Pre-seizure state identified by diffuse optical tomography

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In epilepsy it has been challenging to detect early changes in brain activity that occurs prior to seizure onset and to map their origin and evolution for possible intervention. Here we demonstrate using a rat model of generalized epilepsy that diffuse optical tomography (DOT) provides a unique functional neuroimaging modality for noninvasively and continuously tracking such brain activities with high spatiotemporal resolution. We detected early hemodynamic responses with heterogeneous patterns, along with intracranial electroencephalogram gamma power changes, several minutes preceding the electroencephalographic seizure onset, supporting the presence of a “pre-seizure” state. We also observed the decoupling between local hemodynamic and neural activities. We found widespread hemodynamic changes evolving from local regions of the bilateral cortex and thalamus to the entire brain, indicating that the onset of generalized seizures may originate locally rather than diffusely. Together, these findings suggest DOT represents a powerful tool for mapping early seizure onset and propagation pathways.

Epilepsy is one of the most common and devastating neurologic diseases affecting over 2.5 million Americans. Seizure control with antiepileptic drugs (AEDs) has been proven to decrease morbidity and mortality and remains the only treatment option for the majority of patients. Nevertheless, despite progress in antiepileptic drug development, at least 30% of all epilepsy cases remain resistant to current therapeutics1,2. For patients without complete seizure control, the sudden, unforeseen occurrence represents one of the most debilitating aspects of epilepsy. Amongst the types of epileptic seizures, generalized tonic-clonic seizures are the most dangerous form, which has the potential to cause severe injuries or even death3,4. However, by providing appropriate and timely prevention, the risk factor can be significantly reduced5.

In the absence of completely controlling a patient’s epilepsy, predicting seizures is an important goal of clinical management and treatment. A question of fundamental importance to seizure prediction is to better understand the brain dynamics during the transition from interictal to the seizure state. A promising and widely studied candidate for seizure precursor is high frequency oscillations (HFOs). Recent studies have shown that HFOs occur in advance of impending seizures indicating its strong relationship with the seizure onset zone6,7.

Most seizure prediction methods are based on mathematically extracting specific features from multiscale intracranial electroencephalogram (IEEG) recordings8. Although studies have focused on prospectively testing these methods, no study to date has yet confirmed the ability of any method to predict seizures better than random9. Invasive intracranial electrodes are required to detect oscillations generated in very localized or deep brain regions. Owing to the IEEG’s insufficient spatial sampling, a barrier to obtaining better seizure predictive rates has been the inconsistency in identifying the earliest changes prior to seizure onset. A deeper understanding of the regional interactions at the beginning and during the evolution of a seizure may help identify the earliest seizure susceptible brain regions10-12.

To advance our knowledge about seizure initiation, functional brain imaging techniques like single-photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI) are used to answer questions regarding whether so-called ‘preictal states’ can be distinguished from the ictal period and the functional networks in the brain responsible for seizure generation. With SPECT’s ability to capture a “snapshot” of cerebral blood flow, studies in13,14 observed cerebral blood flow (CBF) increases a few seconds to several minutes preceding clinical electroencephalographic (EEG) seizure onset in temporal lobe partial seizures. With fMRI’s high spatial resolution and its combination with EEG, fMRI studies recently reported that blood-oxygen-level-dependent (BOLD) changes occur minutes prior to seizure onset, either in animals or in humans15,16. However, due to relatively low temporal resolution and lack of portability, such techniques are not suitable for rigorous
statistical seizure prediction analyses, which often require continuous longterm monitoring of the period prior to, during, and after seizures, as well as the continuous interictal state.

Taking advantage of the near-infrared (NIR) window in tissue, optical imaging brings new opportunities to brain studies. Changes in optical spectroscopic properties of brain tissue are often correlated with changes in neuronal activity. Activity evoked optical spectroscopic changes measured in the brain are most likely generated by changes in cerebral hemodynamics. Therefore, such optical properties changes are also the hallmarks of seizure generation and the spread of ictal neural activity. With the ability to provide excellent brain surface maps, intrinsic optical signal (IOS) methodologies have been used in animal models to study seizure foci localization, propagation, and neurovascular coupling. It was also found in an intraoperative procedure that local change of intrinsic signal preceded the seizure onset by approximately 20 seconds. Functional near-infrared spectroscopy (fNIRS) has also been applied to investigate epileptic seizures and other brain disorders, especially in daily clinical use due to its non-invasive and portable merits. A major limitation of IOS and fNIRS is that each fails to provide much depth information. However, depth information can be obtained by using tomographic reconstruction methods such as diffuse optical tomography (DOT). Here, multispectral NIR light is sequentially delivered to the tissue at multiple locations and the diffusely scattered light are measured at multiple positions along the scalp, which are used to reconstruct the spatial distribution of tissue absorption and scattering coefficients at each wavelength through a light propagation model in tissue. The images of absorption spectra are then used to derive the images of functional parameters such as oxy- and deoxyhemoglobin. Indeed, DOT offers sub millimeter resolution within centimeter thick tissue. In the area of neuroimaging, DOT has been applied to study hemodynamic response to motor and visual stimulations in several cortical areas and to quantify cerebral perfusion and blood flow, both in humans and small animals. These studies show that DOT is capable of detecting hemodynamic responses spatially equivalent to fMRI.

The primary goal of this study was to identify hemodynamics prior to and during seizures in a rat model of generalized epilepsy. Our fast three-dimensional (3-D) DOT system, combined with simultaneous IEEG recordings, allowed us to track both changes in hemodynamics and local field potentials (multiunit activity) throughout the brain during Pentylenetetrazol (PTZ)-induced generalized tonic-clonic seizures in rats. We found that hemodynamic changes precede seizure onset by about 1 to 3 minutes, and that seizure zones initiated from a regional onset and then distributed to the entire brain. We also observed the decoupling between local field potentials and hemodynamic changes, accompanied by concurrent scattering changes. For the first time, the whole process of generalized tonic-clonic seizures was recorded in vivo, including the interictal state, the preictal transition state, and the ictal onset state. Our findings suggest that a pre-seizure state can be detected with optical imaging and the “generalized” seizures originate from a regional (focal) onset zone.

Results

Hemodynamic responses elicited during generalized PTZ-induced seizures. Generalized tonic-clonic seizures and corresponding hemodynamic changes were observed in 9/9 seizure rats elicited by i.p. PTZ. Time courses of the EEG gamma power and hemodynamic changes for all nine experiments are shown in supplementary Fig. 1S. A representative (rat #1) co-located neural and hemodynamic response from left primary motor (M1) is shown in Fig. 1. Time series data of local concentration of oxy- [HbO2] and deoxyhemoglobin [HbR] were extracted from the same location as the IEEG recording. Continuous spike and wave discharges occurred abruptly within 4 minutes after i.p PTZ (Fig. 1a). Power spectrum density changes can also be seen on the time-frequency analysis (Fig. 1b). Significant hemodynamic changes (t-test, p < 0.05) are displayed in Fig. 1c. During the resting state, [HbO2] and [HbR] showed no significant changes, only with little fluctuations around their resting state values of ~30 μM and ~10 μM, respectively. Chro-mophore concentrations were calculated from the reconstructed absorption coefficients distribution from three wavelengths. After PTZ injection, a 15 μM decrease of [HbO2], and a corresponding 3 μM increase of [HbR] was observed. The hemodynamic responses were comprised of a large change of [HbO2] with a relatively smaller change of [HbR]. These findings are consistent with other studies of epilepsy using optical methods like NIRS, diffuse optical imaging (DOI), where [HbO2] and the concentration of total hemoglobin [HbT] followed similar trends accompanying by a small variation of [HbR]. Interestingly, IEEG power increase (Fig. 1b) in the fast gamma frequency range (80 ~ 120 Hz) was observed before seizure onset (Pearson Correlation: 0.89) with the hemodynamic change. Similar phenomenon has been reported before by comparing optical intrinsic signals and local field potentials (LFPs). In the age-matched sham saline control experiments, no detectable IEEG spikes or significant hemodynamic changes were observed (Fig. 1d–f).

Hemodynamic changes precede IEEG spikes. We also found that hemodynamic changes began before seizure onset in 9/9 rats, although the pre-seizure time (lead time) for observed changes varied for each seizure. A statistics of such neural and hemodynamic changes for all nine rats experiments is listed in the supplementary Table S1. The lead time represents the interval between the moment when the first significant hemodynamic changes (t-test, p < 0.05) appear in any voxel and the electrographic onset time. A representative result from rat #2 is shown in Fig. 2. Locations of two regions of interests (ROIs), one from the motor cortex and the other from temporal lobe are shown in a coronal slice (Fig. 2a left). By tracking the change of [HbT], whereas a significant hemodynamic decrease (t-test, p < 0.05) in ROI1 (Fig. 2c, top) and increase in ROI2 (Fig. 2c, bottom) occurred about 20 seconds after PTZ injection, the IEEG (recorded at ROI1) seizure onset began 1 minute after PTZ injection (Fig. 2b). The gamma power, having a tight relationship with [HbT] (Pearson correlation, 0.85), started to change almost at the same time as [HbT] (Fig. 2b). In Fig. 2d, the activation maps of [HbT], superimposed on the MRI template, shows the evolution of hemodynamic distribution in the whole brain. We use cool or warm color to specify [HbT] decreasing or [HbT] increasing. [HbT] started to change in the preictal period and remained relatively stable after tonic-clonic seizure onset. In Fig. 2e, we compare the results from all experiments (n = 9) of the time lengths between PTZ injection and occurrence of electroencephalographic seizure onset, significant gamma power changes and significant hemodynamic changes. On average, the hemodynamic change, similar to the gamma power change, leads IEEG seizure onset ~80 seconds.

Decoupling of IEEG and hemodynamic changes. Both neural (local field potentials) and hemodynamic activity (HbT, HbO2, and HbR) from bilateral primary motor cortex (M1), bilateral somatosensory cortex trunk region (S1Tr), bilateral hind limb primary somatosensory cortex (S1HL), and bilateral anterior thalamic regions, were studied using simultaneous IEEG and DOT (Fig. 3a). Results of seizure experiment from rat #2 are shown here. We note that a local dissociation between hemodynamic changes and local field potentials (left M1, S1Tr, Thalamus and right M1) during the preictal period was observed, suggesting a lag between measurable local neural activity (gamma power) and local hemodynamics relative to seizure onset. Moreover, changes of reconstructed local scattering coefficients, as much as 0.3 mm−1 compared to the resting state value of ~1.3 mm−1, occurred concurrently with the hemodynamic changes. These findings further suggest that changes in
hemodynamic activity alter tissue scattering properties, not readily visible with concurrent IEEG.

We also observed early gamma power increase during the preictal period from most IEEG electrodes. This may indicate an early buildup of synchrony as a preparation for the generalized seizure onset. During seizures, we can see that seizure spikes with larger intensities (left side) tend to accompany with larger hemodynamic changes, suggesting a correspondence between local neural and hemodynamic activities as seizures progress into ictal stage. Besides, despite the existence of seizure spikes, the hemodynamic changes in S1HL (left and right) and S1Tr (right) have neither obvious increasing nor decreasing trend in such areas. However, large fluctuations in these areas are still notable, indicating that the hemodynamic activities are not as uniformly distributed as neural activities during the “generalized” seizures.

Evolution and distribution of seizures. Amongst all nine rats, we selected three experiments from rat #2, #3 and #4 with increasing seizure intensities to show the overall seizure evolutions through the whole brain. Fig. 4a gives the positions of five coronal slices selected for activation maps. The coordinates of these slices range from −5 mm to 5 mm relative to bregma. IEEG recordings and statistical activation maps that depict [HbT] increases (warm colors) and decreases (cool colors) compared with the baseline DOT images (t-test, p < 0.05), are shown in Fig. 4b–d.

Seizure activities shown in Fig. 4c were more robust than that depicted in Fig. 4b in term of amplitude and frequency of spikes. Accordingly, larger hemodynamic changes, positive or negative activations were observed in Fig. 4c compared to changes in Fig. 4b. Amongst seizures Fig. 4d shows a high grade seizure in which continuous and high amplitude polyspikes were seen with concomitant behavioral changes twitching limbs and stretching its body even under anesthesia. Furthermore, at the peaks during the preictal period, the activation regions in Fig. 4b,c remained relatively stable during the ictal period. Conversely, in Fig. 4d, as IEEG spike pattern changed strongly during ictal period, the activation regions changed accordingly. We observed large hypoperfusion area in Fig. 4d, possibly due to the excessive energy consummation of the electrical activities during the fierce seizures onset.

Discussion
Multispectral DOT was used to investigate seizure dynamics in a rat PTZ seizure model. Changes in local and global hemodynamics and local field potentials were identified during seizure initiation and propagation. Early hemodynamic changes with concurrent gamma power increase were found several minutes prior to seizure onset supporting the presence of a preictal state. The early changes in optical scattering property offer a possible explanation for the decoupling of local field potentials from early hemodynamic changes during the preictal stage. We also found that generalized seizures initiate with focal hemodynamic changes and subsequently evolve to widespread changes throughout the brain.

There is general agreement that despite pharmacological, neuro-modulation, dietary, and surgical advances in the treatment of chronic epilepsy, seizures cannot be controlled in as many as 25% patients, and therefore there is a need for new therapeutic approaches. At the same time, there is growing awareness that the development of new therapies has slowed, and to move toward new and more effective therapies, novel approaches to therapy discovery...
are needed. A growing body of research indicates that controlling seizures may be possible by employing a seizure prevention closed-loop treatment strategy. For patients this would be a significant breakthrough as they would not be dependent on daily anticonvulsant treatment. Seizure prevention techniques could conceivably be coupled with treatment strategies aimed at interrupting the process before seizures evolve. Treatment would conceivably occur only when needed: on-demand and in advance of an impending seizure.

To develop seizure prevention/intervention models for a specific application, it is necessary to first establish that a pre-seizure state actually exists. The main finding of our experiments is the early changes during the transition from resting interictal state to ictal state involves spatiotemporal changes in hemodynamics, as well as IEEG gamma power, several minutes in advance of overt seizure spikes. Although the mechanisms of seizure generation are still unknown, there is evidence that measurable changes occur prior to seizures. Accumulated experimental evi-
Evidences have suggested that HFOs in the gamma and fast gamma range (40–120 Hz) in IEEG recordings, are not only an electrical signature of focal epilepsy during seizure-free states, but may also play a causal role in the initiation of seizures. Many neocortical seizures were preceded by a build-up of gamma power prior to seizures. On the other hand, works on animals and humans indicate that gamma band neuronal synchronization in electrophysiological recordings is closely related to the BOLD signal. In parallel to these evidences, our findings suggest that DOT can serve as an alternative technique to IEEG to detect preictal activities noninvasively.

Indeed anecdotal reports of preictal changes in brain hemodynamics have been reported with SPECT, fMRI, and optical imaging. The dissociating hemodynamic changes from neural activities have been discussed in a review with several hypotheses. One hypothesis is that such decoupling is due to the inability of scalp EEG or Electrocorticography (ECoG) to detect activities deep in the brain or high frequency local field potential activity. According to our experiment results, with multisite electrode recordings distributed in several regions within the brain, such high frequency changes can be observed in some IEEG channels, and came together with the hemodynamic changes (Fig. 3b). Our findings of the concurrent optical scattering change also support another hypothesis that hemodynamic responses could be triggered by increased activities of glial cells. It has been shown that glial cells play a key role in neurometabolism and neurovascular coupling and cell swelling and shrinkages alter the tissue density, thereby changing the scattering properties of brain tissues. Thus, glial cell volume changes, which could change tissue scattering properties but not detectable in neuronal activity, could induce the hemodynamic activities preceding neuronal activity. Such decoupling suggests that DOT can be more sensitive than IEEG recordings to detect subtle changes.

Generalized epilepsy is characterized by seizures with diffuse, bilateral cerebral involvement, and affects the entire forebrain including both hemispheres of the brain, in contrast to the focal seizures, which originate from focal brain regions and affect only a part of the brain at onset. In generalized epilepsy multifocal
Neocortical regions have the potential to initiate a seizure. Networks or neuronal circuits involved in seizures include the seizure focus, the initiating circuit, the pathways of spread, and the modulatory centers. It has been shown that the interaction between the cortex and several nuclei of the thalamus involved in the key circuit for discharges in the generalized seizures. As can be seen in Fig. 4, during early seizure onset, both the thalamus and bilateral cortex are activated, and as the seizure progresses, adjacent regions are progressively activated.

Despite different patterns of seizures and different origin locations, hemodynamic changes all developed from local regions involving bilateral cortex and thalamus to the larger area. These findings suggest that so-called "generalized" tonic-clonic seizures may be in fact localized, at least during the early phase of seizure onset. Indeed, the concept of a cortical focus has also been previously discussed in other generalized seizure studies. The concept of focal cortical origins during generalized tonic-clonic seizures provides implications for understanding and treating this disorder in human patients, which may ultimately enable targeted therapies including neurostimulation, disconnection procedures, and novel pharmacological treatments directed at involved regions.

In this study, we did not apply statistical group analysis as has been used in other studies. As shown in Fig. 4b–d, for the PTZ-induced generalized tonic-clonic seizures model, seizures can occur in a variety of IEEG manifestation, even with the same PTZ injection due to individual variation for each rat. Such changes in electrophysiology...
accompany with hemodynamic change with distinct patterns in terms of both spatial and temporal distribution. Group analysis could potentially smooth or even remove subtle changes in certain areas.

Heterogeneous patterns of hemodynamic changes have been observed in our experiments. Studies of functional activation using optical techniques have shown the hemodynamic response in newborn infants and a rat model to be highly variable. SPECT studies of both spontaneous secondary generalized and electroconvulsive therapy-induced tonic-clonic seizures reported heterogeneous patterns of changes in CBF during seizures. In IMRI studies, a mixed pattern of focal increased and decreased BOLD activity has been shown in humans and animals during generalized seizures, and relationship between neuronal activity and BOLD signals may depend on brain region and state. One hypothesis is that preictal vasocostriction surrounding the seizure focus could actively shunts blood to the ictal focus to prepare the focus for the impending dramatic increase in neuronal activity and metabolism. However, as shown in Fig. 3, with either increase (left thalamus and S1Tr) or decrease (M1) of [HbO2] all came with significant gamma power increase (t-test, p < 0.05) during the preictal period and spikes strong seizure during ictal period. Moreover, although it is well understood that the increase in CBF caused by the cerebral metabolic rate of oxygen (CMRO2) and glucose comes with increased neuronal activity, it is still not clear if such CBF increase can satisfy local metabolic demands. Depending on the interplay between hemodynamics and metabolism, increase of [HbO2] could arise when the relative increase in CBF is more than the actual need of metabolism. On the other hand, decrease of [HbO2] suggests an excessively high metabolic demand without appropriate compensatory blood flow especially around the seizure focus, rather than a decrease in neuronal activity.

While our study provides information for seizure dynamics, it does have several limitations that are worth noting and should be addressed through future investigation. First, the imaging domain should cover the whole brain. Although a large portion of the rat brain was included in our current study, we cannot exclude possibly important changes in more posterior brain regions. For example, much previous work supports an important role for the brainstem in generalized seizures both in human patients and animals.

Second, the studies should be done continuously and over weeks in freely moving animals. Also, although the PTZ animal model serves the role of a good proof-of-concept model to test our primary hypothesis, future studies should be focused on chronic epilepsy via an animal model with intermittent spontaneous seizures. Third, anatomical information from MRI should be used in the future study. Earlier studies have shown that incorporating subject-specific structural priors into the DOT reconstruction process could improve the location and quantitative accuracy in human head or breast imaging. Besides, subject-specific wearable interface can be built with the MRI-derived anatomical information, and applied to freely moving rats to facilitate the study of chronic epilepsy with spontaneous seizures.

In summary, through the use of a PTZ-induced generalized seizure model, we were able to investigate early seizure initiation and onset as well as seizure propagation patterns. Such studies are essential to understanding ictogenesis and guiding seizure prevention strategies thereof. To this end, DOT offers an opportunity for observing global hemodynamic changes related to neural activities and for capturing the genesis and distribution of generalized seizures. The possibility of seizure prediction, most importantly, has given hope for new warning and therapeutic devices for individuals who cannot be successfully treated with current therapies.

**Methods**

All experimental procedures were approved by IACUC committee at the University of Florida.
collection at 3 NIR wavelengths (660, 780, and 850 nm) for whole brain imaging in 14.4 Hz. The data acquisition for both DOT and EEG recording was synchronized through a controller board (PCI-7811R, NI).

In order to provide reliable data for the image reconstruction and analysis, noise should be firstly considered and reduced. Basically, instrumental noise, experimental errors and systemic physiological artifacts compose the three major sources of noise. A recent system upgrade to DAQ boards (NI-6358) with higher sampling rate allowed an online signal averaging of 100 times for one set of sampling data, which significantly suppressed the random noise as a large part of instrumental noise. A temporal low-pass filter (1 Hz) was applied to attenuate physiological artifacts from movement, heart beating, and breathing.

After the multispectral signals are collected, an effective model-based reconstruction algorithm is critical for accurate image reconstruction in DOT brain imaging. Our DOT algorithm is based on the finite element solution to the following photon diffusion equation coupled with Robin boundary conditions:

$$V \cdot D(r) \nabla \Phi(r) - \mu_s(r) \Phi(r) = -S(r)$$  \hspace{1cm} (1)

$$-D(r) V \Phi_{\text{bc}} = \alpha \Phi$$  \hspace{1cm} (2)

where $\Phi(r)$ is the photon density, $\alpha$ is a coefficient related to the internal reflection at the boundary, $\mu_s(r)$ are the diffusion and absorption coefficients, and $S(r)$ is the source term. The diffusion coefficient can be written as $D(r) = 1/(\mu_s(r) + \mu_t(r))$, where $\mu_t(r)$ is the reduced scattering coefficient. The objective of the DOT reconstruction algorithm is to recover $\mu_s(r)$ and $\mu_t(r)$ at all positions inside the computational domain, achieved through a regularized Newton’s method to update an initial optical property distribution iteratively in order to minimize a weighted sum of the squared difference between computed and measured optical data along its boundary. A calibration method was applied to reduce the errors caused by the use of different source/detection intensities/positions and the system hardware.

Although the continuous wave DOT is theoretically challenged by Arridge and Lionheart, claiming that simultaneous unique recovery of $\mu_s(r)$ and $\mu_t(r)$ cannot be achieved from CW data, our approach is different due to several factors. First, regularization techniques were used to solve the ill-posed inverse problem during reconstruction. The core procedure in our reconstruction algorithms is to iteratively solve the following regularized matrix equation:

$$(f^T J + \lambda I) \Delta \Phi = f^T (\Phi^m - \Phi^0)$$  \hspace{1cm} (3)

where $\Phi^m$ and $\Phi^0$ are the measured and computed photon density; $f$ is the Jacobian matrix consisting of the derivatives of $\Phi$ with respect to $D$ and $\mu_s$ at the boundary measurement sites; $I$ is the identity matrix, and $\lambda$ is the regularization parameter determined by combined Marquardt and Tikhonov regularization schemes ($\lambda = 0.5$ was used in this study). We have found that the quality of inversion was not sensitive to the choice of this parameter. $\Delta \Phi = (\Delta D_1, \Delta D_2, \ldots, \Delta D_m, \Delta \mu_s_1, \Delta \mu_s_2, \ldots, \Delta \mu_s_m)^T$ is the update vector for the optical property profiles, where $m$ is the total number of nodes in the finite element mesh. Second, an optimization procedure was applied to search for the best parameter set of initial $\mu_s(r)$ and $\mu_t(r)$ based on a priori information (biologically feasible ranges of these initial parameters). The optimization procedure seeks best parameter space in the vicinity of the exact one and has allowed the minimization of the object function to be confined in a $(\mu_s(r), \mu_t(r))$ parameter subspace that is close to the exact solution. Using extensive laboratory experiments, we have demonstrated that the scattering and absorption coefficients can be distinguished and the solution is unique in many practical scenarios. These facts have also been corroborated by other researchers.

A three-dimensional cylindrical FE mesh, consisting of 22,764 tetrahedron elements, was used for the image reconstruction. To efficiently process a large amount of data containing thousands of time points for one set of experiment, a GPU (graphic processing unit) based parallel code was implemented using CUDA programming model (NVIDIA), allowing the time consuming image reconstructions for one set of experiment to be processed within one day.

**DOT analysis and rat atlas registration.** Assuming oxy- and deoxy-hemoglobin (HbO$_2$ and Hb$_R$) are the two major chromophores in the rat brain, the absorption coefficients at 660 nm, 780 nm and 850 nm were used to obtain [HbO$_2$] and [Hb$_R$] using the Beer-Lambert law and least square fitting through pseudo inverse matrix calculation. The concentration of total hemoglobin (HbT) was then calculated by summing up [HbO$_2$] and [Hb$_R$] which is proportional to CBV. HbT has been suggested to map cerebral activity in NIRS for its better spatial specificity than [HbO$_2$] or [Hb$_R$] alone. Activation maps were generated by tracking the change of [HbT] that exceeded significant threshold, because HbT contains the information of both [HbO$_2$] and [Hb$_R$] and reflects the local CBV. For statistical analysis, thirty seconds (432 images) consecutive [HbT] images preceding PTZ injection were chosen as a baseline period to which all analysis periods were compared. Several time series images, each covering a 10 seconds time bin (144 images), were selected from the interictal, pre-seizure, and seizure periods. A t-test was performed by comparing each time bin to baseline using a voxel wise 2-sample t-test, with a significant threshold of $p < 0.05$. Activation maps were then overlaid with template MRI images obtained from the Karolinska rat atlas (http://expmr.ki.se/research/ratatlas.jsp) for anatomical guidance.

Activation maps were visualized through the following procedures. First, Amira (Visage Imaging, Inc.) was used to detect the brain surface from the MRI template and generated a brain mask. Then, DOT image coordinate was aligned with the atlas coordinate by comparing the measured bregma position from experiments and its according position in the atlas. Next, DOT images within the brain mask were interpolated to each voxels (1 mm$^3$) in the atlas using Matlab. Results were converted and stored in NIFTI format, which were visualized with MRlcon (http://www. micro.com/).
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Author contributions
T.Z. developed the imaging system, designed and performed experiments, analyzed data and wrote the paper; J.Z. designed and performed experiments; R.J. performed experiments; H.Y. helped system building; P.R.C. guided the experimental design, data analysis, and wrote the paper; H.J. directed the study and wrote the paper.

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