Spatiotemporal dynamics of optogenetically induced and spontaneous seizure transitions in primary generalized epilepsy

Fabien B. Wagner,1,2 Wilson Truccolo,1,3,4 Jing Wang,2 and Arto V. Nurmikko1,2,3
1Department of Neuroscience, Brown University, Providence, Rhode Island; 2School of Engineering, Brown University, Providence, Rhode Island; 3Institute for Brain Science, Brown University, Providence, Rhode Island; and 4Center for Neurorestoration and Neurotechnology, Department of Veterans Affairs, Providence, Rhode Island

Submitted 22 December 2014; accepted in final form 23 December 2014

Wagner FB, Truccolo W, Wang J, Nurmikko AV. Spatiotemporal dynamics of optogenetically induced and spontaneous seizure transitions in primary generalized epilepsy. J Neurophysiol 113: 2321–2341, 2015. First published December 31, 2014; doi:10.1152/jn.01040.2014.—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. In this study, 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.

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; Panayiopoulos 2005). Several studies, however, have revealed focal cortical features in human patients with primary generalized epilepsy (Ferrie 2005; Holmes et al. 2004; Lombroso 1997; Westmijse et al. 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) (Gurbanova et al. 2006; Klein et al. 2004; Lüttjohann et al. 2011; Meeren et al. 2002; Polack et al. 2007, 2009; 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 (Guerrini and Genton 2004; Topalkara et al. 1998). 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 spike-wave discharges (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 (Lüttjohann 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).
In this study, 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-mentioned studies is that electrical stimulation activates cells in a nonphysiological 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 Hayden 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 us 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 micro-electrode array (MEA) recordings (Wang et al. 2012) in freely moving WAG/Rij rats, a well-established model of absence epilepsy (Coenen and van Luijten (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. Animals used in this study were five male WAG/Rij rats (referred to as H2, H10, H11, H12, and H13, and respectively 7, 12, 14, 16, and 17 mo old). We also carried out additional experiments in five male Wistar rats in two age groups (group 1: 6–7 mo, referred to as C1, C2, and C3; group 2, 3–4 mo, referred to as C5 and C6). All animals were acquired commercially (WAG/Rij: Charles River Laboratories Germany; Wistar: Charles River Laboratories USA).

Optogenetic Constructs

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 x 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 [VS-V-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.

Multielectrode Implants

We used commercially available 32-channel “Utah arrays” (1-mm length, 400-μm pitch; Blackrock Microsystems), where one electrode had been laser-ablated, and 64-channel laminar probes (200-μm pitch; NeuroNexus), custom 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 (~1 mm like neighboring electrodes) by visual inspection with a microscope and then fixed it in place using dental cement (C&B Metabond; Parkell). Lamellar 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, and 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 S1 (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) with the use of 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 stereotactic 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 4 wk 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, 4 wk after the injections, to maximize the period of time with both high-quality recordings and opsin expression (subjects H10, H11, H12, C1, C5, and C6). To perform controls, a few subjects were injected and implanted during the same procedure (H2, H13, C2, and C3) 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 4 wk 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

Electrophysiological experiments were performed using a custom data acquisition and control system built with amplifiers from Plexon (32-channel VLSI head stage, 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-6259 or PCl-e6259, M Series DAQ, 32 AI, 48 DIO, 4 AO) and custom software (LabVIEW; National Instruments). Green or blue diode-pumped solid-state lasers (532 nm: LaserGlow Technologies; 561 or 473 nm: OptoEngine) were modulated directly or via an acousto-optic modulator (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 intertrial 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 observations that a seizure could be induced even 4 s after the end of another spontaneous or induced seizure and that seizures lasted on average <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

After a period of 3–6 wk following implantation, subjects were deeply anesthetized with pentobarbital sodium (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 h. Sections (50 μm thick) 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 in which the same stimulation parameters (protocol, optical power, laser) were used. For H13, we pooled together days at 30 mW with 1 day at 80 mW, because there was no difference in optically induced amplitudes and probabilities of seizure induction. The data sets used for H11, H12, and H13 were the same throughout the study. For H10, we used two different data sets. The data set used in Fig. 3 did not have 10-ms pulses. To be consistent across animals, we used another data set 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 artifacts (upper threshold). All thresholds were proportional to the signal median and on a coefficient adjusted to each animal (typically 3–6). Next, if two spikes occurred within a certain time window (typically 250 ms), they were grouped together. Different groups of spikes were further combined if they occurred close enough (typically 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 (respectively end) was the earliest onset (respectively 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 typically 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 or shortly before stimulation, 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 postictal state). We also discarded stimulation trials containing movement artifacts during the time windows of interest.

Perievent LFP and multiunit activity envelope 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 multiunit 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 1 kHz. To remove artifacts, 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, using 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 (“mtspec- trum”) and multitaper time-frequency spectrum (“mtspecgram”) 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 a 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 (see Fig. 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 3 cases). Power spectral densities (PSD; see Figs. 5B and 7A) represent the frequency spectra without normalization (in mV²/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 (see Fig. 7, C and 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 (see Fig. 7D) 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 using analysis based on the Horn-Schunck 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-Schunck or similar) have been used previously to study the spatiotemporal dynamics of neural signals (Leãevre and Baillet 2009; Mohajerani et al. 2013; Slater et al. 2012). Briefly, we normalized LFPs, interpolated spatially, applied a mask at a negative threshold, ran the Horn-Schunck 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 (1–20, 5 ms) around the population peak. More precisely, LFPs were first z-scored on the basis of 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. We then applied a two-dimensional (2D) linear interpolation on a refined grid formed by repeatedly dividing the intervals three 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 two standard deviations. We ran the Horn-Schunck algorithm with 100 iterations and a smoothness parameter \( \alpha = 10 \).

Prediction performance of neural signals. 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 that succeeded (“ind. sz”) or failed (“no sz”) to induce a seizure on the basis of either spectral or amplitude LFP features (for more details about spectral features, see Spectral analysis above) in a period either before or during optical stimulation. We used support vector machine (SVM) classifiers (see e.g., Hastie et al. 2009) with a Gaussian radial basis function (RBF) kernel, i.e., \( K(x, x') = \exp(-||x - x'||^2/2\sigma^2) \). 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 \( (2 \times \text{AUC} - 1) \), 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. The 95% chance levels and \( P \) values associated with the prediction performance \( (2 \times \text{AUC} - 1) \) were computed by permutation tests. Specifically, the labels (ind. sz or no sz) were randomly shuffled, a classifier was trained on the shuffled data, and 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 to obtain data in Figs. 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 simulation protocol. For each surrogate data set, the number of coincidences in the surrogate data (\( n_{\text{ind_MC}} \)) was then compared with the number of coincidences in the observed data (\( n_{\text{ind_obs}} \)), yielding a \( P \) value: \( P = (1 + N)/(N_{\text{MC}} + 1) \), where \( N \) is the number of data sets with \( n_{\text{ind_MC}} \geq n_{\text{ind_obs}} \) and \( N_{\text{MC}} \) is the number of Monte Carlo surrogate data sets.

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).

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 (Fig. 1, A–C). These devices were implanted at the site of viral injections in SI, located in the vicinity of the presumed cortical focus (Fig. 1, D and E). The injection site showed strong expression of the C1V1(T/T)-EFYFP construct as assessed by histology at the end of experiments (Fig. 1F). Despite slight variations between animals, we consistently observed the largest spread in deep layers (5/6), 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 nonhuman primates (Diest 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 (Fig. 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 (Fig. 2A), reminiscent of SWDs during spontaneous seizures (Pollen 1964; Steriade 1974) but with more variable morphologies (Fig. 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 below. Strongly negative LFP deflections were associated with neuronal bursting as seen in the spiking activity, whereas more moderate or positive LFP deflections were associated with irregular spiking (Fig. 2A). We also note that these deflections were physiological and not related to photo-induced artifacts, which affected only a subset of electrodes and could be removed by a custom algorithm (see MATERIALS AND METHODS). Additionally, they were mediated by the opsin and not by nonspecific effects such as heating, as detailed later.

After 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 (Fig. 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 (Fig. 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 SWDs that followed the 1-s stimulation period during induced epileptiform events.

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 METH-
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 3 sessions per animal. The resulting data sets contained hundreds of stimulation trials for each animal and each stimulation parameter. The

<table>
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<th>Subjects</th>
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<th># ind. sz</th>
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<td>5</td>
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<td>H12</td>
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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 dependent tests) for stimulation frequencies of 8, 10, and 12 Hz (Figs. 2C and 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 (Fig. 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, because there was no apparent correlation with the number of days of stimulation.

Finally, optically evoked LFP responses and MUA bursts were mediated by the opsins and not by thermal effects or visual stimulation, as shown by the absence of evoked responses prior to opsin expression and by their progressive increase over 4 wk following viral injections (Fig. 4, A and 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 (Fig. 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 (Fig. 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 (Fig. 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, appearing similar to spontaneous seizures (Fig. 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- to 12-Hz frequency range, with a maximum at 8 Hz (Fig. 5B). Confirming visual inspection, the PSDs of induced and spontaneous seizures were almost perfectly correlated and had similar total powers (Fig. 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 (V\textsubscript{ind}) and spontaneous seizure (V\textsubscript{spont}) groups. We found no statistically significant difference in any of the animals (α = 0.05, Welch’s t-test, FDR correction for independent tests; H10: V\textsubscript{spont} = −0.67 ± 0.0045 mV, V\textsubscript{ind} = −0.66 ± 0.0096 mV; H11: V\textsubscript{spont} = −0.37 ± 0.0028 mV, V\textsubscript{ind} = −0.38 ± 0.0055 mV; H12: V\textsubscript{spont} = −0.53 ± 0.0031 mV, V\textsubscript{ind} = −0.52 ± 0.0067 mV; H13, V\textsubscript{spont} = −0.54 ± 0.0063 mV, V\textsubscript{ind} = −0.54 ± 0.012 mV; means ± SE). Finally, we compared the average durations of induced and spontaneous seizures (Fig. 5, D and E). In two animals, there was no statistically significant difference (α = 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 (Jandó et al. 1995; Vergnes et al. 1982). In all animals, the opsin expressed well and gave rise to strong optically evoked bursting responses. 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 (Fig. 6). These differences seemed to be correlated with the age of the animal (respectively 6–7 mo and 3–4 mo 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 (Fig. 7). For three of the four animals (H11, H12, and 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 for trials without (Fig. 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 with trials that did not (Fig. 7B).

The smaller power in frequency bands below 30 Hz during trials with induced seizures (compared with that during trials without seizure) can seem counterintuitive, because spontaneous seizures were usually preceded by large powers in the frequency bands below 30 Hz, especially in the theta range (4–8 Hz) (Fig. 7A, black). These results are also consistent with the individual examples shown in Fig. 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 (Fig. 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 on the basis of different LFP features. We first computed the power in 8 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 (Fig. 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 (Fig. 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 (Fig. 5).

**Fig. 5.** Induced and spontaneous seizures have the same spectral characteristics and comparable average durations. All examples are from stimulation at 10 Hz with 10-ns pulses (H11, H12, and H13: same data sets as in Fig. 3). A: average LFP multipolar spectrograms for the 3 types of events: stimulation trials with no seizure (no sz; blue), stimulation trials with induced seizures (ind. sz; red), and spontaneous seizures (spont. sz; black) (1 representative electrode from H11: left, 1–300 Hz; right, magnified, 1–30 Hz; n, no. of events). Power for each frequency (P) was normalized by its average value in the 2-s window preceding trials with no seizure (P\textsubscript{no sz}). Solid black lines indicate the beginning and end of stimulation; dotted black lines indicate average duration of induced or spontaneous seizures (computed independently, based on amplitude features). B: electrode-averaged multipolar power spectral density (PSD) computed between 1.5 and 2.5 s after event onset (white dotted lines from A) for trials with no seizure (blue), trials with induced seizures (red), and spontaneous seizures (black). Solid lines indicate mean across trials; shaded areas represent 95% confidence interval of the mean (bootstrap). Dotted lines indicate 5 and 12 Hz. C: summary of PSD similarity analysis between induced and spontaneous seizures for all 4 animals. Left, correlation coefficient between the PSDs of induced and spontaneous seizures was computed (each dot represents 1 animal). The mean across animals (thick horizontal line) is close to 1, indicating a nearly perfect correlation. Right: 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 (ΔP\textsubscript{ind} – ΔP\textsubscript{spont}), expressed as a percentage, is represented (each dot represents 1 animal, thick line indicates mean across subjects, error bars indicate 95% confidence interval of the mean). Note that the 95% confidence interval includes zero. D: comparison of the mean duration of induced and spontaneous seizures (ΔDuration\textsubscript{ind} – ΔDuration\textsubscript{spont}) within each animal. Error bars indicate 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 indicates mean across animals, and error bars indicate the 95% confidence interval of the mean.
7D, “power all bands” values, \( P < 0.05 \) 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 (Fig. 7D, “power alone,” “phase alone,” and “power + phase” values). 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 (Fig. 7C, between vertical 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 (Fig. 8). Figure 8A shows a typical example of the MUA
envelope (eMUA) and LFPs in both cases and across 2 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 10 pulses for each electrode individually (shown for 2 animals, Fig. 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 (Fig. 8B), likely reflecting differences in levels of light activation and in neural excitation.

LFP amplitude differences were consistent across animals ($n = 4$, Fig. 8, $C$ and $D$). To quantify their prediction performance, we trained a classifier as described above (Fig. 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 Figs. 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 That 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 in Fig. 9A. In this animal, the trial-averaged eMUA contained either one or two peaks in response to each light pulse. Sorting these two peaks on the basis of 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 millimeters 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 in Fig. 9B (see also Supplemental Movie 2). In this animal, two types of waves were observed. The first type of wave (Fig. 9B, *top*) started on electrodes near the optical fiber and propagated toward 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 (Fig. 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 Fig. 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 on the basis of 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, Fig. 10A), in terms of direction of propagation.
(angle histograms) or initiation site (amplitude maps). 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 (Fig. 9B). For these animals, we sorted discharges on the basis of 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 (Fig. 10A, compare amplitude map dir2 during stimulation with amplitude map during induced seizures for H10 and H2). Interestingly, they were slightly less synchronized with light pulses than the discharges propagating in a different direction (Fig. 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 Fig. 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 (Fig. 11). SWDs during induced and spontaneous seizures were remarkably similar, in terms of both direction of propagation (Fig. 11A, angle histograms) and relative delays between electrodes (Fig. 11A, delay scatter plots and delay heat maps). 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 (Fig. 11A, compare angle histogram and delay map for induced and spontaneous seizures). 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 Fig. 11, B and C. In particular, these similarities are in sharp contrast with the change in the direction of propagation between stimulation periods and induced seizures (compare Figs. 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 three 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; and 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 (Krook-Magnuson et al. 2013; Paz et al. 2013;
Wykes et al. 2012) and to induce seizure-like afterdischarges in the hippocampus of nonepileptic 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 with 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 ops in expression in these layers), in particular to the thalamic reticular nucleus, which in turn activated a wide variety of cells.

<table>
<thead>
<tr>
<th>A</th>
<th>Prop. dir. during stim.</th>
<th>Amp. map during stim.</th>
<th>Prop. dir. ind. sz</th>
<th>Amp. map ind. sz</th>
<th>mV</th>
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<td>H10 (3 sessions) 23 ind. sz</td>
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<td>dir2</td>
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<tr>
<td>H12 MEA (4 sessions) 40 ind. sz</td>
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<td>dir2</td>
<td>n_spk = 314</td>
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<td>0.3</td>
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<td>H2 (1 session) 5 ind. sz</td>
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<td>dir2</td>
<td>n_spk = 119</td>
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<td>0.5</td>
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<tr>
<td>C1 (2 sessions) 10 ind. sz</td>
<td>dir1</td>
<td></td>
<td>n_spk = 78</td>
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<td>0.1</td>
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<tr>
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<tr>
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<td></td>
<td></td>
<td>n_spk = 273</td>
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<td>0.5</td>
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</table>

**B**

<table>
<thead>
<tr>
<th>H10</th>
<th>H2</th>
</tr>
</thead>
</table>

**C**

Summary prop. change b/w stim. and ind. sz (N = 6 arrays from 5 rats)
An important parameter in these experiments is the volume of direct light activation. This volume depended on the spread of the virus (several millimeters), the optical powers used (30–80 mW), and the alignment between the fiber and injection site, which was only approximate due to the 4-wk 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 a 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 other 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. Because of 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. With the use of 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 (Lütjohann 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 are 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, that are not directly related to the frequency of SWDs during seizures.
Although we did not systematically explore nonperiodic 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,

<table>
<thead>
<tr>
<th></th>
<th>Delays ind. vs spont.</th>
<th>Prop. dir. (ind. sz)</th>
<th>Delay map (ind. sz)</th>
<th>Prop. dir. (spont. sz)</th>
<th>Delay map (spont. sz)</th>
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<td></td>
<td>155 spont. sz</td>
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<td></td>
<td>2 spont. sz</td>
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<td></td>
<td>166 spont. sz</td>
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Fig. 11. SWDs during induced and spontaneous seizures propagate similarly, likely indicating that they are generated by the same epileptogenic network. Data are from same data sets as in Fig. 10. A: scatter plots (far left) depict delays of SWDs during induced (τ_ind) and spontaneous (τ_spont) seizures. Each dot represents mean across SWDs for each electrode (n_spk = total no. of SWDs, detected as negative threshold crossings in the LFPs). Shaded areas indicate 95% confidence regions (bootstrap). The same delays are plotted topographically as heat maps (arrow indicates angular mean of the propagation direction histograms, obtained independently). Angle histograms show 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 1 array; filled and open circles correspond to planar and laminar arrays, respectively. All lie along the diagonal. C: summary of differences between the directions of propagation during spontaneous and induced seizures (Δdirection_ind – spont), represented as in Fig. 10C. Note also that the directional differences here are much smaller than in Fig. 10C.
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 on the basis of 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. The use of 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 (Bernier et al. 1990; Blume et al. 2004; Kalamangalam et al. 2014; Wieser et al. 1979).

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 with MI in WAG/Rij rats, assessed by electically 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, because of the lack of array recordings, the authors did not investigate the precise spatio-temporal 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.

Because of anatomic 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 electrocorticography (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 in vitro (Chervin et al. 1988) to 1 m/s in vivo (Meeren et al. 2002). We found that discharges spread across our arrays (2 × 2 mm and 1.4 × 1.4 mm for planar and laminar arrays, respectively) in about 10 ms, yielding a propagation speed of 0.2–0.3 m/s, likely representing intracortical or intrahalamic 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 since other forms of stimulation, such as electrical, can render even a healthy cortex temporarily prone to seizures, indicating local nonphysiological alterations and generation of nonrealistic epileptiform discharges. 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 introduces 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.
ACKNOWLEDGMENTS

We thank Barry Connors for important discussions, Ilker Ozden for technical expertise and sharing code for heat simulations, David Norton for surgical advice, Saundra Patrick for advice on histology, and Audrey Maertens for feedback and advice in signal processing. We are especially grateful to the Deisseroth laboratory at Stanford University for sharing the optogenetic constructs.

Present address of J. Wang: McGovern Institute for Brain Research, MIT, Cambridge, MA.

GRANTS

This work was supported by Defense Advanced Research Projects Agency REPAIR Award N66001-10-C-3100 (A. V. Nurmikko and W. Truccolo), Epilepsy Foundation Predoctoral Research Fellowship FY12 (F. B. Wagner), National Science Foundation/Emerging Frontiers in Research and Innovation Grant 0937848 (A. V. Nurmikko), National Institute of Neurological Disorders and Stroke (NINDS) Grant R01NS075953 (W. Truccolo), NINDS Career Award K01NS057389 (W. Truccolo), Department of Veterans Affairs Merit Review Award RX000668-01A2 (W. Truccolo), and the Pablo J. Salame ‘88 Goldman Sachs endowed Assistant Professorship of Computational Neuroscience (W. Truccolo).

REFERENCES

F.B.W., W.T., and A.V.N. conceived and design of research; F.B.W. and J.W. performed experiments; F.B.W. and W.T. analyzed data; F.B.W., W.T., and A.V.N. contributed reagents/materials/analysis tools; F.B.W., W.T., J.W., and A.V.N. wrote manuscript; F.B.W., W.T., A.V.N., presented data; F.B.W., W.T., J.W., and A.V.N. approved final version of manuscript.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


