly correlated and had similar total powers (Figure 5C). We also
compared discharge amplitudes between induced and spontaneous seizures. Specifically, we computed
the peak amplitudes of the spike component of SWDs, averaged them across electrodes and discharges
within a seizure, and compared their mean across seizures between induced and spontaneous seizure
groups. We found no statistically significant difference in any of the animals (α = 0.05, Welch’s t-test,
FDR correction for independent tests. Mean ± standard error of the mean: H10: $V_{\text{spont}} = -0.67 \pm 0.0045$ mV, $V_{\text{ind}} = -0.66 \pm 0.0096$ mV; H11: $V_{\text{spont}} = -0.37 \pm 0.0028$ mV, $V_{\text{ind}} = -0.38 \pm 0.0055$ mV; H12, $V_{\text{spont}} = -0.53 \pm 0.0031$ mV, $V_{\text{ind}} = -0.52 \pm 0.0067$ mV; H13, $V_{\text{spont}} = -0.54 \pm 0.0063$ mV, $V_{\text{ind}} = -0.54 \pm 0.012$ mV).

Finally, we compared the average durations of induced and spontaneous seizures (Figures 5D-E). In two animals, there was no statistically significant difference ($\alpha = 0.05$, Welch’s t-test, FDR correction for independent tests). In the other two animals, the average durations still remained on the same order, with reductions of 15 and 35%.

We next asked whether induced seizures were specific to this rat strain. We performed the same experiments in Wistar rats ($N = 4$), from which the WAG/Rij strain was originally inbred. Importantly, seizure susceptibility is also high and increases with age in many outbred rat strains, including Wistar rats (Vergnes et al., 1982; Jandó et al., 1995). In all animals, the opsin expressed well and gave rise to strong optically-evoked bursting responses. Out of these four animals, two showed seizure-like events, both spontaneous and triggered by light. The other two animals did not show any seizure-like event, neither spontaneous nor triggered (Figure 6). These differences seemed to be correlated with the age of the animal (respectively 6-7 months and 3-4 months for the rats with and without seizure-like events). These data indicate that optically-induced seizures were not specific to WAG/Rij rats. Nevertheless, they seemed to occur only in animals that also displayed spontaneous seizures.

**Brain states preceding stimulation and neural excitation in response to stimulation are predictive of seizure induction**

To better understand the mechanisms underlying the induction of seizures, we next searched for neural markers that could predict or explain seizure induction. We first hypothesized that the trial-to-trial variability in seizure induction could be partially explained by changes in brain states characterized by
distinct ongoing dynamics, more precisely by the power of LFP oscillations preceding stimulation (Figure 7).

For three out of the four animals (H11, H12, H13), the power in all frequency bands between 1 and 30 Hz (delta, theta, alpha, beta) during the 1-s period preceding stimulation was consistently lower for stimulation trials with induced seizures than trials without (Figure 7A). This difference was already present between 4 and 3 s before stimulation, although to a smaller extent. For the one animal that did not show this contrast (H10), we nevertheless observed differences in higher frequency bands, above 60 Hz. On average across animals, we observed that the total power below 30 Hz was smaller during the time period preceding trials that induced a seizure, compared to trials that did not (Figure 7B).

The smaller power in frequency bands below 30 Hz during trials with induced seizures (compared to trials without seizure) can seem counter intuitive, as spontaneous seizures were usually preceded by large powers in the frequency bands below 30 Hz, especially in the theta range (4-8 Hz) (Figure 7A, black). These results are also consistent with the individual examples shown in Figure 2, and could potentially result from the destructive interference between optically-evoked responses and ongoing oscillations. Alternatively, seizure transition might be possible only during a transient period at the onset of these oscillations, but not after a prolonged period of oscillatory activity. Indeed, the large powers in bands below 30 Hz preceding spontaneous seizures occurred only during the few seconds preceding seizure onset (Figure 7A, compare black curve at 4-3 s and 1-0 s before seizure onset).

To quantify further the influence of LFP oscillations, we trained a support vector machine (SVM) to predict seizure induction based on different LFP features. We first computed the power in eight frequency bands from 1 to 300 Hz (see Materials and Methods) in a 1-s window at different times around the stimulation, and plotted the evolution of the prediction performance over time (Figure 7C). The prediction performance tended to increase over time before stimulation, increasing above the 95% chance level.
during the 1-s period immediately preceding stimulation onset for all animals (Figure 7C, between -1 and 0 s). After FDR correction for multiple testing, prediction performance remained statistically significant for all animals except H10 during this time period (Figure 7D, table, first row, p < 0.05 after multiple tests correction, 1,000 random permutations). Prediction performance increased during the stimulation period, suggesting that different types of responses discriminated these two types of trials, a feature that we address in more detail next.

To test whether ongoing oscillations at 10 Hz played a particular role in prediction performance, we also trained SVMs based on different features, namely the power and phase at 10 Hz (Figure 7D, rows 2-4). Phase alone was never significantly predictive. Power at 10 Hz was significantly predictive only for H13, and adding phase information did not increase predictability. We conclude that the differences in LFP oscillations that favored seizure induction were broadband and did not involve 10 Hz specifically.

We have shown that fluctuations in brain states, defined as LFP oscillations preceding stimulation, could partially explain if seizure induction was likely to happen depending on the trial. Interestingly, spectral features were discriminative not only before, but also during stimulation, (Figure 7C: between the two solid lines). This led us to investigate further how neural excitation levels, assessed by the amplitude of the response to external stimulation, could explain the variability in seizure induction.

We examined trial-averaged LFPs and MUA during the stimulation period to determine any difference between trials where light stimulation succeeded or failed to induce a seizure (Figure 8). Figure 8A shows a typical example of the MUA envelope (eMUA) and LFPs in both cases and across two consecutive days of recording. Optically-evoked responses / bursts had a larger amplitude in both types of signals for stimulation trials that induced seizures, an effect more or less pronounced depending on the recording day. The variability of response amplitudes across days, like the variability in the number of spontaneous
seizures, probably resulted from day-to-day fluctuations in neural excitation levels. Finally, we note that the amplitude difference between the two types of trials was not present after the first light pulse, but built up over time after a couple of stimuli.

To determine whether these observations were consistent across electrodes, we compared the peak amplitude in response to the first light pulse and the maximum peak amplitude among all ten pulses for each electrode individually (shown for two animals, Figure 8B). For all electrodes, the first peak amplitudes were similar between both types of trials. The maximum peak amplitudes however, were larger for trials with induced seizures. We can also notice a wide distribution of amplitudes across electrodes (Figure 8B), likely reflecting differences both in levels of light activation and neural excitation.

LFP amplitude differences were consistent across animals (N = 4, Figure 8C-D). To quantify their prediction performance, we trained a classifier as described above (Figure 8E). The maximum peak amplitude was indeed an excellent predictor of seizure induction, with predictive values exceeding those obtained previously with ongoing LFP oscillations (compare Figures 7D and 8E). Interestingly, even the first peak amplitude usually contained information about seizure induction, although to a smaller extent and not visible by visual inspection.

Optically-evoked bursting entrains nearby cortical focus into self-sustained SWDs which propagate in the same direction as SWDs in spontaneous seizures

Finally, we asked whether induced and spontaneous seizures were generated by the same cortical regions, and how these regions were recruited. To address this question, we investigated the spatiotemporal dynamics of optically-evoked responses, and of SWDs during induced and spontaneous seizures. We used two types of arrays, planar and laminar, to access these dynamics both in a plane parallel to the cortical surface (1 mm deep) and across cortical layers.
Optically-evoked responses contained contributions from direct light activation and from network effects. These contributions could be difficult to disentangle but are illustrated for one animal on Figure 9A. In this animal, the trial-averaged eMUA contained either one or two peaks in response to each light pulse. Sorting these two peaks based on their latencies, we obtained delay maps of the early (≤ 12 ms after light onset) and late components of the optically-evoked responses. The spatial distribution of the early peak revealed an initial activation affecting a subset of electrodes located a few mms apart, likely reflecting the direct effect of light stimulation. In comparison, the late peak affected a larger number of electrodes and its delays were spatially organized, suggesting propagation of a wave likely generated by indirect network mechanisms.

This observation further motivated us to look at single-trial spatiotemporal dynamics. For single trials, MUA spatial dynamics were noisy and did not reveal obvious propagation patterns. By contrast, LFPs showed clear waves of propagation during single trials, as illustrated on Figure 9B (see also Supplemental Movie 2). In this animal, two types of waves were observed. The first type of wave (Figure 9B, top) started on electrodes near the optical fiber and propagated towards the bottom right corner of the MEA. Characteristic of the optically-evoked responses / bursts, these waves were seen only during the stimulation period, shortly following each light pulse. The second type of waves (Figure 9B, bottom) appeared to propagate in the opposite direction. They occurred mostly during the self-sustained SWDs of induced (and spontaneous) seizures. Sometimes, they were also observed between two consecutive light pulses during the stimulation period, indicating network mechanisms (see also Figure 10B).

In the following analysis, we first compare systematically optically-evoked responses and self-sustained SWDs during induced seizures, to show that the cortical regions involved in the generation of induced seizures were recruited indirectly during stimulation. We then compare SWDs in induced and spontaneous seizures to show that they are generated by the same cortical regions.
To track LFP wave propagation, we developed a method to quantify the direction of propagation of LFP discharges, based on their apparent motion (optical flow, see Materials and Methods). We observed no consistent relationship between optically-evoked responses (during the stimulation period) and induced SWDs (following the stimulation period) (N = 6 arrays from 5 animals, Figure 10A), both in terms of direction of propagation (columns 1 and 3) or initiation site (columns 2 and 4). Only in one array recording (H12-laminar), we observed similar, but not identical, directions of propagation and initiation sites, with an initial activation in the deep and posterior portion of the laminar array. In two animals (H10 and H2), the directions of propagation during stimulation had a bimodal distribution. One of the directions was the same as the one during induced seizures, as mentioned previously for single-trial examples (Figure 9B). For these animals, we sorted discharges based on their direction of propagation and plotted the average initiation site for each direction. The discharges propagating in the same direction as subsequent self-sustained SWDs also had the same initiation site (Figure 10A, compare column 2, dir.2 with column 4). Interestingly, they were slightly less synchronized with light pulses than the discharges propagating in a different direction (Figure 10B), in agreement with the indirect recruitment of a distant region that would then generate discharges at its own frequency. The changes in direction of propagation between the stimulation periods and the subsequent induced seizures are further summarized in Figure 10C.

The above results show that the cortical regions generating induced seizures were entrained by the optically-evoked bursts and started generating their own discharges either during or after stimulation. To determine whether the same regions were involved in spontaneous seizures, we compared the propagation patterns of SWDs during induced and spontaneous seizures (Figure 11). SWDs during induced and spontaneous seizures were remarkably similar, both in terms of direction of propagation (Figure 11A, column 2 vs 4) and relative delays between electrodes (Figure 11A, column 1, and column 3 vs 5). It is important to note that these delay maps are in excellent agreement with the directions of propagation.
obtained independently using the optical flow algorithm (Figure 11A, column 2 vs 3 and 4 vs 5). We also performed a similar analysis based on eMUA propagation, and the results were qualitatively similar between LFPs and eMUA. The similarities in the directions of propagation between induced and spontaneous seizures are further summarized in Figures 11B-C. In particular, these similarities are in sharp contrast with the change in the direction of propagation between stimulation periods and induced seizures (compare Figures 10C and 11C).

We conclude that the seizures induced in the present study originated from the same cortical locations, likely hyperexcitable, as spontaneous seizures, and that these regions were entrained by optically-evoked rhythmic bursting. More precisely, optically-evoked bursts propagated as waves and reached the natural cortical focus. Once this focus became excited enough, it started generating its own self-sustained train of SWDs propagating in the same direction as SWDs during spontaneous seizures, starting either during or after the stimulation period.

DISCUSSION

Overall, our study presents 3 main findings: (1) We provide a causal demonstration that rhythmic population bursting of excitatory neurons in a local neocortical region at particular frequencies is sufficient to trigger primary generalized seizures in absence epileptic rats; (2) We show that the probability of seizure induction is modulated by ongoing brain states, reflected in LFP power spectrum features preceding stimulation, and by neural excitation levels, reflected in the response amplitudes to optical stimulation; (3) We demonstrate that SWDs during induced and spontaneous seizures propagated with the same spatiotemporal dynamics, likely indicating recruitment of the same network, and that this common origin was entrained by network-mediated wave propagation of the optically-evoked bursts. To our knowledge, this is the first study to induce seizures by optogenetic stimulation in an animal model of
primary generalized epilepsy, and to compare the spatiotemporal dynamics of the induced and spontaneous seizures.

Methodological approach

Optogenetics has been used previously to control abnormal seizure activity (Wykes, et al., 2012; Paz et al., 2013; Krook-Magnuson et al., 2013) and to induce seizure-like afterdischarges in the hippocampus of non-epileptic rats (e.g. Osawa et al., 2013). It has never been applied however, to the study of seizure initiation in awake, freely behaving, epileptic animals with primary generalized seizures. Compared to previously used methods of stimulation, optogenetics differs in its physiological effects. Electrical microstimulation is indeed thought to activate a sparse and distributed network of cells by direct stimulation of local processes (Histed et al., 2009), whereas optogenetic modulation affects preferentially cell bodies closer to the optical fiber. Additionally, the CaMKIIα promoter restricts expression to neurons, as opposed to glial cells, with a strong preference for excitatory cells. Furthermore, it is likely that the effects presented in this study were mediated primarily by layer 5-6 excitatory neurons projecting to the thalamus (given strong opsin expression in these layers), in particular to the thalamic reticular nucleus, which in turn activated a wide variety of cells.

An important parameter in these experiments is the volume of direct light activation. This volume depended on the spread of the virus (several mm), the optical powers used (30-80 mW) and the alignment between the fiber and injection site, which was only approximate due to the 4-week delay between injections and device implantation. As an upper bound, Monte-Carlo simulations of light propagation (Ozden et al., 2013) indicate an activation volume of 3-4 mm in diameter for these optical powers, assuming uniform expression of the opsin and a spiking activation threshold of 1 mW/mm².

Electrophysiological data suggested that the sites of direct activation spread over < 2 mm, as assessed by the latencies of eMUA peaks with respect to light stimulation, with delays around 6 and 20 ms.
respectively for direct light activation and network-mediated effects. Importantly, the regions affected by
the < 2-mm stimulation diameter were local in comparison to the bilateral synchronous nature of primary
generalized seizures. As mentioned above, network-mediated effects likely involved thalamocortical
reverberations, and intracortical / intrathalamic propagations.

Frequency-specific rhythmic bursting as a mechanism of ictal transition

Seizures were induced optimally by optical stimulation frequencies around 10 Hz. It is tempting to make
a connection between this optimal frequency and the fact that spontaneous seizures in this absence
epilepsy model also start at ~ 10 Hz, before slowing down to ~ 8 Hz, suggesting the hypothesis of a
resonant network. Interestingly, frequency-dependent responses have also been seen in others forms of
stimulation, such as sensory (visual) or electrical stimulation.

In photosensitivity for example, a condition closely associated with primary generalized epilepsy,
intermittent light stimulation at frequencies of 15-18 Hz (Topalkara et al., 1998) induces
photoparoxysmal responses and can sometimes lead to behavioral seizures. However, this optimal
frequency can differ from the frequency of the subsequent self-sustained discharges, which for example
occur at 3 Hz in childhood absence epilepsy. During cortical functional mapping in epileptic patients
undergoing resective surgery, electrical stimulation can also induce subclinical afterdischarges or
behavioral clinical seizures. For historical reasons, these stimulations typically use frequencies of 50-60
Hz. Additionally, Zangaladze et al. (2008) reported the probability of inducing afterdischarges for 5-, 10-
and 50-Hz electrical stimulation, and found an increase of afterdischarge induction with frequency.
However, these stimulations are usually applied in focal, not primary generalized, epilepsies and affect
different networks, likely accounting for the difference in activation frequency. Due to the photocycle
kinetics of the opsin, optogenetic stimulation is usually limited to frequencies below 50 Hz, making
comparison with electrical stimulation protocols difficult. In our earlier exploration of stimulation
frequency ranges, we noticed that driving LFP oscillations at 50 Hz using 1-s trains did not induce any seizure, rather it seemed to have an inhibitory effect on seizures. Using longer trains, more complex effects arose, including ~ 20 Hz paroxysmal responses during stimulation, sometimes followed by 10-Hz SWDs.

Early work from Steriade and colleagues has also shown that 3-Hz self-sustained trains of SWDs can be elicited by thalamic electrical stimulation in behaving monkeys with spontaneous absence seizures (Steriade, 1974), and by either thalamic or cortical stimulation in cats under light barbiturate anesthesia (Steriade and Yossif, 1974; Steriade et al., 1976). In cats, where repeated stimulation at 7-10 Hz was tested, SWDs followed responses of increasing amplitudes. Such augmenting responses have been reported during both cortical and thalamic stimulation around 10 Hz as a form of short-term facilitation, and occur preferentially during behavioral states of low vigilance (Castro-Alamancos and Connors, 1996; Steriade et al., 1998; Timofeev et al., 2002). Interestingly, the same behavioral dependence is observed in spontaneous absence seizures and electrically-induced afterdischarges (Lüttjohann et al., 2011). In our own experiments, we observed augmenting responses during the first few light pulses, suggesting that a similar form of facilitation might explain the strong frequency tuning we obtained.

As stated earlier, the most epileptogenic stimulation frequency in our experiments, 10 Hz, was also the same as the frequency of SWDs in induced and spontaneous seizures. However, in light of the literature cited above, we would like to be cautious regarding the hypothesis of a resonant network. We note that the match between these two frequencies could be coincidental and that these frequencies might differ in other models, such as the 3-Hz seizures induced by Steriade and colleagues using 10-Hz stimulation. If the frequency match in our experiments is indeed coincidental, the optimal frequency for inducing seizures may be determined by other mechanisms, such as short-term facilitation, which are not directly related to the frequency of SWDs during seizures.
Although we did not systematically explore non-periodic stimulation patterns, it is also important to note that other mechanisms could lead to seizure transition. We have causally demonstrated that rhythmic bursting is sufficient to trigger seizures. It remains, however, an open question whether rhythmic bursting is also necessary for triggering primary generalized seizures.

**Probability of induced ictal transitions is modulated by ongoing brain states and neural excitation levels**

We also demonstrated that ongoing brain states and neural excitation, reflected in the power of ongoing LFP oscillations and the amplitude of response to light stimulation respectively, were predictive of seizure induction. These analyses yield new evidence for the relationship between brain states or excitation levels and the probability of seizure transition.

Using the power of LFP oscillations preceding stimulation, we showed that separate brain states could predict seizure induction. Spontaneous absence seizures and electrically-induced afterdischarges in WAG/Rij rats are known to occur mostly during behavioral states of drowsiness (Lüttjohann et al., 2011). However, predicting the transition to induced seizures / afterdischarges based on neural signals, as opposed to behavior, had not been demonstrated so far. Previous studies in WAG/Rij rats have identified preictal oscillations in the delta and theta range (van Luijtelaar et al., 2011) and network changes assessed by nonlinear Granger causality (Sysoeva et al., 2014) up to 3 s before seizure onset. Our classification results of induced seizures agree with these previous observations, although in our case most changes occurred during the second just before seizure onset.

The best prediction results were obtained using another feature: the amplitude of the optically-evoked responses, i.e. the network response during stimulation. We found that a threshold mechanism separated stimulation trials with and without induced seizures. One potential practical application is the possibility
for active probing of network excitation level. Using brain stimulation to infer parameters of network
dynamics has been conceptually approached by Kalitzin et al. (2010) through computer simulations. In
line with these ideas, our results suggest that measuring the amplitude of the response to repeated external
stimulation at particular frequencies could act as a warning, or as a control signal for seizure prevention
systems. If these results still hold for electrical stimulation, then it can be possible to implement active
probing of network excitation in devices that can both record and stimulate simultaneously.

Network-mediated entrainment of the epileptogenic focus leads to induced seizures with the same
spatiotemporal dynamics as spontaneous seizures

Finally, we showed that the self-sustained SWDs during induced seizures had the same spatiotemporal
dynamics as SWDs in spontaneous seizures. In particular, the switch in the direction of propagation
between the stimulation period and the subsequent self-sustained SWDs reinforces our claim of the
indirect recruitment of the natural epileptogenic network. This was an open issue in previous studies
investigating primary generalized epilepsy and absence seizures in particular. Even in focal epilepsies,
many studies have investigated the mechanisms of electrically-induced afterdischarges or seizures, but the
relationship of these events with respect to spontaneous seizures is still obscure (Wieser et al., 1979;
Bernier et al., 1990; Blume et al., 2004; Kalamangalam et al., 2014).

Our results complement previous studies performed in rat models of absence epilepsy where the
spatiotemporal dynamics of neural signals in response to stimulation was not monitored. For example,
Lüttjohann et al. (2011) previously used double-pulse electrical stimulation to demonstrate the
hyperexcitability of SI compared to MI in WAG/Rij rats, assessed by electrically-evoked potentials and
afterdischarges, which lasted longer when elicited from SI than MI. In that study, it is unknown if
afterdischarges originated from the stimulated region, which could explain the differences in duration
between SI and MI, or if they recruited the natural focus as shown here. Another study by Zheng et al.
2012) has shown that electrical stimulation (2-s trains, 7 Hz) of SI, SII and insular cortex (IC) induces self-sustained SWDs in Genetic Absence Epilepsy Rats from Strasbourg (GAERS), with lower current thresholds for SII and IC. Again, due to the lack of array recordings, the authors did not investigate the precise spatiotemporal dynamics of the induced SWDs. They hypothesized that seizures spread from the SII / IC circuit to SI and other frontal cortices through caudorostral excitatory pathways previously characterized (Fujita et al., 2010). Interestingly, we indeed observed caudorostral propagation in our laminar recordings.

Due to anatomical constraints, it was not possible to implant our planar arrays more laterally. Similarly, in the first study providing evidence for a cortical focus in WAG/Rij rats using an ECoG grid on SI, Meeren et al. (2002) systematically found the leading sites of seizures on the most posterior and lateral electrodes. Still, as shown by Zheng et al. (2012) in GAERS rats, it is not excluded that the focus is actually located in more lateral regions. Our planar MEAs showed variable patterns across animals, probably due to the local somatotopy of SI and to small differences in implantation sites. It is unlikely that these patterns are due to possible depth differences between electrodes, since propagation on the laminar arrays also displayed a strong horizontal component. Propagation speeds of cortical epileptiform events reported in the literature range from 0.06-0.09 m/s \textit{in vitro} (Chervin et al., 1988) to 1 m/s \textit{in vivo} (Meeren et al., 2002). We found that discharges spread across our arrays (2x2 mm and 1.4x1.4 mm for planar and laminar arrays) in about 10 ms, yielding a propagation speed of 0.2-0.3 m/s, likely representing intracortical or intrathalamic propagation reflected in cortex.

In sum, our data provide for the first time evidence that SWDs during seizures induced by rhythmic optogenetic stimulation of excitatory cells in a local neocortical region actually propagate similarly to SWDs in spontaneous seizures. This result is not trivial as other forms of stimulation, such as electrical, can render even a healthy cortex temporarily prone to seizures, indicating local non-physiological alterations and generation of non-realistic epileptiform discharges. Moreover, the switch in the direction
of propagation between the stimulation period and subsequent self-sustained SWDs reinforces our claim of the indirect recruitment of the natural epileptogenic network. Although we did not compare the effect of stimulating different cortical areas or the thalamus, these are important questions for further studies.

Probing spatiotemporal dynamics in healthy and pathological brain networks

Our study also introduced a new approach for investigating cortical network dynamics in response to optogenetic perturbation in animal models of epilepsy. It combines integrated optogenetic stimulation and high-density microelectrode array recordings with computational methods to analyze and visualize these high-dimensional data and complex spatiotemporal dynamics. These methods will be useful to future studies where healthy and pathological spatiotemporal dynamics across neocortex, under locally generated perturbations, need to be investigated.

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DISCLOSURES

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FIGURE CAPTIONS

Figure 1. Combined microelectrode array recordings and optogenetic stimulation in somatosensory cortex of freely moving rats. Implants for simultaneous optical stimulation and multisite recordings, assembled from 32-channel MEAs (A), 64-channel laminar probes (B), or both combined in an orthogonal configuration (C), all integrated with optical fibers (3 in B, 1 otherwise, see bright green spot where light exits the fiber). (D) Chronic implantation into SI cortex of WAG/Rij rats (AP: anteroposterior, ML: mediolateral). Picture from animal H12, implanted with a hybrid MEA / silicon probe. (E) Typical implantation site of planar MEAs (red square) assessed after brain extraction (H10, > 3 weeks after implantation, filled green circle: optical fiber). Superimposed, a somatotopic map of rat SI and SII cortex, adapted with permission from Chapin and Lin, 1984. (F) Histology revealed strong EYFP expression in deep cortical layers and electrode tracks left by array extraction (picture from H10).

Figure 2. Optogenetically-evoked cortical bursting can evolve into self-sustained seizures. (A) Three optical stimulation trials that failed (left and middle) or succeeded (right) to induce a seizure, i.e. self-sustained SWDs (stimulation parameters: 10 Hz, 1-s train, 10-ms pulse width, light pulses indicated by vertical bars, optical power in the brain: ~ 30 mW, data from H12, post-implantation day 14). LFP traces (top two traces) and high-pass MUA (bottom trace) are shown for representative electrodes. For each trial, a 2-s time window around the stimulation period is magnified (bottom). (B) Comparison between induced and spontaneous seizures (same stimulation parameters as in A, optical power in the brain: ~ 50 mW, data from H10, post-implantation day 1). A 1-s period is magnified for optically-evoked responses / bursts, SWDs during the induced seizure (‘ind. sz’), and SWDs during the spontaneous seizure (‘spont. sz’). SWDs during induced and spontaneous seizures were remarkably similar. Evoked bursts have a slightly different morphology. In both panels, data are shown without application of an artifact removal algorithm. (C) Statistics of seizure induction following 10-Hz optical stimulation in 4 animals. Column 2: number of recording sessions (~ 8 h each) that were used for the analysis. Column 3: number of
stimulation trials at 10 Hz. Column 4: number of 10-Hz stimulation trials that induced a seizure. Column 5: probability of seizure induction, defined as the number of stimulation trials with induced seizures divided by the total number of trials (column 4 divided by column 3), expressed as a percentage. Stars indicate a significant probability of seizure induction (p < 0.05, 1,000 Monte-Carlo surrogate datasets, false discovery rate – FDR – correction for multiple independent tests).

Figure 3. Seizures are induced optimally by stimulation frequencies around 10 Hz. (A) Probability of seizure induction (# stimulation trials with induced seizures / # stimulation trials) as a function of stimulation frequency, for 4 animals (1-s train, 10-ms pulse width for H11, H12 and H13 and ~ 30-ms for H10, 6-18 Hz frequency). Data pooled over daylong recording sessions (~ 8 h each), with ~ 100 trials per day per frequency (n_{stim}: total number of trials per frequency, depends on frequency; H10: 566 ≤ n_{stim} ≤ 626; H11: 272 ≤ n_{stim} ≤ 333; H12: 353 ≤ n_{stim} ≤ 385; H13: 370 ≤ n_{stim} ≤ 430). Black stars: significant probability of seizure induction (p < 0.05, 1,000 Monte-Carlo surrogate datasets, FDR correction for multiple dependent tests). Grey stars above brackets: significantly different probabilities of seizure induction between 2 frequencies (p < 0.05, 1,000 random permutations, FDR correction for dependent tests). Only non-redundant significant comparisons are shown. Multiple tests correction includes all tests within figure. Optical powers: 30-80 mW (H10: 80 mW, H11: 50 mW, H12: 30 mW, H13: 30-30-40-80 mW). (B) Summary across subjects. Each grey curve represents data from one of the animals shown in (A). The black curve represents the mean across animals, for frequencies between 6 and 16 Hz.

Figure 4. Optical stimulation evokes bursting and induces seizures only after opsin expression. (A) Trial-averaged LFPs at different times post-injection, for 3 animals injected and implanted within the same surgery, show that optically-evoked responses appeared after 15-30 days (average taken over trials with no seizure). # ind. sz: indicates ‘number of induced seizures / total number of stimulation trials’. H13, day #9: the detected seizure was likely a coincidence, based on visual inspection of the original data. Artifact removal was systematically applied to all data. Shape differences likely come from different
distances and depths with respect to the fiber (H13: silicon probe a few mms away from fiber, C2 and C3: microwires aligned with fiber). (B) Summary showing that LFP responses to optical stimulation were absent shortly after viral injections and developed over time as the opsin expressed. Neural signal energy was defined as the square of the LFP signal, averaged across trials and over time during either the 1-s stimulation period or a baseline period preceding stimulation. The change in energy was defined as the energy difference between stimulation and baseline, divided by the energy during baseline, and expressed as a percentage. All three curves increase by several orders of magnitude over a few weeks post-injections. The curve corresponding to H13 is below the curves for C2 and C3, potentially because the electrodes were located further away from the fiber in H13 compared to C2 and C3. (C) Simulations of light-induced heating. Left: temperature increase at the fiber tip (where heating is largest) as a function of time for different optical powers and pulse widths for 10 pulses delivered at 10 Hz. Right: spatial distribution of temperature changes for the worst scenario on the left (80 mW, 30 ms). The largest temperature changes were restricted to an area of 50 x 50 x 200 μm (fiber diameter: 50 μm) and did not exceed 1-2 °C.

Figure 5. Induced and spontaneous seizures have same spectral characteristics and comparable average durations. All examples are from stimulation at 10 Hz with 10-ms pulses (H11, H12, H13: same datasets as in Figure 3). (A) Average LFP multitaper spectrograms for the 3 types of events: stimulation trials with no seizure (blue), stimulation trials with induced seizures (red), and spontaneous seizures (black) (1 representative electrode from H11, left: 1-300 Hz, right: magnified, 1-30 Hz, n: number of events). Power for each frequency (P) was normalized by its average value in the 2-s window preceding trials with no seizure (P0). Solid black lines: beginning and end of stimulation. Dotted black line: average duration of induced or spontaneous seizures (computed independently, based on amplitude features). (B) Electrode-averaged multitaper power spectral density (PSD) computed between 1.5 and 2.5 s after event onset (white dotted lines from (A)). Solid line: mean across trials. Shaded area: 95% confidence interval of the mean (bootstrap). Shown for trials with no seizure (blue), trials with induced seizures (red) and
spontaneous seizures (black). Dotted lines indicate 5 and 12 Hz. (C) Summary of PSD similarity analysis between induced and spontaneous seizures for all 4 animals. Left: the correlation coefficient between the PSDs of induced and spontaneous seizures was computed (each dot represents one animal). The mean across animals (thick horizontal line) is close to 1, indicating a nearly perfect correlation. Right: the total LFP power was computed by integrating the PSD over frequencies between 1 and 300 Hz. The relative change in power between induced and spontaneous seizures, expressed as a percentage, is represented (each dot: one animal, thick line: mean across subjects, error bars: 95% confidence of the mean). Note that the 95% confidence interval includes zero. (D) Comparison of the mean duration of induced and spontaneous seizures within each animal. Error bars: 95% confidence interval obtained by bootstrap. *p < 0.05 (Welch’s t-test with FDR correction for independent tests). (E) Summary of seizure durations across animals. Each dot represents the mean seizure duration for a given animal, the thick line shows the mean across animals, and the error bars the 95% confidence interval of the mean.

Figure 6. Seizures can also be induced in another rat strain, provided animals have spontaneous seizures. (A) Trial with maximum 5-12 Hz power among trials that failed (‘no sz’, top trace for each animal) or succeeded (‘ind. sz’, bottom trace) to induce a seizure, shown for two rat strains: WAG/Rij (N = 4) and Wistar (N = 4). Two of the Wistar rats did not show any sign of self-sustained trains of SWDs, even for the trial with the highest 5-12 Hz power. Interestingly, these two rats did not show any spontaneous seizure-like event either. (B) Distribution of the 5-12 Hz LFP power (frequency band shown on Figure 5B, 1.5-2.5 s after event onset) for the 3 event types ‘no sz’, ‘ind. sz’ and ‘spont.sz’ (left to right on each graph; n: number of events). Box plots indicate the median, 25th and 75th percentiles of the distribution; whiskers extend to the lowest and highest data within 1.5 times the interquartile range of the lower and upper quartiles; outliers are represented individually. In both WAG/Rij and Wistar rats with seizures, power in this band was a good marker of induced and spontaneous seizures. Note that we were conservative in our definition of induced seizures, as shown by the outliers in the ‘no sz’ category.
Figure 7. Ongoing LFP oscillations before stimulation onset predict whether optical stimulation induces a seizure. Same datasets as in Figure 5. (A) Multitaper PSDs at different times (top vs bottom) preceding stimulation onset or spontaneous seizures, shown for all 4 animals (left to right). (blue: ‘no sz’, red: ‘ind. sz’, black: ‘spont. sz’; n: number of events). (B) Summary of total spectral power differences between trials that succeeded (‘ind. sz’, red) or failed (‘no sz’, blue) to induce a seizure. The total LFP power was computed by integrating the PSD over frequencies between 1 and 300 Hz (each dot: one animal, thick line: mean across subjects, error bars: 95% confidence of the mean). The top and bottom plots correspond to different time periods, respectively 4 to 3 s and 1 to 0 s before stimulation onset. (C) We used support vector machines (SVMs) to determine whether LFP features immediately preceding (ongoing activity) or during the stimulation period (evoked activity) could predict if a seizure would be induced (ind. sz) or not (no sz) after the optical stimulation ended in any given stimulation trial. For completeness, we also assessed how well the trained SVM could discriminate (classify) between induced and no induced seizure states based on the LFP features during the period immediately following the end of the optical stimulation. The solid lines (color indicates the subject H10, H11, …) show the prediction performance, which ranges from 0 to 1, 0 representing random and 1 perfect performance. The prediction performance was assessed at consecutive 1-second time intervals, centered at the times indicated by the filled circles. This prediction performance should be compared to the corresponding 95% chance level (dotted lines, color matched) obtained by randomly shuffling the two categories, induced seizure (ind. sz) and no-seizure (no sz), in 100 random permutations (no multiple test correction here, see Figure 7D for hypothesis testing with FDR correction). Prediction performance was above the 95% chance level for all animals during the 1-second time period immediately preceding the optical stimulation and further increased during the stimulation period itself (denoted by the two vertical lines), indicating that ongoing states and neural excitation reflected in LFP features can predict whether a seizure will be induced or not. In 2 of the subjects (H11, H13), prediction performance was above chance for several seconds before stimulation onset. During the 1-second period immediately following the end of the stimulation, SVMs achieved, not surprisingly, nearly perfect discrimination/classification of whether an induced seizure had
happened or not. The LFP features included the spectral power in 8 frequency bands at different 1-s time periods corresponding to before, during and following stimulation as described above. The 8 frequency bands spanned the 1 to 300 Hz frequency range (the first 4 bands – delta, theta, alpha, beta – are indicated in 7A by the vertical dotted lines) during non-overlapping 1-second long windows. (See also Materials and Methods, Spectral analysis and Neural signals prediction performance, for more details on power estimation and on how SVMs were trained.) The prediction performance at each examined time was defined as $2 \times AUC - 1$, where AUC corresponds to the area under the ROC curve, which is a standard measure used for assessment of prediction and classification performance. Results were obtained with repeated 10-fold cross-validation (10 repetitions). Each filled circle represents the mean of the prediction performance across all repetitions. (D) Prediction performance based on different spectral features during the 1-s period preceding stimulation, associated non-adjusted p-values (i.e. before FDR correction) computed by random permutations tests (1,000 permutations), and after FDR correction for multiple testing (* and yellow highlight: p < 0.05 after FDR correction for dependent tests). 1st row (‘power all bands’): power in all 8 frequency bands as before. 2nd row (‘power 10 Hz’): power in a narrow band between 9 and 11 Hz. 3rd row (‘phase 10 Hz’): phase at the same frequency. 4th row (‘power + phase 10 Hz’): combination of power and phase at 10 Hz.

Figure 8. Increased sensitivity to repeated stimulation at 10Hz is an excellent predictor of seizure induction. Same datasets as in Figures 5 and 7. (A) Trial-averaged LFPs and eMUA (H12, two consecutive days, red: ‘ind. sz’, blue: ‘no sz’, n: number of events). eMUA peaks represent bursts of activity, larger for stimulation trials inducing seizures. Green bars: light stimulation. Shaded areas: 95% confidence interval (bootstrap). (B) Scatter plots of LFP and eMUA amplitudes for different electrodes quantified for the first peak (grey, along diagonal) and maximum peak in the series (orange, above diagonal) (H10 and H12). Each dot: mean across trials for one electrode. Shaded areas: 95% confidence ellipse (bootstrap). (C) Similar analysis for one particular electrode in each animal (LFP only). Electrode with best separation between ‘ind. sz’ and ‘no sz’ for maximum peak is shown. Error bars: 95%
Figure 9. Example illustrating the contributions of direct light stimulation and network mechanisms to optically-evoked bursts and spike-wave discharges during induced seizures. (A) Left: trial-averaged LFPs and eMUA from two electrodes (data from H10). Top electrode: the averaged eMUA has two peaks after the 2\textsuperscript{nd} light pulse, especially during trials with induced seizures (red). Bottom electrode: only one peak for each light pulse. Right: delay maps of early (top) and late (bottom) eMUA peaks, averaged across all light pulses (grey: undefined peak). A peak in the eMUA was defined as early peak if it occurred during the 10-ms light pulse or 2 ms after its end, and was defined as late peak otherwise. Light stimulation affected 7-12 electrodes with short latencies. Network effect was more widespread and originated from a different location. (B) LFP wave examples recorded from H10 during trials with induced seizures. Heat maps: instantaneous LFP amplitudes across the array, at different times (t = 0: negative population peak, arrow: direction of propagation using optical flow algorithm). Top: wave following light stimulation (10-ms pulse starting at t = -15 ms) originating from directly activated regions. Bottom: wave propagating in the opposite direction, recorded during the self-sustained SWDs following stimulation, but sometimes seen during the stimulation period as well (see also Supplemental Movie 2).

Figure 10. Propagation of optically-evoked responses and subsequent spike-wave discharges during induced seizures suggests the indirect recruitment of a hyperexcitable network. Same datasets as before for H10-13. (A) Statistical analysis of LFP wave propagation across animals. Angle histograms summarize directions of propagations across all discharges (n\textsubscript{spk}: total number of discharges, or ‘spikes’,...
detected as negative threshold crossings in the LFPs; area of each bar: number of discharges propagating in a given direction, arrow: angular mean. Average amplitude maps at a particular time (10 to 15 ms) before the global discharge peak are shown. For animals with bidirectional propagation (H10 and H2), results are shown independently for each direction. H12-lam and H13: laminar probes, others: planar MEAs (AP: anteroposterior, ML: mediolateral, DV: dorsoventral). Additional animals included: C1 (epileptic Wistar rat) and H2 (from preliminary experiments, injected with a different virus — see Materials and Methods). Initiation sites and directions of propagation usually differ between optically-evoked responses and subsequent self-sustained SWDs during induced seizures. (B) Histograms of latencies between LFP discharges and light onset for animals with bidirectional propagation during stimulation (H10, H2 as seen in A). Dark green bars: discharges propagating in the direction ‘dir.1’ (‘waves from stimulated area’). White bars outlined in green: direction ‘dir.2’ (‘waves from natural network’). Angle histograms: propagation directions shown as a reminder. In both animals, discharges propagating in the second direction are less phase-locked with the stimulus (more discharges with latencies above 50 ms) than discharges propagating in the first direction, likely reflecting a response of the network, which tends to oscillate at its own frequency. (C) The mean direction of propagation was computed for each array as shown in panel 10A (except for C1 where there was no clear propagation during stimulation). The difference (in absolute value) in the mean direction of propagation between the stimulation periods and the subsequent induced seizures is represented. Each dot: one array (for animals with bidirectional wave propagation, we show one dot for each direction). Thick line: mean across arrays. Error bars: 95% confidence interval. This graph shows that there was an overall change in the direction of propagation between the stimulation periods and the induced seizures. This still holds for C1, where a clear propagation was observed during the induced seizures, but not during stimulation.
Figure 11. Spike-wave discharges during induced and spontaneous seizures propagate similarly, likely indicating that they are generated by the same epileptogenic network. Same datasets as in Figure 10. (A) Column 1: scatter plots showing delays of SWDs during induced ($\tau_{\text{ind}}$) and spontaneous ($\tau_{\text{spont}}$) seizures. Each dot: mean across SWDs for each electrode ($n_{\text{spk}}$: total number of SWDs, detected as negative threshold crossings in the LFPs). Shaded areas: 95% confidence regions (bootstrap). Columns 3 and 5: same delays plotted topographically as heat maps (arrow: angular mean of the propagation direction histograms in columns 2 and 4, obtained independently). Columns 2 and 4: angle histograms of propagation directions, consistent between SWDs during induced and spontaneous seizures. Note origin from deep and posterior sites on laminar probes (H12-lam and H13). (B) Scatter plot comparing the directions of propagation during spontaneous and induced seizures. Each circle represents one array, filled and open circles correspond respectively to planar and laminar arrays. All of them lie along the diagonal. (C) Summary of the differences between the directions of propagation during spontaneous and induced seizures, represented as in Figure 10C. Note also that the directional differences here are much smaller than in Figure 10C.
Supplemental Movie 1. Optically-induced absence seizure. In this example, an optical stimulation trial (14 Hz, 1-s train, 10-ms pulses, data from H11) induced an absence seizure with self-sustained SWDs (LFP trace at the top) clearly associated with a freezing behavior.

Supplemental Movie 2. LFP wave propagation during optical stimulation and subsequent induced seizure. LFP spatiotemporal dynamics during a representative optical trial (10-Hz, 10-ms pulses, 1-s train, data from H10) leading to seizure induction. Left: map of the array on top of a picture of the brain. Colors represent LFP amplitudes, normalized and interpolated across the array (blue: negative polarity, red: positive polarity). Normalization was performed by Z-scoring the data based on the mean and standard deviations computed during the 5 s preceding stimulation across all stimulation trials. Green dot: location of the optical fiber, open: light off, filled: light on. White arrows: optical flow at different locations of the array (shown during detection of an LFP discharge only). Red arrow: optical flow vector spatially averaged across the array. Magenta and brown dots represent the location of the two electrodes detailed on the right. Right: LFP traces are shown for two channels. Green bars indicate light pulses. During the stimulation period, we can see waves propagating in two opposite directions, sometimes interfering with each other. Waves directly evoked by the optical stimulus tended to propagate from the top left to the bottom right corners of the array. Waves spontaneously generated between two consecutive light pulses propagated in the opposite direction. After the stimulation period, during the induced seizure, rhythmic self-sustained SWDs kept propagating in a consistent direction, from bottom right to top left. This pattern of propagation was identical to the one observed during spontaneous seizures.
A

No seizure  No seizure  Induced seizure

LFP 1  LFP 2  MUA 2

No seizure  Induced seizure

B

Stim.  Induced seizure

LFP  MUA

Spontaneous seizure

Evoked resp. / bursts  SWDs (Ind. sz)

SWDs (Spont. sz)

C

Summary 10 Hz stimulation (N = 4 rats)

<table>
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<th>Subjects</th>
<th># sessions</th>
<th># trials</th>
<th># ind. sz</th>
<th>prob. ind. sz (%)</th>
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<td>5</td>
<td>570</td>
<td>55</td>
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<td>3</td>
<td>313</td>
<td>55</td>
<td>17.6 *</td>
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<tr>
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<td>11.2 *</td>
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<td>H13</td>
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<td>6.05 *</td>
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Predicting/classifying trials with and without induced seizures (ind. sz vs no sz) based on spectral power (all frequency bands): Prediction Performance as a function of time.

**Prediction performance (2 x AUC - 1) of different spectral features**

<table>
<thead>
<tr>
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<th>H10</th>
<th>H11</th>
<th>H12</th>
<th>H13</th>
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<td>0.55*</td>
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<td>p=0.035</td>
<td>p=0.001</td>
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<tr>
<td></td>
<td>p=0.097</td>
<td>p=0.05</td>
<td>p=0.081</td>
<td>p=0.008</td>
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Prediction performance (2 x AUC - 1) of peak amplitudes

B

first peak  max peak

H10  LFP (mV)  eMUA (µV)  H12  LFP (mV)  eMUA (µV)

C

H10  H11  H12  H13

Summary (N = 4 rats)

E

Prediction performance (2 x AUC - 1) of peak amplitudes

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<tr>
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<th>H12</th>
<th>H13</th>
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<td>0.73*</td>
<td>0.77*</td>
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</table>
A Wave originating from stimulated area (during stim.)
Wave originating from natural network (during sz and sometimes stim.)

B LFP amplitude maps (examples from H10)

- Light: -14 → -5 ms
- Wave originating from stimulated area (during stim.)
- Wave originating from natural network (during sz and sometimes stim.)

Average traces and eMUA delay maps

H10 (3 sessions)

Ind. sz (n = 23)
No sz: (n = 314)
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<th>Prop. dir.</th>
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B

<table>
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<tr>
<td>dir2</td>
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C

Summary prop. change b/w stim. and ind. sz (N = 6 arrays from 5 rats)

![Graph showing direction change](image)

Latency (ms)
### Table

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<tr>
<th>Region</th>
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<th>Spont. sz</th>
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<tr>
<td>H12 MEA</td>
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<td>C1</td>
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<td>166</td>
</tr>
</tbody>
</table>

### Diagrams

#### A

- **H10**
  - Delays ind. vs spont.
  - Prop. dir. (ind. sz): $n_{spk} = 853$
  - Delay map (ind. sz)
  - Prop. dir. (spont. sz): $n_{spk} = 5728$
  - Delay map (spont. sz)

- **H12 MEA**
  - Delays ind. vs spont.
  - Prop. dir. (ind. sz): $n_{spk} = 944$
  - Delay map (ind. sz)
  - Prop. dir. (spont. sz): $n_{spk} = 5569$
  - Delay map (spont. sz)

- **H2**
  - Delays ind. vs spont.
  - Prop. dir. (ind. sz): $n_{spk} = 144$
  - Delay map (ind. sz)
  - Prop. dir. (spont. sz): $n_{spk} = 71$
  - Delay map (spont. sz)

- **C1**
  - Delays ind. vs spont.
  - Prop. dir. (ind. sz): $n_{spk} = 313$
  - Delay map (ind. sz)
  - Prop. dir. (spont. sz): $n_{spk} = 1450$
  - Delay map (spont. sz)

- **H12 lam.**
  - Delays ind. vs spont.
  - Prop. dir. (ind. sz): $n_{spk} = 1043$
  - Delay map (ind. sz)
  - Prop. dir. (spont. sz): $n_{spk} = 6422$
  - Delay map (spont. sz)

- **H13**
  - Delays ind. vs spont.
  - Prop. dir. (ind. sz): $n_{spk} = 747$
  - Delay map (ind. sz)
  - Prop. dir. (spont. sz): $n_{spk} = 5745$
  - Delay map (spont. sz)

#### B

**Summary propagation spont. vs ind. sz**

(N = 6 arrays from 5 rats)

- Prop. dir. ind. sz vs Prop. dir. spont. sz

#### C

**Summary propagation spont. vs ind. sz**

(N = 6 arrays from 5 rats)

- A direction (ind. spont.)